PROGRAMME

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

XVIIIth International Pathogenic Neisseria Conference (IPNC)

09–14 September 2012

Würzburg

www.ipnc2012.de

XVIIIth International Pathogenic Neisseria Conference (IPNC)

NOVARTIS VACCINES IS PLEASED TO INVITE YOU TO A SYMPOSIUM

An Innovative Step in the Global Fight Against Meningococcal Disease

Tuesday, 11 September 2012
10:30–12:00
Maritim Hotel Würzburg
Franconia Hall
Würzburg, Germany

Welcome and Introduction
Rino Rappuoli and Ulrich Vogel, Programme Chairs

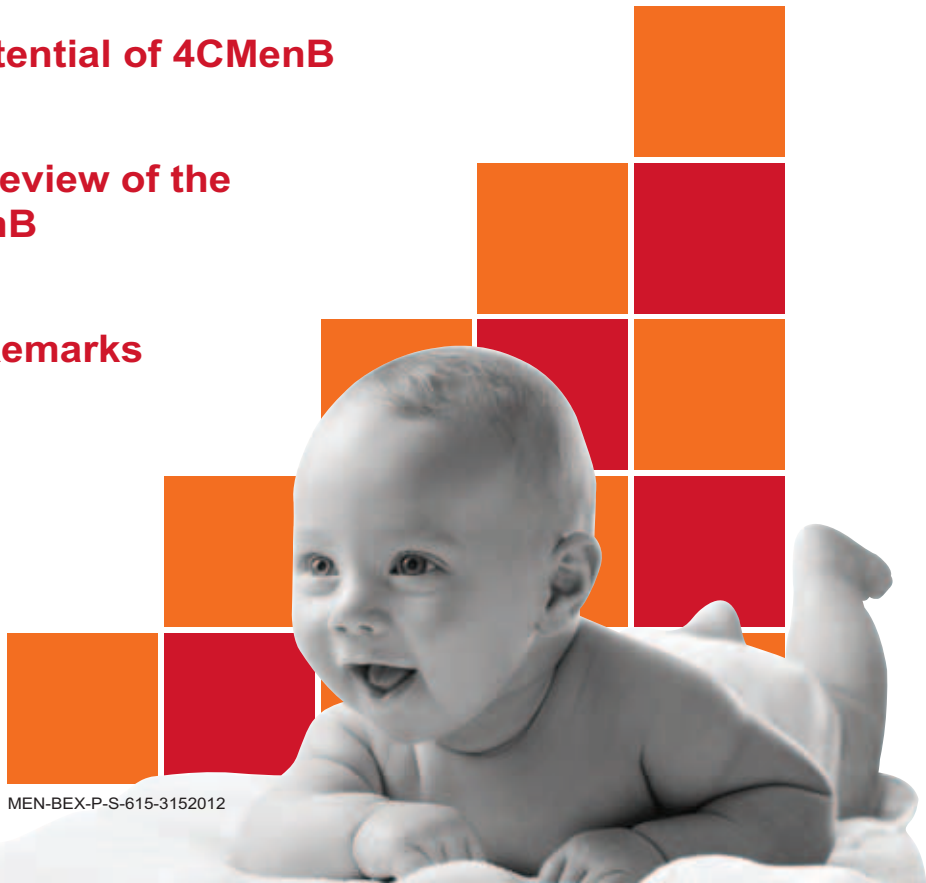
Defining the Multiple Components of an Investigational Serogroup B Vaccine (4CMenB)
Vega Masignani

The Broad Coverage Potential of 4CMenB
Ray Borrow

Fulfilling the Promise: Review of the Clinical Profile of 4CMenB
Peter Dull

Summary and Closing Remarks

Q&A



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We thank the following organisations for their special support:
DGHM – German Society for Hygiene and Microbiology and its Working Groups on
- Microbial Systematics, Infection Epidemiology and Population Biology
- Microbial Pathogenicity

ESCMID – European Society of Clinical Microbiology and Infectious Diseases

FEMS – Federation of European Microbiological Societies

The Igor Stojiljkovic Memorial Fund

Universitätsbund Würzburg

We would like to thank the following media partners for their support:
Berufsverband der Ärzte für Mikrobiologie, Virologie und Infektionsepidemiologie e. V. (Stuttgart/DE)
„Der Mikrobiologe“

Centers for Disease Control and Prevention (Atlanta, GA/US)
“Emerging Infectious Diseases”

Elsevier GmbH (München/DE)
“International Journal of Medical Microbiology”

GIT VERLAG, Wiley-VCH Verlag GmbH & Co. KGaA (Weinheim/DE)
„Management & Krankenhaus“ und „GIT Laborfachzeitschrift“

State at Printing

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Venue and Date
Maritim Hotel Würzburg
Pleichertorstraße 5 • 97070 Würzburg (DE)
09–14 September 2012

Conference Chairs
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Julius-Maximilians-University Würzburg • Institute for Hygiene and Microbiology
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Thomas Rudel
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Ulrich Vogel
Julius-Maximilians-University Würzburg • Institute for Hygiene and Microbiology
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Scientific Advisory Board
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Dan Granoff (Oakland, CA/US)
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Martin Maiden (Oxford/GB)
Xavier Nassif (Paris/FR)
Sanjay Ram (Worcester, MA/US)
Thomas Rudel (Würzburg/DE)
Tone Tønjum (Oslo/NO)
Ulrich Vogel (Würzburg/DE)

Conference Language
The official conference language will be English.

Conference Website
www.ipnc2012.de

Organisation
Conventus Congressmanagement & Marketing GmbH
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Carl-Pulfrich-Straße 1 • 07745 Jena
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ipnc2012(at)conventus.de • www.conventus.de

Design/Layout

Design	www.krea.tif-design.de
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Editorial deadline	20 August 2012



Dear colleagues and friends,

We warmly welcome you to the XVIIIth International Pathogenic Neisseria Conference in Würzburg, Germany. We are convinced that the Neisseria community again will present most interesting new developments in science and innovation. The mere number of submitted abstracts demonstrates impressively the continues high significance of Neisseria research.

For preparation of the meeting and scientific programme we have had a very stimulating meeting with the scientific advisory board, which resulted in the definition of special scientific foci for the conference, and a balanced and well structured programme. Many thanks to all members of the board for their efforts, their expertise, and especially for taking the burden to travel to Germany for a face-to-face meeting.

The discussions at the board meeting brought forward also ideas such as discussion groups on burning questions in the field. These discussion group meetings will be held on Monday and Tuesday and the results of the discussions will be presented to the general audience on Friday morning.

The scientific advisory board also emphasized the importance of gonococcal research in the light of the re-emergence of gonococcal infections and the emergence of multi-drug resistant gonococci. We therefore dedicated one symposium to this special problem.

Besides science, you will have a chance to enjoy the highlights of our small, yet lively University town in the Northern part of Bavaria like culture, architecture, wine and countryside, to name just a few.

We would like to express our thanks to Conventus, especially to Francesca Rustler, for expert help and support in all questions of conference organization and logistics.

We are looking forward to a further IPNC with exciting science, stimulating discussions and exchange and multiple opportunities to initiate collaborations

Matthias Frosch

Thomas Rudel

Ulrich Vogel

Venue and Date
Maritim Hotel Würzburg
Pleichertorstraße 5 • 97070 Würzburg (DE)
09–14 September 2012

Confernce Website
You will find current information on our website at www.ipnc2012.de.

Registration

	Early bird	From 16 July 2012	On site
Graduate Students*	400 EUR	500 EUR	550 EUR
Post-doctoral Fellows*	500 EUR	600 EUR	650 EUR
Regular	600 EUR	700 EUR	750 EUR

* Please provide proof.

Banquet, 13 September 2012 — Regular 50 EUR

Payment/Confirmation of Payment
Registration is subject to available capacities. Conventus GmbH will send you an invoice or confirmation of registration via postal or electronic mail within 14 days after receipt of registration. This invoice is a valid invoice which may be submitted to the local tax and revenue office. All registration fees for the scientific programme and/or social programme will be charged by Conventus GmbH via invoice. Please register any accompanying person(s) for the social programme by name. All fees are due upon receipt of invoice/registration confirmation and should be transferred to the conference bank account and should include participant's name and invoice number. The conference bank account information can be found on the invoice. Payment is also accepted by credit card (MasterCard, VISA, American Express). Should you transfer your invoice amount within 10 days of the start of the event, please present your transfer remittance slip at the check-in desk as proof of payment.

Hotel Reservation/Accommodation
We have reserved a contingent of rooms at special rates in selected hotels in Würzburg. The registration form, rates and information on the hotels can be found on the conference website at www.ipnc2012.de. Please fill out the registration fee and fax it to the fax number provided. Please reference the conference code "IPNC". Please note: The conference organisation acts only as an intermediary party and assumes no liability for reservations. Changes and cancellations should be made directly with the appropriate hotel.

Arrival/Parking

By plane

If you are coming from a non-european country, we recommend flights to Frankfurt International Airport. From there we recommend to take the very comfortable ICE express train leaving directly from the airport every hour.

If you come from Europe the two most comfortable options are:

Frankfurt International Airport (distance to Maritim Hotel Würzburg: 119 km):
Take the ICE train from the airport ("Fernbahnhof") directly to the main station of Würzburg. The journey from Frankfurt airport to Würzburg takes about 1.5 hours.

Airport Nürnberg (distance to Maritim Hotel Würzburg: 107 km):
Take the underground train U2 from Nuremberg airport to the main train station of Nuremberg. From here you drive with the ICE train to the main train station from Würzburg. The journey from the Nuremberg airport to Würzburg takes about 1 hour.

By car

Navigation details: Pleichertorstraße 5 • 97070 Würzburg

Parking

Parking areas ar located near the conference venue. More than 200 underground car parking spaces are available. Parking costs 1.00 EUR per hour and max. 8.00 EUR per day. In addition you can use more than 100 parking spaces directly at the Congress center. The Maritim Hotel Würzburg is directly connected to the Würzburg Congress.

Furthermore you can use the parking place "Am Main" (150 parking spaces, prices similar to the o. m. parkhouse).
On the other side of the river main there is also a free parking possibility called "Talavera", just 5 minutes away by foot.

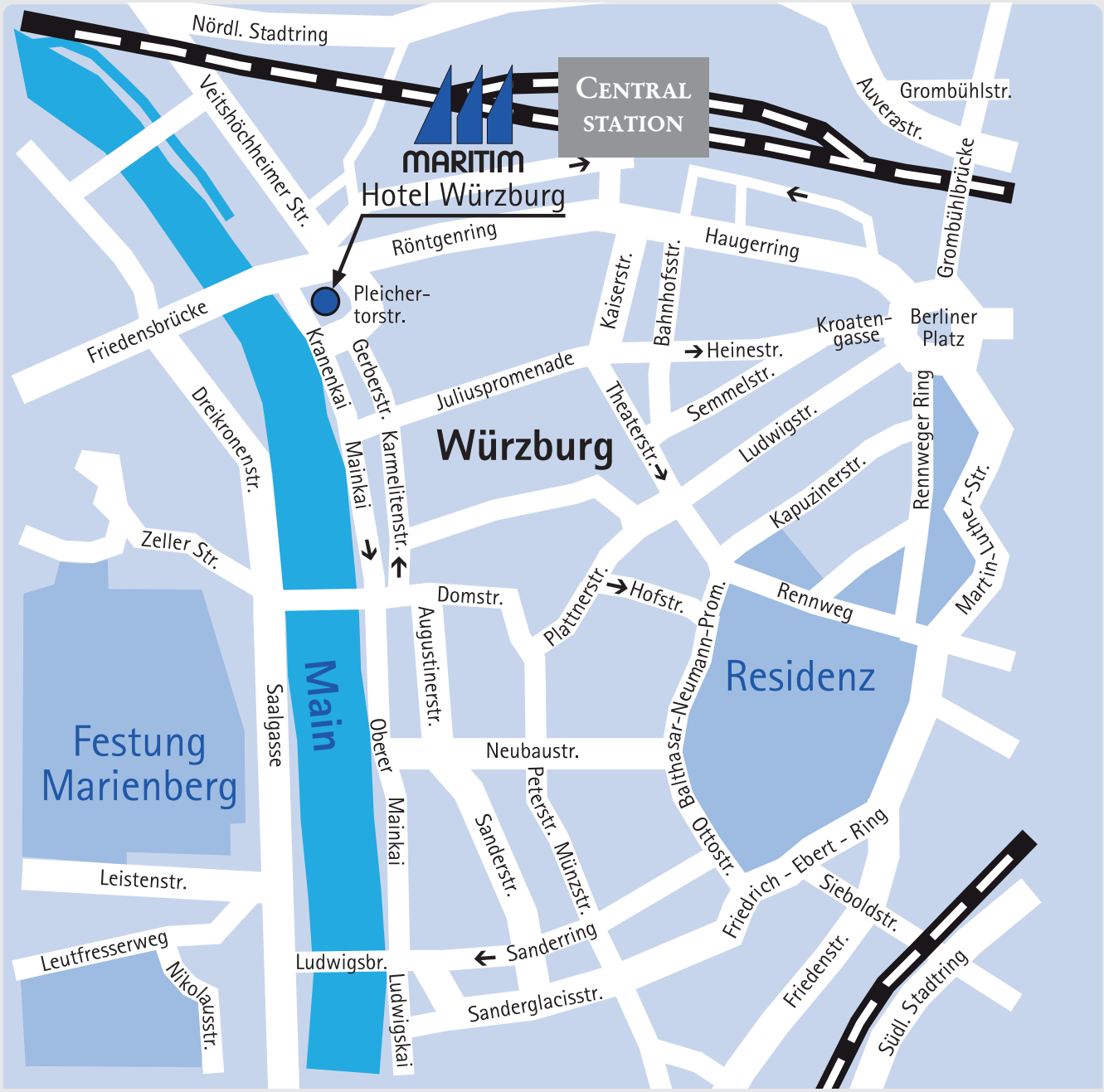
Public Transportation to Venue

From the railway station, you reach the tram stop "Am Congress Centrum" with the lines 2 (Würzburg Hauptbahnhof-Zellerau) or line 4 (Würzburg Sanderau-Hauptbahnhof-Zellerau).

City Plan

Source: Congress - Tourismus – Wirtschaft, Eigenbetrieb der Stadt Würzburg

Route Map


Nördl. Stadtring
Veitshöchheimer Str.
Central Station
Auverastr.
Grombühlstr.
Grombühlbrücke
MARITIM
Hotel Würzburg
Röntgenring
Haugerring
Pleicher-
torstr.
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gasse
Berliner
Platz
Heinestr.
Friedensbrücke
Kranenkai
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Semmelstr.
Ludwigstr.
Rennweger Ring
Dreikronenstr.
Mainkai
Karmelitenstr.
Würzburg
Theaterstr.
Kapuzinerstr.
Martin-Luther-Str.
Zeller Str.
Domstr.
Plattnerstr.
Hofstr.
Rennweg
Augustinerstr.
Residenz
Balthasar-Neumann-Prom.
Main
Saalgasse
Oberer
Mainkai
Neubaustr.
Festung
Marienberg
Peterstr.
Münzstr.
Sanderstr.
Ottostr.
Friedrich - Ebert - Ring
Leistenstr.
Sieboldstr.
Leutfresserweg
Sanderring
Ludwigsbr.
Nikolausstr.
Ludwigskai
Sanderglacisstr.
Friedenstr.
Südl. Stadtring

Cooperation with German Railways (DB)

Good for the environment. Convenient for you. Travel by train to the IPNC 2012.

With the offer of Conventus Congressmanagement & Marketing GmbH and Deutsche Bahn you can save money by visiting the XVIIIth International Pathogenic Neisseria Conference (IPNC)! Get on board and profit from attractive prices and conditions for train travel.

Have a good connection to your venue and to the nature! Compared with a car each journey by train spares the environment two-thirds CO_2 and compared with a plane even respectable 75 percent.

The price for your Event Ticket for a return trip* to Würzburg is:

Offer for conference participants
nationwide uniform fixed price for a specific train (daily) in:
1st class 159 EUR
2nd class 99 EUR

nationwide uniform fixed price without specific train (Mon–Thu) in:
1st class 189 EUR
2nd class 129 EUR

Your ticket is valid from 07–16 September 2012.

Please call our service number **+49 (0)1805 31 11 53**** to book your ticket and quote Conventus as reference. Have your credit card ready please.

We wish you a pleasant journey with Deutsche Bahn!

* Changes and reimbursement before the first day of validity are 15 EUR excluded from the first day of validity onwards. Passengers restrict themselves to a particular train and travel times. For a supplement of 30 EUR in 2nd class resp. 20 EUR in 1st class full flexible tickets are also available.

** The booking line is available from Monday–Saturday 08^{00}–09^{00} hrs. Calls will be charged at 0,14 EUR per minute, the expenses from cell phones max. 0,42 EUR per minute.

Education Credits and Certification

... for attendants from Germany

The certification for the XVIIIth International Pathogenic Neisseira Conference has been requested for the Bayerische Landesärztekammer.

For certification all attendants are required to sign the certification list near the check-in daily for certification. Certificates of attendance may be picked up upon leaving the conference at the check-in.

... for attendants from abroad

The certification for the XVIIIth International Pathogenic Neisseira Conference has been requested for the EACCME – European Accreditation Council for Continuing Medical Education

Certificates of attendance may be picked up upon leaving the conference at the check-in.

List of Attendants

Please remember to sign the list of attendants (with barcode, if required or applicable) which is at the check-in.

Certificate of Attendance

Certificates of attendance are available on the last day of the conference at the check-in.

Name Badge

Attendants and registered accompanying persons will receive a name badge after registration. Admittance to the conference and industrial exhibition is only allowed for those with a name badge. Name badges should be worn at all times. Name badges for exhibitors will be given to the exhibit personnel.

Evaluation

Please return your completed and legible evaluation form to the check-in on the last day. We are always striving to provide high conference quality. This goal can only be reached with your help, your active participation and constructive criticism.

Check-In

Check-in desks are located opposite the main entrance to the Hall Franconia. You will receive all ordered tickets there.

Cloakroom

There is a downstairs cloakroom where you can leave your jackets and your luggage free of charge.

Opening Hours

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
Check-In/Cloakroom	16^{00}–20^{00}	07^{30}–19^{00}	08^{00}–18^{30}	08^{00}–16^{00}	08^{00}–19^{00}	08^{00}–13^{00}
Poster Exhibition		10^{40}–19^{00}	10^{00}–18^{30}	10^{10}–16^{00}	10^{20}–19^{00}	10^{30}–12^{30}
Speakers Ready Room	16^{00}–19^{20}	07^{30}–19^{00}	08^{00}–18^{30}	08^{00}–16^{00}	08^{00}–19^{00}	08^{00}–11^{15}

Internet

There are computers with internet access available at the Baxter Media Lounge. Please follow the signage on site.

Baxter

Publishing of Abstracts

Abstracts which are presented during conference will be published within this programme book (from page 75) and can be picked up at the check-in.

Catering

Catering will be provided during the official programme breaks at the points marked "Catering" (see page 70).

Smoking

Smoking is prohibited inside the conference venue.

Restrooms

Please follow signs or ask at the check-in.

Service for Handicapped Persons

The premises are suitable for the handicapped.

Conference Presentation Types

Invited Oral Presentation

Plenary Sessions
Chairs of plenary sessions are kindly asked to review poster abstracts before the meeting and briefly refer to exciting posters during the plenary sessions. The duration of most invited talks is 15 min plus 5 min discussion, in few cases the time is limited to 10 min plus 5 min discussion. Please note that the plenary sessions comprise 5–10 min for chairpersons to comment on poster sessions.

Scientific Symposium
There will be a scientific symposium entitled "Antibiotic Resistant Gonorrhea – a Call to Action" organized by A. Jerse.

Industrial Symposium
There will be two symposia on vaccine development organized by vaccine manufacturers.

Poster Discussions

Discussion Groups
Discussion groups are workgroup meetings on dedicated topics. We will have 5 discussion groups during the IPNC, for which registration prior to the meeting is required. The results of the discussion groups will be presented to the general audience on Friday morning by the conveners of the groups.
For more information please see also page 22, 25, 31.

Please register online at www.ipnc2012.de.

Venue

Conference Centre
Wine Estate Juliusspital
Klinikstraße 1 • 97070 Würzburg

Legend

supported with a FEMS YSMG

supported with a Igor Stojiljkovic Grant

supported by ESCMID

P – Poster
O – Oral Presentation
* – Presenting Author

Discussion Group 1
Gonococcal Vaccines – Is it Time to Re-visit an Old Idea

Discussion Leaders: Ann Jerse and Peter Rice
Gonococcal infection/disease remains a worldwide public health problem with an incidence of 106 million new cases each year. Gonococcal infection in men also enhances the risk of HIV-1 transmission to female sex-partners five-fold. In recent years, treatment options for gonorrhea have been curtailed severely because of emergence and worldwide spread of strains that are resistant to almost all antibiotics used for treatment. The full impact of antibiotic resistance on the burden of disease has yet to be realized, but there is an urgent need to prevent gonorrhea; uniform vaccination of persons at greatest risk would be an effective deterrent. Choice of immunogen(s) and optimization of vaccine delivery remain enigmatic and immune correlates of protection in the human need better definition. Vaccine/infection models that represent/predict human immune responses will require further development to better understand what is needed to secure a protective immune response to vaccination. The Discussion will focus on these points and will assemble a small group of speakers who will give short presentations to address these issues from a broad perspective.

Discussion Group 2
Working with meningococci in the laboratory

Discussion Leaders: Ed Kaczmarski and Steve Gray
The potential hazards of working with meningococci are well known, specifically those arising from generation of aerosolised organisms. Despite understanding the risk and having the means to mitigate it there continue to be reports of laboratory acquired infections from a variety of settings including routine diagnostic and research. It is important that colleagues working with the organism appreciate that their actions (or inactions) will impact upon fellow scientists around the globe and that sharing experience of incidents, root cause analysis and practical remedial measures will assist in reducing the risks.
Has the real risk of working with the meningococcus been revealed? Do current activities either diagnostic or research, carry an acceptable risk to laboratory workers? Re-categorisation of the meningococcus to a higher containment level would impinge significantly on routine diagnostic laboratory work and greatly limit the ability to perform some assays important for vaccine development and assessment.
The workshop will use topic headings from the UK Meningococcal Reference Unit, "Local Code of Practice for Working with Meningococci" to present questions to the registered participants. It is hoped that participants will feel free to engage in open discussion to achieve the objective of risk reduction by sharing best practice.
Areas for exploring will include: documentation, training, microbiological safety cabinets, personal protective equipment (PPE), liquid manipulations, use of non-viable material, incidents and spillages, incident reporting and staff immunisations. The possibility and practicality of peer review safety audits will be raised. Is this a cost we should all build into our work programmes or conversely, can we afford not to?

Discussion Group 3
Time to revisit meningococcal capsule nomenclature

Discussion Leaders: Odile B. Harrison and Martin C.J. Maiden
Despite the centrality of the capsule to the virulence of the meningococcus, the situation with its nomenclature has not been ideal for many years. Most of us refer to the classic 1987 paper by Neylan Vedros describing the '13 capsular groups'1 but no-one actually uses the nomenclature that he proposed and the article is quite inaccessible. There are also at least two distinct nomenclatures for the capsule genes. On the basis of published and unpublished data and sequence analysis of the capsule region, we have shown that there are in fact only 11 distinct capsular regions known in meningococci (A, B, C, E, H, IK, L, W, X, Y, Z), plus the capsule null sequences. With colleagues, we propose a revised nomenclature for meningococcal serogroups and a new, unified genetic nomenclature for the cps region. This group is a forum for delegates to discuss this proposal.

Discussion Group 4
'From genes to genomes – current status of the Neisseria reference libraries hosted on PubMLST.org'

Discussion Leaders: Keith A. Jolley and Martin C.J. Maiden
The PubMLST Neisseria database has hosted allelic diversity data for multilocus sequence typing (MLST), major antigens, and antimicrobial resistance genes in increasing numbers since 1998 and currently has records for approximately 20,000 isolates sampled from over 100 countries. The database began hosting genomic data, in addition to single and multiple locus data in 2009. The number of meningococcal genome sequences has increased in the past two years and, with initiatives that include the Meningitis Research Foundation Meningococcal Genome Library Project, this will continue at an increasing rate. PubMLST is making assembled annotated sequences available to the community with more than 1600 annotated genes available for all whole genome submissions so far. For this resource to be further developed, members of the Neisseria community are invited to engage with the community annotation process that was started at the IPNC in Banff in 2010.

Discussion Group 5
Infection models – What do we have – What do we need?

Discussion Leaders: Xavier Nassif and Thomas Rudel
This discussion group aims at reviewing and evaluating the various animal models which are presently available for the study of pathogenic Neisseria. Very short presentations will be given for each model (transgenis mice, humanized mice, models using Macaques...) these presentations will serve as the basis for a discussion aiming at identifying the need of the community.

Submitting your Presentation/Technical Information

Please prepare your oral presentation slides in 4:3 aspect ratio.

A presentation notebook with a PDF reader and MS Office PowerPoint 2010/2007 will be provided. We regret that the use of personal notebooks is not possible because it may interrupt the flow of the programme in the lecture hall.

A notebook, presenter and laser pointer are available at the speaker's podium in the lecture hall. A technical supervisor can help you.

Please note: Certain encodings for video and audio files could lead to problems. Please visit our speakers ready room.

Should you wish to use non-digital equipment, please contact us. We can be reached at ipnc2012@conventus.de.

Speakers Ready Room

The speakers ready room/Baxter Media Lounge is also located opposite the main entrance to the Hall Franconia. Please follow the signage on site or ask at the check-in.

Please submit your presentation on the day before your presentation, but no later than 2 hours before the presentation should begin. You may view and/or edit your presentation.

For submission, please use a USB flash drive, CD or DVD disc which should not be protected with software.

Speaking Time

Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt your presentation if you should overrun your time limit.

Posters

Posters should be no larger than DIN A0 (84.1 cm x 118.9 cm; 33.1 inch x 46.8 inch). Poster pinboards are 120cm x 150cm. They are only to be used with the designated pins. Pinboards will be numbered. You will find your poster number in the programme book on page 34. The poster number was provided to presenters upon notification of acceptance.
Posters should be hanging on Monday, 10 September 2012 until 10^{40} a.m. and removed on Friday, 14 September 2012 until 11^{10} a.m. Poster presenters are asked to be present during the respective poster sessions.

Programme Overview

	Sunday, 09/09/2012	Monday, 10/09/2012	Tuesday, 11/09/2012	Wednesday, 12/09/2012	Thursday, 13/09/2012	Friday, 14/09/2012
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09:50			**Coffee break**	**Coffee break**	**Coffee break**	**Coffee break**
10:00						
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10:30			**Novartis symposium** An Innovative Step in the Global Fight Against Meningococcal Disease p. 23			
10:40		**Coffee break**				
10:50				**Session 6** Re-emergence of gonococci: Mechanisms of antimicrobial resistance p. 26	**Session 9** Re-emergence of the gonococcus – Modulation of immune responses p. 28	**Session 11** Population biology (cont'd) p. 32
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14:50			**Coffee break and poster discussion 3** Polysaccharide vaccines (137–155) Tools for studying neisserial infections (156–161) Antibiotic resistance (162–180) Global fight against mening-ococcal disease (181–198) p. 24		**Coffee break**	
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17:10		**Coffee break and poster discussion 2** The surface of *Neisseria* (053–072) Population biology (073–096) Epidemiology (097–136) p. 22				
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17:30						
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18:20	**In memoriam: Harry Smith** J. A. Cole (Birmingham/GB)					
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18:40	**Invited talk** J. Vogel (Würzburg/DE)					
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19:00						
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21:00						

group 1 – Gonococcal vaccines (convener: A. Jerse)
group 2 – Working with meningococci in the laboratory (convener: S. Gray)
group 3 – Meningococcal capsule nomenclature – time for update (convener: O. Harrison)
group 4 – From genes to genomes – current status of the Neisseria reference libaries hosted on PubMLST.org (conveners: M. Maiden & K. Jolley)
group 5 – Infection models – What do we have - What do we need? (conveners: X. Nassif & T. Rudel)

18^{00}–18^{20}	**Welcome address** M. Frosch (Würzburg/DE)
18^{20}–18^{40}	**In memoriam: Harry Smith** J. A. Cole (Birmingham/GB)
18^{40}–19^{20} O1	**Invited talk** An RNA perspective on bad microbes J. Vogel (Würzburg/DE)
19^{20}–20^{30}	**Welcome reception**

08^{30}–10^{30}	**Session 1 • Gene regulation and sRNA in Neisseria**
Chairs	J. Vogel (Würzburg/DE), T. Tønjum (Oslo/NO)
08^{30} O 02	Global Transcriptome Analysis of Neisseria gonorrhoeae unveils a multitude of small regulatory RNAs *M. Albrecht, E. Hausser, T. Rudel (Würzburg/DE)
08^{50} O 03	Repertoire and ex vivo expression of small non-coding RNAs in Neisseria meningitidis B. Joseph, S. Schork (Würzburg/DE), M. Dondrup (Bergen/NO), N. Heidrich J. Vogel, M. Frosch, *C. Schoen (Würzburg/DE)
09^{10} O 04	Small non coding RNAs regulating energy metabolism in Neisseria meningitidis *Y. Pannekoek, R. Huis in 't Veld, K. Schipper, A. van der Ende (Amsterdam/NL)
09^{30} O 05	A cis-acting, non-coding RNA is required to form the guanine quartet structure essential for pilin antigenic variation in Neisseria gonorrhoeae *L. Cahoon, H. Seifert (Chicago, IL/US)
09^{50} O 06	Comparison of the locations of Correia Repeat Enclosed Elements and potential small noncoding RNAs in the Neisseria spp *S. Roberts, L. A. Snyder (Kingston Upon Thames/GB)
10^{10} O 07	Capsule biosynthesis in Neisseria meningitidis is regulated by an RNA thermosensor C. Tang (Oxford/GB)
10^{30}–11^{30}	**Coffee break**
11^{30}–13^{00}	**Session 2 • Interaction of Neisseria with mucosal surfaces – the role of iron**
Chairs	A. Schryvers (Toronto/CA), M.-K. Taha (Paris/FR)
11^{30} O 08	Structure-Function Relationships in the Gonococcal Transferrin-Iron Acquisition System *C. Cornelissen (Richmond, TX/US), N. Noinaj S. Buchanan (Bethesda, MD/US)

11^{50} O 09	Insights into the Neisserial Transferrin Receptor via Structures of the Surface Lipoprotein, TbpB C. Calmettes, R.-H. Yu, J. Alcantara, A. Schryvers, *T. Moraes (Toronto/CA)
12^{10} O 10	The structure, specificity and antigenicity of the FrpB iron transporter from Neisseria meningitidis M. Saleem, S. Prince, S. Rigby, M. Imran (Manchester/GB), H. Patel, H. Chan H. Sanders (Sout Mimms/GB), M. Maiden (Oxford/GB) I. Feavers (Sout Mimms/GB), *J. Derrick (Manchester/GB)
12^{30} O 11	Neisseria gonorrhoeae Modulates Iron-Limiting Innate Immune Defenses in Macrophages to Facilitate Iron Acquisition *S. Zughaier (Atlanta, GA/US)
13^{00}–15^{00}	**Lunch and poster discussion 1** Gene regulation (P 001–P 017) Interaction of Neisseria with mucosal surfaces (P 018–P 052)
15^{00}–15^{30} O 12	**Invited talk** Evolution and function of O-linked protein glycosylation in the genus Neisseria M. Koomey (Oslo/NO)
15^{30}–17^{00} Chairs	**Session 3 • Interaction of Neisseria with mucosal surfaces – pilus und opa protein** M. Koomey (Oslo/NO), T. Rudel (Würzburg/DE)
15^{30} O 13	Structural Characterization of Outer Membrane components of the Type IV pili system in pathogenic Neisseria *M. Nascimento, S. Jain (Marburg/DE, Groningen/NL) K. Mościcka (Groningen/NL), M. Bos (Utrecht/NL), E. Pachulec M. Stuart, W. Keegstra, E. Boekema (Groningen/NL) C. van der Does (Marburg/DE)
15^{50} O 14	Structure and assembly of an outer membrane channel for type IV pili in Neisseria meningitidis J. Berry (Manchester/GB), M. Phelan (Liverpool/GB), R. Collins T. Adomavicius (Manchester/GB), T. Tønjum, S. Frye (Oslo/NO), L. Bird R. Owens (Oxford/GB), R. Ford (Manchester/GB), L.-Y. Lian (Liverpool/GB) *J. Derrick (Manchester/GB)
16^{10} O 15	The gonococcal type IV pilus – a motor with two speeds R. Kurre, K. Ramakrishnan, N. Kouzel, *B. Maier (Cologne/DE)

16^{30} O 16	Type IV pili of Neisseria meningitidis recognize and bind to sialic acid-containing signaling-receptors *M. Coureuil, H. Lécuyer, S. Doly, X. Nassif, S. Marullo (Paris/FR)
16^{50} O 17	Rethinking the Role of Opa in Neisserial Pathogenesis *D. Stein, W. Song, A. LeVan, L. Zimmerman, V. Edwards A. Mahle (College Park, MD/US)
17^{15}–19^{00}	**Coffee break and poster discussion 2** The surface of Neisseria (P 053–P 072) Population biology (P 073–P 096) Epidemiology (P 097–P 136)
19^{30}–21^{00}	**Discussion groups (registration required)**
	group 1 – Gonococcal vaccines – Is it time to revisit an old idea?
Convener	A. Jerse (Bethesda, MD/US), P. A. Rice (Worcester, MA/US)
	group 2 – Working with meningococci in the laboratory
Convener	E. Kaczmarski, S. Gray (Manchester/GB)
	group 3 – Meningococcal capsule nomenclature – time for update
Convener	O. Harrison (Oxford/GB)

08^{30}–10^{00} **Session 4 • Interaction of Neisseria with mucosal surfaces – signaling mechanisms**

Chairs C. R. Hauck (Konstanz/DE), T. F. Meyer (Berlin/DE)

08^{30} O 18 Recruitment of PI3 kinase to caveolin 1 determines the switch from the local to the invasive stage of gonococcal infection
*M. Faulstich (Würzburg/DE), J.-P. Böttcher, T. F. Meyer (Berlin/DE)
M. Fraunholz, T. Rudel (Würzburg/DE)

08^{50} O 19 Neisseria meningitidis hijacks the host cell focal adhesion complex for invasion
H. Slanina, S. Hebling (Würzburg/DE), C. R. Hauck (Konstanz/DE)
*A. Schubert-Unkmeir (Würzburg/DE)

09^{10} O 20 RNAi screening of the human kinome identified RTKs as a missing link between neisserial microcolony and cortical plaque formation
*C. Lange, C. Rechner, P. Braun, I. Bachmann, F. García, M. Kerr
T. F. Meyer (Berlin/DE)

09^{30} O 21 N. gonorrhoeae causes DNA damageand interferes with host cell cycle regulation
*H. Aro (Stockholm/SE)

10^{00}–10^{30} **Coffee break**

10^{30}–12^{00} **Novartis symposium**
An Innovative Step in the Global Fight Against Meningococcal Disease
Welcome and Introduction

Chairs R. Rappuoli (Siena/IT), U. Vogel (Würzburg/DE)

Presentation 1
Defining the multiple components of an investigational serogroup B vaccine (4CMenB)
V. Masignani (Siena/IT)

Presentation 2
The Broad Coverage Potential of 4CMenB
R. Borrow (Manchester City/GB)

Presentation 3
Fulfilling the Promise – Review of the Clinical Profile of 4CMenB
P. Dull (Brookline, MA/US)

Summary and Closing Remarks
Q&A

12^{00}–13^{00}	**Lunch**
13^{00}–13^{30} O 22	**Invited talk** Intimate relations – modeling infection by the human-restricted neisserial pathogens S. Gray-Owen (Toronto/CA)
13^{30}–14^{50} Chairs	**Session 5 • Models for neisserial infection** S. Gray-Owen (Toronto/CA), G. Dumenil (Paris/FR)
13^{30} O 23	Experimental studies of co-infected Neisseria gonorrhoeae-lactobacillus crispatus biofilms *M. Apicella, J. Kirby, J. Hunt, J. Poole (Iowa City, IA/US)
13^{50} O 24	Neisseria meningitidis adhesion to the vascular wall leads to purpura in a new humanized mouse model *K. Melican, P. Michea Veloso, T. Martin, P. Bruneval, G. Duménil (Paris/FR)
14^{10} O 25	Neisseria infection of rhesus macaques – colonization, transmission, persistence and horizontal gene transfer *N. J. Weyand, A. Wertheimer (Tucson, AZ/US) T. R. Hobbs (Portland, OR/US), J. L. Sisko, L. D. Gregston N. A. Taku (Tucson, AZ/US), S. Clary (Portland, OR/US) D. L. Higashi (Tucson, AZ/US), S. L. Planer, A. W. Legasse, M. K. Axthelm S. W. Wong (Portland, OR/US), M. So (Tucson, AZ/US)
14^{30} O 26	Induced nasopharyngeal colonisation with Neisseria lactamica protects against carriage of Neisseria meningitidis in healthy adult volunteers A. Deasy, A. Dale, C. Evans, E. Guccione (Sheffield/GB) N. Andrews (London/GB), A. Gorringe (Salisbury/GB) *R. Read (Sheffield/GB)
15^{00}–17^{00}	**Coffee break and poster discussion 3** Polysaccharide vaccines (P 137–P 155) Tools for studying neisserial infections (P 156–P 161) Antibiotic resistance (P 162–P 180) Global fight against meningococcal disease (P 181–P 198)

17^{00}–18^{45}	**GSK symposium** Recent advances in meningococcal vaccination strategies
17^{00}	Welcome and introduction
Chair	M. Knuf (Wiesbaden/DE)
Co-Chair	S. A. Halperin (Halifax/CA)
17^{05}	**Presentation 1** Polysaccharide and conjugate vaccines – What type of antibody response is induced and how does this affect persistence? J. Findlow (Manchester City/GB)
17^{25}	**Presentation 2** The patchwork of meningococcal vaccination schedules in Canada – How and why? S. Dobson (Vancouver/CA)
17^{45}	**Presentation 3** Future opportunities for meningococcal vaccination of toddlers in Germany – How to bridge epidemiology with vaccine schedules and available vaccination strategies? M. Knuf (Wiesbaden/DE)
18^{05}	**Q&A**
18^{25}	**Concluding remarks** M. Knuf, S. A. Halperin
18^{30}	**Symposium close**
18^{45}–21^{00}	**Discussion groups (registration required)**
	group 4 – From genes to genomes – current status of the Neisseria reference libraries hosted on PubMLST.org
Conveners	M. Maiden, K. Jolley (Oxford/GB)
	group 5 – Infection models – What do we have? – What do we need?
Conveners	X. Nassif (Paris/FR), T. Rudel (Würzburg/DE)

08^{30}–10^{10}	**Symposium** Antibiotic Resistant Gonorrhea – A Call to Action
Chair	A. Jerse (Bethesda, MD/US)
08^{30} O 27	Global action plan to control the spread of antimicrobial resistance in Neisseria gonorrhoeae M. Lusti-Narasimhan (Geneva/CH)
08^{50} O 28	Emergence of Untreatable Gonorrhoea – How, when and crucial actions? M. Unemo (Örebro/SE)
09^{10} O 29	New Antimicrobials as Tools to Combat Multidrug Resistant Gonorrhea T. J. Hiltke (Bethesda, MD/US)
09^{30} O 30	An Alternative Path Forward – Addressing the Challenges of a Gonorrhea Vaccine C. Deal (Bethesda, MD/US)
09^{50}	**Wrap-up** A. Jerse (Bethesda, MD/US)
10^{10}–10^{50}	**Coffee break**
10^{50}–12^{20}	**Session 6 • Re-emergence of gonococci – Mechanisms of antimicrobial resistance**
Chairs	S. Ram (Worcester, MA/US), W. M. Shafer (Atlanta, GA/US)
10^{50} O 31	Resistance to cationic antimicrobial peptides is determined by oxidoreductases in Neisseria sp. *S. Piek (Perth/AU), J. Ganguly (Athens/US), A. Anandan, C. Wanty K. Stubbs (Peth/AU), M. Scanlon (Melbourne/AU), A. Vrielink (Perth/AU) R. Carlson (Athens/US), C. Kahler (Perth/AU)
11^{10} O 32	Sulforaphane induces the expression of antimicrobial peptides that kill Neisseria gonorrhoeae and suppresses inflammation induced by gonococcal lipooligosaccharide *R. Yedery, A. Marinelli (Bethesda, MD/US), W. Shafer (Atlanta, GA/US) A. Jerse (Bethesda, MD/US)
11^{30} O 33	Mechanisms of high-level antibiotic resistance in Neisseria gonorrhoeae M. Duncan, S. Swanson, *R. Nicholas (Chapel Hill, NC/US)

11^{50} O 34	Molecular and structural analysis of penicillin-binding protein 2 from the cephalosporin-resistant Neisseria gonorrhoeae strain H041 – molecular mechanisms underlying treatment failures in the clinic J. Tomberg (Chapel Hill, NC/US), M. Ohnishi (Tokyo/JP) M. Unemo (Örebro, Sweden/SE), R. Nicholas (Chapel Hill, NC/US) *C. Davies (Charleston, SC/US)
12^{20}–13^{00}	**Lunch**
13^{00}–13^{30} O 35	**Invited talk** Progress and perspectives of MenAfriVac, a meningococcal A conjugate vaccine for the African meningitis belt M.-P. Préziosi (Ferney-Voltaire/FR, Geneva/CH)
13^{30}–15^{20} Chairs	**Session 7 • Meningococcal polysaccharide vaccines** M.-P. Préziosi (Ferney-Voltaire/FR, Geneva/CH), A. Pollard (Oxford/GB)
13^{30} O 36	Identifying optimal vaccination strategies for serogroup A Neisseria meningitidis conjugate vaccine in the African meningitis belt *M. Jackson (Seattle, WA/US), S. Tartof, A. Cohn, T. Clark N. Messonnier (Atlanta, GA/US)
13^{50} O 37	The Effects of Conjugate Vaccination on the Population Structure of Neisseria meningitidis – a New Paradigm for Capsule Replacement *E. Watkins, M. Maiden, S. Gupta (Oxford/GB)
14^{10} O 38	Comparison of Innate Regulation of Antibody Responses to Meningococcal Polysaccharide and Meningococcal Conjugate Vaccines in Healthy Adults *N. Rouphael, *D. Stephens, S. Li, S. Duraisingham, H. Nakaya S. Romero-Steiner, G. Carlone, B. Pulendran (Atlanta, GA/US)
14^{30} O 39	Long term persistence of serogroup C meningococcal serum bactericidal antibody and the impact of an adolescent booster dose *P. de Whalley, M. Snape, E. Plested, B. Thompson, E. Nuthall, O. Omar A. Pollard (Oxford/GB)
14^{50} O 40	The Effectiveness of Quadrivalent Meningococcal Conjugate Vaccine (MenACWYD) – a Matched Case-Control Study *J. MacNeil, A. Cohn, R. Anderson, E. Zell, B. Plikaytis, T. Clark N. Messonnier ft. Active Bacterial Core Surveillance (ABCs) Team a. MeningNet Surveillance Partners (Atlanta, GA/US)
15^{20}–20^{00}	**Cultural programme** **(see page 71)**

08^{30}–09^{00} **Invited talk**
O 41 Understanding the basis of anti-fHbp protective antibody provides new approaches for development of more effective second-generation meningococcal fHbp vaccines
D. Granoff (Oakland, CA/US)

09^{00}–10^{20} **Session 8 • Meningococcal vaccines – approaches to combat MenB**
Chairs D. Granoff (Oakland, CA/US), M. Pizza (Siena/IT)

09^{00}
O 42 Bactericidal antibody persistence two years following immunisation with investigational serogroup B meningococcal vaccines at 6, 8 and 12 months and response to a booster dose in 40 month old children
*M. Snape, H. Robinson, S. Kelly, T. John, N. Gossger (Oxford/GB)
A. Kimura, D. Toneatto, C. Kittel, P. Dull (Cambridge, MA/US)
A. Pollard (Oxford/GB)

09^{20}
O 43 PorA -expressing adenovirus vectors as vaccines against serogroup B Neisseria meningitidis
*L. Marsay, C. Dold, Y. Yamaguchi, K. Makepeace, G. Patterson (Oxford/GB)
M. Saleem, J. Derrick (Manchester/GB), I. Feavers (London/GB), M. Maiden
D. Wyllie, A. Hill, A. Pollard, C. Rollier (Oxford/GB)

09^{40}
O 44 The Adhesin Complex Protein (ACP) of Neisseria meningitidis is a new adhesin and invasin with vaccine potential
M.-C. Hung, J. Heckels, *M. Christodoulides (Southampton/GB)

10^{00}
O 45 Meningococcal Opa binding to human CEACAM1 in transgenic mice negatively influences the Opa-specific immune response
*A. Zariri, H. van Dijken (Bilthoven/NL)
G. van den Dobbelsteen (Leiden/NL), P. van der Ley (Bilthoven/NL)

10^{25}–11^{00} **Coffee break**

11^{00}–12^{20} **Session 9 • Re-emergence of the gonococcus – Modulation of immune responses**
Chairs P. Rice (Worcester, MA/US), L. Wetzler (Boston, MA/US)

11^{00}
O 46 Proactive manipulation of host immune responses by Neisseria gonorrhoeae: role of immunosuppressive cytokines and type 1 regulatory T cells
*M. Russell, Y. Liu (Buffalo, NY/US)

11^{20} O 47	Neisseria gonorrhoeae suppression of immune response through antigen presenting cells W. Zhu (Chapel Hill, NC/US), M. Ventevogel (Durham, NC/US) K. Knilans, J. Anderson, M. Hobbs (Chapel Hill, NC/US) G. Sempowski (Durham, NC/US), *J. Duncan (Chapel Hill, NC/US)
11^{40} O 48	Protection against vaginal colonization with Neisseria gonorrhoeae in mouse model by passive (2C7 mAb) and active immunizations using a peptide surrogate of the 2C7 LOS epitope *S. Gulati, B. Zheng, G. Reed, X. Su, S. Ram, P. A. Rice (Worcester, MA/US)
12^{00} O 49	Peptidoglycan degradation and release of toxic peptidoglycan fragments by the pathogenic Neisseria K. Hackett, K. Woodhams, Y. Chan, K. Fisher, *J. Dillard (Madison, WI/US)
12^{25}–13^{20}	**Lunch and poster viewing**
13^{20}–15^{00} Chairs	**Session 10 • Mechanisms of neisserial dissemination** X. Nassif (Paris/FR), M. So (Tucson, AZ/US)
13^{20} O 50	A lipopolysaccharide-deficient meningococcal isolate from a meningitis patient *A. Zariri (Bilthoven/NL), J. Piet, B. van Schaik K. Schipper (Amsterdam/NL), F. Fransen, H. J. Hamstra P. van der Ley (Bilthoven/NL), D. van de Beek A. van der Ende (Amsterdam/NL)
13^{40} O 51	A molecular defect leading to subtotal complement C5 deficiency (C5D) and responsible for increased susceptibility to Neisseria meningitidis *A. Orren (Cardiff/GB), T. Owen, F. Leisegang (Cape Town/ZA) B. Hellerud (Cape Town/ZA, Oslo/NO), T. Mollnes (Oslo/NO) R. Würzner (Innsbruck/AT), P. Potter (Cape Town/ZA)
14^{00} O 52	Presence and expression of Opc in German meningococcal strains from invasive disease and carriage *H. Claus, R. Aumann, J. Elias (Würzburg/DE), W. Hellenbrand (Berlin/DE) U. Vogel (Würzburg/DE)
14^{20} O 53	Immunoglobulin binding, bacterial aggregation and biofilm formation dependent on a protein encoded by prophage DNA linked to invasive meningococcal disease *G. Moe, M. Müller, B. Flitter, M. Cheng (Oakland, CA/US)

14^{40} O 54	Neisseria gonorrhoeae avoids primary granules to survive in human neutrophils *M. B. Johnson, A. Criss (Charlottesville, VA/US)
15^{00}–15^{50}	**Coffee break**
15^{50}–16^{50}	**Session 10 • Mechanisms of neisserial dissemination (cont'd)**
15^{50} O 55	Resistance mechanisms of Neisseria meningitidis against Neutrophil Extracellular Traps *M. Lappann, S. Danhof, F. Günther, C. Schmidt, I. L. Mordhorst U. Vogel (Würzburg/DE)
16^{10} O 56	CD147 is a cellular receptor for pilus-mediated adhesion of meningococci to vascular endothelia S. Bernard (Paris/FR), N. Simpson (London/GB), C. Federici M.-P. Laran-Chich, F. Chretien, O. Join-Lambert, M. Coureuil, F. Nierdergang S. Marullo, P.-O. Couraud, X. Nassif, S. Bourdoulous (Paris/FR)
16^{30} O 57	Neisseria meningitidis targets human endothelial cells in a human skin xenograft transplantation model in SCID mice *O. Join-Lambert, H. Lécuyer, F. Miller, M. Coureuil, P. Pelissier S. Fraitag, X. Nassif (Paris/FR)
16^{55}–19^{00}	**Poster discussion 4** Neisserial dissemination mechanisms and host defense (P 199–P 239) Novel vaccination strategies (P 240–P 308)
19^{30}–24^{00}	**Banquet at the Würzburg Residence** (see page 72)

08^{30}–09^{30} **Presentation of results of discussion groups**
Chair I. Feavers (Potters Bar/GB)

08^{30}
Convener group 1
A. Jerse (Bethesda, MD/US), P. A. Rice (Worcester, MA/US)

08^{45}
Convener group 2
S. Gray (Manchester/GB)

09^{00}
Conveners groups 3 and 4
M. Maiden, K. Jolley, O. Harrison (Oxford/GB)

09^{15}
Conveners group 5
X. Nassif (Paris/FR), T. Rudel (Würzburg/DE)

09^{30}–10^{30} **Session 11 • Population biology**
Chairs M. Maiden (Oxford/GB), A. van der Ende (Amsterdam/NL)

09^{30}
O 58 Exploring the extent of genetic variation during persistent meningococcal carriage and the impact of adaptive host immune responses
*C. Bayliss, F. Bidmos, M. Alamro (Leicester/GB), E. Newton
S. Gray (Manchester/GB), N. Oldfield (Nottingham/GB)
N. Loman (Birmingham/GB), K. Neal, D. Turner
D. Ala'Aldeen (Nottingham/GB), H. Chan, I. Feavers (Potters Bar/GB)
R. Borrow (Manchester/GB)

09^{50}
O 59 Diversity of Multiple-Locus-VNTR-Analysis-(MLVA)-Types in Meningococcal Strains from Different Epidemiological Settings
*J. Elias (Würzburg/DE), P. Kriz, M. Musilek (Prague/CZ), H. Claus
M. Frosch, U. Vogel (Würzburg/DE)

10^{10}
O 60 Intergenic crossover hotspots in meningococcal transformation
*K. Alfsnes (Oslo/NO), O. Harrison, P. Power (Oxford/GB)
S. Bentley (Hinxton/GB), S. A. Frye (Oslo/NO), M. C. J. Maiden
D. Hood (Oxford/GB), T. Tønjum, O. H. Ambur (Oslo/NO)

10^{30}–11^{10} **Coffee break**

11^{10}–12^{30} **Session 11 • Population biology (cont'd)**

11^{10}
O 61
Whole genome epidemiology of the ST-11 Complex 'ET-15' epidemic in the Czech Republic
*D. Hill, O. Harrison, K. Jolley (Oxford/GB), M. Musilek, P. Kriz (Prague/CZ)
M. Maiden (Oxford/GB)

11^{30}
O 62
Genomics of Serogroup Y, ST-23 Carriage and Invasive Neisseria meningitidis in the United States and United Kingdom
*M. Krauland (Pittsburgh, PA/US), J. Dunning Hotopp (Baltimore, MD/US)
J. Nelson (Pittsburgh, PA/US), D. Riley, S. Daugherty (Baltimore, MD/US)
M. Maiden (Oxford/GB), R. Borrow, S. Gray (Manchester/GB), J. Marsh
L. Harrison (Pittsburgh, PA/US)

11^{50}
O 63
Phylogenetics of Neisseria meningitidis Serogroups Rarely Associated with Disease and Their Contribution to the Pan and Core Genomes
*M. Krauland (Pittsburgh, PA/US), J. Dunning Hotopp
D. Riley (Baltimore, MD/US), J. Marsh (Pittsburgh, PA/US)
S. Parankush Das, S. Nadendla, S. Daugherty, L. Tallon, L. Sadzewicz
C. Fraser (Baltimore, MD/US)

12^{10}
O 64
Ribosomal MLST analysis reveals a distinct species of Neisseria, previously identified as Neisseria polysaccharea that is closely related to Neisseria meningitidis
*J. Bennett (Oxford/GB), J. MacLennan, C. Msefula (Blantyre/MW)
S. Bentley, J. Parkhill (Hinxton, Cambridge/GB), M. Molyneux
C. MacLennan (Blantyre/MW), M. Maiden (Oxford/GB)

12^{30}–13^{00} **Concluding remarks and announcement of IPNC 2014 and IPNC 2016**

10^{30}–12^{00} **Novartis symposium**
An Innovative Step in the Global Fight Against Meningococcal Disease

NOVARTIS VACCINES

17^{00}–18^{30} **GlaxoSmithKline symposium**
Recent advances in meningococcal vaccination strategies

gsk GlaxoSmithKline

Gene regulation

P 001 **Small non coding RNAs regulating energy metabolism in Neisseria meningitidis**
*Y. Pannekoek, R. Huis in 't Veld, K. Schipper (Amsterdam/NL)
J. Moir (York/GB), A. van der Ende (Amsterdam/NL)

P 002  **Twin small non-coding RNA molecules involved in feast/famine regulation in Neisseria meningitidis**
*R. Huis in 't Veld, C. Hopman, K. Schipper, D. Speijer, Y. Pannekoek
A. van der Ende (Amsterdam/NL)

P 003 **Rapid quantitation of proteins regulated by the small non-coding RNA FfrR of Neisseria meningitidis assessed by LC-MSE**
*R. Huis in 't Veld, G. Kramer, D. Speijer, Y. Pannekoek
A. van der Ende (Amsterdam/NL)

P 004 **Global identification and characterization of small non-coding RNAs in Neisseria meningitidis in response to multiple stress conditions**
L. Fagnocchi, G. Golfieri , L. Fantappiè, E. Siena, S. Bottini, A. Muzzi (Siena/IT)
V. Scarlato (Siena, Bologna/IT), *I. Delany (Siena/IT)

P 005 **An in-depth conversation between N. gonorrhoeae and the human host as tevealed by RNA-seq**
*R. McClure (Boston, MA/US), B. Tjaden (Wellesley, MA/US)
C. Genco (Boston, MA/US)

P 006 **Gonococcal RNA-seq analysis identifies novel putative small RNAs responding to a variety of signals**
*R. McClure (Boston, MA/US), B. Tjaden (Wellesley, MA/US)
C. Genco (Boston, MA/US)

P 007 **The influence of homopolymeric G tract length and mismatch repair on phase variation in a Neisseria meningitidis model**
*K. Alfsnes, T. Tønjum, O. H. Ambur (Oslo/NO)

P 008 **Novel Fur and FarP regulatory networks implicated in Gonococcal Pathogenesis**
*N. Daou, C. A. Genco (Boston, MA/US)

P 009 **Fur-mediated Transcriptional Activation in the Human Pathogen Neisseria go norrhoeae – a Role for RNA Polymerase and DNA Binding Proteins**
*C. Yu, C. A. Genco (Boston, MA/US)

P 010 **Control of LOS Sialylation in Gonococci by the Transcriptional Regulators Rsp and CrgA**
*K. Matthias (Philadelphia, PA/US), W. Shafer (Atlanta, GA/US)
R. Rest (Philadelphia, PA/US)

P 011 **Stringent response in ex vivo survival of Neisseria menigitidis**
*L. Kischkies, B. Joseph, M. Frosch, C. Schoen (Würzburg/DE)

P 012 **A CRISPR/cas subtype Nmeni/CASS4 system in the human pathogen Neisseria meningitidis**
*N. Heidrich, B. Joseph, C. Schoen, J. Vogel (Würzburg/DE)

P 013 **A Novel Regulatory Switch for increased expression of Neisseria meningitidis NHBA at physiological temperatures found in the human nasopharynx**
*I. Delany, A. Antunes, A. Haag, G. Boccadifuoco, A. Biolchi
B. Brunelli (Siena/IT)

P 014 **Delineating the function of DNA repair helicases in Neisseria meningitidis**
*G. T. Beyene, S. V. Balasingham, E. D. Zegeye, T. Tønjum (Oslo/NO)

P 015 **Transcriptomic changes in meningococci by zinc exposure**
*M.-C. Pawlik, K. Hubert, B. Joseph, H. Claus, C. Schoen
U. Vogel (Würzburg/DE)

P 016 **The regulated transcriptome of Neisseria meningitidis in human blood using a tiling array**
D. Serruto, *A. Muzzi, E. Del Tordello, S. Bottini (Siena/IT)

P 017 **Comparison of RNA extraction methods for effective isolation of bacterial RNA from neisserial cultures**
*S. Roberts, L. A. Snyder (Kingston Upon Thames/GB)

Interaction of Neisseria with mucosal surfaces

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E. Kaczmarski (Manchester/GB), I. Feavers (Blanche Lane, Potters Bar/GB)
C. Bayliss (Leicester/GB)

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*S. Mistry, C. Cornelissen (Richmond, VA/US)

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*K. Ostberg, A. DeRocco, C. Cornelissen (Richmond, VA/US)

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*A. Morgenthau, A. Beddek, A. B. Schryvers (Calgary/CA)

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*O. Harrison, S. Dyball (Oxford/GB), H. Claus, U. Vogel (Würzburg/DE)
J. Derrick (Manchester/GB), I. Feavers (South Mimms/GB)
M. Maiden (Oxford/GB)

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*A. Oki, H. Strange, C. Cornelissen (Richmond, VA/US)

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D. Ala'Aldeen (Nottingham/GB)

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*A. Klimowicz, M. Ramsey, J. Dillard (Madison, WI/US)

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*M. Georgiadou, V. Pelicic (London/GB)

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*N. Biais (New York City, NY/US), D. Higashi (Tucson, AZ/US)
M. Sheetz (New York City, NY/US), M. So (Tucson, AZ/US)

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*C. Thomas (Chapel Hill, NC/US)

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*L. Ball, A. Criss (Charlottesville, VA/US)

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*M. Castagnini, V. Pelicic (London/GB)

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*T. F. Meyer, E. Ziska, V. Brinkmann, R. Hurwitz, M. Kolbe (Berlin/DE)

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F. Alqahatni, J. Mahdavi, L. Wheldon, J. Stoof (Nottingham/GB), A.-B. Jonsson
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*L. Wheldon, J. Mahdavi, N. Oldfield, K. Wooldridge
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*M. Vassey, L. Wheldon, A. Aslam, N. Oldfield, D. Ala'Aldeen
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C. Lange, T. F. Meyer, *C. Rechner (Berlin/DE)

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*D. Ala'Aldeen, A. Khairalla, J. Mahdavi, L. Wheldon (Nottingham/GB)
S. Omer (Sulaimany/IQ), L. Martinez-Pomares, P.-J. Royer (Nottingham/GB)
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P. Solar, P. Rodas (Santiago de Chile/CL), *M. Christodoulides (Southampton/GB)
L. Velasquez (Santiago de Chile/CL)

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K. Vielfort, N. Söderholm, *L. Weyler, D. Håkansson (Stockholm/SE)
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*W. Song, V. Edwards, L.-C. Wang, D. Stein (College Park, MD/US)

P 046 ★ **Gonococcal interactions with polarized epithelial cells disassemble the actin cytoskeleton**
*L.-C. Wang, D. Stein, W. Song (College Park, MD/US)

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*N. J. Weyand, D. L. Higashi, I. Inabathini, M. So (Tucson, AZ/US)

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C. Ott, M. Utech, T. Rudel, *V. Kozjak-Pavlovic (Würzburg/DE)

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T. Brissac, J. Meyer, H. Omer, E. Frapy, P. Martin (Paris/FR)
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P 050 **Oral Lactobacillus plantarum NCIMB 8825 inhibits adhesion, invasion and metabolism of Neisseria meningitidis serogroup B and affords antiinflammatory and cytotoxic protection to nasopharyngeal epithelial cells**
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*X. Ren, J. MacKichan (Wellington/NZ)

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*S. u. Rahman, P. v. Ulsen (Amsterdam/NL)

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*T. Rudel (Würzburg/DE), K. Zeth (Tübingen/DE), V. Kozjak-Pavlovic
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O. Kepp (Berlin/DE)

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*A. Cehovin, V. Pelicic (London/GB)

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*I. Peak, C. Jennings, M. Jennings (Gold Coast/AU)

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*C. Davies (Charleston, SC/US)

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*S. Bartley (Perth/AU), Y.-l. Tzeng (Atlanta, GA/US), K. Heel, C. Lee (Perth/AU)
T. Seemann (Melbourne/AU), C. Peacock (Perth/AU)
D. Stephens (Atlanta, GA/US), J. Davies (Melbourne/AU), C. Kahler (Perth/AU)

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*N. Oldfield (Nottingham/GB), S. Matar (Nottingham/GB, Amman/JO), F. Bidmos
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C. Bayliss (Leicester/GB), D. Ala'Aldeen (Nottingham/GB)

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*M. Alamro, F. Bidmos (Leicester/GB), K. Neal, D. Turner
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*Y.-L. Tzeng (Atlanta, GA/US), L. Martin (Decatur, IL/US)
D. Stephens (Atlanta, GA/US, Decatur, IL/US)

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*F. Veyrier, A. Williams, S. Mesnage, C. Schmitt, M.-K. Taha
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*D. Post (Novato, CA/US), M. Apicella, J. Kirby (Iowa City, IA/US)
B. Gibson (Novato, San Francisco, CA/US)

P 065 **Expression of lptA and phosphoethanolamine decoration of lipid A in Neisseria gonorrhoeae – implications for pathogenesis**
J. Balthazar (Atlanta, GA/US), A. Begum (Atlanta/GB), J. Ganguly, J. Kandler
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*P. I. Rodas, D. Aguayo (Santiago de Chile/CL)
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*A. Antunes, G. Golfieri, S. Guadagnuolo, I. Delany (Siena/IT)

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*F. E. Jen (Gold Coast/AU), A. G. McEwan (Brisbane/AU)
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P 069 **The MisR/MisS Two-Component Regulatory System Influences Expression of lptA and Modulates Phosphoethanolamine Decoration of Lipid A in Neisseria gonorrhoeae**
*J. L. Kandler (Atlanta, GA/US), J. Ganguly, R. W. Carlson (Athens, GA/US)
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*R. Bigwood, I. Vendrell, R. Care, J. Wheeler, C. Vipond (Potters Bar/GB)

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C. John, M. Liu, N. Phillips, Z. Yang, C. Funk (San Francisco, CA/US)
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D. Stein (College Park, CA/US), *G. Jarvis (San Francisco, CA/US)

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N. Tsolakos (Potters Bar/GB), C. Tang (Oxford/GB), *I. Feavers
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*L. Glennie (Bristol/GB)

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*H. Bratcher (Oxford/GB), C. Corton, S. Bentley, J. Parkhill (Hinxton/GB)
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*D. Caugant (Oslo/NO), O. Harrison, H. Bratcher, K. Jolley (Oxford/GB)
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*H. Bratcher, J. MacLennan, M. Maiden (Oxford/GB)

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*E. Siena, S. Censini, R. D'Aurizio (Siena/IT), D. Riley
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*O. Harrison, H. Bratcher, J. Eales (Oxford/GB), S. Gray (Manchester/GB)
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*M. du Plessis, N. Wolter, P. Crowther (Johannesburg/ZA)
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C. Cohen (Johannesburg/ZA), D. van de Beek (Amsterdam/NL)
P. van der Ley (Bilthoven/NL), A. von Gottberg (Johannesburg/ZA)
A. van der Ende (Amsterdam/NL)

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*O. H. Ambur, M. Nilsen, S. Frye, E. Hovland, T. Tønjum (Oslo/NO)

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*M. Nilsen, O. H. Ambur, T. Tønjum, S. A. Frye (Oslo/NO)

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*O. Conchillo-Solé, D. Yero Corona, I. Gibert, X. Daura (Barcelona/ES)

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*D. Yero Corona, O. Conchillo-Solé, P. M. Muñoz, I. Gibert
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*J. Klughammer, M. Dittrich (Würzburg/DE), C. Cicicopol-Boicu
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*O. Harrison, J. Wills, D. Hill, H. Bratcher, K. Jolley (Oxford/GB)
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*K. Jolley, M. Maiden (Oxford/GB)

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*C. Brehony, M. Maiden, K. Jolley (Oxford/GB)

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*M. Krauland (Pittsburgh, PA/US), J. Dunning Hotopp
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*C. Davidson, S. McKeon, M. Faria (Calgary/CA), M. Surette (Hamilton/CA)
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*R. Exley (Oxford/GB), E. Aho (Moorhead, MN/US), M. Woermann, J. Bennett
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*A. Neri, C. Fazio, A. Carannante, P. Mastrantonio, P. Stefanelli (Rome/IT)

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*S. Ladhani, S. Ribeiro, M. Ramsay (London/GB), S. Gray, E. Kaczmarski
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*K. Scott, R. Ure, G. Edwards (Glasgow/GB)

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*G. Norheim (Oslo/NO), M. Sadarangani (Oxford/GB, Vancouver/CA)
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*J. Kozakova, M. Musilek, Z. Vackova, P. Kriz (Prague/CZ)

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*Z. Vackova, M. Musilek, J. Kozakova, P. Kriz (Prague/CZ)

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*I. Koroleva, G. Beloshitskii, I. Zakroeva, A. Melnikova, M. Koroleva, G. Shipulin
K. Mironov (Moscow/RU)

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*X. Wang (Atlanta, GA/US), K. Shutt (Pittsburgh, PA/US), J. Vuong, A. Cohn
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A. Wiringa, K. Shutt, J. Marsh (Pittsburgh, PA/US), A. Cohn
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S. Petit (Hartford, CT/US), M. Farley (Atlanta, GA/US)
K. Gershman (Denver, CO/US), R. Lynfield (St. Paul, MN/US)
A. Reingold (Berkeley, CA/US), W. Schaffner (Nashville, TN/US)
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*D. Barroso, T. Castiñeiras, F. Freitas, É. Fonseca (Rio de Janeiro/BR), M. Tulenko J. Marsh, M. Krauland (Pittsburgh, PA/US), A. Paulo Vicente, M. Rebelo E. Cerqueira (Rio de Janeiro/BR), L. Harrison (Pittsburgh, PA/US)

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*D. Barroso, T. Castiñeiras (Rio de Janeiro/BR), M. Tulenko, J. Marsh M. Krauland (Pittsburgh, PA/US), M. Rebelo (Rio de Janeiro/BR) L. Harrison (Pittsburgh, PA/US)

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*Z. Yin, Y. Li, Z. Shao (Beijing/CN), R. Zhai, F. Zhang (Taiyuan/CN) H. Zhou (Beijing/CN), S. Chang, Q. Zhang, M. Guang, H. Yang, H. Wang S. Yao (Taiyuan/CN), J. Li, L. Xu, X. Liang, H. Luo (Beijing/CN)

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*M. Packiam, A. Begum (Bethesda, MD/US), G. Sempowski (Durham, NC/US)
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*K. Goodmon, C. Genco, P. Massari (Boston, MA/US)

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*A. Platt, X. Liu, M. Mosaheb, L. Wetzler (Boston, MA/US)

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*O. Koeberling (Siena/IT), J. Hauser (Basel/CH), E. Ispasanie (Siena/IT)
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*G. Norheim, G. Tunheim, L. M. Næss, K. Bolstad, Å. K. Fjeldheim (Oslo/NO)
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*C. Rollier (Oxford/GB), J. C. Nagaputra (Oxford/AU)
M. Sadarangani (Oxford/CA), J. C. Hoe (Oxford/GB), O. Mehta (Oxford/AU)
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*L. Shewell (Gold Coast/AU), B. Schulz, S. Ku (Brisbane/AU)
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*H. Claus (Würzburg/DE), R. Borrow (Manchester/GB), M.-K. Taha
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*G. Zlotnick, E. Novikova, L. McNeil, R. Camarda, H.-Q. Jiang, D. Zhu, T. Mininni E. Camposano, S. Logan, X.-J. Zhao, A. Anderson, K. Jansen L. McNeil (Pearl River, NY/US)

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*M. Scarselli, D. Veggi, E. Malito (Siena/IT), F. Cantini (Firenze/IT), I. Ferlenghi F. Giusti, M. A. Gentile, L. Santini (Siena/IT), S. Giuntini D. M. Granoff (Oakland, CA/US), M. Pizza (Siena/IT), L. Banci (Firenze/IT) R. Rappuoli (Siena/IT)

P 282 **Diversity of noncapsular antigens among Neisseria meningitis isolates of serogroups C, W135 and Y in Brazil – implications for vaccine prevention**
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P 284 **Biophysical Characterization of LP2086 Subfamily A Factor H Binding Protein and Its Interactions with Human Factor H**
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P 287 **Heterogeneity of binding of Rhesus macaque factor H to meningococcal factor H binding protein**
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P 290 **Benefit of vaccination against meningococcal capsular group B (MenB), in addition to chemoprophylaxis, for prevention of secondary cases among close contacts of cases and more widely during clusters**
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P 292 **Production and control of a Tailor made Brazilian Meningococcal B vaccine to Phase II trial in children**
*E. Jessouroun, E. C. Figueira, S. A. Fernandes, V. N. Oliveira, A. P. Santos D. A. Nascimento, A. A. Bello, D. M. Silva, I. A. F. Silveira, E. J. Duarte M. L. Leal (Rio de Janeiro, Brasil/BR)

P 293 **Functional impacts of the diversity of the meningococcal factor H binding protein**
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*J. Fegan, R.-H. Yu (Calgary/CA), A. Savchenko (Toronto/CA) A. Schryvers (Calgary/CA)

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*C. Brookes, E. Kuisma, F. Alexander, S. Taylor, A. Gorringe (Salisbury/GB)

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*C. Mattick, R. Care (South Mimms/GB)

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*J. Holst (Oslo/NO), L. Santini, F. Rigat, M. M. Giuliani, A. Genoves, M. Agnusdei G. Frosi, M. Comanducci, J. J. Donnelly, M. Pizza, D. Medini, R. Rappuoli (Siena/IT)

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L. Yan, *M. Akkoyunlu, L. Lee (Rockville, MD/US)

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S. Savino (Siena/IT)

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This short prescribing information is intended for international use and is based on the SPC (summary of product characteristics) for NeisVac-C approved under mutual recognition procedure (MRP) in the European Union under UK/H/0435/001. Please note that the SPC as approved in MRP may not yet have been nationally implemented/approved in the country where you are practicing. Therefore, please always consult your **full** country-specific SPC prior to using the product. **Name and composition:** NeisVac-C 0.5 ml, Suspension for injection in pre-filled syringe, Meningococcal group C polysaccharide conjugate vaccine adsorbed. Neisseria meningitidis group C (strain C11) polysaccharide (de-O-acetylated) 10 micrograms. **Indications:** Active immunisation of children from 2 months of age, adolescents and adults, for the prevention of invasive disease caused by Neisseria meningitidis serogroup C. **Dosage and Route:** For intramuscular injection only. Infants from 2 months to 12 months of age: two doses of 0.5 ml at an interval of at least 2 months between doses. Children 12 months of age and older, adolescents and adults: a single 0.5 ml dose. After completion of the primary immunisation series in infants, a booster dose should be given. The need for booster doses in subjects primed with a single dose has not been established. The use of NeisVac C should be determined on the basis of official recommendations. **Side effects:** *See Summary of Product Characteristics for more details.* Adverse reactions from clinical studies: Very common (≥1/10): in infants/toddlers 2 to <18 months of age: decreased appetite, crying, sedation/somnolence, vomiting, irritability, fatigue, fever, injection site reactions including tenderness/pain, swelling and erythema. In children 3.5 to < 18 years of age and adults: headache, injection site reactions including tenderness/pain, swelling and erythema. Common (≥1/100-<1/10): in infants/toddlers 2 to <18 months of age: pharyngitis/rhinitis, agitations/restlessness, sleep disorder, cough, diarrhea, rash, hyperhidrosis. In children 3.5 to < 18 years of age: pharyngitis/rhinitis, dizziness, sedation/somnolence, cough, nausea, abdominal pain, vomiting, diarrhea, pruritus, ecchymosis, dermatitis, pain in extremity, fever, malaise, fatigue. In adults: vomiting, myalgia, malaise, fever. **Precautions:** Do not give intravenously **Contraindications:** Hypersensitivity to the active ingredient or any of the excipients, including tetanus toxoid. Postpone use in subjects with acute severe febrile illness. **Interactions:** Do not mix with other vaccines in the same syringe. Separate injection sites should be used if more than one vaccine is being administered. Concomitant administration with Hexavac is not recommended. Concomitant administration with combinations containing acellular pertussis components result in lower SBA GMTs compared to separate administrations or to coadministration with whole cell pertussis vaccines. **Overdose:** Unlikely (single dose syringe) **Legal category:** POM **Date of preparation:** October 2011

References: 1. World Health Organization (WHO). www.who.int/immunization/topics/meningitis/en/index.html, accessed on 21 Jan 2011. 2. Summary of Product Characteristics for NeisVac-C (Oct-2011). 3. Kaaijk P et al. Is a single dose of meningococcal serogroup C conjugate vaccine sufficient for protection? Experience from the Netherlands. BMC Infectious Diseases 2012, 12:35 doi:10.1186/1471-2334-12-35

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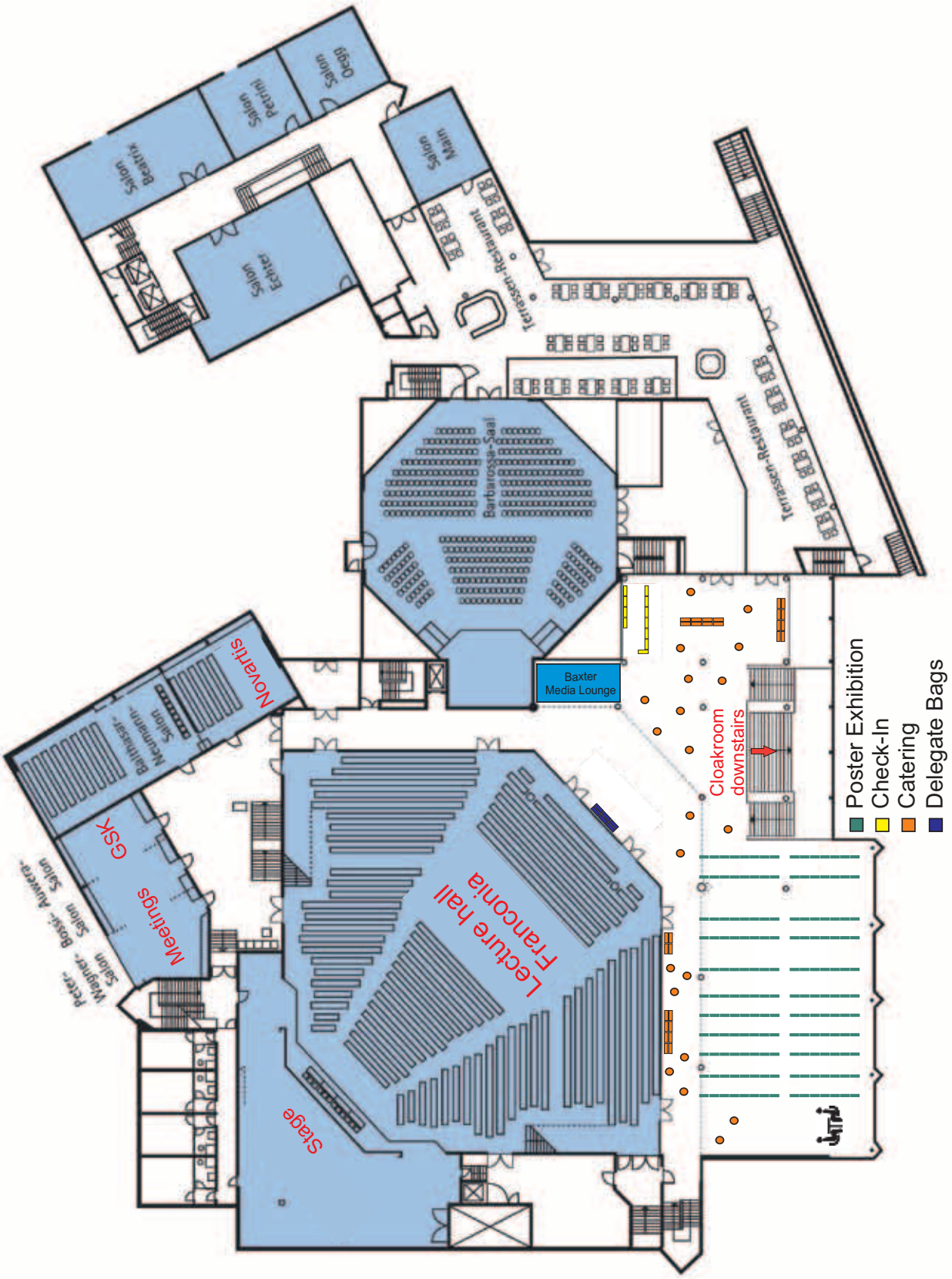

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Social and Cultural Programme

Monday, 10 September and Tuesday, 11 September 2012
Wine Tasting at the Würzburg Residenz
(in parallel with Discussion groups)
Wine tasting in the historic wine cellar of the Residenz begins with a welcome drink and an informative tour through the historic vault. Afterwards four special sorts of wine will be served in the candle lit wine cellar. In addition typical Franconian appetizer will be served to you.
Welcome address by mayor Georg Rosenthal.

Duration	19^{00}–21^{30}
Charge	41 EUR
Nr. of participants	min. 25 persons
Registration	Please register online at the conference website

Wednesday, 12 September 2012
Romantic Road Excursion
Würzburg is the gate to the Romantic Road – one of Germany's best scenic routes. From here, it goes on to Creglingen with its world famous Hergottskirche along the Romantic road by bus. The main attraction of the excursion is the mediaeval Rothenburg ob der Tauber. A guided tour through the historic and unchanged cityscape will complete the excursion.

Duration	15^{30}–20^{00}
Charge	12.50 EUR
Nr. of participants	min. 20 persons
Registration	Please register online at the conference website

Boat Tour to the Palace of Veitshöchheim and the Rococo Gardens
Cruise the hillside vineyards and the monastery Oberzell on the way down the river Main to Veitshöchheim. The former summer residence of the prince-bishops has captivated generations of guests with the Palace and the Rococo Gardens.

Duration	16^{00}–19^{45}
Charge	16.50 EUR
Nr. of participants	min. 20 persons
Registration	Please register online at the conference website

Guided Tour • Old city and UNESCO World Heritage Residenz
The highlight of this guided tour through Würzburg's old city will be a visit to the episcopal Residenz. The Residenz was built by Balthasar Neumann in the beginning of the 18th century. In 1981, the Residenz was added to the UNESCO World Heritage List.

Duration	15^{00}–20^{00}
Charge	12.50 EUR
Nr. of participants	min. 20 persons
Registration	Please register online at the conference website

Social and Cultural Programme

Thursday, 13 September 2012

Banquet at the Würzburg Residenz

We warmly welcome you to join the social evening of the XVIIIth International Pathogenic Neisseria Conference.
The banquet will take place on Thursday, 13 September, 2012, in the magnificent Garden Hall of the Würzburg Residenz.
Food and drinks will be served. The rock band ROSA will entertain the conference covering songs of the past three decades.
Welcome address by mayor Georg Rosenthal.

Duration	19^{30}–24^{00}
Charge	50 EUR

O 01 – Invited speaker

An RNA perspective on bad microbes

J. Vogel[1]

[1]Institute for Molecular Infection Biology, University of Würzburg, Germany

This talk will discuss emerging concepts and mechanisms of gene regulation by small noncoding RNAs (sRNAs) in pathogenic bacteria. Furthermore, I will present examples of how differential RNA deep sequencing (dRNA-seq) has facilitated the global discovery and study of functional RNAs in many bacterial species, and how we have used this approach to uncover a new CRISRP biogenesis pathway in Neisseria.

O 02

Global Transcriptome Analysis of *Neisseria gonorrhoeae* unveils a multitude of small regulatory RNAs

M. Albrecht[1], E. Hausser[1], T. Rudel[1]

[1]University of Würzburg, Department of Microbiology, Würzburg, Germany

Genome annotation of *Neisseria gonorrhoeae* is mainly based on computational approaches and the class of small regulatory RNAs (sRNA) was largely missed in the former annotation. Here we used a high throughput sequencing approach combined with enzymatic treatment of the isolated total RNA to remove fragmented and degraded RNA. Treatment of the RNA with Terminator exonuclease removed RNA molecules without 5'-triphosphate and thereby enriched native transcriptional start sites (TSS). Based on the manual annotation of TSS at single nucleotide resolution a transcriptional map was generated. This data unveiled novel genes including sRNAs and gives insights into the transcriptional organization of polycistronic transcripts. Expression of more than 20 novel sRNAs could be confirmed by Northern blotting. Some of these sRNAs are differentially expressed under changing environmental conditions implying a role in gene regulation mechanism in response to changing external parameters. Based on the mapped TSS the promoter regions were extracted and searched for common motifs. Several promoter motifs have been identified including the major sigma factor binding motif. We will present the gene structure of gonococci and will discuss features of novel translated and non-translated RNAs and their regulation in different environmental situations.

O 03

Repertoire and ex vivo expression of small non-coding RNAs in Neisseria meningitidis

B. Joseph[1], S. Schork[1], M. Dondrup[2], N. Heidrich[3], J. Vogel[3], M. Frosch[1], C. Schoen[1]

[1]University of Wuerzburg, Institute for Hygiene and Microbiology, Wuerzburg, Germany

[2]University of Bergen, Department of Informatics, Bergen, Norway

[3]University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, Germany

Till date, there are only two studies on the role of small non-coding RNAs in meningococcal biology [(1,2)]. In this study, we have elucidated the repertoire of small non-coding RNAs (ncRNAs) in the hyper-invasive serorgroup B strain MC58 (ST-32) and in the carriage serogroup B strain α522 (ST-35) by transcriptome sequencing (RNA-Seq). To compensate for the lack of animal models for meninogococci, we used an ex vivo infection model where the strains were exposed to human saliva, whole blood and cerebrospinal fluid, respectively. Total RNA was isolated from both strains grown in rich medium (PPM+) and after exposure to human saliva, whole blood and cerebrospinal fluid. The cDNA libraries prepared from these RNA samples for each strain were sequenced using Illumina/Solexa technology. Although genome sequences of the two strains used in this study were found to be very similar, our results from the RNA-seq data revealed the presence of strain specific and niche specific expression of small non-coding RNAs, which were confirmed by Northern Blot analyses. Overall, 1119 and 1073 transcriptional start sites were identified from the RNA-Seq data in the strain MC58 and alpha522 respectively. Based on the identified transcriptional start sites, 339 operons were identified in the strain MC58. 45 potential ncRNAs could be detected in both the strains and 21 were found to be specific for the strain MC58 while seven candidates could be detected only in the strain α522. Over 2/3 of these candidate ncRNAs were found to be differentially expressed between both strains and conditions including amongst others the 6S RNA and NrrF. However, most of the differentially expressed ncRNAs had no RFAM homologs and therefore represent novel genetic factors involved in meningococcal ex vivo fitness and therefore potentially also virulence in vivo. The implications of these novel ncRNAs in the biology and pathogenesis of meningococci will be discussed.

References:[1]Mellin JR, Goswami S, Grogan S, Tjaden B, Genco CA. A novel fur- and iron-regulated small RNA, NrrF, is required for indirect fur-mediated regulation of the sdhA and sdhC genes in Neisseria meningitidis. J Bacteriol. 2007 May;189(10): 3686-94.
[2]Huis in 't Veld RA, Willemsen AM, van Kampen AH, Bradley EJ, Baas F, Pannekoek Y, van der Ende A. Deep sequencing whole transcriptome exploration of the σE regulon in Neisseria meningitidis. PLoS One. 2011;6(12)

O 04

Small non coding RNAs regulating energy metabolism in *Neisseria meningitidis*

Y. Pannekoek[1], R. Huis in 't Veld[1], K. Schipper[1], A. van der Ende[1]

[1]Academic Medical Center, Center for Infection and Immunity Amsterdam (CINIMA), Department of Medical Microbiology, Amsterdam, the Netherlands.

In prokaryotes small non-coding RNAs (sRNA) are involved in post-translational regulation of protein expression. NrrF is one of the sRNAs identified in *Neisseria meningitidis* and was previously shown to be under control of the ferric uptake regulator (Fur).
To predict sRNAs and mRNA targets, we combined biocomputional prediction analyses. RNA-sequencing was used to detect the presence and expression levels of sRNAs in meningococci. Potential sRNA-mRNA interactions and their effect on protein expression were assessed using a *gfp* reporter system in *Escherichia coli*. sRNA deletion and overexpression mutants of *N. meningitidis* were created and LC-MSE proteomic/mass spectrometry analyses were performed to identify differentially expressed proteins.
We identified two sRNAs with targets functioning in the general metabolism of meningococci.
We found that Nrrf targets mRNAs of *petABC* encoding the cytochrome bc_1 complex functioning in respiration. Direct interaction between NrrF and the 5'-untranslated region (5'-UTR) of the mRNAs of *petABC* was assessed and validated *in vivo* by using the *gfp* reporter system. Proof for the *in silico* predicted interaction between NrrF and *petABC* was obtained *in vivo* by mutagenesis of the predicted region of interaction. Mutations in the site of interaction in NrrF abrogated the down regulation of *petABC* 5'-UTR-*gfp* fusion product. Compensatory mutations in the 5'-UTR of *petABC* restored the down regulation.
In addition, we identified two structurally nearly identical sRNAs located in their immediate vicinity, with 70% sequence identity (twin sRNAs). Assessment of a twin sRNA deletion mutant by LC-MS^E proteomic/mass spectrometry showed increased expression of proteins involved in the tricarboxylic acid (TCA) cycle or in gluconeogenesis and down regulation of proteins involved in oxidative phosphorylation or Substrate-level phosphorylation activity. Direct translational control by one of the twin sRNAs was proven for 6 enzymes involved in the TCA cycle and for two enzymes involved in propanoate metabolism connected to the TCA cycle. Overexpression of the twin sRNAs did not impair meningococcal growth in nutrient-rich medium or in fresh human blood, whilst growth was drastically inhibited in medium with glucose as the sole carbon source or in human CSF.
In conclusion, in meningococci the two main energy generating systems are regulated by two different sRNAs. For the first time evidence is provided, revealing the mechanism by which components of the respiratory chain are regulated by Fur via NrrF. In addition, the twin sRNAs targeting the TCA cycle are the first example of sRNAs in bacteria involved in switching between feast/famine growth conditions. We propose to name these sRNAs feast/famine regulator RNA (FfrR).

O 05

A *cis*-acting, non-coding RNA is required to form the guanine quartet structure essential for pilin antigenic variation in *Neisseria gonorrhoeae*

L. Cahoon[1], H. Seifert[1]

[1]Northwestern University, Dept of Microbiology-Immunology, Chicago, USA

To evade the host immune responses, pathogens have evolved mechanisms to provide genetic diversity in targets of immune surveillance. Antigenic variation (Av) systems rely on cellular recombination machinery to catalyze dedicated high-frequency reactions without leaving delirious effects on the genome. Previously, we defined a guanine quartet (G4) structure in the strict human pathogen *Neisseria gonorrhoeae* that is required for initiating the homologous recombination reactions leading to pilin Av (Cahoon, L. A. & Seifert, H. S. Science **325**:764, 2009). G4 structures have been implicated in many biological processes, the mechanisms allowing their formation within a chromosome has not been elucidated. In this work, we show a direct link between transcription of a small RNA (sRNA) and pilin Av. sRNAs have emerged as important regulatory molecules in both eukaryotes and prokaryotes, but none have been proven to be responsible for the formation of a G4 structure. Here, we establish that blocking transcription of the sRNA blocks pilin Av and occurs upstream of recombination factors required for pilin Av. The sRNA transcript initiates within in thepilE G4 forming sequence. The sRNA promoter sequence and location is conserved in all sequenced gonococcal strains and all variable meningococcal strains. Substitution of thepilE G4-associated sRNA promoter with a T7 phage promoter allowed for wild-type levels of pilin Av, only when the T7 polymerase was expressed. Expression of thepilEG4-associated sRNA at a distal site on the gonococcal chromosome did not rescue a sRNA promoter mutant showing it must act in-*cis*. We postulate that transcription of this sRNA is required to unwind the DNA containing the G4 sequence to allow formation of thepilE G4 structure. We anticipate that reliance of G4 structure formation on transcription is a mechanism used by other systems that rely on this alternative DNA structure.

O 06

Comparison of the locations of Correia Repeat Enclosed Elements and potential small noncoding RNAs in the *Neisseria* spp

S. Roberts[1], L. A. S. Snyder[1]

[1]Kingston University, Life Sciences, Kingston Upon Thames, United Kingdom

Small noncoding RNAs do not code for proteins, but have been shown to perform regulatory roles in the cell, including the regulation of processes such as secretion and virulence. A small noncoding RNA,nrrF, that mediates Fur-dependant regulation of succinate dehydrogenase has been previously identified in *Neisseria meningitidis*. Deep sequencing of transcriptomes has revealed small noncoding RNAs in both *N. meningitidis* and *Neisseria gonorrheoae*. A small noncoding RNA, Hfq found in *N. meningitidis* is up-regulated during incubation in blood suggesting that noncoding RNAs might also play a role in *N. meningitidis* infection. Analysis of differential expression in intergenic regions showed enhanced transcription of a novel non-coding RNA molecule of 74 nucleotides, involved in the riboregulation of target mRNAs. Research on Correia Repeat Enclosed Elements in the *Neisseria* spp. has shown that many of them are 5' of genes and possess functional promoter elements. A comparison of available neisserial genome sequences was undertaken to see if Correia Repeat Enclosed Elements were found at similar positions to predicted and previously identified small noncoding RNAs. A high correlation between the locations of small noncoding RNAs and Correia Repeat Enclosed Element was seen in some strains.

O 07

Capsule biosynthesis in Neisseria meningitidis is regulated by an RNA thermosensor

C. Tang[1], E. Kugelberg[2], E. Loh[2], B. Gollan[2], Q. Zhang[2], C. M. Tang[2]

[1]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom

[2]Centre for Molecular Microbiology and Infection, Imperial College London, London, United Kingdom

To survive in the nasopharynx, the obligate human pathogen Neisseria meningitidis needs to constantly sense environmental stimuli encountered in the host and modify its gene expression accordingly. Capsule expression in the meningococcus is governed by a 134 bp intergenic region (IGR) between thesiaandc-troperons responsible for capsule synthesis and export respectively.

We have identified mutations within the IGR which lead to an increase in capsule biosynthesis and enhanced resistance against complement mediated killing, providing a mechanism for avoidance of this key aspect of innate immunity. Loss of an 8 bp repeat sequence immediately upstream of the ribosome binding site of the siaoperon leads to an increase in serum resistance and elevated cellular SiaA without changes in siaA mRNA levels. Inspection of the 5'-untranslated region of the siaA transcript revealed a predicted secondary structure indicative of an RNA thermosensor, which is destabilised by the loss of one copy the 8 bp repeat. Examination of SiaA levels in bacteria grown at 30, 37 and 42°C and in vitro transcription/translation assays confirmed that the siaoperon is precisely regulated by ambient temperature, but not after loss of the 8 bp repeat. RNA toe printing revealed reduced access of the 30S ribosomal subunit to the Shine Delgarno sequence in the 5' UTR.

Although the deletion is found in meningococcal disease isolates, it is almost invariably accompanied by a substitution of two bases in the remaining 8 bp sequence which re-establishes the thermosensor; these substitutions are only present in strains which have the 8 bp deletion. These findings emphasise the importance of thermal regulation of capsule biosynthesis in the meningococcus.

O 08

Structure-Function Relationships in the Gonococcal Transferrin-Iron Acquisition System

C. Cornelissen[1], N. Noinaj[2], S. Buchanan[2]

[1]Virginia Commonwealth University, Microbiology and Immunology, Richmond, USA
[2]National Institutes of Health, Molecular Biology, Bethesda, MD, USA

Neisseria gonorrhoeae can employ a variety of human proteins as sources of the necessary nutrient, iron. Transferrin, lactoferrin and hemoglobin can all be accessed by *N. gonorrhoeae* in a mechanism that involves surface exposed receptors and TonB-derived energy. The first of these iron uptake systems to be defined in molecular detail was the *N. meningitidis* transferrin-iron uptake system (Noinaj et al. 2012 Nature. 483:53-58). Using these structures as templates, we developed homology models for the gonococcal transferrin receptor proteins, TbpA and TbpB. We previously generated a two-dimensional topology model of TbpA based upon sequence diversity and surface exposure of a foreign epitope. Our 2D model turned out to be remarkably accurate in terms of predicting the structure of TbpA, which includes 22 transmembrane β-strands, 11 surface-exposed loops and an amino-terminal plug domain that occludes the inner βarrel oriface. The structure of TbpA in complex with human transferrin demonstrates that a helix finger within loop 3 directly interacts with the C-lobe of human transferrin, near the iron binding cleft. This is consistent with our observation that loop 3 is critical for transferrin binding and iron uptake function in the gonococcus. We demonstrated that a conserved motif within the plug is necessary for iron uptake function and that this sequence was capable of low affinity iron binding. The crystal structure of TbpA reveals a putative iron pathway through the barrel and plug which includes this conserved, iron-binding motif. The crystal structure of TbpB reveals two homologous lobes; however all in vitro binding data indicate that transferrin interacts exclusively with the N-lobe of TbpB. Structural data on complexes of TbpB-hTf and TbpA-TbpB-hTf support this model. In contrast, our in vivo data with gonococcal mutants indicate that the C-lobe also contributes to binding and iron extraction functions. Overall, the structure of TbpA is largely consistent with our functional analyses in *N. gonorrhoeae*. Interestingly, the TbpB crystal structure is not completely congruent with functional studies in the gonococcus, which suggests that the native structure of TbpB, as presented on the gonococcal outer membrane with the lipid anchor, might assume multiple conformations and/or associate with transferrin in ways not observed by crystallography. Models will be presented in which the various stages of transferrin-iron uptake in the gonococcus are depicted.

O 09

Insights into the Neisserial Transferrin Receptor via Structures of the Surface Lipoprotein, TbpB

C. Calmettes[1], R.-H. Yu[1], J. Alcantara[1], A. Schryvers[1], T. Moraes[1]

[1]University of Toronto, Biochemistry, Toronto, Canada

Pathogenic *Neisseriaceae* survive the iron-restricted host environment by over-expressing two outer-membrane proteins that consist of a transmembrane iron transporter (TbpA) and a surface-exposed lipoprotein (TbpB). Transferrin-binding protein (Tbp) A and B are two synergic receptors that together function to acquire iron from the host by sequestering and stripping iron from the host glycoprotein, transferrin (Tf). Herein we present the crystal structures of the prominent vaccine target TbpB from *Neisseria meningitidis*, alone and complexed with human transferrin (hTf). These structures give insight into the mechanism of iron acquisition. In particular, TbpB N-lobe's cap area binds the Tf C-lobe and stabilizes the holo form of the Tf C-lobe. A comparison with the human Tf receptor (hTfR) complex illustrates that hTfR binds to regions on human Tf that are also bound by TbpB, suggesting that the receptors directly compete for the same binding site. Together the recent structures of Neisseria meningitidis TbpB-Tf (Calmettes *et al.*, 2012) and TbpA-Tf (Noinaj *et al.*, 2012) give us a good approximation of the mechanism of iron acquistion via the bacterial Tf receptor, but there are still many questions to be addressed. The intimate details of the tenary complex of TbpA:TbpB:Tf will be discussed in addition to vaccine potential for the bacterial transferrin receptor.

References: Calmettes, C., Alcantara, J., Yu, R.-h., Schryvers, A. B., and Moraes, T. F. (2012) The structural basis of transferrin sequestration by transferrin-binding protein B. Nature Structural & Molecular Biology 19, 358-360. Noinaj, N., Easley, N .C., Oke, M., Mizuno, N., Gumbart, J., Boura, E., Steere, A.N., Zak, O., Aisen, P., Tajkhorshid, E., et al.(2012). Structural basis for iron piracy by pathogenic *Neisseria*. Nature 483, 53-58.

O 10

The structure, specificity and antigenicity of the FrpB iron transporter from Neisseria meningitidis

M. Saleem[1], S. Prince[1], S. Rigby[1], M. Imran[1], H. Patel[2], H. Chan[2], H. Sanders[2], M. Maiden[3], I. Feavers[2]
J. Derrick[1]

[1]The University of Manchester, Manchester, United Kingdom
[2]National Institute for Biological Standards and Control, Sout Mimms, United Kingdom
[3]The University of Oxford, Oxford, United Kingdom

Question: FrpB (also called FetA) is an outer membrane protein which is subject to a high degree of antigenic variation and is a promising vaccine component. It is a TonB-dependent transporter (TBDT) but its precise substrate specificity and the structural basis for antigenic variation are unclear.
Methods: We have used a combination of X-ray crystallography, electron paramagnetic resonance (EPR) spectroscopy and binding studies.
Results: We solved the crystal structures of two antigenic variants of FrpB, F3-3 and F5-1. A peak in the electron density map, verified by anomalous scattering measurements, identified a bound ion as Fe, located above the plug region in the central part of the 22-stranded beta barrel (Figure 1). The Fe atom is coordinated by side chains from Y132 and H133 from the plug domain, and Y347 and H573 from external loops 4 and 8. Comparison of two crystal forms, corresponding to the Fe-bound and unbound states, showed an alteration in the N-terminal plug region on Fe binding. Direct addition of Fe(III) to FrpB , followed by SEC and measurement of iron content by ICP-MS, demonstrated that the transporter binds iron with high affinity. EPR spectra of the bound Fe(III) atom confirmed that its chemical environment was consistent with that observed in the crystal structure. An H133A mutant showed a weaker Fe(III) EPR signal, which was abolished completely when spectra were collected for the H133A/Y347F double mutant. FrpB orthologs were identified in other Gram-negative bacteria which showed absolute conservation of the Fe-coordinating residues, suggesting the existence of a defined TBDT sub-family dedicated to the transport of iron. The region of antigenic variability lies in a separate sub-domain which extends outwards from the rest of the protein and consists of a short helix, followed by an extended strand which packs against a second, C-terminal helix (Figure 2). Binding of a monoclonal antibody, specific for the F3-3 variant, was only marginally affected by the binding of Fe(III).
Conclusions: The results are consistent with a single, high affinity binding site for Fe(III) in FrpB. The binding of Fe(III), apparently associated with the transporter function of FrpB, and recognition of the antigenic region by antibody are independent. We infer that the antigenic sub-domain has arisen separately as a result of immune selection pressure, apparently to distract the immune response from the primary function of the transporter.

figure 1

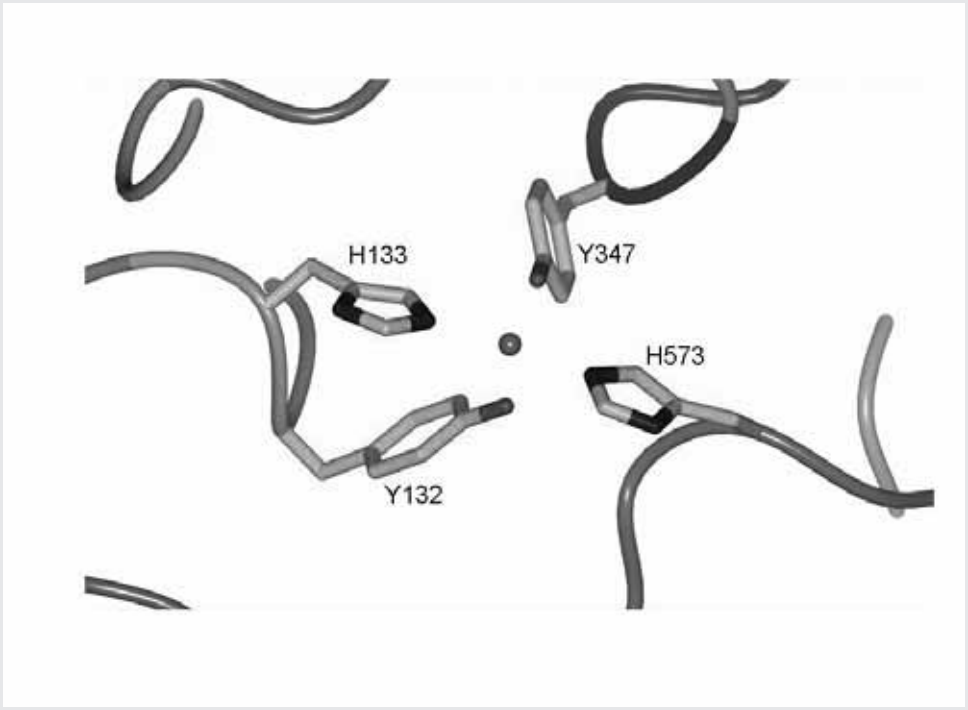
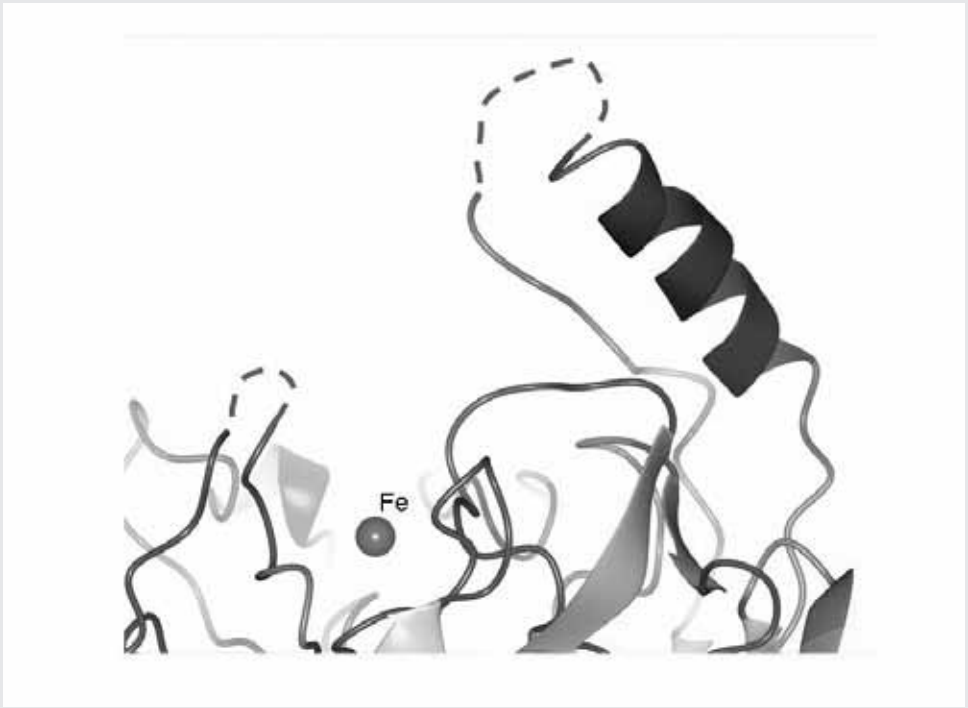


figure 2



O 11

Neisseria gonorrhoeae Modulates Iron-Limiting Innate Immune Defenses in Macrophages to Facilitate Iron Acquisition

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Background: *Neisseria gonorrhoeae* is a strictly human pathogen that causes sexually transmitted disease. Since iron is essential for bacterial growth, upon infection the host limits the bioavailability of iron by up-regulating expression of hepcidin, the master iron regulating hormone, which limits iron uptake from the gut and retains iron in macrophages. The host also secretes the antibacterial protein NGAL which sequester bacterial siderophores and therefore, inhibits bacterial growth. This host defense strategy is known as the iron-limiting innate immune defense. *N. gonorrhoeae* survives intracellulary although it does not produce siderophores. The aim of this study is to investigate whether infection with live *N. gonorrhoeae* affects expression of hepcidin and other iron-regulated proteins in macrophages in order to facilitate intracellular iron acquisition.
Methods: Human macrophage-like cell lines MM6 and THP-1and murine RAW264 macrophages were infected with live *N. gonorrhoeae* strain FA19 at MOI of 10 and incubated at 37°C for 16 hrs. Cellular responses were assessed for iron retention, induction and release of cytokines and nitric oxide. Expression of iron-regulating genes was assessed by quantitative RT-PCR.
Results: Infection with live FA19 gonococci induced the release of TNFα, IL-6, IL-1β, MCP-1 and IP-10 from human macrophages and nitric oxide release from murine RAW264 macrophages indicating the recognition of gonococci by the innate immune system. This infection induced significant up-regulation of hepcidin expression in human and murine macrophages as determined by qRT-PCR. The up-regulation of hepcidin led to increased iron retention in macrophages as measured by the Calien-AM fluorescent probe. Further, live infection with FA19 gonococci also up-regulated expression of NGAL, the iron carrier protein, and NRAMP1 in human and murine macrophages. NRAMP1 controls iron export from phagosomes and late endosomes and therefore plays a very important role in cellular iron homeostasis and host defense. In contrast, infection with FA19 gonococci down-regulated the expression of BDH2 enzyme that catalyzes the production of the mammalian siderophore, which is required for chelating and detoxifying labile intracellular iron and therefore, plays an important role in intracellular iron homeostasis. The data suggest that gonococci induced expression of hepcidin, which causes iron retention and increase labile iron pool in macrophages to facilitate iron acquisition. In addition, infection with live FA19 gonococci suppressed the expression of LL-37 in human THP-1 and MM6 macrophage-like cells to potentially support intracellular survival.
Conclusions: *N. gonorrhoeae* drastically modulated iron-limiting innate immune defenses to facilitate iron acquisition and promote intracellular survival in macrophages.

O 12 – Invited speaker

Evolution and function of O-linked protein glycosylation in the genus Neisseria

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The broad-spectrum O-linked protein glycosylation (pgl) system expressed by species within the genus Neisseria provides a unique model system in which to study the structure and function of protein-associated glycans as well as the underlying genotype – phenotype relationships. Genetic and biochemical analyses together with mass spectrometric- and serotyping-based glycan characterization have established that broad-spectrum pgl systems are found in all species within the genus but that pgl gene content and associated levels of glycan diversity may vary. Three major deviations in pgl genotype have been observed: the replacement of pglB by pglB2 (in N. meningitidis (Nm) and commensals), the loss of ORF2 / pglH (in N. gonorrhoeae (Ng) and Nm) and the absence of pglA and pglE in commensals. Based on these findings, a phylogenetically informative model for protein glycan evolution will be presented.
From a host-pathogen/symbiont perspective, two major gaps in knowledge relate to the forces driving protein glycan diversification and the functions glycosylation itself might have. Although most attention has been focused on modification of the type IV pilus pilin PilE, it is now clear that this system targets a large number of extracytoplasmic proteins as well. Although many of these ancillary targets are not predicted to be surface exposed, they are both periplasmically-localized and membrane-tethered as is PilE is in unassembled / disassembled state. Thus, it seems likely that protein glycosylation might serve a common, intracellular role. However, pgl null mutants display few if any distinct phenotypes. We found that expression of a particular hexa-histidine-tagged PilE was associated with growth arrest. By studying intra- and extragenic suppressors, we found that this phenotype was dependent on pilus assembly and retraction. Based on these results, we developed a sensitive tool to identify factors with subtle effects on pilus dynamics. Using this approach, we found that glycan chain length has differential effects on the growth arrest that appears to be mediated at the level of pilin subunit–subunit interactions and bidirectional remodelling of pilin between its membrane-associated and assembled states. Pilin glycosylation thus plays both an intracellular role in pilus dynamics and potential extracellular roles mediated through type IV pili. Together, these studies provide new perspectives on the functional correlates of bacterial protein glycosylation and glycoform diversification.

O 13

Structural Characterization of Outer Membrane components of the Type IV pili system in pathogenic Neisseria

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The obligate human pathogen Neisseria gonorrhoeae colonizes mucosal tissue in the urogenital tract causing the sexual transmitted disease gonorrhea. To adhere to epithelial cells Neisseria gonorrhoeae uses Type IV pili, which are also involved in twitching motility and natural DNA transformation. The main outer membrane component of this system is the secretin complex. Secretins form large oligomeric ring like structures, forming the gateway to the extracellular milieu, and are found in e.g. Type II and Type III secretion systems, filamentous bacteriophages and in Type IV pili. Here we show the analysis of the secretin PilQ of N. gonorrhoeae visualized by electron microscopy and single particle analysis. Secretin complexes of N. gonorrhoeae showed 14-fold symmetry with novel features like a peripheral ring with 14-fold symmetry and 7 extending spikes. In contrast, the secretin of N. meningitides showed no or only partial peripheral ring structures with 19-fold symmetry. The additional features of the N. gonorrhoeae secretin complex were chracterized by different mutants of the Type IV pili system. Deletion mutants of the adhesion PilC and the lipoprotein PilW showed no effect on the structural domains, whereas deletion of the assembly ATPase PilF lead to a lost of spikes but not in change of symmetry. Changes of 14-fold to 19-fold symmetry and also lost of spikes were shown for mutants with deleted pilin subunit pilE and lipoprotein pilP. Our secretin complex analysis of the Type IV pili system of Neisseria showed that it forms a large multidomain complex with novel features which have not been detected before.

O 14

Structure and assembly of an outer membrane channel for type IV pili in Neisseria meningitidis

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Question: PilQ is a member of the secretin superfamily, and forms a channel in the outer membrane (OM) for the passage of type IV pili. The secretins are also found in type II and type III secretion systems, but the way in which they mediate the passage of such a wide range of substrates across the OM is unclear. Here we present the structure of the N. meningitidis OM translocon, formed from a complex of the PilQ dodecamer bound to the PilP lipoprotein.

Methods: Solution state NMR was used to determine the 3D structures of individual domains from the N-terminal and central portions of PilQ. Single particle averaging was applied to generate a 3D structure of the entire PilQ dodecamer by cryoelectron microscopy. A combination of NMR chemical shift mapping, modelling and computationally-driven docking procedures were then used to reconstruct the PilQ:PilP oligomeric complex.

Results: The PilQ N-terminus consists of two beta-type domains which are unique to the type IV pilus-dependent secretins and have not been structurally characterised before. The second beta domain adopts an eight-stranded β-sandwich structure which is a novel variant of the HSP20-like fold (Figure 1). The central part of PilQ consists of two alpha/beta fold domains: the structure of the first of these forms a similar fold to domains from other secretins, but with an additional alpha helix which links it to the second alpha/beta domain. The PilP C-terminal domain binds specifically to the first alpha/beta domain in PilQ, in a similar fashion to GspC:GspD recognition in type II secretion system secretins. The PilQ dodecamer forms a large cage-like structure, sealed at both ends; specific regions were identified which corresponded to the individual PilQ domains. Individual domain structures were then docked into the cryoelectron microscopy density map, hence allowing reconstruction of the PilQ: PilP dodecameric assembly (Figure 2).

Conclusions: The PilQ assembly needs to disassemble substantially to accommodate the pilus fibre, and the beta domains must play a critical role in gating access to the secretin chamber. PilP appears to play a central function in stabilising the PilQ assembly during the transit of pili across the OM and forming a link between the inner and outer membrane components of the type IV pilus biogenesis system.

Figure 1 Structure of the second beta domain.

Figure 2 Reconstruction of the PilQ:PilP dodecameric complex, viewed from above.

figure 1

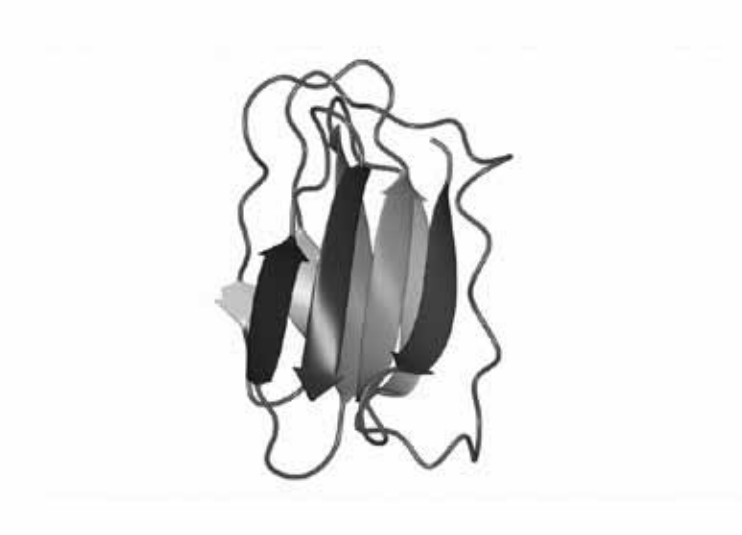

figure 2

O 15

The gonococcal type IV pilus – a motor with two speeds

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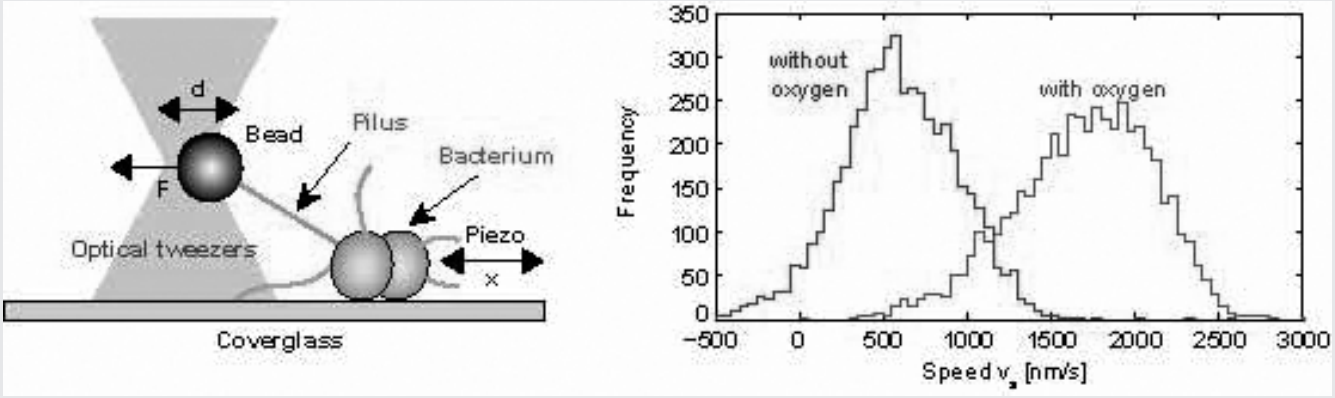
Type IV pili are polymeric bacterial appendages that affect host cell interaction, motility, biofilm formation, and horizontal gene transfer. They are molecular motors that can generate high force by retraction. We found that the motor can work at two distinct speeds, either at 2 μm/s or at 1 μm/s. This property is unusual for a molecular motor. We therefore addressed the question which regulatory input controlled motor speed.

Using a combination of image analysis and surface analytics, we simultaneously monitored the speed of twitching motility and the concentration of oxygen. While oxygen was detectable, Neisseria gonorrhoeae moved in the high speed mode. Upon full depletion of oxygen, gonococci simultaneously switched into the low speed mode. Speed switching was complete within seconds, independent of transcription, and reversible upon oxygen restoration. Using laser tweezers, we found that oxygen depletion triggered speed switching of the pilus motor at the single molecule level. Depletion of oxygen is likely to affect proton motive force and potentially the intracellular ATP concentration. We addressed the two components of the proton motive force as well as the effect of ATP depletion on speed switching. We conclude that gonococci implement a "power saving mode" of pilus dynamics in the absence of oxygen.

Figure legend: The speed of single type IV pilus retraction depends on oxygen. Left: setup for measuring speed of single pilus retraction. Right: Speed distribution of single pilus retraction in the presence and absence of oxygen.

figure 1



O 16

Type IV pili of Neisseria meningitidis recognize and bind to sialic acid-containing signaling-receptors

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The tropism of *N. meningitidis* for the wall of blood vessels is due to the expression, by the endothelial cells, of specific receptors of the type IV pili (tfp). This tfp-receptors interaction allows the adhesion and the localized remodeling of the sub cortical cytoskeleton, leading to the formation of endothelial membrane protrusions that anchor bacterial colonies at the luminal face of the endothelial cell membrane, allowing a better resistance to blood flow. Lately, *Neisseria meningitidis* recruits the polarity complex Par3/Par6/aPKC that re-routes junctional molecules at the site of bacterial cell interaction thus opening a paracellular route for bacteria to cross the endothelial barrier.
We previously report that *Neisseria meningitidis* specifically recruits and stimulates the β2-adrenoceptor β-arrestin signaling pathway in human brain endothelial cells (hCMEC/D3) and primary peripheral endothelial cells (HUVECs and HDMECs). We showed that type IV pili interact with the N-terminal domain of the β2-adrenoceptor. This domain contains two close N-glycosylation sites on Asn6 and Asn15. Here we demonstrate that the sugar moiety located on the β2-adrenoceptor N-terminal domain is necessary for *Neisseria meningitidis* induced signaling through this receptor and that the proximity of the two Asn is crucial for *Neisseria meningitidis* induced signaling. Moreover, we show that lectins specific for Sialic Acid inhibit *Neisseria meningitidis* induced recruitment of the cortical plaque. These results unrevealed the lectin property of tfp and represent a new way to activate the β2-adrenoceptor by interacting with its sugar moiety.

O 17

Rethinking the Role of Opa in Neisserial Pathogenesis

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To better understand the role of Opa in *Neisseria gonorrhoeae* (GC) pathogenesis, we generated a derivative of MS11 that had all 11 *opa* genes deleted and an isogenic set of 11 strains that individually expressed each Opa in a non-phase variable state. MS11Δopa and Opa-expressing MS11 [MS11(Opa+)] grew at the same rate. Suspensions containing comparable turbidity readings from non-piliated MS11(Opa+) and MS11Δopa consistently produced a log lower CFU measurement, even after vigorous vortexing. MS11Δopa rarely formed clumps of greater than a few bacteria when grown in broth with vigorous shaking, while MS11(Opa+) always produced large aggregates. MS11Δopa also lost the ability to bind to purified LOS. Using flow cytometry analysis, we demonstrated that MS11Δopa produced a homogeneous population of bacteria that failed to bind Mab 4B12, a Mab specific for Opa. MS11(Opa+) consisted of a two predominant populations, where ~85% bound significant levels of Mab 4B12; the other population bound very little. About 90% of bacteria isolated from a phenotypically Opa-negative colony (a colony that failed to refract light) failed to bind appreciable amounts of Mab 4B12; the remaining 10% bound varying degrees of Mab. Piliated MS11Δopa formed dispersed microcolonies on ME180 and HEC-1-B cells that were visually distinct from those of piliated MS11(Opa+). When MS11(Opa+) was inoculated onto the apical surface of polarized T84 cells, the rare bacterium recovered in the basolateral media after 6 hours was Opa-negative. When MS11Δopa was added to the apical surface, thousands of CFU were recovered from the basolateral media, in periods of time as short as 4 hours. Confocal microscopy indicated that these bacteria could be visualized in the paracellular space.

The data indicate that Opa expression facilitates invasion into epithelial cells. In the absence of Opa expression, GC are more likely to invade into tissues. A pathogen like *N. meningitidis*, which can express far fewer Opa variants, would have a greater likelihood of having Opa-negative bacteria in a colonized individual, and these bacteria would be capable of paracellular invasion into tissue. Taken in toto, this could provide one explanation as to why meningococci are more invasive. The data suggest that Opa contributes differently to different steps of infection. For invasive diseases that require GC transmigration across polarized epithelia, Opa expression may limit pathogenesis, rather than enhance disease.

O 18

Recruitment of PI3 kinase to caveolin 1 determines the switch from the local to the invasive stage of gonococcal infection

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Neisseria gonorrhoeae predominantly causes local infections of the urogenital tract but occasionally invades into the blood stream initiating disseminated gonococcal infections (DGI). The bacterial type 4 pili are important colonisation factors that facilitate the initial adherence to epithelial cells and the same time stabilize the local infection by inducing anti-invasive signals. The outer membrane porin $PorB_{IA}$ is associated with DGI and facilitates the highly efficient invasion of gonococci into a variety of different cell types. Here we demonstrate that both, type 4 pilus-mediated inhibition of invasion and $PorB_{IA}$-triggered invasion depend on formation of membrane rafts and phosphorylation of caveolin 1 at tyrosine 14 for these opposing cellular events. The pilus-mediated inhibition of invasion is achieved by interaction of phosphorylated caveolin 1 with Vav2 and RhoA-dependent accumulation of actin underneath lipid rafts In case of $PorB_{IA}$ triggered invasion we identified the p85 regulatory subunit of PI3K/Akt as new and critical interaction partner of phosphorylated caveolin-1 recruited to lipid rafts. By the additional activity of PLCy1 – another newly identified interaction partner of phosphorylated caveolin 1- PI3 kinase is activated and leads to PorBIA-dependent invasion. Active PI3K/Akt then induces the uptake of invasive gonococci via the unconventional protein kinase C μ (PKCμ/PKD1) and Rac1-induced actin reorganisation. Our data describe the PI3K/Akt- PKCμ/PKD1-Rac1 as a novel route of bacterial entry into epithelial cells and offer a first mechanistic basis for the switch from the extracellular to the invasive status of gonococcal infection.

O 19

Neisseria meningitidis hijacks the host cell focal adhesion complex for invasion

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Focal adhesions are large dynamic protein complexes which mediate the connections of the cellular cytoskeleton with the extracellular matrix and are involved in cell adhesion, cell motion and endocytosis. In this study we show that *N. meningitidis* targets signalling components in focal adhesions to subvert the host cytoskeleton machinery to promote its uptake.
We demonstrate that both the Src protein tyrosine kinase (PTK) and the focal adhesion kinase (FAK) are activated in response to contact with *N. meningitidis*. Selective pharmacological inhibition of Src by use of the Src inhibitor PP2 as well as inhibition of FAK by use of specific FAK inhibitor PF 573882 reduced meningococcal uptake into human brain microvascular endothelial cells (HBMEC) and human embryonic kidney (HEK) 293T cells up to 90%. Moreover, the dominant-negative version of FAK (FRNK) and overexpression of the cellular Src antagonist C-terminal Src kinase (CSK) also reducedN. meningitidis invasion. Importantly, Src-deficient as well as FAK-deficient mouse embryonic fibroblasts were significantly less invaded by *N. meningitidis*. In addition, binding of pathogenic *N. meningitidis* triggered the recruitment of the focal adhesion associated proteins vinculin, paxillin and zyxin to the site of bacterial attachment. Using a variety of approaches including siRNA and expression of dominant negative derivates we furthermore demonstrated a crucial role of the dual kinase complex FAK/Src in the activation of actin-binding protein cortactin. Based on immunoprecipiation experiments we observed that Src activity alone is sufficient to activate cortactin, whereas FAK cannot compensate for the loss of Src activity.
Mutation of critical amino acid residues within cortactin, that encompass Arp2/3 association and dynamin binding, significantly interfered with uptake of *N. meningitidis* suggesting that both domains are critical for efficient meningococcal invasion into eukaryotic cells.
Together, these data suggest a model in which the dual kinase complex FAK/Src Src is exploited by *N. meningitidis* to promote recruitment and activation of cortactin leading to transient actin cytoskeleton reorganization required for bacterial engulfment.

O 20

RNAi screening of the human kinome identified RTKs as a missing link between neisserial microcolony and cortical plaque formation

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Aim of the study: Type IV pili (Tfp) are key factors mediating initial attachment of pathogenic *Neisseria* spp. to human cells. It is well known that bacterial microcolonies are formed during infection, with the actin cytoskeleton and other cellular proteins subsequently recruited to attachment sites. However, key regulators of pathways underlying this remain enigmatic. Therefore, to elucidate the nature and identity of the *N. gonorrhoeae* pilus receptor,and additional factors involved in the infection process, we aim to perform a genome-wide loss-of-function (RNAi) screen in host cells. Here, we present the results of a pre-screen using a small, specified RNAi library which was carried out to test the assay set-up in terms of experimental procedures, microscopic assays and data analysis pipelines.
Methods: The kinome RNAi loss-of-function analysis was done in 384-well format using a robotic screening platform (Beckman). ME-180ActRFP cells, a human cervical epithelial cancer cell line, stably expressing LifeAct-RFP, were transfected with siRNAs using HiPerFect reagent *(*SKV2 Validated Kinase Library V4.1, 2 siRNAs per gene*;* QIAGEN*).* After three days of incubation, cells were infected with a piliated, Opa-negative and GFP-expressing *N. gonorrhoeae* strain (derivative of MS11F3 strain) for three hours. Subsequently, non-adherent bacteria were removed from cells. Fixed cell read-out was based on automated fluorescence microscopy and image analysis software Scan^R (both Olympus). We monitored (i) *N. gonorrhoeae* attachment, (ii) microcolony formation by count and measurement of area, (iii) actin cytoskeleton recruitment beneath microcolonies and (iv) invasion rate. The complex data set was analysed using different bioinformtic tools, e. g. KNIME (Konstanz Information Miner).
Results: The pre-screen demonstrated that the established assay set-up is robust and reliable. Silencing of *ezrin*, used here as positive control, which is known to be recruited to the site of infection and to be involved in the process of cortical placque formation, reduced actin recruitment remarkably. Furthermore, a notable number of proteins of the diverse receptor tyrosin kinase (RTK) and Src kinase families were found to be involved not only in actin recruitment, but also in microcolony formation. We are currently completing follow-up work and it is anticipated that the realisation of the genome-wide RNAi screen will further clarify the interplay between epithelial host cells and *N. gonorrhoeae* during infection.

O 21

N. gonorrhoeae causes DNA damage and interferes with host cell cycle regulation

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Prolonged local gonococcal existence and survival in the epithelial cell lining can be achieved by slowing down the progression of the cell cycle, leading to reduced epithelial renewal and exfoliation to the detriment of mucosal barrier fidelity. The protected niche created by gonococcal infections is a favorable environment for bacteria, but its persistence also causes DNA damage to the reproductive tract. We reveal that gonococcal infection causes DNA damage with at least 1000 additional strand breaks per cell per hour in non-tumor vaginal epithelial cells. Infected cells exhibited elevated levels of DNA double-strand breaks, as indicated by a more than 50% increase in cells expressing DNA damage-response protein 53BP1-positive foci that co-localized with phosphorylated histone H2AX (γH2AX).
As consequence, increased levels p21 and p27 in a p53 independent manner, *N. gonorrhoeae* slowed down the host cell cycle by impairing progression through the G2 phase leading to a G1 arrest after 24 hours of infection. In G1 phase, growth factors, such as amphiregulin, are active to stimulate cellular growth and cell cycle progression. Amphiregulin is belonging to the epidermal growth factor (EGF) family and is upregulated in different forms of cancers. Upon infection, a massive up-regulation of amphiregulin mRNA is seen. The protein changes its subcellular distribution and is also alternatively cleaved at the plasma membrane, which results in augmented release of an infection-specific product from the surface of human cervical epithelial cells that likely contribute to cellular abnormalities during *N. gonorrhoeae* infections.
We highlight the importance of studying the role of the gonococci in the regulation of host cell growth and proliferation that will lead to a greater understanding whether *N. gonorrhoeae* infections could predispose host cellular malignancies.

O 22 – Invited speaker

Intimate Relations – Modeling infection by the Human-restricted Neisserial Pathogens

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The single greatest challenge to understanding infection, immunity and pathogenesis by Neisseria sp. is the lack of a tractable model that can be exploited to understand neisserial life within the mucosa. Established infection models with wild type mice have provided important insight into certain aspects of infection and disease by both N. meningitidis and N. gonorrhoeae. However, these cannot consider the exquisite adaptation of Neisseria to life in humans since neisserial virulence factors are often specific for the human form of their target cell receptors or serum proteins. This has prompted our efforts to develop humanized transgenic mouse lines that encode up to eight different human proteins known to be targeted by neisserial virulence factors. This effort has proven fruitful, allowing prolonged infection involving an intimate association between Neisseria and the urogenital (gonococci) or nasopharyngeal (meningococci) mucosa. The humanized mouse models have allowed us to compare the relative contribution of humoral and cellular immune control of mucosal colonization, and to correlate specific immune memory responses with adaptive protection. Our findings from these studies will be discussed in the context of our ongoing effort to dissect the relative contribution of these and other interactions to neisserial infection and disease.

This work has been supported by operating grants from the Canadian Institutes for Health Research and the Alberta Heritage Foundation for Medical Research Team in Vaccine Design and Implementation.

O 23

Experimental studies of co-infected *Neisseria gonorrhoeae-lactobacillus crispatus* biofilms

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Lactobacillus crispatus (*Lc*) is a common commensal organism inhabiting the normal vaginal flora. It produces H_2O_2 and has been recommended as a natural biocide to maintain normal vaginal flora. Our studies with biopsies from patients with cervical gonorrhea suggest that *Neisseria gonorrhoeae* (*Ng*) forms mixed biofilms with other organisms in the genital tract flora. To study this experimentally, we developed methods to co-culture *Ng* with *Lc* and demonstrated microscopically that these organisms can form a mixed biofilm. Our studies suggest that mature *Ng* biofilm formation is controlled at least in part by the product of the *Ng luxS* operon. Using ComStat analysis, we have shown significant reduction in biofilm height and mass in an *Ng*1291 *luxS* mutant compared to biofilms formed by *Ng*1291 wildtype. In addition, the morphology of the biofilm is substantially different. In order to determine if the product of *Ng*1291 *luxS* operon also impacted *Lc* physiology, we examined the effect of sterile 6 and 24 hour *Ng* broth culture supernates on H_2O_2 production by *Lc*. Our studies showed that supernates from both the 6 and 24 hour *Ng*1291 wildtype cultures inhibited H_2O_2 production to a significantly greater degree than *Ng* 1291 *luxS* supernates. Heating of the *Ng* 1291 supernates to 100 C° resulted in almost complete ablation of the inhibitory activity. To determine if *Ng* catalase might be a factor involved in reducing the H_2O_2, we studied the effect of a *Ng*1291 *kat* mutant and found that there was a significant decrease in the ability to reduce H_2O_2 compared to *Ng* 1291 wildtype. However, the loss of H_2O_2 by the *Ng*1291 *kat* mutant was significantly less than that seen with the *Ng* 1291 *luxS* supernate suggesting that either other *Ng* enzymes were involved or there was direct suppression of H_2O_2 production in *Lc* by the product of *Ng luxS*. In conclusion, our studies suggest 1) *Ng* can form biofilms in the presence of *Lc*, 2) the product of the *Ng* l*uxS* operon plays a role in biofilm formation, 3) *Ng* can reduce H_2O_2 produced by *Lc* and 4) the product of the *Ng luxS* operon may be modulating factors produced by both *Ng* and *Lc* during biofilm formation.

O 24

Neisseria meningitidis adhesion to the vascular wall leads to *purpura* in a humanized mouse model

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Septic shock caused by the *Neisseria meningitidis* is typically rapidly evolving and often fatal despite antibiotic therapy thus requiring further understanding of the mechanisms of disease. Postmortem samples from the characteristic *purpuric* rashes of meningococcemia show bacteria in close association with microvessel endothelium but the species specificity of *N. meningitidis* has previously hindered the development of an *in vivo* model to study the role of adhesion on disease progression. We introduced human dermal microvessels into SCID/Beige mice by xenografting human skin. Bacteria injected intravenously exclusively adhered to the human vessel endothelium in the skin graft. Adhesion led to the secretion of human inflammatory cytokines and the recruitment of inflammatory cells. Importantly, adhesion also led to local vascular damage with hemostasis, thrombosis, vascular leakage and finally *purpura* in the grafted skin replicating the clinical presentation. The type IV pili of *N. meningitidis* were found to be the main mediator of adhesion *in vivo*. Bacterial mutants with altered type IV pili function did not trigger inflammation or lead to vascular damage, demonstrating the importance of local bacterial adhesion in the development of *purpura* and disease progression. This work shows how adhesion of *N. meningitidis* to the vascular wall determines vascular dysfunction in meningococcemia.

O 25

Neisseria infection of rhesus macaques – colonization, transmission, persistence and horizontal gene transfer

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The strict tropism of pathogenic *Neisseria* for man hampers the development of animal models that recapitulate microbe-host interactions such as colonization, transmission and persistence. We have developed a rhesus macaque model of infection using *Neisseria* species indigenous to that animal. We report that rhesus macaque *Neisseria* naturally persist in their monkey host for at least 6 years. They recolonize animals after laboratory passage, persist in the animals for at least 70 days, and are transmitted between animals. They are naturally competent and transfer genetic markers to each other *in vivo*. We conclude that the rhesus macaque infection model provides an excellent system for studying the molecular mechanisms of *Neisseria* colonization, long-term persistence, transmission and horizontal gene transfer. Rhesus macaque *Neisseria* encodes orthologs of current or candidate vaccine antigens. This model may therefore be further developed for use in preclinical testing of vaccine candidates.

O 26

Induced nasopharyngeal colonisation with *Neisseria lactamica* protects against carriage of *Neisseria meningitidis* in healthy adult volunteers

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Question: Nasopharyngeal carriage of *Neisseria meningitidis* is a pre-requisite for meningococcal disease. During childhood, carriage of *N. meningitidis* rises in inverse proportion to the carriage of *Neisseria lactamica*. This suggests that carriage of *N. lactamica* protects against *N. meningitidis* carriage and disease, though the mechanism of this remains undetermined. We previously showed that intranasal administration of *N. lactamica* to young adult volunteers generates mucosal and systemic immunity against *N. lactamica*, and generates weak, broad cross reacting opsonophagocytic activity against strains of serogroup B *N. meningitidis* but no specific bactericidal antibody response (SBA) (Evans C.*et al*, Clin Infect Dis 2011). We also previously showed that intramuscular administration of *N. lactamica* OMVs generates large rises in *N. lactamica* OMV specific IgG, but a modest rise in SBA titres to *N. meningitidis* (Gorringe A.*et al*, CVI 2009). Carriage of *N. lactamica*, but not *N. meningitidis* appeared to be inhibited after vaccination with *N. lactamica* OMVs, suggesting that the natural relationship between *N. lactamica* and *N. meningitidis* carriage is not due to cross reacting humoral immunity.
Methods: In the current study, 310 college students, aged 18-25, were recruited in October 2011. 1ml 10^4 cfu *N. lactamica* was administered intranasally to 149 individuals, and 161 participants received 1ml PBS control solution. Posterior pharyngeal swabs for *Neisseria* culture were collected at baseline, with repeat swabs at 2, 4, 8, and 16 weeks. At week 26 all individuals received a further inoculation of 1ml 10^4 cfu *N. lactamica*,and swabbed two weeks later.
Results: At baseline, carriage of *N. meningitidis* was similar between the groups with 24.2% (95%CI 17.2%- 31.0%) carriage in the challenge group compared to 21.1% (95%CI 14.8%- 27.4%) in the control group (p= 0.587). Two weeks after inoculation 33.6% (95%CI 25.8%- 41.3%) of participants in the challenge group were successfully colonised with *N. lactamica* and carriage of *N. meningitidis* in this group was 14.7% (95%CI 8.9-20.5) compared 23.6% (95%CI 16.9-30.2) in the control group (p=0.058). The observed trend of reduced *N. meningitidis* carriage in the challenge compared to control group continued at each visit, at weeks 8 (challenge:13.4%;Control 26.1% p=0.0062) and week 16 (challenge: 19.0%;Control:33.6% p= 0.0068). This effect on *N. meningitidis* colonisation was due to a reduction in carriage and a reduction in acquisition of *N. meningitidis* both in the group colonised with *N. lactamica* and those challenged but not colonised.
Conclusion: We conclude that inoculation of *N. lactamica* into the nasopharynx of young healthy adults prevents natural acquisition o f *N. meningitidis*, but this is not due to cross-reacting humoral immunity

O 27

Global action plan to control the spread of antimicrobial resistance in Neisseria gonorrhoeae

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O 28

Emergence of untreatable gonorrhoea – How, when and crucial actions?

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The new superbug Neisseria gonorrhoeae has retained resistance to antimicrobials previously recommended for first-line treatment and now shown its capacity to develop in vitro and clinical resistance to the extended-spectrum cephalosporin ceftriaxone, the last remaining option for first-line empiric treatment of gonorrhoea. An era of untreatable gonorrhoea may be approaching, which represents an exceedingly serious public health problem. In the talk, the evolution, origin, emergence and spread of AMR and genetic resistance determinants (focus on extended-spectrum cephalosporins) in N. gonorrhoeae; the current situation regarding verified treatment failures with extended-spectrum cephalosporins, and suggested future treatment options will be presented and discussed. Essential actions to combat the emergence of multidrug resistant and possibly untreatable gonorrhoea will also be highlighted, such as implementing the recently published WHO Global Action Plan and national or regional action/response plans, e.g., the ECDC Response plan for the European Union and the CDC Response Plan for the USA; enhancing surveillance of gonococcal antimicrobial resistance, treatment failures and antimicrobial use/misuse; and improving prevention, early diagnosis and treatment of gonorrhoea. Ultimately, novel effective treatment strategies, antimicrobials (or other compounds), and ideally a vaccine must be developed!

O 29

Challenges to a gonococcal vaccine – an alternative path forward

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Over the last 100 years, vaccines have proven to be one of the most important public health tool in the control and prevention of infectious diseases. A vaccine to prevent gonococcal infection would be a significant advance in the world wide effort to reduce the morbidity and mortality associated with these infections, in particular the disproportionate impact that they have on women, adolescents and infants. However, the development of a vaccine is a multi-step process with significant scientific, clinical, and manufacturing challenges. From the initial selection of the vaccine indication, all stages of the product development process are designed and focused on supporting that indication. Stages of the product pipeline, including selection and evaluation of target antigens, considerations for advancing vaccine candidates, product formulation and manufacturing will be discussed. Finally, a discussion of potential vaccine efficacy estimations and how those inform the statistical calculationsused in the design and sample size estimation for the clinical efficacy trials will be presented.

O 30

New antimicrobials as tools to combat multidrug-resistant Neisseria gonorrhoeae

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Multidrug-resistant N. gonorrhea is a worldwide health emergency that deserves immediate attention. In the U. S. only one class of antibiotic (the cephalosporins) is recommended to treat gonorrhea, and the development of resistance to these drugs may render the disease untreatable. Up to the present time treatment decisions have relied on the availability antimicrobial products that were not initially indicated and licensed for gonorrhea but nonetheless have favorable minimal inhibitory concentrations, adequate tissue distribution and acceptable safety profiles in target populations.As the utility of these products dwindle it is perhaps time to consider antimicrobials that are specifically developed for a gonorrhea indication. In this seminar, broad concepts in modern therapeutics development will be covered including preclinical components such as target product profiles, target identification, and chemistry, manufacturing and control in the choice of formulation and route of administration. Critical clinical components will also be discussed and will include Phase I safety studies and a hypothetical design for a pivotal Phase III trial for gonorrhea including potential public health implications. Finally, current efforts by NIAID to support the development of products to treat gonorrhea will be presented.

O 31

Resistance to cationic antimicrobial peptides is determined by oxidoreductases in *Neisseria sp.*

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Multi-drug resistant (MDR) strains of *Neisseria gonorrhoeae* have arisen and result in the failure of antibiotics to successfully treat infections. A second line treatment drug used in life threatening Gram negative infections is polymyxin (PxB). However, neisserial strains are intrinsically resistant to PxB, a phenotype which is determined by substitution of the lipid A headgroups of lipooligosaccharide (LOS) with phosphoethanolamine (PEA) by the LOS PEA transferase, LptA. To understand this mechanism of PxB resistance in *Neisseria sp.,* we have solved the crystal structure of LptA and identified a mechanism of control which is dependent upon enzyme stability in the periplasm.

We have solved the crystal structure of the soluble fragment of LptA which consists of an alkaline phosphatase fold surrounding an active site which binds at least one metal. The protein also contains four disulphide bonds which are located on the surface of the protein. Oxidoreductases are required to introduce disulphide bonds into proteins, and we examined which of the three neisserial oxidoreductases, DsbA1, DsbA2 or DsbA3 were responsible for the stability of the enzyme. By examining the amount of PEA substitution of lipid A expressed by the various oxidoreductase mutants, we showed that the stability of the enzyme was dependent upon the oxidoreductase DsbA3 but not DsbA1 or DsbA2.

In conclusion, LptA contains disulphide bonds which are essential for stability and function of the enzyme. Since oxidoreductases maintain the oxidation of LptA in the periplasm, these enzymes are also an important in determining resistance to PxB in MDR gonococci.

O 32

Sulforaphane induces the expression of antimicrobial peptides that kill Neisseria gonorrhoeae and suppresses inflammation induced by gonococcal lipooligosaccharide

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Questions: Novel therapeutic approaches are needed to treat and control the spread of gonorrhea due to the rapid emergence of antibiotic resistant strains. Sulforaphane (SFN) is a dietary histone deacetylase inhibitor (HDACi) that is known to induce increased transcription of innate immunity genes and suppress pathogen-induced inflammation. Here we asked whether SFN would induce the synthesis of antimicrobial peptides (APs) with activity against Neisseria gonorrhoeae (Gc) in human genital tract cells. We also asked whether SFN could suppress Gc-induced inflammation, as inflammation can damage tissue and is also an undesirable consequence of immunomodulatory therapies against gonorrhea.
Methods: Human ME180 (cervical) and Hec1B (endometrial) cells were treated with 5, 10 or 20 μM SFN and the expression of nine innate effector genes was measured by real time RT-PCR after 24 hours of incubation. Gc strains FA1090, MS11 and F62 were incubated with supernatants from SFN-treated and untreated cells and the number of viable bacteria was determined by quantitative culture. Susceptibility of Gc to synthetic human defensins HBD-2 and HD-5 and to secretory leukocyte protease inhibitor (SLPI) was tested similarly. The capacity of SFN to control Gc-induced inflammation was measured by incubating Gc lipooligosaccharide (LOS) with SFN-treated and untreated NF-κB reporter cell lines.
Results: Expression of SLPI, HBD-2 and HD-5 was increased in SFN-treated ME180 and Hec1B cells in a dose-dependent manner. Supernatants from ME180 or Hec1B cells treated with 20 μM SFN exhibited maximum killing against strains FA1090 and F62, while strain MS11, which naturally over-expresses the mtrCDE operon due to mutations in the mtrR locus, was resistant to killing. SLPI was bactericidal against Gc strain FA1090 (see abstract, A. Marinelli) and HBD-2 and HD-5 were bactericidal against strains FA1090 and F62 bacteria (effective dose, ED50: 1.25 - 2.5 μg/ml). In contrast, strain MS11 and an mtR mutant of strain FA1090 were resistant to HBD-2 and HD-5 (ED50 > 5 μg/ml), and therefore, HBD-2 and HD-5 appear to be substrates of the mtrCDE active efflux pump. Finally, pretreatment of mouse embryonic fibroblast NF-κB reporter cells with SFN suppressed Gc LOS-mediated activation of NF-κB, the pathway utilized in the production of proinflammatory cytokines.
Conclusion: SFN induces changes in host gene expression that result in increased production of one or more soluble factors that kill Gc. Human defensins HBD-2, HD-5 and SLPI, a bactericidal protein with immunomodulatory activity, were identified as the potential effectors responsible for this observation. This work supports the potential of SFN, a candidate immunomodulatory agent, as a therapy for gonorrhea. Further development should also include compounds that induce host-mediated protection against strains that naturally overexpress the mtrCDE operon.

O 33

Mechanisms of high-level antibiotic resistance in *Neisseria gonorrhoeae*

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Chromosomally mediated resistance in *Neisseria gonorrhoeae* to β-lactam antibiotics is complex and to date, four determinants have been identified at the molecular level: *penA* (mutations in penicillin-binding protein 2 [PBP 2]), *mtrR* (overexpression of the MtrC-MtrD-MtrE efflux pump), *penB* (mutations in $PorB_{1b}$), and *ponA* (mutation in PBP 1). These determinants can be readily transferred from a penicillin-resistant donor (FA6140) to a susceptible recipient strain (FA19) by transformation and homologous recombination. However, despite repeated attempts, transformation to high-level penicillin resistance equivalent to the donor strain has not been achieved, indicating the existence of a fifth, uncharacterized mechanism. Bioinformatics studies analysis revealed 57 open reading frames found in two high-level penicillin-resistant strains (FA6140 and 35/02) that were absent from FA19 and FA1090, but introduction of these genes into a 4^{th}-level transformant (FA19 penA mtrR penB ponA) had no effect on the MIC_{pen}. Reversion of each of the known determinants in FA6140 back to wild-type in a step-wise fashion revealed that the phenotype of increased resistance is expressed even in the absence of the four known determinants. To examine whether this increased resistance is specific to β-lactam antibiotics or also includes other antibiotics, we determined the MICs of a broad range of antibiotics against FA6140 and the highest-level transformant for each individual antibiotic. These studies demonstrated that FA6140 has increased resistance to all bactericidal, but not bacteriostatic, antibiotics compared to the highest-level transformant of FA19. This result is strikingly reminiscent of studies in *E. coli* whereby bactericidal antibiotics, no matter which pathway they target, kill bacteria through Fe^{2+}-mediated generation of hydroxyl radicals. Treatment of gonococci with a hydroxyl-radical scavenger or an iron chelator prevented bactericidal antibiotic-induced killing, suggesting that these antibiotics also induce the same mechanism of killing in *N. gonorrhoeae*, and that high-level resistant strains, such as FA6140, display pan resistance to bactericidal antibiotics by preventing or delaying hydroxyl radical formation until higher levels of antibiotics are present. Taken together, our results show that high-level resistance in *N. gonorrhoeae* is even more complex than anticipated and appears very different from the highly specific mechanisms underlying the known determinants.

O 34

Molecular and structural analysis of penicillin-binding protein 2 from the cephalosporin-resistant *Neisseria gonorrhoeae* strain H041 – molecular mechanisms underlying treatment failures in the clinic

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The emergence of *Neisseria gonorrhoeae* strains that exhibit resistance to expanded-spectrum cephalosporins may soon herald an era of untreatable gonorrhea. One such strain is H041 (Ohnishi *et al.* Antimicrob Ag Chemother. 2011), for which the MICs of cefixime and ceftriaxone far exceed the established breakpoints for these antibiotics. Increased resistance to β-lactams has been driven in large part by mutations in *penA*, the gene that encodes the essential transpeptidase, penicillin-binding protein 2 (PBP 2). When *penA* from H041 (*penA41*) is transformed into the penicillin-susceptible strain FA19, the MICs for cefixime and ceftriaxone rise to 1.7 and 0.36 μg/ml, respectively, beyond the breakpoints of 0.25 μg/ml. Our previous work identified a subset of mutations in *penA* critical for intermediate resistance to cephalosporins in N. gonorrhoeae strain 35/02 (Tomberg *et al.* Biochemistry. 2010). Comparison of *penA* sequences shows that H041 contains 13 additional mutations compared to 35/02. To evaluate the respective contribution of these additional mutations to the higher levels of resistance in H041, the effect of each individual mutation on the resistance of FA19 was determined. The mutations were incorporated into the *penA35* gene from 35/02, and the resulting mutant genes were transformed into FA19. The MICs of cefixime were doubled to 0.25 μg/ml for each of the individual mutations A311V, T316P and T483S, whereas other mutations had little to no effect. A double A311V/T316P mutant raised the MICs additively to 0.5 μg/ml. The triple mutant (A311V/T316P/T483S), however, raised the MIC to 1.5 μg/ml, which is only slightly below the MIC conferred by *penA41*. Similar increases in the MICs of ceftriaxone were also observed. These data indicate that the increased resistance in H041, compared to 35/02, is conferred primarily by three of the additional 13 mutations found in *penA41*. Mapping these onto the crystal structure of PBP 2 shows that two mutations reside on the same helix as the serine nucleophile (Ser310), which is critical for the catalytic function of PBP 2, while the T483S mutation lies on a loop close to the active site. Using these data and those from our previous study on the important mutations in *penA35*, we have now identified 8 mutations, which, when incorporated into the wild-type *penA* gene, confer essentially all of the resistance bestowed by *penA41*. Structural investigations to determine how these mutations impact the structure and mechanism of PBP 2 are ongoing.

O 35 – Invited speaker

Progress and perspectives of MenAfriVac, a meningococcal A conjugate vaccine for the African meningitis belt

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Major recurrent group A meningococcal epidemics have posed a major public health problem for over a century in the African meningitis belt where outbreaks occur annually with large epidemics striking at intervals ranging between 8 and 12 years. The Meningitis Vaccine Project (MVP) is a partnership between the World Health Organization (WHO) and PATH, funded by the Bill & Melinda Gates foundation. It was established in 2001 with the mission to eliminate epidemic meningitis as a public health problem in sub-Saharan Africa through the development, testing, licensure, introduction, and widespread use of affordable meningococcal conjugate vaccines.

Following global international standards, an affordable monovalent group A conjugate vaccine, MenAfriVac (Serum Institute of India, Ltd), was developed through an innovative public private partnership. After Indian market authorization (December 2009) and WHO prequalification (June 2010), MenAfriVac was introduced at public-health scale in countrywide vaccination campaigns with a single dose among 1- to 29-year-olds. The introduction started in 2010 with three meningitis belt countries (Burkina Faso, Mali, and Niger). In 2011, three new countries (Cameroun, Chad, and Nigeria) launched their national campaigns starting with regional vaccine introductions. The 2011 and 2012 epidemic seasons were characterized by a dramatic fall in cases of group A meningococcal disease in the three initial countries and ended with no reported case in any of the 57 million vaccinated individuals to date.

The high vaccine coverage achieved augurs well for further rollout of the vaccine in Cameroun, Chad, Nigeria, and in the additional 19 countries that constitute the African meningitis belt. Continuing surveillance for cases of meningitis and monitoring of vaccination coverage and safety will be crucial to confirm the effects of the vaccine as it is introduced across the entire belt. An important challenge after the initial mass immunizations campaigns will be the protection of new birth cohorts. Vaccination strategies are being evaluated, and current research results indicate this could be achieved through immunization starting in late infancy.

O 36

Identifying optimal vaccination strategies for serogroup A *Neisseria meningitidis* conjugate vaccine in the African meningitis belt

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Question: A low-cost conjugate vaccine (MenAfriVac) against serogroup A *Neisseria meningitidis* (MenA) has recently become available to countries in the African meningitis belt. The optimal long-term vaccination strategies to provide the population-level protection against MenA are unknown. We developed an age-structured mathematical model of MenA transmission, colonization, and disease in the African meningitis belt, and used this model to explore the impact of various vaccination strategies.
Methods: The model stratifies the simulated population into groups based on age, infection status, and MenA antibody levels. We defined model parameters (such as birth and death rates, age-specific incidence rates, and age-specific duration of protection) using published data and maximum likelihood estimation. We assessed the model's validity by comparing simulated incidence of invasive MenA and prevalence of MenA carriage to observed incidence and carriage data.
Results: The model fit well to observed age- and season-specific prevalence of carriage (mean pseudo-R^2 0.84) and incidence of invasive disease (mean R^2 0.89). The model is able to reproduce the observed dynamics of MenA epidemics in the African meningitis belt, including seasonal increases in incidence, with large epidemics occurring every eight to twelve years. Following a mass vaccination campaign of all persons 1-29 years of age, the most effective modeled vaccination strategy is to conduct mass vaccination campaigns every 5 years for children 1-5 years of age. Less frequent campaigns covering broader age groups would also be effective, although somewhat less so. Introducing MenAfriVac into the EPI vaccination schedule at 9 months of age results in higher predicted incidence than periodic mass campaigns.
Discussion: We have developed the first mathematical model of MenA inAfricato incorporate age structures and progressively waning protection over time. Our model accurately reproduces key features of MenA epidemiology in the African meningitis belt. This model can help policy makers consider vaccine program effectiveness when determining the feasibility and benefits of MenA vaccination strategies.

O 37

The Effects of Conjugate Vaccination on the Population Structure of *Neisseria meningitidi* – A New Paradigm for Capsule Replacement

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The use of conjugate vaccines to control meningococcal disease has raised concerns about the possibility of capsule replacement, in which strains not targeted by vaccines expand to fill the niches left vacant by vaccine-eliminated strains. Such post-vaccination replacement has been widely observed for *Streptococcus pneumoniae*. Despite concerns however, this phenomenon is yet to be observed on a widespread scale among meningococci. A paradox thus exists among encapsulated bacteria: why has replacement occurred among pneumococci but not meningococci?

We present a novel evolutionary framework to explore the phenomenon of capsule replacement, based on selection acting upon multiple loci in the meningococcal genome. The framework was built upon that of Buckee *et al*. (PNAS 2008 105:15082-7), which investigated effects of virulence, transmissibility and immune selection in two separate models. Here, we integrate these three genetic properties into one deterministic framework, combining: (i) an antigenically variable region; (ii) metabolic genes conferring transmission fitness; (iii) a combination of loci conferring either an increased or decreased likelihood of invasive disease.

Immune selection acting on variable antigenic determinants causes populations to segregate into non-overlapping associations to evade population immunity (Gupta *et al.* Nat Med 1996 2:437-442). In the model, these are represented among meningococci by diverse outer membrane proteins (e.g. PorA), and among Gram Positive pneumococci, which lack an outer membrane, by the capsule. The relatively low number of meningococcal serogroups (~13) compared to the pneumococcus (>90) precludes the amenability of the meningococcal capsule to the segregating forces of immune selection. Our model shows that virulence and metabolic alleles from vaccine strains transfer to non-vaccine antigens and then increase in prevalence; this may reflect capsule replacement in pneumococci but outer membrane antigen replacement in meningococci.

The model provides a cohesive framework incorporating interactions between strains, structural differences between membranes, and evolutionary pressures at a number of sites in the genome, to study the emergence of virulence and predictions of capsule replacement.

O 38

Comparison of Innate Regulation of Antibody Responses to Meningococcal Polysaccharide and Meningococcal Conjugate Vaccines in Healthy Adults

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Question: Innate responses to the quadrivalent (A, C, Y, W-135) meningococcal polysaccharide vaccine (Menomune®) and meningococcal conjugate vaccine (Menactra®) were evaluated in vivo.
Methods: Healthy adults were vaccinated with Menomune (n=13) or Menactra (n=17). Gene expression microarrays of PBMCs obtained at days 0, 3 and 7 were evaluated. Serum bactericidal activity (SBA) to serogroup C *Neisseria meningitidis* was assessed in sera collected at days 0, 30, 180 and 730.
Results: Menactra induced a stronger transcriptomic response in day 7 that was highly enriched for B cell signatures. Early (e.g., CDC42 signaling events) and late (e.g., FoxM1 transcriptase factor network) pathways were identified for both vaccines. For example, PLK1 signaling leading to suppression of IFN was significantly upregulated by Menactra at days 3 and 7, but not by Menomune. These responses were compared to "antibody predictor" genes previously identified for yellow fever (YF-17D) and influenza vaccines (Flu TIV and LAIV) (Querec et al., *Nature Immunol* 2009; Nakaya et al., *Nature Immunol* 2011). Menactra and Menomune highly upregulated genes such as TNFRSF17 (tumor necrosis factor receptor superfamily, member 17), shown to be a predictor of antibody responses for these other vaccines. Comparison of integrated networks reveals 76 genes common in the transcriptomic response for Menomune, Menactra, YF-17D, Flu TIV and LAIV.
Conclusions: Both Menomune and Menactra induce significant increases in SBA titers against meningococcal serogroup C *N. meningitidis* at days 30 and 180. Meningococcal polysaccharide and conjugate vaccines provide a framework for evaluating similarities and differences in innate signatures generating specific antibody responses to these vaccines and to other antibody-inducing vaccines.

O 39

Long term persistence of serogroup C meningococcal serum bactericidal antibody and the impact of an adolescent booster dose

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Background: The incidence of serogroup C meningococcal (MenC) disease fell sharply after the introduction of glycoconjugate MenC vaccines with a catch-up campaign. However, serum bactericidal antibody (SBA) levels wane after vaccination in early childhood and it is likely that population protection relies on herd immunity which may be dependent on immunity in adolescents and young adults. Some countries have recently recommended routine vaccination in adolescence to maintain herd immunity in the face of waning immunity. However, the duration of protection after an adolescent booster is unknown.
Methods: This study followed a cohort of English schoolchildren who received their first dose of MenC CRM glycoconjugate vaccine in 2000, at 9-12 years of age. Some of them were randomised in 2003-04 (at 13-15 years of age) to receive a booster dose of either MenC CRM glycoconjugate vaccine (GC group), or bivalent MenAC polysaccharide vaccine (PS group); each vaccine contained 10μg MenC polysaccharide. The control group received no booster. In 2011-12, a blood sample was obtained and analysed for MenC SBA (using rabbit complement), 11 years after the initial immunisation.
Results: 134 of the original cohort of 531 took part in the current study. 7 participants were excluded from the analysis because they had received additional doses of MenC vaccine; an inadequate blood sample was obtained from 3 participants. 124 participants were included in the analysis (GC group 26, PS group 44, controls 54). Median age at priming was10.61 years (range 8.93-12.46). Median age at boosting was 14.42 years (range 13.01-15.73). Median age at blood sampling was 22.11 years (range 20.40-24.11). Geometric mean titre (GMT) for MenC SBA: GC group 1373 (95%CI 971-1942); PS group 1024 (695-1509); controls 284 (169-477). SBA titres were <8 in 4/54 (7.4%) of controls, and in 0/70 (0%) of those who had received a booster dose of vaccine.
Conclusions: Immunisation with a MenC glycoconjugate vaccine in late childhood provides much longer lasting protective antibody than vaccination in infancy. A booster dose of either polysaccharide or glycoconjugate vaccine given in adolescence produces a long-lasting elevation of SBA titre. MenC vaccination schedules which include routine immunisation of adolescents are likely to provide better overall protection for the population than schedules in which only infants and young children are immunised.

O 40

The Effectiveness of Quadrivalent Meningococcal Conjugate Vaccine (MenACWY$_D$) – A Matched Case-Control Study

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Background: In 2005, a quadrivalent (serogroups A, C, Y, and W-135) meningococcal conjugate vaccine (MenACWY$_D$) was licensed in the United States and recommended for adolescents as a single dose. We initiated a case-control study in January, 2006 to evaluate vaccine effectiveness (VE). This analysis provides estimates of VE 0-6 years after vaccination.
Methods: Cases of meningococcal disease (serogroups A, C, Y, or W-135) in persons aged ≥11 years and born on or after January 1, 1986 were identified through Active Bacterial Core surveillance (ABCs) and MeningNet enhanced surveillance sites. Controls were matched to cases by age and geographic area. We calculated VE estimates using both conditional logistic regression and the generalized estimating equations (GEE), controlling for underlying conditions (cancer, complement deficiency, immune deficiency, kidney disease, diabetes, sickle cell, asplenia) and smoking. The GEE approach allowed us to harvest information from case-control sets that were incompletely matched or had concordant vaccination statuses, and should produce vaccine effectiveness estimates similar to those obtained from traditional conditional logistic regression but with lower standard errors.
Results: As of May 1, 2012, 151 cases and 200 controls were enrolled. Serogroup C accounted for 73 (48%), serogroup Y 69 (46%), and serogroup W-135 9 (6%) of enrolled cases. Twenty-seven (18%) cases and 72 (36%) controls were vaccinated with MenACWY$_D$. Using conditional logistic regression, the overall VE was 66% (95% CI 24-84); VE was 67% (95% CI 5-89) against serogroup C and 65% (95% CI -39-91) against serogroup Y. Using GEE, the overall VE was 60% (95% CI 35-75); VE was 79% (95% CI 47-92) at <1 year, 73% (95% CI 34-89) at 1-<2 years, 44% (95% CI -17-74) at 2-<3 years, and 41% (95% CI -19-71) at 3-<6 years.
Conclusions: MenACWY$_D$ is highly effective in the first year after vaccination but wanes quickly. While data are not available on the VE of the second conjugate vaccine, MenACWY$_{CRM}$, these estimates informed the Advisory Committee on Immunization Practices (ACIP) in their decision to add a booster dose of MenACWY in adolescence, and will be monitored to inform future decisions to improve the US vaccination program.

O 41 – Invited speaker

Understanding the basis of anti-fHbp protective antibody provides new approaches for development of more effective second-generation meningococcal fHbp vaccines.

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Recombinant factor H binding protein (fHbp) is part of two promising vaccines for prevention of meningococcal disease caused by capsular group B strains. Anti-fHbp antibodies bind to the bacterial surface, activate the classical complement pathway, and inhibit binding of the complement down-regulating molecule, factor H (fH) to fHbp. With less bound fH, the organism becomes more susceptible to bacteriolysis via amplification of complement activation by the alternative pathway. For vaccine evaluation, fHbp amino acid sequence variants can be subdivided into two sub-families or three variant groups. In general, anti-fHbp antibodies confer protection only against strains expressing fHbp variants within a sub-family or variant group that is matched to the vaccine. Within a sub-family or variant group, however, strains can be resistant to anti-fHbp bactericidal activity when there is low fHbp expression. Strains with moderate fHbp expression also can be resistant when there are small differences in amino acid sequence identity between the vaccine and target antigen (Poster P 288). We also have observed differences in anti-fHbp bactericidal susceptibility among strains with similar expression of an identical fHbp sequence variant; the underlying mechanism is not known. Several approaches are being investigated to improve the breadth of protection elicited by fHbp vaccines. These include native outer membrane vesicle vaccines from mutants with attenuated endotoxin and over-expressed fHbp, and chimeric fHbp molecules that contain epitopes from more than one variant group. Mutant fHbp molecules engineered not to bind fH also appear to be promising since in human fH transgenic mice binding of fH to fHbp vaccines impairs immunogenicity. One potential limitation of fHbp as a vaccine antigen is that meningococcal isolates that lack a functional fHbp gene can cause invasive meningococcal disease. As shown in Poster P 286, these invasive mutant strains can bind human fH via alternative ligands such as NspA, survive in human serum, and cause bacteremia in human fH transgenic rats. Thus, fHbp is not an essential virulence factor in all strains, and immune pressure from widespread use of fHbp vaccines may select for circulating strains that do not require fHbp for fH binding and evasion of host defenses. Inclusion of additional vaccine targets in meningococcal vaccines containing fHbp may prevent or delay emergence of strains with diminished or absent fHbp expression.

O 42

Bactericidal antibody persistence two years following immunisation with investigational serogroup B meningococcal vaccines at 6, 8 and 12 months and response to a booster dose in 40 month old children

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Question: In a previous study 60 infants receiving either a serogroup B meningococcal vaccine containing recombinant-proteins alone (rMenB) or the proteins with an outer-membrane vesicle (4CMen B) at 6, 8 and 12 months of age produced serum bactericidal antibody (SBA) responses against multiple meningococcal strains. We studied persistence of this response.
Methods: In this extension study, rMenB and 4CMenB recipients had SBA titres evaluated before and after booster doses of their respective vaccines at age 40 months. Men B naïve age-matched children served as a control group. Sera were assessed against 4 reference strains: 44/76 -SL (assessing vaccine antigen fHbp), 5/99 (NadA), NZ98/254 (OMV/PorA) and M10713 (NHBA). Bactericidal activity against four additional strains (UKP1.7-2.4, GB101, GB355 and GB364) was also assessed.
Results: Prior to the booster doses, the proportions of 4CMenB recipients with SBA titres ≥ 1:4 were 36% (n=14, 95% C.I. 13-65%) for strain 44/76-SL, 100% (77-100%) for 5/99, 14% (2-43%) for NZ98/254 and 79% (49-95%) for M10713. These percentages were 14 to 29% for rMenB recipients (n=14), except for 5/99 (93%, 66-100%). For controls (n= 40) these proportions were ≤ 3% for all strains except M10713 (53%, C.I. 36-68%). For the additional four strains the percentages of 4CMenB recipients with SBA titres ≥ 1:4 were at least 69% except for one strain lacking vaccine antigens (GB355), where this proportion was 8% (0-36%). One month after a booster dose, ≥ 92% of 4CMenB recipients had SBA titres ≥ 1:4 for all strains except GB355 (31% (9% -61%)). For controls receiving their first dose of 4CMenB, 23% (11-39%) had SBA titres ≥ 1:4 for NZ98/254, 11% for GB355 (3-25%) and 100% for GB101 (91-100%). For the remaining strains these proportions were 59% to 87%.
Conclusion: These data suggest waning of bactericidal antibodies following infant immunisation with rMenB or 4CMenB, but an anamnestic response to a booster-dose. Booster doses of 4CMenB may be required to maintain immune protection through childhood and adolescence.

O 43

PorA -expressing adenovirus vectors as vaccines against serogroup B Neisseria meningitidis

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Question: A detailed understanding of serogroup B N. meningitidis(MenB) epidemiology strongly suggests that judicious combination of appropriate allelic variants of two well-established vaccine candidates, Porin A (PorA) and Ferric enterobactin transport protein A (FetA), could provide a high level of coverage against invasive MenB strains. Adenovirus vectors could provide a potent delivery system for bacterial protein antigens since they induce both innate and adaptive immune responses in mammalian hosts. Adenovirus vectors have been shown to induce T-cell and antibody responses after a single immunization for rabies, malaria and Ebola. In this study we evaluated the potential of adenovirus vectors as vaccine candidates against MenB.

Methods: Genes encoding either the full-length proteins, or chimeric constructs encoding only the known bactericidal epitopes, were inserted into replication-deficient adenovirus. Expression of the antigens was confirmedin vitrousing an immuno-fluorescence assay. Groups of mice were immunized with the menB antigens-expressing adenovirus vectors or outer membrane vesicles (OMV), and the antibody response assessed by ELISA.

Results: A single immunization with PorA expressing adenovirus induced IgG responses against recombinant protein and OMVs, largely exceeding the response obtained after one injection of a homologous OMV. The antibody concentration remained high up to 32 weeks after a single dose. Bactericidal capacity of the antibodies is currently being evaluated.

Conclusion: These data show that adenovirus vectors can express neisserial proteins in mammalian cells and indicate that such constructs could be used as vaccines against MenB.

O 44

The Adhesin Complex Protein (ACP) of *Neisseria meningitidis* is a new adhesin and invasin with vaccine potential

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Question: A goal for effective vaccine development is to identify antigens capable of inducing cross-protective bactericidal antibody responses. In the current study, we investigated the role of the Adhesin Complex Protein in meningococcal-host cell interactions and whether it showed any promise as a candidate vaccine antigen.
Methods: The *acp* gene encoding the 13-kDa Adhesin Complex Protein (ACP) from *Neisseria meningitidis* serogroup B strain MC58 was cloned and expressed in *E. coli*, and the purified recombinant (r) ACP was used for immunization of mice and rabbits. ACP amino acid sequence variation and protein expression was investigated in meningococci. Humoral immune responses were investigated with ELISA and western blotting and surface-exposure of ACP on meningococcal cells examined with FACS. The role of ACP in pathogenesis was investigated by comparing the interactions of wild-type MC58 and MC58ΔACP mutant bacteria with epithelial, endothelial and meningeal cells. The ability of antibodies to induce complement-mediated bactericidal activity was investigated against a variety of strains expressing different ACP proteins.
Results: Analysis of the ACP amino acid sequences from 13 meningococcal strains, isolated from patients and colonized individuals, and 178 strains in the Bacterial Isolate Genome Sequence (BIGS) Database showed only three distinct sequence types (I, II, and III) with high similarity (>98%). By contrast, gonococci encoded for 2 different ACP proteins, which showed ~95% similarity with meningococcal ACP proteins. Immunization of mice with type I meningococcal rACP in detergent micelles, liposomes and in saline alone induced high levels of serum bactericidal activity (SBA titres of 1/512) against the homologous strain MC58 and killed strains of heterologous sequence types II and III with similar SBA titres (1/128 to 1/512). Levels of expression of type I, II or III ACP by different meningococcal strains were similar. FACS analysis using rabbit anti-ACP sera demonstrated that ACP was exposed on the surface of meningococci. ACP functioned as an adhesin, as demonstrated by reduced adherence of *acp* knockout (MC58ΔACP) meningococci to human cells *in vitro* compared with wild-type (ACP^+) meningococci ($p<0.05$)
Conclusions: In summary, the newly-characterized ACP protein is an adhesin-invasin that induces cross-strain bactericidal activity and merits serious consideration for incorporation into the next generation of serogroup B meningococcal vaccines.

O 45

Meningococcal Opa binding to human CEACAM1 in transgenic mice negatively influences the Opa-specific immune response

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Outer membrane vesicles (OMVs) have been extensively investigated as meningococcal vaccine candidates. Among their major components are the opacity (Opa) proteins, a family of surface-exposed outer membrane proteins which are important for bacterial adherence and entry into host cells. Many of the Opa-dependent interactions are mediated through the carcinoembryonic antigen cell adhesion (CEACAM) family of receptors. Importantly, binding of Opa to CEACAM1 has been reported to suppress human CD4 T cell proliferation in vitro in response to OMV preparations. This suppressive capability raises the question whether OMV vaccines should contain Opa proteins at all, considering these may interfere with the immune response against OMV antigens by their inhibition of CD4 T cell proliferation. Until now it has been difficult to answer this question, as the proposed immunosuppressive effect was only demonstrated with human cells in vitro, while immunization experiments in mice are not informative because the Opa interaction is specific for human CEACAM1. Therefore in the present study we have used Opa+ and Opa- OMVs for immunization experiments in a human CEACAM1 transgenic mouse model. Transgenic mice with a genomic insertion of the complete human CEACAM1 sequence were generated. Tissue expression patterns were tested by immunohistochemistry, and were found to be similar to the human pattern. OMVs were prepared from a meningococcal strain H44/76 variant expressing the CEACAM1-binding OpaJ protein, and from an isogenic variant in which all opa genes have been inactivated. Both the CEACAM1 expressing transgenic mice and their congenic parent lacking it were immunized twice with the OMV preparations, and the sera were analyzed for bactericidal activity and ELISA antibody titers. Total IgG antibodies against the OMVs were similar in both mouse strains, but in the case of IgG antibodies specific for purified OpaJ protein significantly lower titers were found with the mice expressing human CEACAM1 as compared to the nontransgenic mice. This was reflected in the SBA, where lower bactericidal titers were raised with the Opa+ OMVs against an OpaJ+ H44/76 variant in the transgenic vs. nontransgenic animals, whereas no such difference was found against the Opa-negative H44/76 variant. Overall, these data do not support an Opa-CEACAM1 mediated immunosuppressive effect in vivo against OMV proteins in general, but only against Opa protein itself. Based on these results we conclude that while there is no need to deliberately exclude Opa proteins from OMV preparations, if one wants to specifically use Opa as a target for bactericidal antibodies it would be best to select variants which cannot bind to CEACAM1.

O 46

Proactive manipulation of host immune responses by *Neisseria gonorrhoeae* – role of immunosuppressive cytokines and type 1 regulatory T cells

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We have previously demonstrated in the mouse model of vaginal gonococcal infection (Jerse, 1999) that *N. gonorrhoeae* selectively induces Th17-governed innate immune defenses and suppresses Th1/Th2-governed adaptive immune responses through TGF-β-dependent mechanisms that can be reversed by treatment with anti-TGF-β antibody. TGF-β is a pleiotropic cytokine involved in T cell regulation, including the generation and function of T regulatory (Treg) cells. However, *N. gonorrhoeae* only slightly increases the expression of the Foxp3 transcription factor that is associated with induced Treg cell functional differentiation. Finding that *N. gonorrhoeae* induces very high levels of IL-10, but not IL-2 or IL-4, in murine lymphocytes, we hypothesized that the type 1 subset of regulatory T cells (Tr1) is involved. Tr1 cells are known to suppress Th1 and Th2 responses. Flow cytometry studies showed that IL-10 induced by *N. gonorrhoeae* was produced by $CD4^+$ T cells that were $IL\text{-}4^-$ and $Foxp3^-$, which is characteristic of Tr1 cells found in other mucosal tissues. Iliac lymph node cells or vaginal explants cultured in vitro with *N. gonorrhoeae* also released large amounts of IL-10, but not IL-2, IL-4, or IL-12. We have previously shown that, in mice infected with *N. gonorrhoeae*, treatment with anti-TGF-β antibodies accelerates clearance of the primary infection, alleviates suppression of Th1/Th2-driven adaptive immune responses, and generates immune memory such that secondary gonococcal challenge leads to anamnestic anti-gonococcal antibody responses and resistance to re-infection. IL-10-knockout mice, or mice treated with anti-IL-10 antibody showed accelerated clearance of vaginal gonococcal infection and the establishment of anti-gonococcal Th1/Th2 memory responses. Treatment of mice with both anti-TGF-β and anti-IL-10 antibodies increased the effect. Gonococcus-specific Tr1 cells were generated by prolonged culture of murine $CD4^+$ T cells (plus antigen-presenting cells) with *N. gonorrhoeae*. When these Tr1 cells were adoptively transferred into mice that were infected with *N. gonorrhoeae*, the development of adaptive immune responses and clearance of gonococcal infection were delayed. These findings strengthen our overall hypothesis that *N. gonorrhoeae* selectively and proactively induces Th17-governed innate responses that it can resist, and concomitantly suppresses Th1/Th2-governed adaptive immune responses that might be capable of eliminating it.

O 47

Neisseria gonorrhoeae suppression of immune response through antigen presenting cells

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N. gonorrhoeae is a pathogen that is highly adapted to the human genital tract. Despite causing a localized inflammatory response, infected individuals do not develop protective adaptive immune responses to *N. gonorrhoeae*. Reinfection with the same strain of *N. gonorrhoeae* is common. *N. gonorrhoeae* has acquired the capacity to evade host immunity through multiple mechanisms. *N. gonorrhoeae* is known to engage immunosuppressive signaling in B and T lymphocytes. We have now found that *N. gonorrhoeae* also suppresses adaptive immune response through effects on antigen presenting cells. Using primary dendritic cells and lymphocytes from mice and humans, we show that *N. gonorrhoeae*-exposed dendritic cells fail to elicit antigen-induced CD4+ T lymphocyte proliferation. *N. gonorrhoeae* exposure causes upregulation of a number of immunosuppressive factors in dendritic cells including both secreted and surface proteins, particularly Interleukin-10 (IL-10) and Programmed Death Ligand 1 (PDL1). We also show that the capacity of *N. gonorrhoeae* to maximally suppress dendritic cell-induced T cell proliferation requires IL-10 and PDL1. Our data now suggest that *N. gonorrhoeae* is able to influence host immune response through interactions with host antigen presenting cells. We further demonstrate that *N. gonorrhoeae* can suppress dendritic cell-mediated T cell proliferation through the release of soluble pathogen derived factors. Combined, these findings suggest that gonococcal factors involved in host immune suppression may be useful targets in the design of vaccines or therapies for this pathogen in order to stimulate host immune response.

O 48

Protection against vaginal colonization with *Neisseria gonorrhoeae* in mouse model by passive (2C7 mAb) and active immunizations using a peptide surrogate of the 2C7 LOS epitope

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The emergence of ceftriaxone-resistant strains of *Neisseria gonorrhoeae* may herald an era when gonorrhea will be untreatable with conventional antibiotics. There is an urgent need to develop vaccines against this infection. The 2C7 epitope is a conserved oligosaccharide (OS) structure, a part of lipooligosaccharide (LOS) on *N. gonorrhoeae*. The epitope is expressed by 94% of gonococci residing in the human genital tract (in vivo) and may represent a potential candidate for an anti-gonococcal vaccine.

BALB/c mice were administered intraperitoneally (ip), either purified mAb 2C7 or IgG3 λ (isotype control) and then inoculated intravaginally with *N. gonorrhoeae* strain FA1090. A repeat experiment was also performed. Median times to clearance in each experiment were 4 days in 2C7 vs 6 days in IgG3 λ (n=16 mice in each group) and 6 days in 2C7 vs 9 days in IgG3 λ (n=18 mice in each group) (p=0.027 and p=0.008 respectively).

To circumvent the limitations of saccharide immunogens in producing long lived immune responses, previously we developed a peptide mimic (called PEP1) as an immunologic surrogate of the 2C7-OS epitope by selecting candidate peptides from a peptide library using mAb 2C7 and reconfigured one of these into a multi-antigenic form (MAP), called MAP1. To test efficacy of MAP1 as a vaccine candidate, female BALB/c mice were immunized either with MAP1 or an irrelevant MAP control (called MAP2) together with monophosphoryl lipid A (MPL) used as adjuvant. Mice immunized with MAP1 developed a T_H1 biased anti-LOS IgG antibody response that together with human complement was bactericidal; immunization with the MAP2 control peptide did not yield an anti-LOS response. Immune mice were challenged with live *N. gonorrhoeae* in 2 separate experiments. Median times to clearance were 5 days in the MAP1 groups (n=24 mice in the two experiments) vs 9 days in the MAP2 controls (n=21 mice) (p=0.0001 and p=0.0002, respectively). Bacterial burden over the course of infection was also lower in MAP1 immunized mice ($p \leq 0.0001$).

The OS epitope defined by mAb 2C7 may represent an effective vaccine target against gonorrhea, which is rapidly becoming incurable with currently available antibiotics.

O 49

Peptidoglycan degradation and release of toxic peptidoglycan fragments by the pathogenic Neisseria

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Neisseria gonorrhoeae and *Neisseria meningitidis* release immunomodulatory peptidoglycan (PG) as they grow. The PG fragments released by *N. gonorrhoeae* are known to cause the death and sloughing of ciliated cells of the human fallopian tubes, mimicking the pathology of pelvic inflammatory disease. The mechanisms involved in production and release of PG fragments are not known for either of these pathogens. We previously identified two PG-degrading lytic transglycosylases LtgA and LtgD that are responsible for the production of monomeric PG fragments active in fallopian tube toxicity. We purified LtgA and LtgD and examined their function in vitro. Both enzymes degraded gonococcal sacculi to 1,6 anhydro-PG monomers, and each enzyme showed greater activity on non-acetylated PG than on sacculi from a wild-type, acetylating strain. Characterization of PG fragments released by *N. gonorrhoeae* suggests that gonococci manipulate the types of PG fragments released. The majority of PG fragments released are 1,6 anhydro-PG monomers carrying a tripeptide. By contrast the sacculus contain a majority tetrapeptide side chains. We identified LdcA as a potential carboxypeptidase capable of converting liberated PG fragments in the periplasm from tetrapeptide monomers to tripeptide monomers. *Neisseria* are unusual in that LdcA carries a signal sequence for transport to the periplasm, whereas in most bacteria, LdcA is a cytoplasmic enzyme. Mutation of *ldcA* was found to result in virtually all monomers released having the tetrapeptide side chain. This difference may affect host cell responses, as human Nod1 responds to Dap-containing tripeptide, and not tetrapeptide, PG fragments. The fallopian tube organ culture model is being used to examine the effects of PG fragment release mutations on gonococcal infection. Peptidoglycan fragment release by *N. meningitidis* has not been explored. Therefore we used metabolic labeling with ^{3}H-DAP to follow release of PG fragments from this pathogen. Mutations in *ampG*, *amiC*, and *ldcA* were used to examine the amounts and types of PG fragments released by meningococci. The profile of released PG fragments was similar to that of gonococci, although free peptide release appeared greater in meningococci. PG fragment recycling occurs in meningococci and a majority of liberated fragments are recycled. An *ldcA* mutant showed increased PG dimer release and a shift in the size of released monomers, likely to all tetrapeptide monomer release. The similarities and differences in PG fragment release by *N. gonorrhoeae* and *N. meningitidis* may reveal mechanisms used by these two pathogens for manipulation of the immune response.

O 50

A lipopolysaccharide-deficient meningococcal isolate from a meningitis patient

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Lipopolysacccharide (LPS) of Neisseria meninigitidis consists of a core oligosaccharide and a membrane-anchoring lipid A part, where the latter is recognized by mammalian immune cells through the TLR4/MD-2 receptor complex in the initiation of an inflammatory response. Most N. meningitidis strains contain 6 acyl chains in their lipid A, which is the optimal number for TLR4 activation. However in 5-10 % of clinical isolates LPS is found with only 5 acyl chains due to mutations in the lpxL1 gene. These strains induce a strongly reduced cytokine response in vitro, and the disease course in patients infected with lpxL1 strains is less severe and associated with less coagulopathy. During screening of clinical isolates for this reduced cytokine-inducing phenotype, we came across a strain in which low IL-6 induction was not associated with any change in lpxL1 sequence. The strain (992008, serogroup B, ST-41/44 complex) was isolated from a 19-year old man presenting to the emergency department with thunderclap headache. Upon analysis of the strain 992008 outer membrane by SDS-PAGE and silver staining, no LPS could be detected, and a panel of LPS-specific mononclonal antibodies failed to react in whole-cell ELISA. The strain induced far lower amounts of pro-inflammatory cytokines IL-6 and IP-10 compared to wildtype N. meningitidis, but the cytokine profile was slightly different from an lpxL1 mutant. In order to define the responsible mutation, the 992008 genome was sequenced and LPS biosynthesis genes were screened for possible mutations. With the exception of lpxH, all appeared to be normal without mutations that could effect their function. In the amino acid sequence of lpxH, which encodes the GlcN-diacyl-UDP hydrolase, a single glycine to aspartic acid substitution at position 95 was found compared to all other known neisserial lpxH sequences. A Gly at this position is conserved not only in neisserial lpxH but also in all other available bacterial lpxH genes. Cloning and expression of the Asp95 lpxH gene in E.coli resulted in growth impairment, whereas no such effect was seen with the wildtype gene showing that this single amino acid substitution has a functional effect. We conclude that the mutant Asp95 lpxH allele is responsible for the LPS-deficient phenotype of strain 992008. We have previously shown that N. meningitidis in which the lpxA gene has been inactivated is viable without LPS in vitro; we now show that meningococci lacking LPS due to lpxH mutation can also cause invasive disease.

O 51

A molecular defect leading to subtotal complement C5 deficiency (C5D) and responsible for increased susceptibility to Neisseria meningitidis

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Meningococcal disease (MD) remains endemic in the Western Cape (WC), South Africa and complement deficiency is a predisposing factor. One in 1800 WC individuals are homozygous C6 deficient (C6Q0) (1). C5 deficiency (C5D) is less common. Two adults presented with recurrent MD and C5D. A woman (P1) had a known exon 1 defect (Q19X) (2), and an apparently non-pathogenic SNP A252T. We searched extensively (genomic and mRNA) for a recognisably pathogenic second defect, but failed to find one. The 2nd patient died age 27 of MD. No samples were available. However, his mother was heterozygote for R1476X (2); his 2nd defect was not identified as the father was not available.

We screened 107 new cases of MD for four C6 defects and for the three C5 defects (3). C6Q0 was found in 10% of cases. We did not find the two defects described by Wanget al. (2). However, we did find homozygous C5 A252T in 3% of cases and concluded A252T is pathogenic. These cases (P2, P3 & P4) were all Black children. P4 died of MD but had a homozygous A252T sibling who, along with P2 and P3, is receiving penicillin prophylaxis. Serum C5 levels in P2 and P3 levels are less than 1 μg/ml (normal mean conc. 70 μg/ml).

A possible functional activity of trace amounts of C5 protein present in serum of patients P1, P2 and P3 was then tested by measuring formation of the soluble terminal complement complex (TCC) by zymosan activation. Zymosan activation of normal serum produces in our protocol per definition 1000 AU/mL. In comparison, activated sera from P1, P2 and P3 contained 3-4 AU/mL. Thus, it seems that the very low amount (<0.5% of normal) of functionally active C5 is not sufficient to protect against MD.

In conclusion, we describe a C5 defect associated with very low levels of functionally active protein. Unlike subtotal C6 deficiency, this C5 subtotal deficiency is associated with susceptibility to MD.

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O 52

Presence and expression of Opc in German meningococcal strains from invasive disease and carriage

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Opc is a major meningococcal outer membrane protein that targets cellular integrins via extracellular matrix proteins. Opc is immunogenic in humans and induces bactericidal antibodies (Rosenqvist et al. 1993). It has therefore been used in outer membrane vesicle vaccines by stabilized expression (Keiser et al. 2010). Opc is attractive as a vaccine candidate as it is relatively conserved. However, gene expression is variable due to phase-variable transcription depending on a poly-C stretch within the promoter region. The number of C's ranges from 6 to >15 with 11-14 C's resulting in Opc expression (Sarkari et al. 1994). The opc gene is absent in MLST sequence type (ST)-11 and ST-8 complex strains.
In this study we intended to quantify the presence of the opc gene and protein expression in large strain collections from invasive disease and carriage. A subset of German meningococcal invasive disease isolates from the years 2002 through 2010 with known clinical outcome data was selected (n=1149). Carriage isolates (n=822) had been collected in winter 1999/2000 in Bavaria (Claus et al. 2005). PCR, DNA sequencing and ELISA using Mab B306 (Achtman et al. 1988) were applied.
The opc gene was absent from 29% of the disease isolates which mostly belonged to the ST-8, ST-11, ST-213, ST-231 and ST-334 complexes. Opc-negative carriage isolates (23%) belonged to the same clonal complexes and additionally to the ST-53 complex. The proportion of strains harbouring a poly-C stretch with 11-14 residues associated with Opc expression was lower in disease than in carriage isolates (16 vs. 29%, $p<0.0001$, Chi-square test). Whilst Opc expression was comparable in disease and carriage isolates of the ST-32 and ST-41/44 complexes, the higher rate of opc expression in carriage isolates was due to other opc-positive lineages over-represented in carriage. Surprisingly, not all isolates with 11-14 Cs in the promoter region expressed the Opc protein. Of note, 1/3 of the isolates with 11-14 Cs showed additional intragenic slipped strand mispairing, which we identified as a further mechanism for variation of opc expression. Strains lacking the opc gene predominated among non-meningitis invasive isolates compared to meningitis-causing isolates (62.9% vs. 37.1%). This finding is in line with experimental evidence showing that Opc promotes binding to brain microvascular endothelial cells (Unkmeir et al. 2002). However, multivariate analysis of our dataset is still in process.
In conclusion, Opc was expressed in only 21% of all isolates tested. There was a tendency to more frequent expression of Opc in carriage isolates. Evidence for additional intragenic phase variation is provided. The data need to be interpreted with care as a definite conclusion about expression in vivo is not possible with cultured isolates. Nevertheless, the use of Opc as vaccine antigen might be hampered by very low expression rates.

O 53

Immunoglobulin binding, bacterial aggregation and biofilm formation dependent on a protein encoded by prophage DNA linked to invasive meningococcal disease

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Objective: Immunoglobulin binding proteins (Igbps) are common among human pathogenic bacteria, though less prevalent in Gram-negative species. We have shown that recombinant T and B cell stimulating protein B, TspB, encoded by Neisserial prophage gene *ORF6* binds Igs with a preference for human Ig (Müller MG et al, submitted). Studies comparing case/carriage isolates showed the prophage DNA and *ORF6* in particular was associated invasive meningococcal disease (Bille et al 2005, J Exp Med 201:1905). The objective of this study was to characterize the function of TspB expressed by *Neisseria meningitidis* (Nm) strains cultured in the presence of human serum and serum fractions.
Methods: Nm strains from A, B, C, W135, X and Y capsular groups were cultured in Mueller-Hinton alone or chemically defined medium supplemented with 5% human serum or serum fractions produced using the Cohn method. Ig-binding activity and characteristics of cultured bacteria were observed by flow cytometry and fluorescence microscopy. Production of TspB was determined by Western blot with murine anti-TspB polyclonal serum. Three known *ORF6* genes were knocked out in Nm strain H44/76 by homologous recombination.
Results: Production of TspB was independent of culture media. However, anti-TspB reactivity and Ig binding activity exhibited by bacteria were dependent on human serum suggesting that serum factors affect exposure or release of already existing TspB. Ig binding/TspB surface exposure were correlated with formation of large (>50 μm) bacterial aggregates enveloped in a biofilm containing TspB, Ig, and complement proteins not directly associated with the bacterial surface. Bacteria cultured in 5% Cohn Fraction IV also formed large aggregates that were reactive with anti-TspB even though the medium contained less than 5 μg/ml IgG and no measurable IgM. Knocking out known *ORF6* genes abrogated aggregate/biofilm formation by bacteria cultured in 5% human serum.
Conclusion: TspB may contribute to Nm pathogenesis by promoting bacterial aggregation to inhibit opsonophagocytosis by macrophages and producing biofilm in the presence of Ig that non-productively consumes complement.

O 54

Neisseria gonorrhoeae avoids primary granules to survive in human neutrophils

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Infection with *Neisseria gonorrhoeae* (gonococcus, Gc) results in a potent neutrophil (polymorphonuclear leukocyte, PMN)-driven inflammatory response. PMNs store antimicrobial peptides and proteases in cytoplasmic granules which upon PMN activation fuse at the plasma or phagosome membrane to release their contents extracellularly or intracellularly, respectively. Despite the variety of mechanisms used by PMNs to kill microbes, a fraction of Gc are resistant to PMN killing, and viable Gc can be cultured from human gonorrhea exudates. Here we report that most Gc-containing phagosomes in primary human PMNs fail to fuse with primary granules and mature into phagolysosomes, as compared toStaphylococcus aureusphagosomes. We confirmed the observation that Gc avoid fusion with primary granules *in vivo* by examination of human gonorrhea exudates. Experiments investigating Gc viability and enrichment for primary granule proteins demonstrate that viable Gc display reduced primary granule enrichment compared to non-viable Gc. Moreover, increasing primary granule fusion with Gc-containing phagosomes via treatment with lysophosphatidylcholine (LPC) reduces Gc survival inside PMNs. Inhibition of PMN proteases prior to increasing primary granule fusion with LPC rescues gonococcal survival in PMNs to control levels. These data demonstrate PMN primary granule proteases have antigonococcal activity, however avoidance of primary granule fusion with Gc phagosomes allows Gc to remain viable in PMNs. In contrast to bacteria which require ATP-dependent secretion of bacterial effector proteins to modulate phagolysosome formation, we show the in the absence of active Gc biological processes Gc phagosomes still avoid primary granule fusion. We also demonstrate that IgG opsonization of Gc enhances primary granule fusion with the Gc phagosome and so decreases Gc viability. This observation demonstrates that an effective antibody-based vaccine against Gc may be sufficient to allow PMN clearance of the bacteria in the absence of antibiotics. From our data we conclude that avoidance of PMN primary granule fusion with Gc phagosomes is a central mechanism used by Gc to survive inside human PMNs.

O 55

Resistance mechanisms of *Neisseria meningitidis* against Neutrophil Extracellular Traps

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Neisseria meningitidis (N.m., meningococci) is a major cause of septicemia in young children. The rapid progression of disease suggests that N.m. effectively overcomes innate immunity in the circulation. Here we show that meningococcal cells and outer membrane vesicles (OMVs) induce the formation of Neutrophil Extracellular Traps (NETs) from human neutrophil granulocytes, which are thought to trap and kill pathogenic bacteria, fungi and parasites (1). NETs are grid-like three-dimensional structures composed of DNA and a complex mixture of antimicrobials such as histones, neutrophil elastase, or LL-37 (2). We here demonstrate that human NETs trap meningococci and decelerates their proliferation, but that they do not significantly kill the bacteria. OMVs blocked binding of meningococci to NETs. OMVs were shown to co-localize with NET structures. We hypothesize that OMVs block binding sites for life meningococci. Consecutively, we analyzed the resistance mechanisms of meningococci to bactericidal activity of NETs. Seven meningococcal systems previously associated with resistance towards antimicrobial peptides were addressed. The polysaccharide capsule, the efflux systems MtrCDE and FarABmtrE, and the modification of lipopolysaccharide (LPS) with acyl residues (LpxL1) did not seem to contribute to NET resistance. In contrast, LPS modification with phosphoethanolamine (mediated by LptA) was shown to be indispensable. Cathepsin G was identified as the major antimicrobial for killing an *lptA* mutant. Finally, we also addressed resistance to nutritional immunity. NETs have been assumed to catch divalent cations essential for microbial growth. In this study, meningococcal mutants of the Zinc uptake component D (ZnuD) were sensitive to NET mediated killing in contrast to the wild type. The increased sensitivity of *znuD* mutants was neutralized by addition of external Zn^{2+} and by dissolution of NETs by the nuclease DNase I. Taken together we provide evidence that *Neisseria meningitidis* and its OMVs stimulate NET formation. OMVs block meningococcal binding to NETs. LptA and ZnuD contribute to meningococcal NET resistance.

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O 56

CD147 is a cellular receptor for pilus-mediated adhesion of meningococci to vascular endothelia

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Neisseria meningitidis is an important and devastating human pathogen, a worldwide cause of epidemic meningitis and of rapidly progressing fatal shock. Adhesion to brain vascular endothelium is the first step in bacterial traversal of the blood-brain barrier and invasion of the meninges, whereas adhesion to peripheral endothelial cells leads to septic shock, vascular leakage and extensive necrotic purpura. Although it is well established that these interactions rely upon meningococcal Type IV pili, both host factors and the precise pilus components involved remain unknown. Here we identified the multifunctional immunoglobulin superfamily protein CD147 (also called EMMPRIN or Basigin) as a critical host receptor for *N. meningitidis* adhesion to human brain and peripheral endothelial cells. The primary attachment of *N. meningitidis* to human endothelial cells was potently inhibited by CD147 knock-down, addition of soluble CD147 and of antibodies targeting the membrane proximal domain of CD147. Furthermore, piliated meningococci specifically bound to immobilised CD147 molecules. Among pilus components, we found that two pilins, the major pilin PilE and a minor pilus component PilV, could directly interact with CD147 and competitively inhibit meningococcal adhesion to brain endothelial cells. Remarkably, *ex-vivo* infection of human brain sections revealed that meningococci specifically adhere to CD147-expressing endothelial cells. Consistently, this adhesion was dependent on PilE and PilV expression and was inhibited by anti-CD147 antibodies. This finding represents a major breakthrough in our understanding of the initial events in meningitis aetiology and provides a new target for treatment and prevention of meningococcal infection.

O 57

Neisseria meningitidis targets human endothelial cells in a human skin xenograft transplantation model in SCID mice

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Background: *Neisseria meningitidis* (Nm) is a strict human pathogen. Mouse models of meningococcal infections are appropriate to study virulence factors associated with Nm sepsis but can not be used to study bacterial – endothelial cells interactionsin vivo as Nm only interacts with human cells. We established a mouse model of meningococcal infection in 10 weeks old female SCID mice transplanted with human skin grafts.
Methods: mice were infected one month after transplantation with a lethal dose of 2 x 10^6 colony forming units (CFUs) of Nm strain 2C43 or with a 2C43 *ΔpilE* isogenic mutant by intraperitoneal route. Human holotransferrin (10 mg) was co-injected to promote bacterial growth *in vivo*. Bacteremia was assessed by quantitative blood cultures and mice were euthanized one day after infection for tissue analysis for an histopathology and immuno-histology study.
Results: the histopathological study demonstrated that the wild type strain specifically targets the intraluminal surface of human endothelial cells in the graft and can forms colonies that end up in obliteration of the lumen of infected vessels. Infection with the *ΔpilE* mutant resulted in a similar level of bacteremia (10^6 CFUs/ml). However, the *ΔpilE* bacterial load of the graft one day after infection was significantly decreased (1000 times fewer) as compared to that of the wild type strain, and no bacteria could be detected adhering to endothelial cells within the graft.
Conclusion: these data demonstrate that Nm specifically interact with human endothelial cells i*n vivo* in a pilus dependant manner.

O 58

Exploring the extent of genetic variation during persistent meningococcal carriage and the impact of adaptive host immune responses

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Introduction: Meningococcal genomes contain multiple mechanisms for generation of genetic variation. These mechanisms have evolved to facilitate asymptomatic carriage of meningococci in the upper respiratory tract of humans. This study set out to explore the extent of genetic variation occurring during persistent carriage of meningococci and the impact of adaptive immune responses on these genetic variants.
Methods and Results: Multiple meningococcal isolates (~20/time point) and concomitant serum and mucosal samples were obtained from three to four time points covering a six-month period from volunteers persistently colonized with a single meningococcal strain. High levels of antigenic variation were detected in the PilE gene both within and between time points. Seven outer membrane proteins, subject to phase variation due to alterations in simple sequence repeats, were examined in 20 carriers and frequent alterations in repeat number were observed between time points. In contrast, the variable regions of PorA and FetA exhibited only low levels of variation. A pair of isolates of the same strain from one carrier were subject to whole genome sequencing and SNPs were detected in only 7 genes during the six months of carriage of this strain. Phenotypic expression of the serogroup Y capsule indicated stable expression of this capsular antigen for up to six months of carriage. Serological investigations of 33 serum samples (carriers and non-carriers) detected high levels of antibodies specific for the Y capsular antigen in all persistent carriers and rapid induction of antibodies upon gain-of-carriage. A similar pattern was detected for variant-specific PorA antibodies using a multiplex bead-based assay. We are currently examining whether PorA specific-antibody levels influence phase variable changes in PorA expression.
Conclusion: We conclude that meningococcal persistence in carriers is characterized by high levels of genetic variation in specific regions of meningococcal genomes but that specific immune responses against the Y capsule have no impact on capsule expression. Whilst further immunological studies and whole genome sequence analyses are required, we have reached an initial overview of how persistence of meningococci in the human nasopharynx may influence genetic variation. These findings will provide information critical to understanding the spread of meningococci within a population and a baseline for characterization of whether specific genetic changes are required for meningococcal disease.

O 59

Diversity of Multiple-Locus-VNTR-Analysis-(MLVA)-Types in Meningococcal Strains from Different Epidemiological Settings

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Multiple Locus VNTR [Variable Number of Tandem Repeats] Analysis (MLVA) is a cheap, portable, and highly discriminatory typing method for meningococci, which assesses the variation at several different VNTR loci within the genome. While for meningococci MLVA's discriminatory power is higher than that of Multi-Locus-Sequence-Typing (MLST), little is known about the increase of variation with the number of transmissions and hence its usefulness for the tracing of transmissions. We therefore attempted to describe the extent of diversity across five epidemiological settings differing in the presumed number of transmissions: A) strains from long-term carriers obtained over a period of up to 6 months; B) strains from cases with invasive disease and their close contacts; C) strains from a cross-sectional study of carriers; D) strains from finetype-specific spatio-temporal clusters of invasive disease (computed with SaTScan version 5.1.3); and E) strains from sporadic cases of invasive disease. Genetic background was defined by finetype (PorA- and FetA-sequence-type) and Multi-Locus-Sequence-Type. Strains from above categories A-E were further grouped according to carrier, index-contact group, genetic background, finetype-specific cluster, and genetic background, respectively. Categorical distances were computed based on MLVA-profiles of strains within a group. Variation of MLVA profiles within a group differed significantly across settings ($p<0.001$, Kruskal-Wallis test); it increased from settings B to E, suggesting accumulation of changes with increased number of transmissions. Interestingly, a similar extent of within-group variation was observed between settings A (long-term carriers) and B (cases and close contacts) with 6 of 26 carriers and 2 of 19 index-contact groups showing changes in at least one locus ($p=0.269$, Fisher's Exact Test). Observed overall variation was considerably smaller than predicted by a computer model assuming random change of repeats. Specifically, cluster analysis of all MLVA profiles based on categorical distances revealed groupings overlapping with clonal complexes, suggesting that variation is possibly restricted by the genetic makeup of a strain. In summary, this is the first study investigating transmission-dependent variation of tandem repeats in meningococci. Given the relatively high rate of variation, it seems questionable that MLVA can be used to trace transmissions of invasive disease.

O 60

Intergenic crossover hotspots in meningococcal transformation

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The identification of crossover hotspots from homologous recombination in the meningococcus (Mc) and other bacteria is enigmatic. A particular challenge in crossover identification is to distinguish between common ancestry and true recombinational hotspots in sampled genetic sequences. Furthermore, since sequence divergence itself influences the termination of homologous recombination events, obtaining a sufficient resolution of polymorphisms to accurately identify the crossover point may not be straightforward. Here, for the first time, the crossovers formed during Mc transformations were identified by whole genome DNA sequencing and single nucleotide polymorphism (SNP) analysis. Crossover hotspots were identified in regions with reduced sequence similarity between the donor DNA and recipient chromosome, generally in intergenic locations. Furthermore, inverted repeat sequences were frequently identified in close proximity to hotspots alluding to a role in determining the extent of homologous recombination events. These data show that during transformation of Mc, intergenic regions are recombinationally suppressed relative to the conserved coding regions. Therefore, the evolutionary significance of transformation, in which homologous recombination is essential, may be allelic reassortment rather than the acquisition of heterologous DNA encoding novel traits. This is the first study to identify crossover hotspots, in this context referring to the positions on the meningococcal (Mc) chromosome where recombination breakpoints had clustered, by complete genome sequencing and single nucleotide polymorphisms (SNP) analysis following several Mc transformations. The intergenic location of these crossover hotspots provides a new understanding of the magnitude and dynamics of homologous recombination in transformation. From an evolutionary perspective, the crossover hotspots identified suggest that the control of allelic reassortment and genetic linkage is fine-tuned to a higher resolution than previously thought.

O 61

Whole genome epidemiology of the ST-11 Complex 'ET-15' epidemic in the Czech Republic

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Background: The Czech Republic 1993 ST-11 epidemic was investigated at high resolution with whole genome data. MLEE and serological typing had found 1993 ST-11 meningococci to be distinct from any previously isolated in the Czech Republic; all were electrophoretic type-15 ('ET-15') due to a variant *fumC* allele, and strain type C:2a:P1.5,2. All but one were indistinguishable by seven locus MLST.
Methods: The genomes of all 21 disease isolates and a further 33 carriage isolates were Illumina sequenced. Reads were assembled *de-novo* with VELVET and uploaded to a local installation of BIGSdb for annotation and comparison of content. Core and accessory genome phylogenies were drawn using 'whole genome MLST' and using an un-annotated, reference-free approach.
Results: Czech Republic ST-11 meningococci from 1993 differed from older, non-'ET-15', ST-11 isolates at more than 853 core genome loci, including nine coding for ribosomal protein genes, 47 coding for conserved hypothetical proteins, and many coding for metabolic enzymes such as those involved in DNA repair. The epidemic isolates were more closely related to each other than to a 1993 'ET-15' disease-causing isolate from the UK, from which they were differentiated by 190 coding regions comprising pilin genes, enzymes involved in transcription, and DNA repair. Czech Republic 'ET-15' isolates shared 94% of the genome including 258 variable coding sequences. Hypothetical proteins and those with virulence-associated functions (pilin proteins, capsule modification enzymes, TspA and LbpA) were the most variable. Meningococci isolated from 12 carriers at the same time and location were unique; four TspA alleles and up to 10 variants of a single locus were present. There was a heterogeneous distribution of 'core-genome types' across the country, with two clusters coincident with two major cities. Phylogenetic analysis suggests that 'ET-15' meningococci entered the Czech Republic via Prague.
Conclusion: Even with the maximum genetic resolution possible and complete epidemiological data, it is not always possible to track individual meningococcal transmission events. Our data suggest that individuals were colonised by more than one variant of the 'ET-15' strain type, and that these different variants underwent multiple transmission events between cases. It is likely that a population of 'ET-15' variants entered the Czech Republic in a single introduction.

O 62

Genomics of Serogroup Y, ST-23 Carriage and Invasive Neisseria meningitidis in the United States and United Kingdom

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Question: Although serogroup Y, ST-23 *Neisseria meningitidis* causes approximately one third of meningococcal disease in the USA, serogroup Y disease has been relatively rare in the UK, despite presence of serogroup Y, ST-23 carriage. We performed comparative whole genome sequence analysis of UK and US serogroup Y, ST-23 carriage and invasive isolates to identify genetic factors that may contribute to the low rates of meningococcal disease in the UK.
Methods: Five serogroup Y, ST-23 *Neisseria meningitidis* isolates from the US (one carriage, two invasive) and UK (one carriage, one invasive) were sequenced using the 454 Titanium protocol with a 3kb library. Reads were assembled, aligned to a reference genome and subjected to automated gene prediction and annotation. Genomes were visualized using the Sybil comparative genomics browser. Similarity analysis and alignments were accomplished using freely available software and custom Perl scripts.
Results: By whole genome comparison, the five strains were highly similar. The UK carriage isolate was most similar to a US invasive strain of a type common in the early 1990s (100% nucleotide identity in 73% of shared single-copy open reading frames (ORFs)). The UK invasive isolate and the US carriage isolate were most similar to a US strain which emerged and became established in the late 1990s (100% nucleotide identity in 84% and 100% nucleotide identity in 88% of shared single-copy ORFs, respectively). Presence/absence of 164 putative virulence factors was identical among all five genomes, except for eight ORFs found only in the UK carriage isolate, contained in an ~11 kb region and not found in other sequenced serogroup Y, ST-23 genomes. The UK carriage genome contained an additional ~43kb region spanning 50 ORFS, whose insertion point disrupts an ORF encoding tRNA dihydrouridine synthase A. This region was also absent in other serogroup Y and other sequenced genomes, except WUE2594, a transformable serogroup A ST-5 invasive strain isolated in Germany in 1991. The UK carriage and invasive isolates and the US carriage isolate have a single silent *pilS* cassette, while the US invasive strains have three or four. The most divergent genes among the five strains include those encoding the iron uptake and utilization proteins, transferrin binding protein B and lactoferrin binding protein B, and the pilus assembly protein, PilC.
Conclusions: Sequenced carriage and invasive serogroup Y strains obtained in the US and UK during the same time period had highly similar genomes, with a limited number of divergent genes. There were no clear differences in presence or absence of putative virulence factors, except for the unexpected finding of additional virulence related genes in the UK carriage isolate and a decrease in the number of unexpressed *pilS* cassettes. Disruption of tRNA dihydrouridine synthase A could negatively impact modification of tRNA, but the role of this enzyme is not well established. Gene conversion in the *pilE/pilS* locus is responsible for antigenic variability of the expressed pilin subunits. A reduction in the number of silent *pilS* cassettes available for recombination decreases the potential for antigenic variation in the major pilin subunit. This reduction could contribute to a reduced ability to cause disease by decreasing invasiveness in these strains.

O 63

Phylogenetics of *Neisseria meningitidis* Serogroups Rarely Associated with Disease and Their Contribution to the Pan and Core Genomes

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Question: Invasive *Neisseria meningitidis* strains almost universally produce a polysaccharide capsule, which provides the basis for serological typing. However, of the 13 capsule types, a few (A, B, C, Y, and W135) are responsible for the majority of invasive disease cases. The remaining serogroups exist mainly as human commensals along with *N. meningitidis* lineages which do not produce a capsule (non-groupable). Non-invasive strains can participate in horizontal gene transfer with invasive lineages and therefore their genomes are a reservoir of genes and alleles. We sequenced 15 strains of serogroups rarely associated with disease to investigate evolution and the basis for invasiveness in *N. meningitidis*.
Methods: Hybrid assemblies of 15 genomes from serogroups H (1), I (1), X (1), Z (5), 29E (3) and non-groupable strains (4) were obtained using a combination of 454 and Illumina technologies. Using whole genome alignments generated in Mugsy and the Sybil comparative pipeline, the core and pan-genomes were determined. ClonalFrame was used to generate a phylogenetic tree from the Mugsy alignments for the 15 genomes and 22 other meningococcal genomes available in Genbank.
Results: The core genome of *N. meningitidis* was estimated to contain ~1602 unique genes. The pan-genome size was estimated to be ~2546 genes. The calculated power law regression slope of -1.1 indicated that as more genomes are sequenced, new genes can be estimated to be contributed to the pan-genome at a relatively slow rate. The pan-genome plot showed slow growth, with a slope of 0.0606. These data suggest that *N. meningitidis* has a borderline closed pan-genome. Four US serogroup Z strains collected within a 7 year period clustered closely together but were phylogenetically distant from a US serogroup Z strain collected > 25 years earlier. The remaining 11 newly sequenced strains represent distinct lineages.
Conclusion: The 15 newly sequenced genomes are highly diverse phylogenetically and are a valuable resource for investigating evolution and invasiveness in *N. meningitidis*. Analysis of the core and pan-genomes of 37 whole genomes of diverse *N. meningitidis* isolates suggests that the species has a closed pan-genome. These data predict that as more genomes are sequenced, few new genes will be discovered. Prediction of a borderline closed pan genome despite the high phylogenetic diversity of the 15 newly sequenced genomes suggests that lateral gene transfer from outside of the species may be more rare than might be expected, given the propensity of *N. meningitidis* for DNA uptake. This project has been funded in whole or part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under contract number HHSN272200900007C.

O 64

Ribosomal MLST analysis reveals a distinct species of *Neisseria*, previously identified as *Neisseria polysaccharea* that is closely related to *Neisseria meningitidis*

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Ribosomal MLST (rMLST) exploits next generation sequencing data to classify bacteria based on a subset of core gene sequences, namely the 53 ribosomal protein subunit (*rps*) genes. This method broadly agrees with *16S rRNA* phylogenetic trees, but has the added advantage of providing strain resolution within a given species.

Recently an examination of the taxonomic relationships of 55 representative members of the genus *Neisseria* revealed groupings that were largely, but not completely congruent with current species designations. The analysis suggested some minor changes in nomenclature and the reassignment of a few isolates to different species.

In particular, these data showed that isolates classified as *N. polysaccharea* are polyphyletic and probably include more than one taxonomically distinct organism. Subsequent genome sequencing and rMLST of 77 unidentified commensal *Neisseria* collected during a meningococcal carriage study in Malawi, Africa, showed that 17 of these isolates clustered phylogenetically with the type strain of *N. polysaccharea*. Another 57 isolates clustered with a strain previously identified as *N. polysaccharea* but phylogenetically distinct from this species. The suggested name for this species is "*Neisseria bergeri*", in recognition of Ulrich Berger who first described the strain in 1985.

Frequent carriage of these organisms, both closely related to *N. meningitidis* may have implications for meningococcal vaccine design and development, considering the potential for horizontal genetic exchange of nucleotide sequences coding for antigenic determinants among the *Neisseria*.

P 001

Small non coding RNAs regulating energy metabolism in *Neisseria meningitidis*

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Small non-coding RNAs (sRNA) are present among the genomes of prokaryotes and involved in riboregulation. NrrF is one of the three identified small s RNAs in *Neisseria meningitidis* and was previously shown to be under control of the ferric uptake regulator (Fur).
We identified two sRNAs, one them being them NrrF, involved in the regulation of two energy generating pathways. We combined a biocomputional sRNA and mRNA target prediction with experimental approaches among which proteomics and agfpreporter system for translational control and target recognition of sRNAs in *E. coli*.
mRNAs of *petABC* and *cycA*, encoding for the cytochrome bc1 complex and cytochrome c4 respectively and both functionally involved in respiration, were identified as putative targets of NrrF. Using heme-stained protein blots we show that expression of both proteins decreased in meningococci overexpressing NrrF. Furthermore, NrrF mediated down regulation of the expression of CcoO, CcoP, cytochrome c5 (cycB), also components of the respiration chain, and of the NO detoxification component CycP, was observed. Direct interaction between NrrF and the 5'- untranslated region (5'-UTR) of the mRNAs of *petABC* ,*cycA*, *cycB*, *cycP*, and *ccoNOQP* was assessedin vivo by using the gfp reporter system and validated for *petABC*. Ultimate proof for the in silico predicted interaction between NrrF and *petABC* was obtained i*n vivo* by mutagenesis of the predicted region of interaction. Mutations in the site of interaction in NrrF abrogated the down regulation of *petABC* 5'-UTR-*gfp* fusion product. Compensatory mutations in the 5'-UTR of *petABC* restored the down regulation.
In addition, we identified two structurally nearly identical (70% sequence identity) sRNAs located in their immediate vicinity. A twin sRNA deletion mutant showed differential expression of seven proteins among which four proteins involved in the tricarboxylic acid (TCA) cycle. Direct translational control of either *in silico* predicted targets or of proteins differential expressed after deletion of the twin sRNAs was assessed *in vivo* using thegfpreporter system and confirmed for the 5'-UTR of the mRNA of *sdhC*, *gltA*, *sucC* and for *acnB*, all encoding enzymes functionally involved in the TCA cycle, and for *prpB* and *prpC*, encoding enzymes involved in metabolism of Succinyl-CoA, a carbohydrate component of the TCA cycle.
In conclusion, the two main energy generating systems are regulated by two different sRNAs. Our results provide for the first time insight into the mechanism by which components of the respiratory chain are regulated by Fur and the first description of a small non-coding RNA involved in the regulation of the respiratory chain in prokaryotes. In addition, riboregulation of five different targets functional in the TCA cycle by one particular sRNA represent a novel and extremely efficient mechanism of adaption of meningococci to metabolic challenges.

P 002

Twin small non-coding RNA molecules involved in feast/famine regulation in *Neisseria meningitides*

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Introduction: Small non-coding RNA molecules (sRNAs) are present across the genome of prokaryotes. They are involved in riboregulation where they function as quick and economical regulators of protein expression through interaction with mRNA molecules. This interaction of sRNAs with their mRNA targets can be enhanced by Hfq, a chaperone protein which has the ability to stabilize these complexes and influence degradation. The aim of this study is to discover and characterize sRNAs of *Neisseria meningitidis* in order to better understand its physiology which might open new paths to future therapeutic options.
Methods: Bioinformatic prediction and confirmation by RNA-sequencing were used to detect the presence and expression levels of sRNAs in N. meningitidis. Putative targets of discovered sRNAs were consequently predictedin silico. These potential sRNA-mRNA interactions and their effect on protein expression were assessed using agfpreporter system for translational control and target recognition of sRNAs in *Escherichia coli*. Furthermore, sRNA deletion and overexpression mutants of *N. meningitidis* were created and proteomic/mass spectrometry analyses were performed to identify differentially expressed proteins. Growth curves were determined in nutrient rich and carbohydrate limited medium.
Results: Investigation into the existence and function of sRNAs in N. meningitidis revealed two structurally nearly identical sRNAs located in their immediate vicinity, with 70% sequence identity (twin sRNAs). A twin sRNA deletion mutant showed differential expression of seven proteins. Complementation by both sRNAs restored the wild type, while complementation by either of the two partially restored wild type protein levels. Four of these proteins are directly involved in the tricarboxylic acid (TCA) cycle: AcnB, GltA, Icd, and SucC. All four were previously shown to be regulated by Hfq. Direct translational control by one of the twin sRNAs was proven for mRNAs encoding the TCA cycle enzymes GltA, AcnB, SucC, and SdhC, and for PprC and PrpB; the latter two enzymes are involved in propanoate metabolism which feeds into the TCA cycle through Succinyl-CoA. Constitutive overexpression of both twin sRNAs does not impair growth in nutrient-rich medium (GC broth) and in fresh human blood, whilst growth was drastically inhibited in medium with glucose as the sole carbon source and in fresh human CSF.
Conclusions: Twin sRNAs in *N. meningitidis* is an inhibitor of the TCA cycle during logarithmic growth in rich medium (feast). Overexpression of twin sRNAs in meningococci growing in poor medium (famine) inhibits growth due to inhibition of the TCA cycle. This is the first example of a sRNA involved in regulation of protein expression in bacteria switching between feast/famine growth conditions. We propose to name this sRNA feast/famine regulator RNA (FfrR).

P 003

Rapid quantitation of proteins regulated by the small non-coding RNA FfrR of *Neisseria meningitidis* assessed by LC-MS^E

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Introduction: The small non-coding RNA FfrR of *N. meningitidis* is involved in switching between feast/famine growth conditions by the regulation of the tricarboxylic acid (TCA) cycle.
Methods: Whole cell lysates of *N. meningitidis* H44/76 wt and H44/76Δ*ffrr* were subjected to tryptic digestion. The resulting peptide mixtures were separated by reversed phase liquid chromatograpy (LC) prior to analysis by data independent alternate scanning mass spectrometry (MSE). Mass spectral data were matched to the *N. meningitidis* MC58 proteome database. Proteins are quantified by normalizing the sum of the three most abundant peptides of each protein on those of a reference protein digest added to each sample.
Results: Of all 2063 annotated ORFs in MC58, approximately 500 proteins (24%) could be identified. Of these, 359 yielded accurate quantification of relative expression between H44/76 wt and H44/76Δ*ffrr*. The dynamic range of differentially expressed proteins was within 13 fold up- and 3 fold downregulation. Using a 1.5 fold up- and downregulation as a cutoff for differential expression, a total of 27 up- and 20 downregulated proteins were identified. Of these, 6 up- and 1 downregulated proteins were also detected by SDS-PAGE/peptide mass fingerprinting.
As expected from the role of the sRNA FfrR in repression of the TCA cycle many of the newly identified upregulated proteins in H44/76Δ*ffrr* are catalysts involved in the TCA cycle like Succinyl CoA ligase, which catalyzes the reaction of succinyl-CoA to succinate generating NTPs by substrate-level phosphorylation. Others are involved in synthesis of TCA enzymes like pantoate-beta-alanine ligase (used in Coenzyme A biosynthesis). Also, increased expression of malate oxidoreductase, converting malate to oxaloacetate (a key component in gluconeogenesis), is observed. Among the downregulated proteins is phosphoenolpyruvate synthase, indicating an increased entry of pyruvate into the TCA cycle, while surprisingly, pyruvate kinase catalyzing the transfer of a phosphate group from phosphoenolpyruvate to ADP, generating ATP, is also downregulated. Moreover, Rieske iron-sulfur protein of cytochrome b6f (part of the oxidative respiratory chain), and ATP synthase (generating ATP during oxidative phosphorylation) show downregulation.
Conclusions: Almost 25% of the theoretical protein coding content of *N. meningitidis* could be identified through fast analysis by LC-MSE. Together the results indicate that in meningococci lacking FfrR, metabolism switches to increased TCA, gluconeogenesis, and substrate-level phosphorylation activity, acting as a quick source of ATP independent of external electron acceptors and respiration. Overexpression of FfrR downregulates the TCA cycle and gluconeogenesis and switches off substrate-level phosphorylation, befitting its role as an inhibitor of the TCA cycle during logarithmic growth in rich medium.

P 004

Global identification and characterization of small non-coding RNAs in *Neisseria meningitidis* in response to multiple stress conditions

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Background: *Neisseria meningitidis* (Nm), the leading cause of bacterial meningitis, is able to adapt to different host niches during human infection. Bacterial small regulatory RNAs (sRNAs) are post-transcriptional regulators involved in adaptive response which control gene expression by modulating the translation or stability of their target mRNAs often in concert with the RNA chaperone Hfq. It has been demonstrated that in Nm Hfq plays a major role in virulence and adaptation to stresses, suggesting the presence of Hfq-dependent sRNA activity. The first two sRNAs in Nm (NrrF and AniS) have been identified and characterized. Here we report the use of a tiling array technology, in order to identify sRNAs that are induced in response to physiologically relevant stress signals.

Results: Total RNAs from Nm cultures exposed to different stress conditions (heat shock, oxidative stress, iron and carbon source limitation) were compared to a reference RNA by performing 2-color hybridization on a tiling microarray. The expected expression profiles of the two known sRNAs, NrrF and AniS, were detected in the microarray data, demonstrating the potential of this tool to identify sRNAs. Lists of putative novel sRNA were obtained by further analyses of the transcriptome data. Northern blot and primer extension techniques were used to validate some of the novel sRNAs and candidates were studied in an attempt to characterize their physiological role and regulatory circuits.

Conclusions: Our results suggest the presence of a global network of sRNA-mediated gene regulation in Nm. New possible regulatory mechanisms for the already known NrrF and AniS sRNAs were suggested and multiple new sRNAs were identified, induced in different stress conditions, which may coordinate downstream regulatory circuits in response to the stress signals. Further studies will focus on the characterization of these sRNAs and their targets.

P 005

An In-Depth Conversation Between *N. gonorrhoeae* and the Human Host as Revealed by RNA-seq

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The sexually transmitted disease gonorrhea represents the second most common reportable disease in the U.S. and is widespread around the world. In addition, new strains of *N. gonorrhoeae* are emerging which are resistant to third-line antibiotics. Consequently, new analyses of both host and pathogen are necessary to provide alternative strategies to combat this disease. Here we present the first global analysis of both the host and pathogen global transcriptome during *in vitro* infection of transformed human endocervical epithelial cells utilizing RNA-seq. By sequencing all RNA expressed during infection, this method provides a more complete and accurate picture in comparison to a traditional microarray. Our analysis identified 664 differentially expressed human genes when comparing RNA isolated from epithelial cells which were incubated *in vitro* with *N. gonorrhoeae* as compared to RNA isolated from epithelial cells grown in media alone. We also detected over 1,400 instances of transcription in human cells that did not correspond to any annotated gene with several of these novel transcripts regulated via gonococcal infection. Our preliminary analysis of gonococcal gene expression identified 130 differentially expressed protein-coding genes responding to infection. In addition, hundreds of noncoding RNA and antisense RNA transcripts were detected in the gonococcus with many of these transcripts also regulated via infection. These experiments represent the first RNA-seq analysis of both the human and gonococcal transcriptional response to the early stages of *in vitro* infection.

P 006

Gonococcal RNA-seq Analysis Identifies Novel Putative Small RNAs Responding to a Variety of Signals

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In the last several years, bacterial gene regulation via small RNA (sRNA) transcripts has become a rapidly expanding field. Hundreds of characterized sRNAs have been identified in a variety of both Gram-negative and Gram-positive bacteria. However, only two sRNAs have been fully analyzed in the gonococcus. Here we present data obtained from carrying out RNA-seq analysis of *N. gonorrhoeae* grown under iron replete and iron deplete conditions as well as a preliminary analysis of *N. gonorrhoeae* during *in vitro* infection of transformed human endocervical cells to identify additional sRNAs in this organism. In addition, we compared two methods to isolate sRNAs. Iron responsive RNA was size selected prior to sequencing whereas RNA from infection experiments was not. Analysis of RNA isolated from *N. gonorrhoeae* grown in iron replete and deplete conditions identified 176 putative sRNAs. Additional analysis including Northern blot, primer extension and qRT-PCR were carried out on several sRNAs. At least one was found to be repressed by the Ferric Uptake Regulator (Fur) protein; however, this sRNA was not regulated via iron. This is in agreement with emerging studies showing that the Fur protein can respond to signals other than iron. Analysis of RNA isolated from *N. gonorrhoeae* during infection of human epithelial cells identified 86 putative sRNAs. Preliminary experiments showed that 58 of these putative sRNAs are regulated via infection including the above mentioned Fur regulated sRNA. These experiments contribute to the effort of defining the sRNAome of *N. gonorrhoeae* and highlight the advantages of RNA-seq in identifying novel transcripts.

P 007

The influence of homopolymeric G tract length and mismatch repair on phase variation in a *Neisseria meningitidis* model

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Background: Phase variation (PV) caused by slipped-strand mispairing during replication of DNA repeats allows rapid stochastic shifts in expression (ON/OFF) of several proteins in the human pathogen *Neisseria meningitidis* (the meningococcus, Mc). Here, the genetic instability of a panel of polyG tracts inside the spectinomycin resistance gene *aadA* was monitored in two different ways; in a PV reporter assay and by colony size. The PV assay determined the frequency of OFF-ON switching of spectinomycin resistance, whereas the colony size characteristic exhibited the ability to retain the ON-state during replication cycles. The instability of a comprehensive panel of polyG tracts covering the 5-13 nucleotide range was monitored in a wildtype (wt) Mc strain and in a mismatch repair (MMR) deficient background.
Results: Both G-tract length and MMR deficiency strongly influenced PV. The comparison of the PV frequencies for the polyG tracts from G_5 to G_8 revealed an increase in OFF-ON switching by more than three orders of magnitude. Tract lengths $>G_8$ did not lead to a relative increase in switching, and a tendency for reduced OFF-ON switching was observed for tracts up to G_{13}. Under the influence of spectinomycin selection favouring the ON-state during growth, the colony sizes of individual strains were inversely correlated to tract length, demonstrating that shorter tracts were more stable than longer tracts. Frequent back-switching (ON-OFF) was documented. By both methodological approaches the stability of the short G-tracts was reduced in MMR deficient strains. The influence of MMR on tract instability was found to decrease with increasing tract length.
Conclusions: The instability of Mc polyG tracts was confirmed to be influenced by tract length and MMR. In the PV assay, a balance between tract expansion and contraction appeared to be tuned by the ability and advantage ofinstabilityto generate many resistant colonies and the advantage ofstabilityto avoid back-switching to antibiotic sensitivity. This characterization of polyG tract instability in Mc thus elucidates how the level of PV can be adapted by tract length and MMR, features under the scrutiny of natural selection.

P 008

Novel Fur and FarP Regulatory Networks Implicated in Gonococcal Pathogenesis

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted disease gonorrhea, can infect and colonize multiple mucosal sites. The ability to cope with different environmental conditions requires tight regulation of gene expression. We have recently defined a novel regulatory network involving the global regulatory protein Fur and a newly identified secondary repressor FarP which is activated by Fur. Initial studies demonstrated that Fur and FarP compete for a single overlapping binding site within the promoter region of genes transcribed in an operon and regulated by both proteins. GonococcalfurandfarPmutants exhibited increased adherence and invasion of human endocervical epithelial cells as compared to the wild type strain. Likewise, both the gonococcalfurandfarPmutants exhibited enhanced colonization in a gonococcal mouse model suggesting that Fur and FarP function to repress genes important for Neisserial pathogenesis. To define the Fur and FarP regulatory networks on a global level Chip-seq analysis was performed. Fur and FarP flagged proteins were inserted in the genome ofN. gonorrhoeae fur and far P mutant respectively and their expression in both mutants was confirmed by Western blot analysis using anti-flag antibodies. This analysis has begun to define on a global level the regulatory networks of these transcription factors as well as to define the cross talk between both Fur and FarP. Collectively, our studies have defined a novel gonococcal regulatory network involving the global regulatory protein Fur and a secondary repressor FarP that functions to control and coordinate transcription of genes implicated in gonococcal pathogenesis.

P 009

Fur-mediated Transcriptional Activation in the Human Pathogen *Neisseria gonorrhoeae* – a Role for RNA Polymerase and DNA Binding Proteins

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It is well established that theFerricuptakeregulatory protein (Fur) functions as a transcriptional repressor in numerous microorganisms. Recent studies demonstrated that Fur also functions as a transcriptional activator. In the human pathogen *Neisseria gonorrhoeae* we have established that Fur plays a major role in the regulation of genes required for gonococcal invasion and adherence to human epithelial cells. We have also determined that Fur directly activates transcription of 16 gonococcal genes, some of which were shown to play roles in inducing antibody protection and protecting the pathogen from oxidative stress conditions. These genes have variable transcriptional patterns in a gonococcal *fur* mutant strain, which correlated with different positions of Fur binding sites within the promoter regions.

Question: Based on these results we postulated that Fur may activate transcription by either recruiting RNA polymerase to the promoter region or by outcompeting a repressor protein. RNA polymerase typically functions together with several different sigma factors including the extracytoplasmic function (ECF) sigma factor and σ32. DNA binding proteins, such as IHF, DbhA, Fis, Lrp and ArsR also function as negative transcriptional modulators.

Methods:In this study, we purified these gonococcal proteins and analyzed their interaction with the promoter regions of Fur-activated genes using a gel shift assay.

Results:We demonstrated that each protein bound to a specific subset of Fur-activated genes, suggesting the involvement of these proteins in Fur-mediated direct transcriptional activation.

Conclusions:Collectively, these studies have established that Fur functions in conjunction with other factors to directly activate gene transcription through several different mechanisms.

P 010

Control of LOS Sialylation in Gonococci by the Transcriptional Regulators Rsp and CrgA

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Question: There is no vaccine to help prevent the 62 million cases of gonorrhea in the world each year, and gonococci resistant to all clinically useful antibiotic classes are being isolated with increasing frequency. These sobering facts highlight the urgency for continued analysis of the host-pathogen interactions that promote gonococcal virulence in order to identify therapeutic targets. Lipooligosaccharide (LOS) sialyltransferase (Lst) is a critical surface-expressed virulence determinant of *Neisseria gonorrhoeae* (*Ng*) that catalyzes LOS sialylation and promotes *Ng* survival *in vivo*. Whereas LOS sialylation increases virulence by enhancing *Ng* resistance to complement and neutrophil killing, it decreases *Ng* association with urogenital epithelial cells, which may be necessary for disease. Hypothesizing that Lst expression is controlled by niche microenvironments *in vivo*, we analyzed *Ng lst* expression under several *in vitro* conditions. As predicted, cell-associated *Ng* expressed less *lst* than did *Ng* grown in medium alone.
Methods: Using a *Himar1 mariner* transposon library to screen for increased resistance to serum killing by sialylated *Ng* (suggesting altered Lst expression), we identified a fused two-component periplasmic *regulator-sensor protein* (*rsp*).
Results: Both cell-associated and medium-grown *Δrsp* gonococci exhibited increased *lst* transcript levels compared to wt, as well as enhanced Lst protein expression by both plate- and broth-grown bacteria. We also examined *lst* expression by *Ng* deleted for *crgA* (*contact-regulated gene A*), since CrgA directly represses expression of surface proteins by cell-associated *N. meningitidis*. Like *Δrsp*, *ΔcrgA* gonococci exhibited increased *lst* transcription, Lst protein expression, and resistance to serum killing compared to wt.
Conclusions: These data suggest that *rsp* and *crgA* contribute to *lst* regulation during cell-associated and planktonic growth. *rsp* may also exhibit a second level of control by repressing *crgA* expression. Ongoing studies include an investigation of whether *rsp* and *crgA* bind directly to the *lst* or *crgA* promoters and the identification of other genes associated with the control of Lst expression.

P 011

Stringent response in ex vivo survival of Neisseria menigitidis

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Previous transcriptome comparisons of the invasive meningococcal serogroup B strain MC58 (ST-32 cc) and the serogroup B carriage strain α522 (ST-35 cc) in an ex vivo infection model exposing both strains to human saliva, whole-blood and cerebrospinal fluid, respectively, revealed that relA encoding the monofunctional (p)ppGpp synthetase RelA was up regulated in the hyperinvasive strain MC58 compared to α522 specifically in blood. (p)ppGpp is a key alarmone in the stringent response pathway of proteobacteria and was shown to play a central role also for virulence in a number of proteobacterial pathogens. Since nothing is known so far about the stringent response in meningococci and its potential contribution to meningococcal virulence we further analyzed the role of RelA in stress resistance and ex vivo virulence in Neisseria meningitidis.

Comparisons of the relA genomic locus in both the strains revealed the presence of an AT rich repeat (ATR_{relA}) element in the promoter region of relA only in strain α522 but not in MC58. Combined computational and PCR analyses of 29 strains comprising 22 clonal complexes further suggest that it is absent in the relA upstream regions only of strains belonging to ST-32 cc, ST-53 cc and ST-18 cc. Finally, relA deletion mutants as well as ATR_{relA} knock-in and ATR_{relA} knock-out mutants were generated in both strains and were characterized in the ex vivo infection model. The results suggest a pleiotropic effect of ATR_{relA} on ex vivo survival by affecting relA expression and therefore the stringent response pathway in cis, and the strain specific manner of this effect suggests epistatic interactions between ATR_{relA} and the genetic background of the respective strain.

These results provide the first evidence that the stringent response is likely to play a key role also in meningococcal virulence and that it might contribute to virulence differences observed among the different clonal complexes.

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P 012

A CRISPR/cas subtype Nmeni/CASS4 system in the human pathogen *Neisseria meningitidis*

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A class of repetitive DNA elements denoted CRISPR (clustered regularly interspaced short palindromic repeats) confers sequence-based immunity against virus infections and plasmid conjugation in prokaryotes and is known as CRISPR interference. The pathogen, *Neisseria meningitidis*, carries a type II CRISPR system (subtype Nmeni/CASS4), and we could recently show that certain CRSIPR-associated (Cas) proteins are significantly linked only with carriage but not hyperinvasive strains [1]. Type II CRISPR systems are marked by an intriguing maturation pathway of small CRISPR RNAs (crRNAs) that relies on *trans*-encoded small RNA (tracrRNA) and RNase III as a host factor for CRISPR RNA processing [2].

The production of small crRNAs from full-length CRISPR RNA precursors (pre-crRNAs) is regarded as a hallmark of CRISPR-based immunity in all three types of CRISPR systems. We have focused our efforts on four strains of the *Neisseriae spp.* group: *N. meningitidis* alpha14, *N. meningitidis* WUE2594, *N. meningitidis* Z2491 and *N. lactamica* 020-06, each of which harbor several physically unlinked CRISPR loci. Importantly, the Nmeni/CASS4 system of CRISPR01 of all strains encodes only three Cas proteins, and thus lends itself for the study of a minimal CRISPR/Cas system.

Here we have used a differential RNA deep-sequencing (dRNA-seq) approach to determine where CRISPR transcripts start and end, and to map precisely where the RNA precursors are processed into small crRNAs [3]. Total RNA from all *Neisseria* strains was isolated and enriched for primary transcripts by terminator exonuclease treatment (TEX) that degrades 5' mono-phosphate but not 5' tri-phosphate RNA (treated library). The two libraries for each strain were converted to cDNA and analyzed by Solexa sequencing. Small crRNAs as well as tracrRNA were detected as abundant class of transcripts suggesting the existence of an intact and active CRISPR/cas system for each strain. Most striking was the observation that the number of reads obtained for every small crRNA was strongly increased for the TEX-treated libraries, implying that crRNAs are synthesized from single transcriptional units, i.e. from own promoters located in each CRISPR repeat. Single deletions of RNase III in *N. meningitidis* WUE2594 and *N. lactamica* 020-06 confirmed the nature of small crRNAs as primary transcripts.

We show that small crRNAs that act as a guide to specify a target in the CRISPR interference pathway are produced as single transcriptional units from their own promoters in the human pathogen *N. meningitidis*. Our findings add another novel characteristic to the minimal type II CRISPR/cas system and redefine CRISPR RNA processing to generate mature crRNAs as a general hallmark of CRISPR-mediated adaptive immunity in prokaryotes.

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P 013

A Novel Regulatory Switch for increased expression of Neisseria meningitidis NHBA at physiological temperatures found in the human nasopharynx

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Background: Neisserial Heparin Binding Antigen (NHBA), also known as GNA2132, is part of a multicomponent protein vaccine againstNeisseria meningitidis (Nm) serogroup B (4CMenB). NHBA, a surface-exposed lipoprotein present in all Nm strains analyzed so far, induces a cross-bactericidal response in humans and is recognized by sera of patients recovering from meningococcal disease. NHBA binds heparin through an arginine-rich region, a property that correlates with better survival of Nm in human serum and might facilitate increased adherence to host tissues. NHBA is cleaved by the meningococcal NalP and the human lactoferrin proteases, yet the role of NHBA in Nm pathogenesis remains to be fully characterized.

Results: In vitro, Nm is routinely grown at 37°C, the temperature that the bacterium finds when it infects the human bloodstream and causes septicaemia. However, we recently discovered that NHBA expression is increased when Nm is grown at reduced temperatures of 30 and 32°C, a range which reflects the nasopharyngeal niche. By analysing a panel of strains from different clonal complexes and containing different NHBA peptide variants, we determined that temperature-controlled expression of NHBA was conserved among Nm and that this regulation was neither dependent on the presence of a CREN sequence nor NalP processing. While only minor effects of temperature on transcript levels were measurable, substitution of the regulatory regions upstream of GNA2132 coding sequence abrogated temperature-dependent control of NHBA expression, suggesting that the regulation is due to a post-transcriptional regulatory switch.

The potential of diverse Nm strains to be killed by 4CMenB vaccine-induced bactericidal antibodies is assessed using the Meningococcal Antigen Typing system (MATS). For this assay, Nm strains are typically grown at 37°C, a condition with reduced NHBA expression. However, when Nm strains are grown at 30°C, increased NHBA expression is detected on the bacterial surface by FACs and the relative potency value of NHBA in the MATS assay increases significantly, which correlates to a predicted increase in killing by anti-NHBA antibodies in serum bactericidal assays.

Conclusions: We have uncovered a novel temperature-dependent regulatory switch which exquisitely controls NHBA expression and ensures that the protein is expressed at higher levels at temperatures found in the nasopharynx. This suggests that the true functional role of NHBA is in this specific niche, possibly in the colonization and persistence of Nm in its host environment. Furthermore, the current MATS assay might underestimate the contribution of NHBA towards the 4CMenB vaccine coverage as an increased NHBA expression in the nasopharynx could result in more efficient killing by anti-NHBA antibodies in this niche and could contribute to a decrease in strain carriage which could consequently lead to herd immunity.

P 014

Delineating the function of DNA repair helicases in *Neisseria meningitidis*

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Question: Helicases are ubiquitous enzymes vital to all living organisms. They are involved in various aspects of cellular processes including DNA repair, recombination, replication, transcription and RNA processing. Helicases are motor proteins that move directionally along the nucleic acid phosphodiester backbone separating two annealed nucleic acid strands using energy from nucleoside triphosphate hydrolysis. The product of the *recG* gene of *E. coli* is needed for normal recombination and DNA repair and has been shown to enable processing of Holliday junction intermediates to mature products by catalysing branch migration. The DinG helicase in *E. coli* is a damage-inducible, SOS-regulated, structure-specific enzyme, related to the human helicases XPD and BACH1, *Saccharomyces cerevisiae* Rad3 and *Schizosaccharomyces pombe* Rad15. XPD in eukaryotes is an integral subunit of the transcription factor TFIIH. In this study, we address the neisserial DNA repair profile and perform biochemical and mutant analysis of selected helicases.
Methods: We have cloned, overexpressed and purified the *Neisseria meningitidis* helicases RecG and DinG to homogeneity. The enzymes are biochemically characterized with regard to their activity on model DNA substrates to delineate their role in genome maintenance. The function of these helicases in transformation and survival under stress is also studied.
Results: RecG and DinG biochemical characterization including DNA binding and unwinding show that they are DNA helicases with ATPase activity. RecG and DinG null mutants will reveal the effects of these components on transformation efficiency and recombination. Mutant fitness for survival and behavior under genotoxic stress will also be assessed.
Conclusions: DNA repair helicases RecG and DinG play a prime role in the defense against mutations. Elucidation of the structure-function relationship of these components is fundamental for the understanding of neisserial DNA repair. In turn, these studies will lead to improved understanding on neisserial genome (in)stability, fitness for survival and pathogenesis. Also, helicases represent potential drug targets.

P 015

Transcriptomic changes in meningococci by zinc exposure

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Zinc is essential for bacterial growth and metabolism, but also toxic at high amounts (1). Not surprisingly, intracellular zinc concentrations are tightly controlled in many bacteria, e.g. by zinc uptake regulators (Zur) (2). *Neisseria meningitidis* expresses ZnuD, a TonB-dependent outer membrane receptor involved in the uptake of zinc at zinc-limited conditions (3). *znuD* was postulated to harbour a putative Zur responsive element in the promoter region and a knockout of the meningococcal Zur homologue resulted in increased ZnuD expression. Besides of that, not much is known about the meningococcal Zur and regulatory processes in response to zinc. To elucidate the response of meningococci to zinc, we analysed transcriptomic changes of the *N. meningitidis* stain MC58 using a common reference design of microarray analysis and verified results by qRT-PCR. cDNA obtained from MC58, either grown at low zinc or high zinc conditions, was hybridised against the common reference, a MC58 *zur* knockout mutant grown at low zinc condition. This design allows for future extension of the experiment and permits deduction of side regulations not depending on Zur but on *zur* deletion or zinc addition by bioinformatical analysis. We identified 15 genes that were repressed by high zinc and two genes that were activated. We detected putative Zur binding motifs in all their promoter regions, which supports the assumption that the meningococcal Zur regulon was at least partially deciphered. We established a meningococcal consensus Zur motif from the sequence data. The 23 bp binding motif harbours a conserved central palindrome consisting of six-meric inverted repeats seperated by three nucleotides (TGTTATDNHATAACA). *In vitro* binding of meningococcal Zur to Zur binding motifs of three genes was shown for the first time using electrophoretic mobility shift assays. Binding of meningococcal Zur to DNA depended specifically on zinc as previously shown for other bacterial zinc uptake regulators. Mutations in the palindromic sequence constrained Zur binding to the DNA motif. Amongst the Zur-repressed genes we found genes involved in zinc uptake, tRNA synthesis, and ribosomal assembly. A subset of Zur-regulated genes codes for hypothetical proteins with yet unknown function that will be investigated in future studies.

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P 016

The regulated transcriptome of *Neisseria meningitidis* in human blood using a tiling array

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Neisseria meningitidis (Nm) is the major cause of septicemia and meningococcal meningitis. During the course of infection, the bacterium must adapt to different host environments as a crucial factor for survival and dissemination. We recently showed that Nm alters the expression of 30% of ORFs of the genome during incubation in human whole blood and suggested the presence of a fine regulation at gene expression level, in order to control this step of pathogenesis. In this work, we used a customized tiling oligonucleotide microarray to define the changes in the whole transcriptional profile of Nm in a time course experiment of ex vivo bacteremia. The application of a newly-developed bioinformatic tool to the tiling array data set allowed the identification of new transcripts – small intergenic RNAs, cis-encoded antisense RNAs, mRNAs with 5' and 3' untranslated regions (UTRs) and operons – differentially expressed in human blood. Here we report a panel of expressed small RNAs, some of which can potentially regulate genes involved in bacterial metabolism, and we show, for the first time in Nm, an extensive antisense transcription activity. This analysis suggests the presence of a circuit of regulatory RNA elements used by Nm to adapt to proliferate in human blood that is worth to further investigate.

P 017

Comparison of RNA extraction methods for effective isolation of bacterial RNA from neisserial cultures

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RNA is an important macromolecule isolated in many areas of research to look at biological reactions, gene expression, cellular signals, processing, and more. However, in order to look into specific areas of interest, purification is required before any type of downstream transcriptomics work, such as sequencing, microarrays, and quantitative RT-PCR, to ensure reliability and success. In this research, three different RNA extraction methods were compared using both widely available reagents as well as commercial kits. Each method was compared to establish how efficient and effective it was in extracting pure RNA using the Gram-negative bacterial species, *Neisseria gonorrhoeae* strain NCCP11945. RNA was extracted from cultures using the Ambion® RiboPure™ Kit (Life Technologies™), the RNeasy Kit (QIAGEN®) and a previously reported combined method using TRIzol® Reagent (Life Technologies™) followed by the RNeasy Kit. Evaluation of RNA quantity and purity was carried out using the Nanovue® spectrophotometer and quality and integrity was established using the Agilent 2100 Bioanalyzer and agarose gels. There was little success with the Ambion® RiboPure™ Kit for extraction of *N. gonorrhoeae* RNA. Although the TRIzol/RNeasy-based extraction was successful at producing high yields it used hazardous chemicals and required further purification methods. It was concluded that the RNeasy kit used on its own was proven to be the best method of extraction in terms of RNA yield, quality, reproducibility, and convenience.

P 018

Transferrin and haemoglobin binding proteins are required for growth of meningococci in human blood

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Question: Acquisition of iron is essential during the intravascular phase of meningococcal infections. Meningococci can acquire iron from host proteins through expression of surface proteins involved in binding transferrin (Tf), lactoferrin (Lf) and haemoglobin (Hb). With the hypoferremic response to the infection significantly reducing the amount of iron-bound Tf molecules in the blood, the meningococcus is hypothesized to utilize Hb and Hb-complexes via the phase variable receptors HpuAB and HmbR for survival. Previous studies highlighting an over-representation of *hmbR* in disease isolates of hyper-invasive lineages[1], predicted ON phase variants of one or both receptors in the majority of disease isolates[2] and attenuation of an *hmbR*-null mutant in an infant rat model[3] suggest a crucial role for the presence and expression of *hpuAB* and/or *hmbR* in human blood. Using an *in-vitro* whole blood model of meningococcal bacteraemia, this study will test the ability of a strain incapable of utilizing Hb to survive in human blood. **Methods:** Deletion mutations were constructed in the *hmbR* and *tbp* loci of strain MC58 (*hpuAB* negative) to give single mutants MC58Δ*hmbR*, MC58Δ*tbp* and a double mutant, MC58Δ*hmbR*Δ*tbp*. To compare survival/growth rates in blood, meningococci grown to mid-log in Mueller-Hinton broth supplemented with 20 μM CMP-NANA were added to freshly collected heparinised human blood. Blood cultures were incubated at 37°C, 5% CO_2 and samples taken at hourly intervals were plated onto BHI agar. Blood samples were collected from two healthy MBL-deficient volunteers, V1 and V2. **Results:** Phenotypic assays showed that MC58Δ*hmbR* utilized Tf but not Hb and the converse for MC58Δ*tbp*. The double mutant was unable to utilize Tf or Hb but showed similar doubling times with wild-type and single mutants in iron-replete conditions. Significant reductions in CFU counts from 0 to 120 minutes were noted in V1 but not V2 blood samples before growth was restored. No significant differences in growth rate between wild-type and MC58Δ*hmbR* were observed in either blood sample but the double mutant showed an inability to grow after 180 minutes. **Conclusions:** An ability to utilize Tf may contribute to the stable growth seen with wild-type and the Δ*hmbR* mutant. Future experiments will investigate the effect of deletion of *tbp* alone, of the *hpu* receptor gene and iron starvation on growth in human blood. Our findings indicate the importance of Hb and Tf as iron sources for the systemic phase of meningococcal infections.

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P 019

Characterization of potential interactions between transferrin binding proteins in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is an obligate human pathogen and the causative agent of sexually transmitted infection, gonorrhea. *Neisseria gonorrhoeae* requires iron for survival and establishment of infection in the human host. Pathogenic *Neisseriae* have evolved a repertoire of high-affinity iron acquisition systems to facilitate iron uptake in the human host. This requires specific outer membrane receptors and energy harnessing cytoplasmic membrane proteins. The transferrin receptor proteins of *Neisseria gonorrhoeae* are necessary for iron uptake from transferrin in the host. The iron uptake system consists of two transferrin binding proteins, (Tbp) A and B. TbpA is an integral outer membrane, TonB-dependent transporter that forms the pore for iron internalization. TbpB is a surface exposed lipoprotein that makes the iron internalization process more efficient. We hypothesize that the presence of TbpA impacts the exposure or conformation of TbpB. In this study, we have utilized photoactivable cross-linkers to assess the effect of TbpA on TbpB in live gonococcal cells and studied it in presence of ligand and TonB derived energy. We employed insertion mutants, in which TbpA and TbpB contained the hemagglutinin (HA) epitope tag, to probe for impact of TbpA on TbpB. Our results demonstrate that photo-cross-linking altered TbpB size and migration and was dependent on the presence of TbpA. HA epitope insertion mutants in surface exposed loops of TbpA did not impact the mobility of cross-linked TbpB. With TonB mutants, we observed that the de-energized state resulted in wildtype TbpA and TbpB migration after cross-linking. Addition of human transferrin to the de-energized mutant caused a change in TbpB migration after cross-linking. This result indicates that when ligand is bound tightly and irreversibly to TbpA, the surface accessibility and perhaps conformation of TbpB is altered. Recent structural studies of TbpA-TbpB-ligand triple complex illustrate that Tbps bind ligand through unique, non-over lapping binding sites such that TbpA and TbpB do not interact. Our *in vivo* studies suggest that in presence of ligand, de-energized TbpA does not impact TbpB. Models will be presented that depict the possible topologies of TbpA and TbpB that would explain these findings. Currently, we are pursuing chemical cross-linking coupled with co-immunoprecipitation, to identify potential interaction between the Tbps.

P 020

Conserved Regions of Gonococcal TbpB Are Critical for Surface Exposure and Transferrin Iron Utilization

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The transferrin binding proteins of *Neisseria gonorrhoeae* comprise a bipartite system to obtain iron from host transferrin. In this system, the surface-exposed lipoprotein TbpB facilitates, but is not required for, TbpA-mediated iron acquisition. While TbpB has been shown to be lipidated and expressed on the bacterial surface, the importance of lipidation of TbpB on transferrin iron utilization has not been investigated. Additionally, TbpB possesses a well-conserved string of 4 glycine residues 2 bp downstream of the lipidation site. The significance of these conserved residues is unclear. To evaluate the functional contributions of these two regions, we generated site-directed mutants and analyzed the effects on TbpB expression, surface exposure, and transferrin iron utilization. To allow for transport of TbpB into the periplasm while removing the site of lipidation, the signal II peptidase cleavage site "LSAC" was replaced with a signal I sequence. To assess the role of the glycine-rich region, a mutant was created in which these residues were deleted. Western blot analysis and palmitate labeling indicated that TbpB was not lipidated in the LSAC mutant. Furthermore, the protein was not surface-accessible as determined by dot blot and protease accessibility analyses. Absence of lipidation resulted in the release of TbpB into the culture supernatant, while wild-type TbpB remained cell-associated. In contrast, the glycine deletion mutant was similar to the wild-type strain in both TbpB lipidation and surface expression. When these mutations were combined with a previously constructed TbpA mutant that requires TbpB for iron acquisition, growth on transferrin was completely abrogated in the LSAC mutant and was dramatically diminished in the glycine deletion mutant. We conclude that while lipidation is not necessary for transport of TbpB across the outer membrane, it is required for anchoring of the protein to the bacterial surface. Surface localization appears to be required to compensate for a known defect in TbpA. Furthermore, deletion of the downstream glycine-rich region also compromises TbpB's compensatory function in a TbpA mutant. Overall, these results provide important insights into the functional roles of two conserved regions of TbpB, furthering our understanding of this critical iron uptake system.

P 021

The Role of Lactoferrin Binding Protein B in Pathogenic *Neisseria* Species

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The human pathogens *Neisseria meningitidis* and *N. gonorrhoeae* express a receptor capable of binding to, and removing iron from, the host glycoprotein lactoferrin. Similar to the transferrin binding protein complex, the lactoferrin receptor consist of an integral outer membrane protein, lactoferrin binding protein A (LbpA), and a surface exposed bi-lobed lipoprotein, lactoferrin binding protein B (LbpB). Human gonococcal infection models have shown that possession of either lactoferrin or transferrin binding proteins are essential for survival, while possession of both receptors provides a competitive advantage during co-infection. As we have shown previously, LbpB is also capable of providing protection against human lactoferricin, a short cationic peptide derived from human lactoferrin. In the current study we demonstrate that the protection by LbpB is mediated by two clusters of negatively charged amino acids that localize to exposed loops in the C-terminal lobe of LbpB. We also demonstrate that the protection provided by LbpB in our assay is underestimated, due to the activity of the NalP, an outer membrane protein mediating proteolytic release of LbpB from the surface. Thus an isogenic mutant lacking NalP activity has substantially enhanced protection in our assay, which is likely more representative of the activity of LbpB *in vivo*. In this study we also explored the role of the negatively charged capsular polysaccharide in mediating protection against lactoferricin, and determined that a modest degree of protection was only observed at lower concentrations of lactoferricin. Experiments are currently underway to determine whether the protection mediated by LbpB extends to other cationic antimicrobial peptides. Thus our results to date indicate that LbpB provides substantial protection against the antimicrobial peptide derived from human lactoferrin and may provide effective protection against elements of the innate host defense mechanisms.

P 022

Delineating the interactions involved in formation of a ternary complex between human transferrin and the transferrin receptor proteins from Neisseria meningitidis

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Pathogenic Neisseria species acquire iron from human transferrin (Tf) in a process mediated by a surface receptor composed of transferrin-binding proteins A and B (TbpA and TbpB). TbpA is an integral outer membrane protein that functions as a gated channel for the passage of iron into the periplasm. TbpB is a surface exposed lipoprotein that facilitates the iron uptake process. In this study we demonstrate that the region encompassing amino acids 2 to 37 of N. meningitidis TbpB is required for forming a complex with TbpA, and that the formation of the complex requires the presence of human Tf. Site-directed mutagenesis of TbpB and TbpA was used to probe the interaction between TbpB, TbpA and Tf. The site-directed mutants were used to validate a structural model of the ternary complex derived from the TbpB-Tf (1) and TbpA-Tf (2) structures and computational docking and modeling of the anchor peptide region. The structural model differs from the published structure (2) in the orientation of TbpB relative to the TbpA-Tf complex. These results are consistent with a model in which TbpB is responsible for the initial capture of iron-loaded Tf and subsequently forms a ternary complex mediated by interactions between Tf and TbpA, Tf and TbpB and TbpB and TbpA.

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P 023

Diversity of the meningococcal transferrin and lactoferrin receptors

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Background: *Neisseria meningitidis* is able to acquire iron from human transferrin and lactoferrin through the expression of high-affinity receptors specific for each glycoprotein. These receptors consist of two iron-repressible outer membrane proteins: the transferrin or lactoferrin binding proteins A (TbpA or LbpA) and B (TbpB or LbpB). This work set out to determine the diversity of these receptors linking these findings to information documented on their functionality and structure. In doing so, selection pressures acting upon these proteins were revealed.

Methods: Nucleotide sequences encoding the lactoferrin and transferrin binding proteins were derived from Illumina sequenced genomic data contained in a local installation of the Bacterial Isolate Genome Database ($BIGS_{DB}$). Sequence definitions were generated for each *lbpA, lbpB, tbpA* and *tbpB* gene in the sequence definition database and seeded with corresponding reference nucleotide sequences. Nucleotide sequence homology searches using BLAST with a default word size of 15 and sequence identity of 70% were undertaken using the scan function within the database curator interface. Thus, isolates were selected and sequentially screened for each gene giving rise to a list of exact or partial matches detailing percent sequence identity score, alignment length and E-values for each gene searched. New alleles arising from partial matches and typically consisting of at least 70% sequence identity were manually checked for correct start and stop codons and aligned with known alleles before assignment. Selection pressures acting on lactoferrin or transferrin proteins were investigated with a maximum likelihood method, with the characterisation of selection in the presence of recombination carried out using the OMEGAMAP software package.

Results and Conclusions: The recent publication of TbpA and TbpB crystal structures enabled positively selected sites to be mapped onto the structures. Immune selection was found to occur on the larger surface exposed loops from LbpA and TbpA. Due to their surface exposed structure, TbpB and LbpB were found to be extremely variable with very little clonal complex association observed contrary to other surface-exposed antigens. In addition, two families of *tbpA* were found: one comprised solely of *N. meningitidis* isolates belonging to the ST-11 clonal complex, previously found to harbour the smaller *tbpB* gene; the other contained all of the other lineages.

P 024

Characterizing iron induced invasion in *Neisseria gonorrhoeae*

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Iron is an essential nutrient that is sequestered by iron-binding proteins in the human host resulting in a hostile environment for microbes. *Neisseria gonorrhoeae*, however, can utilize numerous iron-binding proteins including transferrin and hemoglobin to acquire this nutrient. Consequently, the gonococcus likely encounters conditions of high iron at some stages in the course of natural infection. During initial infection, gonococci can acquire iron via transferrin and lactoferrin present in semen and vaginal fluids, as well as from hemoglobin during menses or in blood during disseminated infections. Most studies investigating invasion represent an iron deplete environment as bovine transferrin present in fetal bovine serum chelates free iron in an inaccessible form for the gonococcus. Thus, potentially significant contributions made by iron to the gonococcal invasion process have not been fully appreciated. Our invasion studies show that the presence of excess iron enhances the invasive capabilities of gonococci into cervical epithelial cells by more than a hundred-fold. We propose that iron-induced proteins are responsible for the observed phenomenon. To identify such gonococcal proteins, we characterized the gonococcal iron-induced surface proteome by isolating outer membrane proteins from both iron replete and deplete cultures and comparing protein profiles using 1D and 2D analyses and then identified iron induced proteins by mass spectrometry. Mutations were generated in proteins likely to participate in this phenomenon and the strains were tested for defects in invasion. Our first candidates were an Opa-like integral outer membrane protein, NspA, and a TonB-dependent transporter, TdfJ. Although a significant defect in iron induced invasion was not detected in either the *nspA* or the *tdfJ* mutant, a double mutant lacking both proteins resulted in a strain which was significantly defective in iron induced invasion compared to the parent strain. In conclusion, our data suggests that multiple proteins participate in this phenomenon including NspA and TdfJ which may be important for interacting with host cells during natural infection.

P 025

Three-dimensional obstacles for bacterial surface motility

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Many bacterial species live at surfaces. For surface colonization they have developed mechanisms which allow them to move while remaining attached to surfaces. The most ubiquitous mode of surface motility is mediated by type IV pili. These polymeric cell appendages mediate motility through cycles of pilus polymerization, adhesion, and depolymerization. Natural adhesion surfaces, including mammalian host cells, are not flat. It is unknown, however, how the topography of a surface influences bacterial surface motility. Here, we show that the roundNeisseria gonorrhoeae (gonococcus) was preferentially reflected from barriers with a height of 1 μm but not by lower barriers. Gonococcal motility was confined to grooves whose dimensions were on the order of the size of the bacteria and the dynamics of movement was in agreement with a tug-of-war model. Likewise, the motility of the rod-likeMyxococcus xanthus(myxococcus) was confined to grooves. In summary, the data demonstrate that surface-motile bacteria „feel" the topography of the surface and that their movements are guided by microscopic elevations.
Claudia Meel *et al.*, Small 2011

P 026

Meningococcal TspA interacts with cell envelope components including the type IV pilus machinery and porins

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Question: T-cell stimulating protein A (TspA) is a highly conserved, immunogenic protein with homologues in many Gram negative bacteria which elaborate type IV pili. The precise functions of meningococcal TspA and other members of the TspA family, including FimV of *Pseudomonas aeruginosa*, are unclear, but may relate to type IV pili (T4P). Here, we aimed to gain insight into the function(s) of meningococcal TspA by identifying its interacting partner proteins.
Methods: TspA-interacting proteins were identified using a re-tagging technique followed by MS/MS. Recombinant derivatives of the identified interacting proteins were expressed and purified. ELISA and surface plasmon resonance techniques were used to further explore the interactions between sub-fragments of TspA and its interacting proteins.
Results: TspA-interacting proteins of approximately 80, 45, and 40-kDa were detected using re-tagging. MS/MS analysis identified these to be the outer membrane PilQ protein (NMB1812), the cytoplasmic PilT-1 protein (NMB0052) and the outer membrane protein PorA (NMB1429), respectively. TspA could bind recombinant PilQ, PilT-1 or PorA derivatives in ELISA assays and binding could be significantly reduced in the presence of the cognate soluble ligand. SPR was used to further examine the TspA-PorA interaction; the data confirmed that the proteins could interact and that pre-treatment of TspA with soluble PorA could inhibit the subsequent binding of TspA to immobilized PorA in a dose-dependent manner. To define which regions of TspA were responsible for the observed interactions, two sub-fragments of TspA: TspA59-371 (corresponding to the N-terminal, periplasm-localized region of TspA) and TspA406-875 (C-terminal, cytoplasm-localized region) were expressed and purified. The N-terminal region bound all three ligands, whilst the C-terminal TspA fragment did not. Finally, mutating TspA did not affect the levels of PilQ multimers formed in the meningococcal outer membrane.
Conclusions: The work presented here suggests that TspA interacts with important meningococcal envelope proteins: PorA; PilQ and PilT-1 through its N-terminal, presumably periplasm-localized domain. However, although TspA interacts with PilQ, it is not required for PilQ multimer formation or stability, as has been shown for the TspA-homologue, FimV, in *P. aeruginosa*.

P 028

Regulation of the gonococcal type IV secretion system

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Neisseria gonorrhoeae produces a type IV secretion system (T4SS) that secretes chromosomal DNA into the extracellular environment and thereby provides DNA for natural transformation. The T4SS also affects interactions with host cells, including conferring the ability to acquire iron by a TonB-independent mechanism during intracellular growth. Little is known about the regulation of expression of the T4SS. Outer membrane proteins of the T4SS apparatus are undetectable by Western blot, unless overexpressed. Quantitative real-time PCR analysis indicates that transcript levels of the T4SS gene traK are very low in the wt strain, but can be upregulated by the addition of 4 bp mutations in the predicted promoter region. TraK protein is also detectable in this strain. These results suggest that the T4SS is expressed at low levels in culture. Previous work in our lab suggests that type IV secretion of DNA might be controlled by expression of traD, which encodes the putative coupling protein and is divergently transcribed from the T4SS structural genes. To investigate the regulation of traD expression, we constructed a traD::lacZ translational fusion. A time course analysis of beta-galactosidase activity showed that traD::lacZ expression increased during log-phase growth, similar to the time course observed for secreted DNA. We performed chemical and transposon mutagenesis on the traD::lacZ reporter strain and screened for up- and down-regulated mutants on medium containing X-Gal. We identified blue, gray, and white chemical mutants, some of which were linked and others unlinked to the kanamycin resistance gene immediately downstream from traD::lacZ. From the transposon library we identified a mutant that was white when grown on plates containing low glutamine and X-Gal. Sequence analysis showed the transposon was inserted in glnD, which in other bacteria is known to encode a bifunctional uridylyltransferase/uridylyl-removing enzyme. Complementation of the glnD mutant restored the blue phenotype. In E. coli and other Gram-negative bacteria, GlnD acts as the primary nitrogen sensor of the cell by sensing the level of intracellular glutamine. Under nitrogen limitation, GlnD uridylylates PII signal transduction proteins which in turn regulate the activity of various target proteins. N. gonorrhoeae has a single PII protein, a GlnB homolog. We found that a glnD deletion strain grown under low glutamine still had uridylylated GlnB, suggesting that an alternative protein is able to modify GlnB and that GlnD does not regulate traD expression via GlnB in N. gonorrhoeae. These results also suggest that gonococcal GlnD uses a novel method to regulate traD expression. Further analysis of our chemical and transposon mutant libraries is expected to identify additional factors involved in T4SS regulation.

P 029

Large-scale study of the interactions between proteins involved in type IV pilus biology in *Neisseria meningitidis* – characterization of a sub-complex involved in pilus assembly

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The functionally versatile type IV pili (Tfp) are one of the most widespread virulence factors in bacteria. However, despite generating much research interest for decades, the molecular mechanisms underpinning the various aspects of Tfp biology remain poorly understood, mainly because of the complexity of the system. In the human pathogen *Neisseria meningitidis* for example, 23 proteins are dedicated to Tfp biology, 15 of which are essential for pilus biogenesis. One of the important gaps in our knowledge concerns the topology of this multi-protein machinery. Here we have used a bacterial two-hybrid system to identify and quantify the interactions between 11 Pil proteins from *N. meningitidis*. We identified 20 different binary interactions, many of which are novel. This represents the most complex interaction network between Pil proteins reported to date and indicates, among other things, that PilE, PilM, PilN and PilO, which are involved in pilus assembly, indeed interact. We focused our efforts on this subset of proteins and used a battery of assays to determine the membrane topology of PilN and PilO, map the interaction domains between PilE, PilM, PilN and PilO, and show that a widely conserved N-terminal motif in PilN is essential for both PilM-PilN interactions and pilus assembly. Finally, we show that PilP (another protein involved in pilus assembly) forms a complex with PilM, PilN and PilO. Taken together, these findings have numerous implications for understanding Tfp biology and provide a useful blueprint for future studies.

P 030

Impact of the PilU Protein on Multiple Stages of Meningococcal Colonization and Infection

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Expression of type IV pili (Tfp) is a prerequisite for meningococcal microcolony formation, adherence to host cells and subsequent colonization. The meningococcal PilT protein is a hexameric ATPase that mediates pilus retraction. The PilU protein, produced from the pilT-pilU operon, shares a high degree of homology with PilT. While the function of PilT in Tfp biology has been extensively studied, the role of PilU remains poorly understood. We investigated the importance of the meningococcal PilU protein in several stages of infection, including the formation of microcolonies and the ability to cause systemic disease. The pilU knock-out mutant exhibited delayed microcolony formation compared to wild-type, both the in presence and absence of host cells, indicating that bacteria-bacteria interactions are affected. PilU seems to negatively affect survival of bacteria in normal human serum whereas, in an animal model of meningococcal disease, the survival of mice infected with the pilU mutant was significantly increased. To conclude, these data suggest that PilU promotes timely microcolony formation and that both PilU and PilT are required for full bacterial virulence.

P 031

Type IV pili as host adaptation factors

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The role of Type IV pili (Tfp), the long (up to tens of microns) and thin (6 nm diameter) filamentous appendages bore by both Neisseria gonorrhoeae and Neisseria meningitidis, as virulence factors promoting infection of these two human pathogens has been long demonstrated. More recently, specifically in the case ofNeisseria gonorrhoeae, the importance of the dynamics of Tfp and the forces they can generate on human epithelia have been pinpointed as crucial elements for infection. We have recently shown that the commensalNeisseria elongata also bears Tfp. This provides a unique opportunity to better understand the role of Tfp in the interaction of bacteria with human cells. Using an array of force measuring techniques, we have measured the force generated by the Neisseria elongata Tfp as well as have studied the mechanical and chemical differences and similarities between Tfp from pathogens and commensals. This comparison starts to unveil the role of Tfp as host adaptation factors more than virulence factors across the Neisseria genus.

P 032

Effects of Calcium Binding on the *Neisseria gonorrhoeae* adhesin, PilC1

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PilC1, a type IV pilus adhesion protein in the infectious bacterium, *Neisseria gonorrhoeae*, is a member of the PilC family of adhesins found in other *Neisseria* species and *Pseudomonas* genera. Previous attempts to characterize the role of *N. gonorrhoeae* PilC's role in adhesion have been hampered by difficulties in producing significant amounts of protein. Here, we define a method to produce recombinant full length PilC1 from *E. coli* and demonstrate that this protein can inhibit *N. gonorrhoeae* binding to human cells. In addition to adhesion, PilC1-like proteins also control pilus biogenesis. Recently, the PilC1-like protein PilY1 from *Pseudomonas aeruginosa* was shown to control pilus biogenesis through a C-terminal calcium binding site. Calcium-bound and -unbound states of PilY1 control pilus extension and retraction, respectively. We have identified a homologous calcium binding site in the C-terminal half of *N. gonorrhoeae* PilC1. Here, using recombinant protein, we confirm that the putative calcium binding site is capable of binding calcium. Furthermore, we show that calcium binding at this site affects adhesion though regulating functional pilus biogenesis. Taken together, we establish that ion binding plays a critical role in PilC1 function.

P 033

Creation and Characterisation of Opaless FA1090 *Neisseria gonorrhoeae*

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The opacity-associated (Opa) proteins constitute a family of integral outer membrane proteins that bind to receptors on human cells including heparin sulphate proteoglycans or members of the CEACAM superfamily. While Opa proteins are important in human infection by *Neisseria gonorrhoeae* (Gc), their contribution to virulence is not well understood. Investigating Opa mediated effects upon human cells is complicated by "translucent" Opas, Opa proteins that confer an Opa- colony morphology in Gc and are only detectable by western blot. Therefore, a culture of phenotypically Opa- Gc, may in fact contain a subset of translucent Opa+ bacteria. To address this problem, we created a derivative of Gc strain FA1090 in which the genes encoding the translucent Opas OpaB, E, G and K were deleted in frame. The remaining seven *opa* genes in the FA1090 genome were then deleted to produce another derivative, which is consistently Opa-. "Opaless" FA1090 shows no reversion to an Opa+ state after twenty nonselective passages on agar and no defect in growth on agar or in liquid culture when compared to phenotypically Opa- FA1090 parental Gc or Δ*opaKEGB*. Like phenotypically Opa- Gc, Opaless bacteria exhibited significant survival after exposure to neutrophils and the ability to suppress the neutrophil oxidative burst. Intriguingly, ~50% of Opaless Gc associated with human neutrophils were internalised, indicating a route of entry of Gc into neutrophils that is opsonin- and Opa-independent.
The Opa- derivatives of FA1090 described here will allow for discrimination of Opa- dependent parameters during infection in a defined Gc background. Moreover, the derivatives provide genetic backbones for reintroducing Opas, including engineered *opa* genes that cannot phase vary.

P 034

Attempts at reconstituting a minimal meningococcal type IV pilus assembly machinery in a heterologous host

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Type IV pili (Tfp) are one of the most widespread virulence factors in bacteria, an the only pilus found both in Gram negative and Gram positive species. Their biogenesis relies on a complex multi-protein machinery composed of as many as 15 components in the meningococcus (PilC, PilD, PilE, PilF, PilG, PilH, PilI, PilJ, PilK, PilM, PilN, PilO, PilP, PilQ and PilW). These proteins are conserved in Tfp-expressing bacteria, even in phylogenetically distant species, which suggests that a common mechanism is involved. However, how the filaments are assembled remains largely unknown. We have previously shown, using a genetic approach, that piliation could be restored in the absence of eight of the above 15 proteins when pilus retraction is abolished by a concurrent mutation in the PilT molecular motor, suggesting that pilus assembly is simpler than expected and may require "only" PilD, PilE, PilF, PilM, PilN, PilO and PilP. Since this "minimal" system might be easier to characterize, we are trying to reconstitute it in *E. coli* by co-expressing the corresponding genes. An update on our efforts will be presented. The eventual assembly of Tfp by a minimal system in a heterologous host would have important consequences on our understanding of the biology of these fascinating organelles.

P 035

Follow-up news on the PilC tip-located adhesin of the *Neisseria* type 4 pilus

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We have generated a monoclonal antibody that specifically interacts with PilC protein from *N. gonorrhoeae* strain MS11. Here, we present recent data obtained with the use of this antibody showing co-localization of distal pili ends with gold-conjugated secondary antibody in gonococcal cells. We also succeeded in purifying native PilC protein from a gonococcal overproducing strain in amounts, sufficient to generate protein crystals for x-ray diffraction experiments. Together, our observations corroborate our previous observations on PilC, functioning as a tip-located adhesion and being essential for pathogenic *Neisseria* species to adhere to human epithelial cells (1).

(1) Rudel, T., Scheuerpflug, I., and Meyer, T. F. *Neisseria* PilC protein identified as type-4 pilus tip-located adhesin (1995) Nature 373(6512), 357-359.

P 036

Host galectin-3 binds meningococcal PilE and PilQ, and promotes bacterial invasion

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Question: We recently demonstrated thatNeisseria meningitidis targets the 37/67kDa laminin receptor (LR) on the surface of human brain microvascular endothelial cells, and identified the outer membrane proteins, PorA and PilQ, as the meningococcal ligands involved (1). The relationship between the 37-kDa and 67-kDa forms of LR is not completely understood, but previous observations have suggested that 37-kDa LR and galactin-3 (Gal-3) can hetero-dimerise to form 67-kDa LR. Gal-3 is the only member of the chimera-type group of galectins, and has one C-terminal carbohydrate recognition domain (CRD) that is responsible for binding the b-galactoside moieties of mono- or oligosaccharides on several host and bacterial molecules, including neisserial lipo-oligosaccharide (LOS) (2). The aim of this study was to characterise the role of Gal-3 in the meningococcal-host cell interaction and identify the non-LOS meningococcal molecules that bind Gal-3.

Methods: Expression of Gal-3 was examined in the brain of CD46 expressing mice by immunofluorescence staining. Meningococcal invasion assays were carried out in Neuro-2a (N2a) cells (which do not express Gal-3) and transfected N2a cells expressing Gal-3. Lactose-liganded Gal-3 and its C-terminal CRD were used in ELISA to examine their binding to N. meningitidis. Meningococcal Gal-3 ligands were identified by re-tagging and MALDI-ToF.

Results: Infection of CD46-expressing transgenic mice with meningococcal strain MC58 significantly increased Gal-3 expression across the brain. Transient expression of Gal-3 in mouse N2a cells significantly enhanced meningococcal invasion when compared with non-transfected cells. Direct ELISA and inhibition assays, using immobilized lactose-liganded Gal-3 and CRD, showed that all (n=25) meningococcal isolates bound the host molecules. Binding was not CRD-restricted, indicating that the N-terminal domain of Gal-3 was also involved. A meningococcal mutant lacking the glycosyltransferase required for chain elongation from the core lipid A-$(KDO)_2$-Hep_2 showed reduced binding to lactose-liganded Gal-3, but binding was not abolished indicating that the meningococcal-Gal-3 binding was not entirely LOS-dependant. Using a re-tagging approach, meningococcal PilQ and PilE proteins were identified as Gal-3 binding ligands. Mutation of the genes encoding either of these two molecules in strain MC58 led to a significant reduction in Gal-3 binding. PilQ is not known to be glycosylated, therefore its interaction with Gal-3 is likely to be protein-mediated. PilE is post-translationally glycosylated and deletion of the pilin glycosylation genespglC and/or pglL dramatically reduced bacterial-Gal-3 binding.

Conclusions: We demonstrated that Gal-3 is important in the meningococcal-host cell interaction and that it targets PilQ and PilE, as well as the previously known LOS. This interaction may be part of host-cell defence against the organism, and/or, conversely, it may be part of a strategy adopted by the organism to modulate the host response and facilitate its invasion.

References:

(1) Orihuela, C. J., J. Mahdavi, et al. (2009). "Laminin receptor initiates bacterial contact with the blood brain barrier in experimental meningitis models."J Clin Invest119(6): 1638-46.

(2) Paola, Quattroni., Yanwen, L., et al (2010). Binding of Neisseria meningitidis to Galectin-3.17th international pathogenic Neisseria conferenceBanff, Canada.

P 037

Meningococcal PorA and PilQ provoke localized actin rearrangement and recruitment of 67kDa laminin receptor and early trafficking marker rab5A

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Question: Recent work identified the meningococcal proteins PorA and PilQ as being critical for adhesion to endothelial cells of the blood-brain barrier via the 37/67kDa laminin receptor (LamR). PorA is a multifunctional trimeric outer membrane porin and the secretin PilQ is an essential component of the type IV pili (tfp). Meningococcal adhesion and host cell-induced signalling rely on tfp, whilst porins are among the most abundant proteins in the outer membrane of Gram negative bacteria and are increasingly recognised as being important virulence factors. Whilst meningococci are known to engage multiple host cell receptors and molecular linkers, and to induce localized actin polymerisation to form cortical plaques thereby promoting adherence, cell invasion and the creation of a paracellular invasion route, many details of the molecular mechanism of initial adherence and colonisation leading to invasion remain unclear. This study aimed to investigate the roles of PorA and PilQ in early events of cortical plaque formation and host cell-induced signalling in human microvascular endothelial cells (hBMECs).
Methods: Confocal microscopic analysis was used to investigate: the effect of recombinant PorA and PilQ on localised actin polymerisation; the localization of the 67kDa non-integrin Laminin receptor; and the recruitment of early trafficking markers, rab5A and EEA1.
Results: Our data demonstrates that both PorA and PilQ induce assembly of rab5A-associated 67kDa LamR-rich plaques in hBMECs with concomitant depletion of EEA1-associated 67kDa LamR. However, only PilQ induced efficacious cortical actin rearrangement. Subsequent investigation withN. meningitidis strain MC58 demonstrated both 37kDa and 67kDa LamR recruitment to the cortical plaque whilst rab5A was recruited to, and found within, meningococcal colonies.
Conclusions: In devising strategies for therapeutic intervention, a clear understanding of the contribution from both the meningococcus and responding host cells are critical. Here we show that individual adhesins may exhibit pleiotrophism and functional redundancy whilst mimicking characteristics of the whole organism.

P 038

Mapping the laminin receptor binding domains of *Neisseria meningitidis* PorA and *Haemophilus influenzae* OmpP2

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Question: *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* are major bacterial agents of meningitis. To access the central nervous system they first adhere to the cerebral vascular endothelium prior to crossing it. They each bind a common carboxy-terminal recognition site on 37/67-kDa laminin receptor (LamR) via the surface protein adhesins: meningococcal PilQ and PorA; *H. influenzae* OmpP2; and pneumococcal CbpA. Understanding the structural basis for the ability of these ligands to bind LamR could facilitate the design of therapeutic interventions which could prevent or disrupt the interaction and thus engender broad protection against bacterial meningitis. We have previously reported that a surface-exposed loop of the R2 domain of CbpA bound to LamR. The aim of this work was to identify the LamR-binding regions of PorA and OmpP2.
Methods: Recombinant *N. meningitidis* MC58 PorA and *H. influenzae* Rd KW20 OmpP2 and several sub-fragments of each were expressed, purified and tested for LamR-binding activity in ELISA assays. Synthetic peptides corresponding to specific extra-cellular loops of PorA and OmpP2 were also tested for LamR-binding and the ability to block LamR-binding by ligands. Finally, meningococcal and H. influenzae strains expressing PorA or OmpP2 lacking specific extra-cellular loops were generated and tested for LamR binding.
Results: Using truncated recombinant proteins we showed that LamR-binding was dependent on the regions encompassed by amino acids 171-240 and 91-99 of PorA and OmpP2, respectively, which are predicted to localize to the fourth and second surface exposed loops, respectively, of these proteins. Synthetic peptides corresponding to the loops bound LamR and could block LamR-binding to bacterial ligands in dose dependant manner. Meningococci expressing PorA lacking the apex of loop 4 and *H. influenzae* expressing OmpP2 lacking the apex of loop 2 showed significantly reduced LamR binding.
Conclusions: We have identified the principal LamR-binding regions within meningococcal PorA and *H. influenzae* OmpP2. These regions correspond to the apical regions of the fourth and second extracellular loops, respectively. Both loops are hyper-variable, suggestive of the molecular basis for the diverse range of LamR-binding capabilities previously reported for meningococcal and *H. influenzae* strains of different lineages.

P 039

Extracellular loop 4 of the meningococcal outer membrane protein PorA effects cell cycle and migration of human epithelial cells

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Question: PorA is a multifunctional protein found within the outer membrane of *Neisseria meningitidis*. This trimeric protein serves as a porin, adhesin and is used as the basis for part of the meningococcal typing system. Porins are among the most abundant proteins in the outer membrane of Gram negative bacteria and are increasingly recognised as being important virulence factors in addition to their primary roles. Recent work demonstrated that meningococci bind to cells of the blood brain barrier via the 37/67kDa laminin receptor and identified PorA, as one of two meningococcal ligands for this protein, the other being the secretin, PilQ. Further studies utilising recombinant PorA-sub-fragments and synthetic peptides localised the LR-binding domain to the variable, surface-exposed fourth extracellular loop of PorA. This study aims to investigate the role of PorA, and in particular extracellular loop 4, in the host pathogen interaction between *N. meningitidis* and human epithelial cells.
Methods: A linear wound healing assay was used to investigate the *in vitro* effect of PorA loop 4 on human epithelial cell migration. Cell cycle analysis was carried out, using flow cytometry with cells stained with propidium iodide (PI) after 24h treatment.
Results: Monitoring migration over time showed that wound closure was significantly impaired when cells were treated with synthetic PorA loop 4. In contrast, recombinant PorA or synthetic loop 1 peptide had no effect on the closure of wounds compared to the untreated controls. Analysis of the cell cycle after treatment with PorA loop 4 showed a significant reduction in the number of cells that transitioned through the G1 checkpoint after 24 h. However, there was no effect on the cell cycle after treatment with loop 1 compared to untreated controls.
Conclusions: Our data suggest that meningococcal PorA loop 4 has a significant effect on cell migration and cell cycle control which is potentially mediated through the laminin receptor. This effect appears to be cytostatic and not cytotoxic and may play an important role in the interaction between meningococci and human epithelial cells *in vivo*.

P 040

Neisseria meningitidis and *Neisseria gonorrhoeae* overcome endothelial cell monolayers via a paracellular pathway that is independent of type IV pili

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Objective and methods: *N. gonorrhoeae* (*Ngo*) and *N. meningitidis* (*Nme*) share numerous virulence factors, namely type IV pili (Tfp) and Opa proteins. It has been well reported that both *Ngo* and *Nme* cross epithelial cell monolayers by transcytosis, without affecting barrier integrity. Additionally, recent work has suggested that *Nme* strains cross endothelial monolayers via a paracellular route, through recruitment and/or destruction of cell-cell-contact forming proteins; however, there are inconsistencies in the overall description of these processes. Therefore, we employed an *in vitro* infection model to further elucidate the underlying molecular mechanisms of this paracellular pathway. Primary human umbilical vein endothelial cells (HUVECs) expressing endothelial cell characteristics were used in combination with CellZscope (nanoAnalytics), an automated cell-monitoring system, to determine changes in the transendothelial electrical resistance (TEER) of polarised cell monolayers upon infection in real-time.
Results: The system reported is stable and exhibits good physiological reliability as determined by the immediate, dose-dependent, transient loss in barrier integrity following addition of thrombin, which could be inhibited by antibody. Both *Ngo* and *Nme* caused specific, temporal loosening of the tight cell-cell contacts of HUVEC monolayers, detected by reduced TEERs. This effect started approximately 3-4 h p.i., was maximal 8-12 h p.i. (lowest TEERs), and was followed by full recovery around 24-46 h p.i. The effects were specific as neither heat-inactivated bacteria nor the addition of bacterial antigens, *e.g.* LPS, reproduced phenotypes. Furthermore, Tfp were dispensable for the observed phenotypes as Δ*pilE1/2* mutants expressing Opa proteins also decreased TEERs. Moreover, there was a 3 h delay to TEER reduction induced by Opa-negative selected Δ*pilE1/2* mutants, which correlated with an intensive Opa-switch visible in colony phenotype. Thus, we hypothesize that Tfp first establish host cell contact, and then Opa proteins induce a loss in barrier integrity, which allows the paracellular crossing of endothelial cell monolayers. The ongoing work is focused on the assumed key factors of the regulatory host-cell signalling networks and utilises inhibitor studies and immunofluorescence microscopy.

P 041

Internalisation, nuclear translocation and molecular interactions of meningococcal App and MspA autotransporters in human dendritic cells

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Question: We have previously shown that the adhesion and penetration protein (App) and meningococcal serine protease A (MspA) mediate adherence to host cells. The role of the secreted passenger domains of these proteins is unknown. Here, we provide experimental evidence to support the hypothesis that during invasive meningococcal disease, secreted App and MspA bind specific host cell receptors, escape into the cytoplasm and translocate to the nucleus, where they modulate host cell responses that may contribute to pathogenesis.
Methods and Results: Using confocal microscopy, we confirmed that the passenger domains of App and MspA were internalised and translocated into the nucleus of dendritic cells (DCs). Re-tagging and cross-linking experiments, in conjunction with MALDI-TOF, showed that App and MspA bound specifically to several host cell surface and intracellular molecules, including the Mannose Receptor (MR), transferrin Receptor (TfR), heat-shock protein 70 (HSP70) and histone proteins (H1, H2A, H2B, H3.1 & H4). These interactions were confirmed using ELISA, confocal microscopy, inhibition assays and Far-Western overlay assays. Analysis of the molecular structure and function of App and MspA demonstrated that the two proteins are *O*-glycosylated, a feature that is likely to be required for the MR and TfR-mediated uptake as well as HSP70-mediated nuclear translocation.
We have confirmed the predicted serine protease activity of App and MspA and shown that they belong to the trypsin-like group of serine proteases. Furthermore, they are both capable of specifically cleaving the tail of histone H3; similar activity has recently been described for an endopeptidase of *Sacharomyces cerevicea* which has been suggested as a mechanism to release histone-mediated repression of gene expression. Both autotransporter proteins modulate the cytokine responses of DCs and inhibit phagocytic activity in these cells.
Conclusion: The two meningococcal autotransporters are multi-functional proteins, playing significant roles in the host-pathogen interactions.

P 042

Intracellular fate and biological impact of meningococcal App and MspA autotransporters

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Question: Neisseria meningitidis secretes a number of proteins that mediate numerous interactions with host cells including adhesion and invasion of various human cells. Two such proteins, the adhesion and penetration protein (App), and meningococcal serine protease A (MspA) belong to type Va autotransporters. Each protein comprises biologically active N-terminal passenger and C-terminal transporter domains. We previously showed that App and MspA mediate adherence to host cells. However, the intracellular fate and biological impact of App and MspA are not knonw.

Methods and Results: Here, in this study, we demonstrate that:

• CD46 expressing mice survived better when challenged with a meningococcal mutant lacking both MspA and App, compared wild type parent strain.

• The two proteins reduced uptake of FITC-labelled dextran and modulated cytokine production (particularly IL-10 & IL12) by host dendritic cells in-vitro.

• Confocal microscopy showed that purified recombinant passenger domains of App and MspA were internalised and translocated into the nucleus of endothelial and dendritic cells.

• Re-tagging and cross-linking experiments, in conjunction with MALDI-TOF, showed that App and MspA bound specifically to several host cell surface and intracellular molecules, including the Mannose Receptor (MR), transferrin Receptor (TfR), heat-shock protein 70 and histone proteins (H1, H2A, H2B, H3.1 & H4). These interactions were confirmed using ELISA, confocal microscopy, inhibition assays and Far-Western overlay assays.

• By probing immunoblots for glycoproteins we showed that both App and MspA are glycoproteins

• Using protease assays we confirmed that both App and MspA, exhibit serine protease activities, and demonstrated that they are able to cleave histone tailsin-vitro.

Implications: We provide evidence to support the hypothesis that during invasive meningococcal disease, secreted App and MspA adhere to host cell surfaces, bind specific domains of the two receptors, MR and TfR, escape into the cytoplasm, bind HSP70 and translocate to the nucleus, where they cleave histone tails and modulate host cell responses that may contribute to pathogenesis. The nature of the response may be cell-specific and vary between the two autotransporters.

P 043

Identification of potential extracellular matrix-associated proteins (EMAPs) involved in bacterial invasion of the human Fallopian tube by *Neisseria gonorrhoeae*

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Question: The extracellular matrix (ECM) layer that covers the mucosal surface of the human Fallopian tube (FT) is regulated by ovarian steroids and a complex network of paracrine mediators such as matrix metalloproteinases (MMPs) and tissue extracellular matrix inhibitors (TIMPs). In addition, the MMPs/TIMPs system is involved in bacterial infection of FT byNeisseria gonorrhoeae. However, potential participation of other extracellular matrix-associated proteins (EMAPs) in gonococcal infection of the FT has not been yet identified.
Methods: Real time-PCR array was used to analyze the expression of 96 genes related to the EMC proteins during the follicular, ovulatory and luteal phase of the menstrual cycle by use of human FT samples. Bioinformatic analysis was used to identify and determine the cellular interaction between the EMAP-selected genes and the genes selected from others models of *Neisseria* host invasion.
Results: All the genes identified revealed that they interact within four cellular pathways: i) ECM receptor interaction pathway; ii) the leukocyte transendothelial migration pathway; iii) cell adhesion molecules; and iv) bacterial invasion of epithelial cells. All pathways are involved in gonococcus pathogenesis and are described in other experimental models. Twelve genes have been previously described in the pathogenesis of *N. meningitidis*:
Cd44, Icam-4, Selp (cell surface antigens);*Itga5, Itgb1, Itgb2* (cell adhesion proteins);*CTNNB1*(B-catenin) and *Ncam1* (adherent junction-associated proteins); and *Sele, Icam1 and Vcam1* (leukocyte migration proteins). In addition, *Cd105, ezrin, Cam* and *PI3K* which are known to respond to gonococcal infection in other experimental models, showed extensive interactions with identified EMAP genes.
Conclusions: Our results suggest that the EMAP genes *Cd44, Icam-4, Selp,Itga5, Itgb1, Itgb2, CTNNB1, Ncam1, Sele, Icam1 and Vcam1* together with *Cd105, ezrin, Cam* and *PI3K* may play a role during bacterial invasion of the human Fallopian tube by *N. gonorrhoeae*. Further studies are in progress in order to determine the participation of these EMAPs in FT tissue during infection and whether the gonococcus may modulate their expression. The study is supported by the Wellcome Trust (090301) and FONDECYT (1120712).

P 044

Neisseria gonorrhoeae infection causes DNA damage and affects the expression of p21, p27, and p53 in non-tumor epithelial cells

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The constant shedding and renewal of epithelial cells maintain the protection of epithelial barriers. Interference with the processes of host cell-cycle regulation and barrier integrity permits the bacterial pathogen *Neisseria gonorrhoeae* to effectively colonize and invade epithelial cells.
Here, we show that a gonococcal infection causes DNA damage in human non-tumor vaginal VK2/E6E7 cells with an increase of 1100 DNA strand breaks per cell per hour as detected by an alkaline DNA unwinding assay. Infected cells exhibited elevated levels of DNA double-strand breaks, as indicated by a more than 50% increase in cells expressing DNA damage-response protein 53BP1-positive foci that co-localized with phosphorylated histone H2AX (γH2AX). Furthermore, infected cells abolished their expression of the tumor protein p53 and induced an increase in the expression of cyclin-dependent kinase inhibitors p21 and p27 to 2.6-fold and 4.2-fold of controls, respectively. As shown by live-cell microscopy, flow cytometry assays, and BrdU incorporation assays, gonococcal infection slowed the host cell-cycle progression mainly by impairing progression through the G2 phase.
Our findings show new cellular players that are involved in the control of the human cell cycle during gonococcal infection and the potential of bacteria to cause cellular abnormalities.

P 045

Neisseria gonorrhoeae breaches the apical junction of polarized epithelial cells for transmigration by hijacking EGFR

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Gonorrhea, a common sexually transmitted infection, is caused by the gram-negative bacterium *Neisseria gonorrhoeae*. In the female reproductive tract, gonococci initiate infection at the apical surface of columnar epithelial cells. These cells provide a physical barrier against mucosal pathogens by forming continuous apical junctional complexes between neighboring cells. The apical junction controls both the permeability between epithelial cells and the lateral movement between the apical and basolateral membrane to maintain cell polarity.

This study examines the impact of gonococcal interaction on the barrier function of polarized epithelial cells. We show that viable but not gentamicin killed gonococci preferentially localize at the apical side of the cell-cell junction in polarized endometrial and colonic epithelial cells, HEC-1-B and T84. Confocal fluorescence microscopic analysis shows that this colonization results in a loss of junctional proteins, ZO-1 and occludin, from the cell periphery and the redistribution of the junction-associated protein β-catenin from the apical junction to the cytoplasm. This result indicates the disruption of the apical junction by gonococcal colonization. However, gonococcal inoculation does not change the overall cellular level of junctional proteins. Using live cell imaging, we found an increase in the movement of an apically labeled membrane dye into the basolateral membrane in gonococcal-inoculated polarized HEC-1-B cells. The data indicate a decrease in the barrier function of the apical junction against the lateral movement between the apical and basolateral membrane as well as cell polarity. However, gonococcal inoculation does not significantly increase the permeability of the polarized epithelial monolayer to small fluorescent molecules. Disruption of the apical junction by removing calcium dramatically increases gonococcal transmigration across the epithelial monolayer. Gonococcal inoculation induces the phosphorylation of both EGFR and β-catenin in polarized epithelial cells. Inhibition of EGFR phosphorylation significantly reduces both gonococci-induced β-catenin redistribution and gonococcal transmigration.

Therefore, gonococci are capable of weakening the apical junction and the polarity of epithelial cells via activating EGFR, which facilitates GC transmigration across the epithelium.

P 046

Gonococcal interactions with polarized epithelial cells disassemble the actin cytoskeleton

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The obligate human pathogenNeisseria gonorrhoeae is a gram negative diplococcus that causes the sexually transmitted infection gonorrhea. Since gonococcal infections are typically asymptomatic in females, they often remain untreated and can lead to more serious complications such as pelvic inflammatory disease, disseminated gonococcal infections and arthritis. Infections can also result in fallopian tube scarring that can predispose women to ectopic pregnancies or sterility.

Invasive disease in women requires that gonococci not only colonize the surface of the female reproductive tract, but also invade into and transmigrate across the polarized epithelial barrier. Epithelial cells generate a protective monolayer by forming continuous apical junction complexes between neighboring cells. The apical junction controls the permeability and polarity of epithelial cells and its barrier function is controlled by actin and its motor myosin.

Gonococcal invasion into non-polarized cells requires actin recruitment. In this study, we investigate the impact of gonococci on the actin cytoskeleton in polarized epithelial cells and the role for actin in gonococcal transmigration. The data indicate that when gonococci interact with polarized T84 cells, rather than recruiting actin to the site of interaction, excludes actin. In polarized cells, non-muscle myosin II (NMII) is recruited to gonococcal adherent sites at the apical surface. Inhibition of the motor activity or activation of NMII by treating polarized epithelial cells with either NMII ATPase inhibitor or myosin light chain kinase inhibitor blocked gonococcal induced actin exclusion and reduced gonococcal transmigration, but increased gonococcal invasion. This indicates that the interaction with polarized epithelial cells is quite different from that in non-polarized epithelial cells, and the different natures of actin remodeling facilitate gonococcal invasion into or transmigration across the epithelium.

These findings suggest that gonococcal-induced host responsesin vivocan be quite different from those observed inin vitrostudies.

P 047

Evaluating the interplay of autophagy and CD46 proteolytic processing upon *Neisseria* invasion

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CD46 is a type I transmembrane protein targeted by numerous viruses and bacteria. CD46 plays a role in innate immunity via its complement regulatory functions. CD46 cyt1 cytoplasmic tail isoforms have immune effector functions that promote clearance of intracellular pathogens by autophagosomes. CD46 cyt1-dependent autophagy is dependent upon interaction of the cyt1 tail with the autophagosome formation complex. We evaluated if *Neisseria gonorrhoeae* (Ng) infection of epithelial cells induces autophagy. We report the Ng infection of epithelial cells promotes maturation of LC3, an event associated with autophagosome accumulation and maturation. Time course experiments have shown that peak accumulation of mature LC3 coincides with infection-induced proteolytic processing of CD46 by Presenilin/gamma-secretase (PS/γS). The impact of CD46 proteolysis on autophagosome maturation and function is unknown. Interestingly, inhibition of PS/γS with chemical inhibitors promotes Ng invasion. To test if the invasion phenotype is dependent upon PS/γS processing of CD46 we first identified amino substitutions in the CD46 transmembrane segment that block PS/γ-S cleavage. Stable expression of uncleavable CD46 cyt1 in epithelial cells promoted Ng invasion. Thus, both chemical inhibition and a mutational approach block CD46 cyt1 cleavage by PS/γS and lead to higher levels of Ng invasion. We are working to evaluate how Ng-induced CD46 proteolysis influences invasion. Since autophagy can clear invading microbes this work may identify new mechanisms for evading host defenses.

P 048

Targeting of neisserial Omp85 to the outer mitochondrial membrane depends on the carboxy-terminus and POTRA domains

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Omp85 proteins are evolutionary conserved and found exclusively in the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts. Omp85, itself a β-barrel protein, functions primarily in the membrane assembly of other β-barrel proteins, such as porins. Omp85 from Neisseria gonorrhoeae (Ngo) and its enterobacterial homologue YaeT/BamA have high sequence similarity and are both similar to their mitochondrial counterpart Sam50. We have shown, however, that whereas Ngo Omp85 is imported and integrated into the outer mitochondrial membrane (OMM), where it forms fully functional complexes, this is not the case with BamA, which is not taken up by human mitochondria at all1. In order to pinpoint the region in Omp85 required for its mitochondrial targeting, we exchanged domains of Omp85 and BamA and monitored the mitochondrial localization of such fusion proteins. At the same time, we removed the amino-terminal polypeptide-transport-associated (POTRA) domains of Ngo Omp85, to determine which of them are required for the formation of functional OMM complexes. Our data indicate that the fourth and the fifth POTRA domains are necessary for the protein to form membrane complexes in the OMM, but their removal does not affect mitochondrial localization of Ngo Omp85. We also conclude that the extreme carboxy-terminal of Ngo Omp85 is necessary for its mitochondrial import. We could not mutate the enterobacterial BamA protein in any way that would direct it to the mitochondria of human cells. Therefore, neisserial β-barrel proteins remain the only group of bacterial β-barrel proteins so far that, according to our data, successfully target human mitochondria. This may reflect the close co-evolution of Neisseria sp., some of which are obligate human pathogens, and their host.

1. Kozjak-Pavlovic V, Ott C, Götz M, Rudel T (2011) Neisserial Omp85 is selecively recognized and assembled into functional complexes in the outer membrane of human mitochondria.J. Biol. Chem.286:27019-27026.

P 049

Genomic organization and characterization of MDAΦ, a temperate filamentous bacteriophage of *Neisseria meningitidis* associated with the meningococcal disease

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Neisseria meningitidis has the capacity in a small proportion of colonized people to invade the host tissue and to cause life-threatening disease, meningitis and septicaemia. Why in some circumstances the bacteria become invasive is not well understood, but this is likely to rely on both host and bacterial factors. Comparison of the genomes of strains belonging to invasive clonal complexes with non-hypervirulent clonal complexes of *Neisseria meningitidis* by whole genome DNA arrays revealed the presence of an 8kb island in strains of invasive complexes. Further epidemiological investigations have shown that this island appears to be directly associated with the disease (Bille *et al.* 2005; Bille *et al.* 2008). This island was subsequently designated MDA for Meningococcal Disease Associated.
The island contains ten open reading frames. It is integrated in the bacterial chromosome into dRS3 repeat sequences. Further sequence analysis revealed similarities with bacteriophages of the type Ff of *Escherichia coli* and CTXΦ of *Vibrio cholerae*. Subsequent molecular investigations showed that the MDA island has indeed the characteristics of a filamentous prophage. Electron microscopic examination of phage preparation identified structures compatible with a filamentous phage. This was confirmed by immunogold labelling using antibodies raised against the major capsid protein. The same antibodies used on whole bacterial cells revealed that only a few bacterial cells within a population are capable of producing this phage.
Data will be presented on the characterization of this phage and its role in meningococcal invasiveness.

Bille E, Ure R, Gray SJ, Kaczmarski EB, McCarthy ND *et al.* (2008) Association of a bacteriophage with meningococcal disease in young adults. PLoS ONE 3(12): e3885.
Bille E, Zahar JR, Perrin A, Morelle S, Kriz P *et al.* (2005) A chromosomally integrated bacteriophage in invasive meningococci. The Journal of experimental medicine 201(12): 1905-1913.

P 050

Oral *Lactobacillus plantarum* NCIMB 8825 inhibits adhesion, invasion and metabolism of *Neisseria meningitidis* serogroup B and affords anti-inflammatory and cytotoxic protection to nasopharyngeal epithelial cells

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Our objective was to investigate the potential for oral strains of Lactobacilli (LB) to afford innate protection against the nasopharyngeal pathogen *Neisseria meningitidis* serogroup B(NmB).
Oral isolates of *L. plantarum* (NCIMB 8825), *L. salivarious* (NCIMB 11975), *L. casei* (NCIMB 8822), *L. rhamnosus* (NCIMB 6375), *L. gasseri* (NCIMB 8819) and gut probiotic *L. rhamnosus* GG were compared for their ability to suppress nasopharyngeal epithelial inflammatory responses to pathogenic NmB. The specificity of attenuation was examined against TLR 2 ligand, Pam3Cys, and early response cytokine IL1 β; and the mechanism of attenuation was explored using heat-killed organisms and conditioned medium. Pro-inflammatory IL-6 and TNFα cytokine secretion was quantified by ELISA and associated cell death was quantified by PI staining and LDH release. NmB adhesion, invasion and growth were determined using standard gentamicin protection with viable counts and confirmed using bioluminescence.
L. plantarum and *L. salivarious* significantly suppressed both IL-6 and TNFα secretion from NmB-infected nasopharyngeal epithelial cells (Fig 1, $p<0.01$). These organisms did not need to be alive and could suppress using secretions, which were independent of TLR2 or IL1β signalling. *L. plantarum*, in particular, also reduced NmB-induced necrotic cell death of epithelial monolayers and furthermore reduced NmB adhesion and abolished NmB invasion (Fig 2). Using bioluminescence as a reporter of pathogen metabolism, *L. plantarum* and its secretions were found to inhibit NmB metabolism during cell invasion assays.
We conclude that oral *L. plantarum* NCIMB 8825 and its secretions could be used to help reduce the burden of meningococcal disease through innate effects at the mucosal barrier.

figure 1

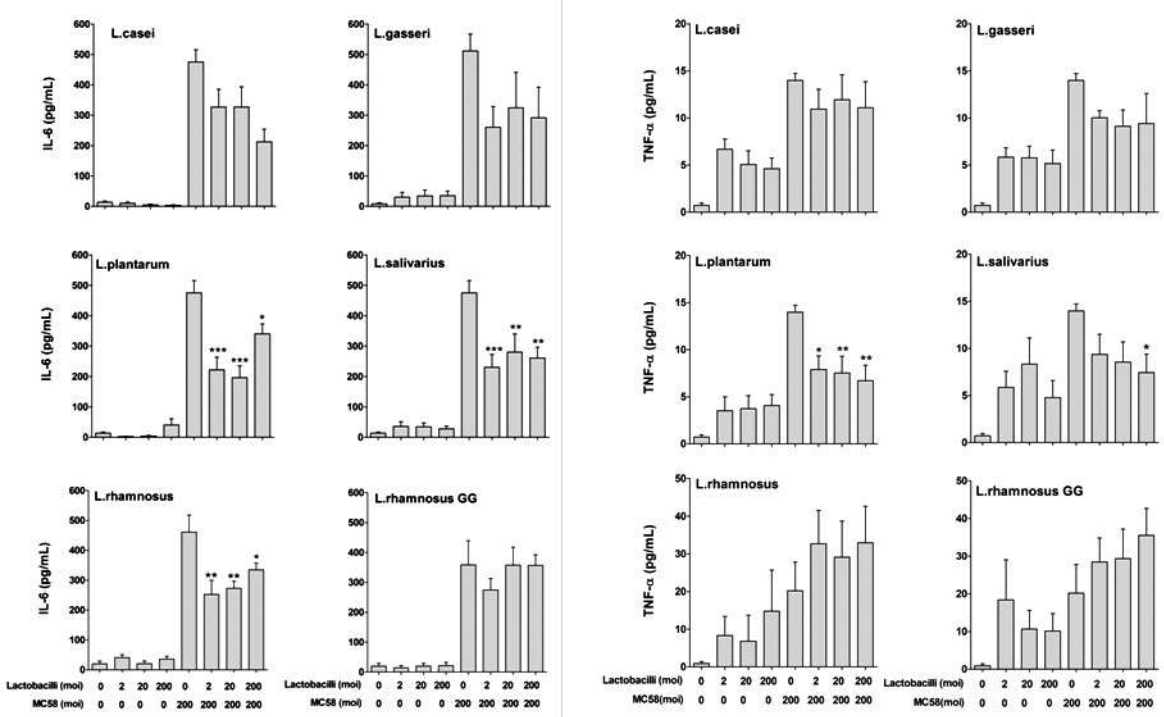

Figure-1 Suppression of epithelial inflammatory cytokine secretion by NmB infected cells when pre-incubated with oral *Lactobacillus plantarum* and *L. salivarious* *Cells were pre-incubated for 3h with Lactobacilli then challenged with NmB for a further 3h. Cells were washed and incubated with antibiotics for 21h . Cytokines were quantified from cell culture supernatants by ELISA. Means of 3 independent experiments each performed in triplicate.* *p<0.05, **p<0.01

figure 2

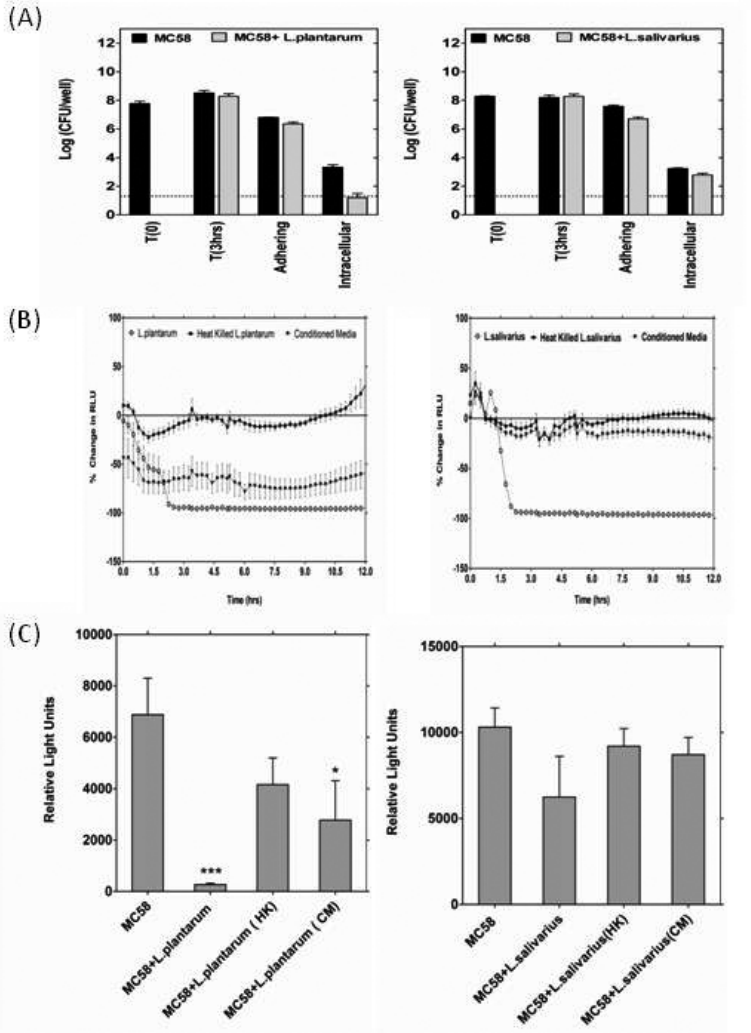

Figure 2 ***L. plantarum*** **reduces adhesion and prevents invasion of viable** ***N. meningitidis*** **in nasopharyngeal epithelial cells by inhibiting intracellular pathogen metabolism.** (A) *L. plantarum* and *L. salivarious* reduce Nm adhesion and invasion, using standard gentamicin protection assay and viable count. (B) *L. salivarious, L. plantarum* and *L. plantarum* secretions inhibit metabolism of MC58, determined by light output (relative light units) of a bioluminescent construct. (C) Both *L. plantarum* and its secretions (CM) but not heat killed (HK) organisms were also able to inhibit pathogen metabolism inside gentamicin treated host cells. Error bars indicate standard errors of the mean. * $p<0.05$, *** $p<0.001$

P 051

Enhancement of adaptive immunity to *Neisseria gonorrhoeae* by local intravaginal administration of microencapsulated IL-12

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We have previously shown how *Neisseria gonorrhoeae* proactively subverts the normal course of an immune response to infection for its own benefit, by suppressing Th1/Th2-governed specific adaptive immunity while eliciting Th17-governed innate responses that it can resist. We have further demonstrated that this pattern of responsiveness can be reversed by systemic treatment with TGF-β-neutralizing antibody (Ab), which permits the induction of Th1 and Th2 cells, development of anti-gonococcal Abs, and the establishment of immune memory, leading to accelerated clearance of primary vaginal gonococcal infection and increased resistance to subsequent challenge. However, systemic treatment with anti-TGF-β Ab is unlikely to be a viable treatment option for gonococcal infection, and we have therefore explored alternative approaches to intervention aimed at restoring protective immune responses against *N. gonorrhoeae*. Treatment of mice by vaginal instillation of biodegradable poly-lactic acid microspheres to give sustained local release of IL-12 during primary vaginal infection with *N. gonorrhoeae* significantly accelerated the clearance of the infection. Control microspheres, or vaginal instillation of soluble IL-12, were without effect. Moreover, when mice that had been treated with microencapsulated IL-12 during primary infection were allowed to recover, treated with antibiotics to ensure elimination of the infection and rested for 1 month, and then re-infected with *N. gonorrhoeae* without further treatment with IL-12, the course of infection was significantly shorter than in controls. Control groups included mice that were infected and treated with control microspheres or treated with soluble IL-12, or sham-infected mice that were treated with microencapsulated IL-12, all of which showed the same course of infection as age-matched mice that were infected with *N. gonorrhoeae* for the first time without additional treatment. Treatment with microencapsulated IL-12 during primary infection permitted the development of Th1 cells that secrete IFN-γ, and led to the production of circulating and vaginal anti-gonococcal Abs. Similar vaginal instillation of microspheres containing neutralizing Ab to IL-10 or to TGF-β during primary gonococcal infection also led to accelerated clearance of the infection. These results indicate a novel approach to the treatment of gonorrhea based on redirecting the host's immune response against *N. gonorrhoeae*, and having both therapeutic and prophylactic potential.

P 052

Household contact *Neisseria meningitidis* disease-carriage pairs – a tool to dissect virulence

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Introduction: Beginning in 1991, New Zealand suffered from a *Neisseria meningitidis* epidemic, with about 85% of disease cases caused by a single serogroup B strain type (defined as B:4:P1.7-2,4). With the introduction of a tailor-made strain-specific vaccine in 2004, the number of cases has been greatly reduced. Various studies have found that 8-25% of the population carry meningococcus; during the New Zealand epidemic, one study found 12% of adolescents were carriers, with 1.4% carrying the epidemic strain (M. Horsfall, personal communication). These data suggest the NZ epidemic strain type is highly virulence but with low transmissibility. The genetic basis of this high virulence has not been identified.
Question and Methods: Between 1996 and 1998 the Auckland Healthcare Public Health Protection Service and Institute of Environmental Science & Research (New Zealand) carried out a carriage study among household contacts of meningococcal disease patients (Simmons et. al, Eur J Clin Microbiol Infect Dis 2001). This study identified several groups where members of a single household were colonised by the same meningococcal strain type which caused invasive illness in one member of the family. Further analysis of the isolates by MLST confirmed instances where healthy household members were colonised by an isolate indistinguishable from the one isolated from the patient. We have selected these related isolates, collected from different anatomical sites and individuals, to dissect genetic and phenotypic differences between closely related isolates. Our aim is to identify bacterial factors that may contribute to virulence, as well as factors that may contribute to survival in specific anatomical sites, such as the mucosal epithelium or blood and CSF.
Results and Conclusion: Using bronchial epithelial cells, we have demonstrated that there are key differences in how these isolates interact with the host. Several mucosa-derived isolates elicited a different epithelial cytokine response than the associated invasive isolate, which may be due to differences in ability to adhere to and invade epithelial cells. We also identified other differences in the cellular response to infection, i.e., effects on cadherin junctions and actin dynamics. We are in the process of analyzing the genome of one household contact group of strains to dissect genetic differences among these isolates.

P 053

Target selection by the TpsB-transporter of multiple co-existing Two- partner Secretion Systems in *Neisseria meningitidis*

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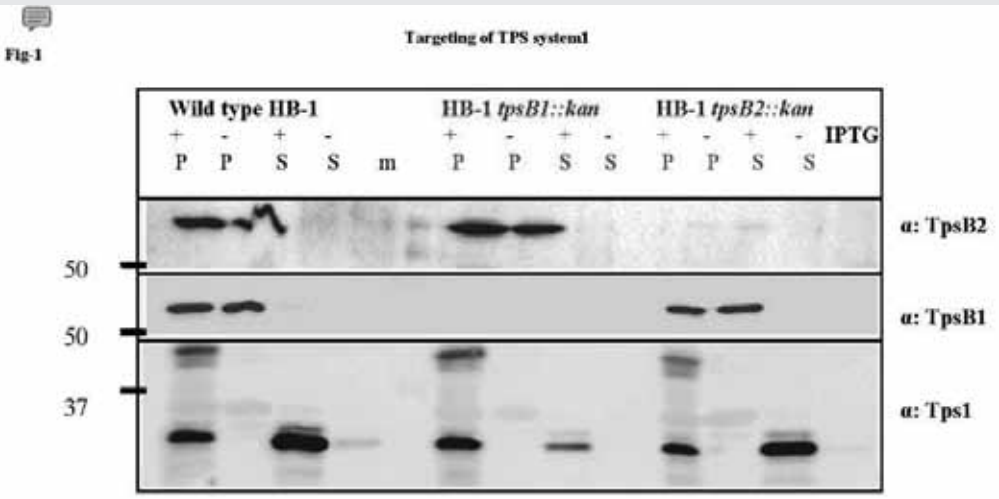
Neisseria meningitides (NM) is a common cause of human diseases sepsis and meningitis and initiates its infection by colonizing the upper respiratory tract. *N. meningitidis* expresses multiple Two-partner Secretion Systems (TPS) to colonize and invade the epithelial cells. *In vitro*, the meningococcal TPS system 1 has been found in all strains tested to date, contributes to intracellular survival and escape from infected cells.

TPS systems are composed of two components, a secreted exoprotein (TpsA) and a β-barrel outer membrane protein (TpsB) that is thought to transport the exoprotein across the outer membrane. Recognition of the TpsB transporter requires the presence of a TPS domain at the N-terminus of a TpsA. This recognition is thought to be system specific and restricted to cognate partners, often organized in an operon. Replacing the cognate TpsB with an heterologous TpsB resulted into blocked secretion. However, three distinct TPS systems have been identified in meningococci with a different organization. Amongst them, systems 1 and 2 contain more than one TpsA and a single TpsB each, while system 3 contains a singular TpsA protein without a cognate TpsB translocator. We have investigated the targeting and possible redundancy in the neisserial TpsBs. Truncated TpsAs, containing signal peptides and TPS domain, "the minimal secretable form" were expressed in NM wild type and isogenic-tpsB mutants. The culture supernatants of the induced cells, expressing different TPS systems, were collected and analysed through western immuno-blot using specific antibodies against the TPS components. Here we report that the neisserial system-2 TpsB showed a reduced specificity, which enables it to recognize and secrete the TPS domain of system 3 and 1 as well as more distantly related TPS domains of *N. lactamica*, but not of other bacterial species. We then, constructed a series of mutants in neisserial TpsBs to determine the domains/parts of TpsBs involved in substrate specificity, selection and subsequent recruitment for efficient secretion. Altogether our data provide insights in the target selection by a membrane-bound transporter protein involved in toxin related secretion.

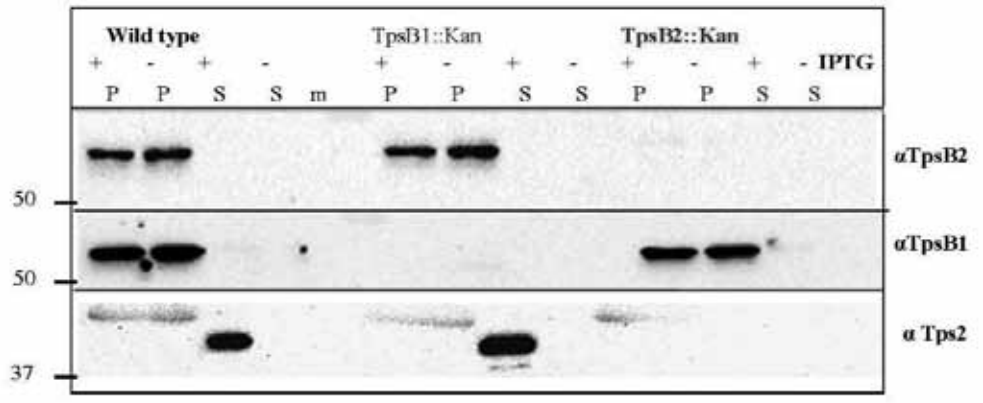
Results

figure 1



Western immuno blot analysis of whole cell lysate (P) and concentrated supernatant (S) from *Neisseria meningitidis* H44/76 wild type (WT), *tpsB1::Kan* and *tpsB2::Kan*, expressing TPS domain of TpsA1a of system 1 (pEN1000). Anti TPS1 antibody was used to detect TPS domain while, anti TpsB2 and TpsB1 were used to detect TpsB2 and TpsB1 respectively.
+ indicates induced with IPTG (250mM) while, – indicates uninduced. Each lane of pellet was loaded with 0.05 OD while each lane of supernatant was loaded with 0.35 OD
Nos to the left side indicate marker size. Symbols used; α, anti.

figure 2



Western immuno blot analysis of whole cell lysate (P) and concentrated supernatant (S) from *Neisseria meningitidis* H44/76 wild type (WT), *tpsB1::Kan* and *tpsB2::Kan*, expressing TPS domain of TpsA2a of system 2 (pEN1200. Anti Tps11 antibody was used to detect TPS domain while anti TpsB2 and TpsB1 were used to detect TpsB2 and TpsB1 respectively).
+ indicates induced with IPTG (250mM) while, – indicates uninduced. Each lane of pellet was loaded with 0.05 OD while each lane of supernatant was loaded with 0.35 OD
Nos to the left side indicate marker size. Symbols used; α, anti.

P 054

Structure and Function of the PorB Porin from invasive N. gonorrhoeae

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The outer membrane of Gram-negative bacteria contains a large number of channel forming proteins, porins, for the uptake of small nutrient molecules. N. gonorrhoeae porins of the serotype A ($PorB_{IA}$) are associated with disseminated diseases and mediate a rapid bacterial invasion into host cells in a phosphate sensitive manner. To gain insights into this structure-function relationship we analyzed $PorB_{IA}$ by X-ray crystallography in the presence of phosphate and ATP. The structure of $PorB_{IA}$ in complex solved at a resolution of 3.2 Å displays a surplus of positive charges inside the channel. ATP ligand binding in the channel is coordinated by positively charged residues of the channel interior. These residues are ligating the aromatic, sugar and pyrophosphate moieties of the ligand. Two phosphate ions were observed in the structure, one of which is kept by arginine residues (R92 and R124) localized at the extraplasmic channel exit. A short b-bulge in the b2-strand together with the long L3 loop narrow the barrel diameter significantly and further support substrate specificity through H-bond interactions. Interestingly, the structure also comprised a small peptide as remnant of a periplasmic protein which physically links porin molecules to the peptidoglycan network. To test the importance of R92 on bacterial invasion the residue was mutated. In vivo assays of bacteria carrying the R92S mutation confirmed the importance of this residue for host cell invasion. Furthermore, systematic sequence and structure comparisons of $PorB_{IA}$fromNeisseriaceae indicated the R92 residue to be unique in disseminated N. gonorrhoeae thereby possibly distinguishing invasion-promoting porins from other neisserial porins.

P 055

Identification of a protein receptor that enables competent Neisseria to recognize their own DNA during natural transformation

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Natural transformation, which plays a key role in the extreme genetic variability exhibited by *Neisseria* species, is a dominant force in bacterial evolution. This widespread process relies on two complex machineries to transport free DNA from the extracellular milieu into the bacterial cytoplasm. Type IV pili (Tfp) or evolutionarily related pseudopili are involved in DNA uptake, i.e. the transport of DNA to the translocase located in the cytoplasmic membrane. How this occurs and how model competent Gram-negative species, among which pathogenic *Neisseria* species, manage to selectively take up DNA containing a specific uptake sequence (DUS) remains enigmatic. We will present evidence, obtained using a range of approaches, that meningococcal Tfp bind DNA through an associated protein that has an exquisite preference for DUS, which is virtually identical in *N. meningitidis*, *N. gonorrhoeae*, *N. lactamica* and *N. polysaccharea*. By identifying a DUS receptor that has remained elusive for more than 30 years, our findings help illuminate how Tfp mediate the earliest step in natural transformation.

P 056

Generation of *Neisseria meningitidis* lacking both PorA and PorB

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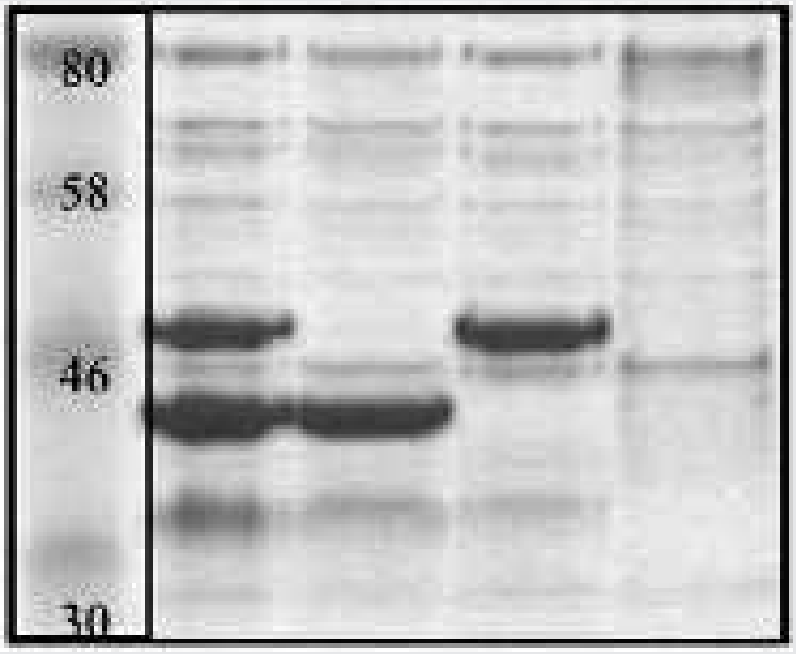
Introduction: Strains of *Neisseria meningitidi*s almost all express two major porins. PorB has anion and cation translocation pathways, as well as small sugar uptake and a purine nucleotide sugar binding region (Tanabe *et al* 2010 PNAS **107**:6811-6), PorA is reported to be cation-selective at pH>6 (Cervera *et al*, 2008, Biophys J. **94**:1194-202). Both proteins are 16 strand beta barrel structures with 8 extracellular loops. These loops are variable between strains, particularly the longest, most exposed. PorA is reported to be an immunodominant protein, eliciting strong bactericidal antibodies, most of which are directed at the two major variable regions. PorA expression is also variable by the mechanism of phase variation leading to strains expressing lower levels, or no PorA (van der Ende *et al* 2003 J Infect Dis.**187**:869-71). There have been no reports of disease or carriage isolates lacking expression of PorB, but PorB mutants can be easily made, with slight growth defects *in vitro*. PorA deficient strains exhibit no growth defects at least underin vitroconditions. PorB, but not PorA mutants exhibit increased antibiotic resistance to tetracycline, chloramphenicol, cephaloridine, and cefsulodin (Tommassen *et al* 1990 Infect Immun **58**:1355-9). However, at least one of these major porins was believed to be required for viability. **Questions:** are both major porins required for meningococcal viability? Does PorA play a role in antibiotic resistance? **Methods:** The genes encoding PorA and PorB were separately amplified and cloned, and insertionally inactivated with selectable markers. Plasmids were linearised and transformed into *meningitidis. MICs* were undertaken in liquid media in twofold serial dilutions of 13 antibiotics. **Results:** Mutants:We have obtained strains of *N. meningitidis* lacking expression of PorA and PorB. Approximately 10 fold fewer transformants were obtained when the Δ*porA::tet* allele was introduced into a *ΔporB::cm* strain than into its respective parental strain. MICs to 13 antimcrobials were compared for parental, *Δ*PorA, *Δ*PorB, and *Δ*PorA*Δ*PorB strains. The double mutant had similar MIC profile to the *Δ*PorB strain, confirming the role of PorB in susceptibility to tetracycline and cephalosporins. MIC profile of PorA expression did not alter suggesting that PorA does not allow ingress of antimicrobials. Interestingly, the *Δ*PorB and double mutants showed increased susceptibility to vancomycin, cloxacillin, and rifampin relative to parental and *Δ*PorA strains. **Conclusions:** PorA/PorB double mutants are viable. PorA appears not to have a role in altering antimicrobial resistance. These mutant strains may serve as a useful tool to further dissect the biology of *N. meningitidis*, and for vaccine studies.

Figure Legend: Fig 1.30μg sarkosyl OMVs of *N. meningitidis* were separated on 8-12% Bis-Tris acrylamide gel. Lane 1, Molecular weight markers (indicated in kDa): Lane 2, WT: Lane 3, *Δ*PorA: Lane 4, *Δ*PorB; Lane 5, *Δ*PorA*Δ*PorB strains.

figure 1



P 057

N-acetylmuramyl-L-alanine amidase from *Neisseria gonorrhoeae* is a bifunctional autolysin

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Strains of *Neisseria gonorrhoeae* that are resistant to the last antibiotics of choice for treating gonoccocal infections are now emerging, a precarious position that demands the development of new antimicrobials directed against this pathogen. The success of β-lactam antibiotics, which target enzymes required for cell-wall synthesis, suggests that other enzymes of peptidoglycan metabolism could also be viable targets for drug development. One enzyme involved in peptidoglycan breakdown is *N*-acetylmuramyl-L-alanine amidase, known as AmiC. Mutation or deletion of AmiC results in improper cell separation during division and increases susceptibility to a number of antimicrobial agents. We are investigating the biochemical and structural properties of AmiC to evaluate its potential as a target for antimicrobials with the goal of identifying novel inhibitors of the enzyme. AmiC comprises two domains and when cloned and expressed individually, the C-terminal domain exhibits zinc-dependent amidase activity, as shown by turbidity, zymogen and fluorescent peptidoglycan assays. It is believed that the N-terminal domain serves to target the catalytic domain toward its peptidoglycan substrate at the septum and, indeed, pull-down assays show that a proportion of it co-sediments with insoluble peptidoglycan. However, using the same biochemical assays employed to measure the autolytic activity of the C-terminal domain, we have made the surprising finding that the N-terminal domain also breaks down peptidoglycan and therefore appears to be a hydrolase. HPLC separation of peptidoglycan fragments after incubation with the N-terminal domain, followed by analysis of the breakdown fragments by ESI-MS, indicates that the domain functions as a glycosidase. Overall, our findings reveal that AmiC is a bifunctional autolysin, exhibiting both amidase and glycosidase activities, and paves the way for development of novel antimicrobials directed against both active sites. Structural studies of each domain of AmiC are ongoing.

P 058

The role of variable surface encapsulation and bacterial cell size in the attachment and invasion of *Neisseria meningitidis* into host cells

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Strains of *Neisseria meningitidis*, the causative agent of epidemic meningitis and septicaemia, are grouped into hypervirulent lineages composed of clonal complexes (cc) of different sequence types (ST) which are characterised by novel genetic content. We compared exemplar strains from two hypervirulent clonal complexes, strain NMB-CDC from ST-8/11 cc and strain MC58 from ST-32/269 cc, in host cell attachment and invasion. Surprisingly, the diplococci of strain NMB-CDC were shown to have a two-fold increase in surface area compared to strain MC58 by flow cytometry and high throughput microscopy. In addition, strain NMB-CDC attached to and invaded host cells at a significantly greater frequency than strain MC58. Type IV pili retained the primary role for initial attachment to host cells for both isolates regardless of pilin class and glycosylation pattern. In strain MC58, serogroup B capsule was the major inhibitory determinant affecting both bacterial attachment to and invasion of host cells. However, removal of either serogroup B capsule or LOS sialylation in strain NMB-CDC increased bacterial attachment indicating equal inhibitory roles for these components in this strain. In both strains, the level of transcription of the capsule synthesis locus and regulation of capsule expression by the two-component response regulator MisR were the same. When compared to strain MC58, strain NMB-CDC was found to express similar levels of surface capsule, but was more hydrophobic by hydrophobicity chromatography. These results were interpreted to indicate that the capsule of NMB-CDC was less dense than that of strain MC58 as it was distributed over a larger surface area. The less dense capsule on the bacterial surface of strain NMB-CDC was correlated with improved invasiveness of this isolate in the absence of phase variation of pili or LNT-bearing LOS. For the first time, this study shows that there is diversity in bacterial cell surface area within *N. meningitidis* and that this phenotype can influence steps in meningococcal pathogenesis.

P 059

Phase variation of *nalP* and *mspA* occurs during meningococcal carriage

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Question: Phase variation is the reversible switching ON or OFF of bacterial surface molecules; a process often mediated by slipped-strand mispairing during DNA replication at repeat tracts located within the promoter or coding sequence of a gene. Many meningococcal surface structures undergo phase variation; a process which may allow the bacterium to evade adaptive immune responses. NalP [NMB1969] and MspA [NMB1998] are conserved antigenic outer membrane autotransporter proteins with roles in virulence. Expression of both is modulated by polyC tracts within their coding sequence. To help understand the contribution of these autotransporters to meningococcal interactions with the host, we determined the presence and phase variation status of both NalP and MspA in a collection of recent carriage isolates.
Methods: Carriage isolates were obtained from first year undergraduate students at Nottingham University, UK during 2008-09 [Bidmos et al., 2011. J. Clin. Microbiol. 49: 506-512]. The presence of *nalP* and *mspA* was determined by PCR using primers specific to conserved regions. Length of repeat tracts was examined by DNA sequencing and gene scanning, thus allowing a prediction of the ON or OFF status of each gene. Expression in representative stains was confirmed by immunoblot analysis.
Results: *nalP* was present in 94% of 215 strains tested; only one isolate lacked *mspA*. Examination of *nalP* deletion loci revealed the presence of differing numbers of residual dRS3 repeats, and in one strain, the presence of an IS*1655* transposon. Tract lengths ranged from 7-13 bp (mode = 10; phase ON) in *nalP*, and 6-14 bp (mode = 9, phase ON) in *mspA*. Overall, 57% of strains had NalP ON, whilst 47% had MspA ON. Taken together: 22% both OFF; 22% both ON; 24% NalP OFF/MspA ON; 32% NalP ON/MspA OFF. Examination of paired isolates with identical typing characteristics and obtained from the same individual several months apart, revealed in some cases, differences in *nalP* and/or *mspA* tract lengths.
Conclusions: Both genes were highly prevalent in the strains tested, yet only 22% of strains were expressing both proteins, whilst only 56% of strains were expressing either NalP or MspA, suggesting that expression of neither protein is required for nasopharyngeal carriage. Multiple strains lacking *nalP* were detected; characterisation of their deletion loci suggested multiple, independent *nalP* deletion events. For the first time, we demonstrate phase variation of *nalP* and *mspA* occurring *in vivo* during nasopharyngeal carriage. Determining the phase variation status of NalP in carriage isolates is important since NalP is a cell-surface protease known to cleave several putative vaccine candidates including MspA, LbpB and GNA2132/NhbA; therefore its expression status may influence isolate susceptibility to vaccine-induced immune responses.

P 060

Frequent Phase Variation of Five Outer Membrane Proteins During Persistent Meningococcal Carriage

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Introduction: Meningococcal carriage rates are in the range of 10-15% but can increase in closed and semi-closed populations. Meningococci can persist in a carrier for 6-9 months and this persistence may be enabled by antigenic or phase variation of outer membrane antigens. Phase variation of the five surface proteins, FetA, Opc, NadA , HpuA and PorA, are associated with changes in repeat tract lengths. The genesfetA,opcandporAhave polyG tracts located in the core promoter whilst thenadArepeats (TAAAs) are located upstream of the core promoter.ThehpuApolyG repeats are in the open reading frame. The aim of this study is to determine whether these five genes exhibit frequent alterations in repeat length during persistent carriage.

Methods: Multiple colonies were isolated of persistent nasopharyngeal meningococcal carriage strains obtained from university students over a 6 month period at three/four time points. PCR and sequencing methodologies were using to characterise the capsular group, fetA, porAand MLST types. DNA was extracted from 6 colonies for each time point. Length variation of the repeat tract was measured by GeneScan analysis and direct DNA sequencing.

Results: Five different meningococcal strains (CC174, CC167, CC23, CC60, CC32) from 21 volunteers were examined for changes in repeat tract length of 5 genes in 368 isolates. Tract lengths ranged between 8-13, 8->15,9-14, 9-14 and 10-20 forfetA, opc, nadA, hpuA, and porA, respectively. The CC60 strain did not exhibit changes in thefetArepeat length (6G) during carriage. The nadAgene was absent in CC60, CC167 and CC23 strains but exhibited an ON-to-OFF switch in 4 of 8 CC174 carriers. A 9G tract was observed for fetAat the first time point in all carriers of strains CC174 and CC32 and exhibited switches to other lengths in 6 of 9 of these carriers. Overall phase variation occurred in 11/21, 13/21, 4/9, 11/21 and 11/21 of volunteers for fetA, opc, nadA, hpuA and porA, respectively.

Conclusion: This study indicates that high frequencies of phase variation occur during persistent meningococcal carriage. We are currently investigating the expression status of these phase variants but speculate that the phase variable changes in the proteins encoded by these genes are affected by different conditions in each individual and may be mediating avoidance of host immune responses.

P 061

Analysis of the effect of antigenic diversity of the *Neisseria meningitidis* outer membrane porin B protein (PorB) on strain fitness

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The current meningococcal polysaccharide vaccine does not confer protection against *Neisseria meningitidis* serogroup B. Since the capsule of *N. meningitidis* serogroup B strains is poorly immunogenic, outer membrane proteins are ideal candidates for investigation as potential vaccine antigens. The porin B (PorB) is an essential outer membrane protein that is immunogenic and does not exhibit phase variability. However, PorB, along with other outer membrane proteins, is antigenically diverse among serogroup B strains complicating its development and assessment as vaccine component. Molecular analysis of *porB* sequences shows evidence of population structuring that is not explained by immune pressure. Therefore, we are investigating fitness and structure-function effects of diversification of PorB to determine if antigenic diversity, which is the roadblock to prophylactic intervention, can be addressed by identifying regions that are antigenically relevant and partially functionally restricted. In order to examine this aim, four different *porB* genes were isolated from *N. meningitidis* MC58, CU385, Ch501, or BB1350 and cloned into an isogenic strain (MC58Δ*porA*) to replace its *porB* gene. Growth rate results indicate that PorB variability might influence strains' growth and fitness. Rabbit immunizations using detoxified Outer Membrane Vesicles (dOMV) purified from transformed strains showed that antibody responses against each PorB were directed against one or more of the outer membrane variable loops. Interestingly, the specific targets of individual animal responses were diverse and not predicted by PorB type. Serum bactericidal assay using human complement and rabbit anti-dOMV antisera showed high titers of functional (bactericidal) antibody irrespective of the PorB type. In addition, these anti-OMV antisera exhibited bactericidal activities against heterologous strains. Our results suggest that PorB antigenic variability might be an outcome that is driven not only to escape host immune pressure but also to enable strain fitness and survivability within different hosts.

P 062

Encapsulation does not enhance survival of *Neisseria meningitidis* to environmental desiccation

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Background and question: *Neisseria meningitidis*, an exclusive pathogen of humans carried in the nasopharynx, is unique among the major bacterial agents of meningitis in that it causes epidemic as well as endemic disease. Epidemic meningococcal disease has been linked to transition from the rainy to dry season. Meningococci are transmitted by direct contact with nasal or oral secretions or through inhalation of large droplets. Capsular polysaccharide is a major meningococcal virulence factor. Serogroup A *N. meningitidis* expresses a homopolymericN-acetyl mannosamine-1-phosphate capsule, while serogroup B and C capsular polysaccharides are sialic acid homopolymers of (α2→8) and (α2→9) linkages, respectively, with serogroup C capsule being partially O-acetylated. Capsular polysaccharides are believed to prevent desiccation and provide antiadherent properties, thereby promoting meningococcal transmission and survival outside the human host. We investigate whether encapsulation of *N. meningitidis* indeed enhanced survival under environmental desiccation and examine the capacity of meningococci to persist in the environment.
Methods: One representative strain from serogroups A, B and C and their non-encapsulated derivative grown overnight on GCB agar plates were suspended in PBS and aliquots of ~10^8 CFUs were dried onto the surface of microtiter plates. The plates were kept at room temperature in the dark, and bacteria recovered at various time points were plated for viable CFU counts. The survival rates were normalized to the initial input CFUs. *Streptococcus pneumoniae* strain D39, which has been found to be desiccation tolerant, and *Acinetobacter baumannii* known for its ability to survive in the environment were also examined under identical conditions.
Results: The kinetics of meningococcal survival was similar between encapsulated and non-encapsulated strains in all three serogroups, with ~6-8 logs of killing in 24 hours. *S.pneumoniae* showed a significant higher survival with ~ 2-3 logs killing, while *A. baumannii* demonstrated the best survival with only ~ 1 log killing in 24 hours. No differences in meningococcal survival were seen with plastic, glass or metal surfaces.
Conclusions: *N. meningitidis* is comparatively susceptible to environmental desiccation. Encapsulation, in contrast to the common belief, does not appear to provide survival advantage against desiccation to meningococci.

P 063

Regulation of *Neisseria meningitidis* cell size and virulence by chemical modification of peptidoglycan

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Peptidoglycan O-acetylation is a modification found in many bacteria. Although its physiological role has remained elusive, it has been shown to contribute to the virulence of Gram-positive pathogens by conferring resistance to host lysozyme, a major component of the innate immune response. In Gram-negative pathogens, the contribution of this modification to physiology and virulence is unknown. In this work, we analyzed the peptidoglycan O-acetylation pathway in the major human pathogen *Neisseria meningitidis* (Nm). We elucidated the respective contribution of *patA*, *patB* and *ape1* using genetic expression of all possible combinations of the three genes in *E. coli* and *N. meningitidis*. We showed that PatA and PatB are required for O-acetylation, while ApeI is an esterase that removes the O-acetyl group. ApeI was active on all O-acetylated muropeptides produced by PatA and PatB during heterologous expression in *E. coli* and in vitro using purified peptidoglycan. Interestingly, we showed that in N. meningitidis ApeI was specifically de-O-acetylating muropeptides with tripeptide stems (GM3), suggesting that its activity is highly regulated. Accordingly, we showed that de-O-acetylation of GM3, enriched at the end of the glycan chains, regulates glycan chain elongation and consequently the cell size. Finally, we show that the virulence of Nm lacking ApeI is drastically reduced in a mouse model of infection, indicating that regulation of glycan chain length by O-acetylation contributes to bacterial fitness in the host. Altogether, our results suggest that ApeI represents an attractive target for new drug development.

P 064

Assessing the role of protein acetylation and phosphorylation in *Neisseria gonorrhoeae* biofilm formation

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Neisseria gonorrhoeae, the causative agent of gonorrhea, has typically been effectively treated with antibiotics. However, the identification of multi-antibiotic-resistant strains has led to concerns about the long-term efficacy of antibiotics. *N. gonorrhoeae* has been shown to produce biofilms both *in vivo* and *in vitro*. Organisms present in biofilm communities exhibit unique properties, such as resistance to antibiotics and clearance by the host immune system. Biofilms are typically regulated by quorum sensing, where a certain number of bacteria must be present before a two-component regulator is activated which then triggers a cascade of pathways involved in biofilm formation. Gene array and non-targeted proteomic studies are unable to directly assess the role of post-translational modifications (PTMs) in biological systems. PTMs are highly regulated, reversible modifications known to be involved in modulating protein activities and protein-protein interactions. The experiments presented here are the first studies to assess the role of protein acetylation and phosphorylation in *N. gonorrhoeae* biofilm formation. In these studies we utilize a *luxS* mutant, which is defective in its ability to form mature biofilms, for comparisons with wild-type strain 1291. Both the *luxS* and the wild-type strains were grown, isolated, washed, and frozen until ready for use. Bacteria were resuspended in a 6 M Urea lysis buffer containing phosphatase and de-acetylase inhibitors. Cells were ruptured using a sonicator microtip. Cellular debris was removed, and the total protein concentration was determined. An in-solution trypsin digestion was performed and the quality of each of the samples was assessed by analyzing them by reverse-phase HPLC-ESI-MS/MS using a quadrupole time-of-flight TripleTOF 5600 mass spectrometer (AB SCIEX). These studies showed that we were able to identify 594 and 603 proteins from the wild-type and *luxS* samples with 95% confidence, respectively. Once we verified the quality of our samples, the enrichment protocols were performed. Acetylated peptides were targeted using an anti-acetylation antibody. In a single experiment we were able to identify 746 unique acetylated peptides from the wild-type and the mutant. Of these, 293 peptides were found in both strains, including peptides from pilus biosynthesis proteins, OMP III, and some ABC-transporter components. Proteins involved in metabolic pathways, DNA and protein synthesis, and stress response all had high levels of acetylation. We utilized TiO2 tips for enrichment of phosphopeptides. Studies analyzing these samples are currently underway. As we analyze additional biological replicates we will assess, both quantitatively and qualitatively, the role that protein acetylation and phosphorylation have in *N. gonorrhoeae* biofilm formation.

P 065

Expression of *lptA* and phosphoethanolamine decoration of lipid A in *Neisseria gonorrhoeae*: implications for pathogenesis

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Questions: We asked if expression of *lptA* in *Neisseria gonorrhoeae* strain FA 1090 is needed for phosphoethanolamine (PEA) decoration of lipid A at the 4′ position, resistance of gonococci to cationic antimicrobial peptides (CAMPs) and if it impacts fitness in vivo. We also questioned if a poly-T tract in the *lptA* coding sequence is subject to phase variation.
Methods: Standard methods of bacterial genetics and molecular biology were used to construct a defined deletion in the l*ptA* coding sequence. Spontaneous variants were identified by replica plating onto GCB agar with or without polymyxin B. PCR and DNA sequencing were used to confirm or identify mutations. Chemical and structural analyses of lipid A were performed by established methods. In vivo fitness assays were performed using the female mouse model of lower genital tract infection in mice dually infected with mutant and wild type strains.
Results: In order to establish the importance of *lptA* expression in strain FA 1090 with respect to CAMP resistance, we constructed an internal deletion in its coding sequence. Chemical composition and structural analyses of isolated lipid A from parent, mutant and complemented strains confirmed that deletion of l*ptA* resulted in loss of PEA modification of lipid A at the 4′ position. The mutant also displayed important biologic differences from the parent and complemented strains: it was hypersusceptible to a model CAMP (polymyxin B [PB]) and exhibited a significant fitness defect in the female mouse model of lower genital tract infection. Since the nucleotide sequence of *lptA* has a T8 tract in the coding region, we questioned if it might be a site for phase variation, which could result in changes in PEA decoration of lipid A, CAMP resistance and in vivo fitness. To test this possibility we screened individual colonies of strain FA 1090 for spontaneous variants exhibiting increased susceptibility to PB. We identified a PB susceptible variant at a frequency of approximately 3x10-4; in contrast, spontaneous resistance to erythromycin occurred at a frequency of 5x10-8. Using a spontaneous PB-sensitive variant, we recovered spontaneous variants (frequency of 5x10-4) that expressed wild type levels of PB-resistance. DNA sequencing studies revealed that the PB-susceptible variant had a single nucleotide (T) insertion in the poly-T tract within *lptA* that would prematurely truncate LptA. The *lptA* sequence of two PB-resistant revertants had the 8T tract seen in FA1090, which would be consistent with their production of a full-length LptA.
Conclusions: We conclude that expression of *lptA* in strain FA 1090 is important in CAMP-resistance and in determining fitness during an experimental infection. We propose that isoforms of lipid A produced by gonococci could be generated by phase variable expression of *lptA* due to high frequency mutations in a T8 tract within the coding sequence. We predict that phase-on variants would have a fitness advantage over phase-off variants during infection due, in part, to their increased resistance to CAMPs. However, since other studies by us indicate that *lptA* mutants have a low pro-inflammatory potential, *lptA* phase-off variants may be found in patients with asymptomatic infections.

P 066

In silico analysis of the putative ADP-ribosylating toxin NarE of *Neisseria gonorrhoeae*

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Question: The NarE protein of *Neisseria gonorrhoeae* is uncharacterised. The homologue in meningococci is an ADP-ribosiltransferase toxin (ADPRT), which is also able to bind a single iron or zinc through an iron/zinc-sulfur center (Fe/Zn-S). In *N. gonorrhoeae* strain FA1090, *narE* gene was annotated as an open reading frame encoding a hypothetical protein (NGO563, 96 aa), but previous reports suggested that it is a pseudogene due to duplication of a tetra nucleotide (TTAT) 12 bases downstream from the predicted ATG site.
Methods: An *in silico* analysis by sequencing, bioinformatics and molecular modelling was used to characterise the gonococcal *narE* gene and its predictive protein product.
Results: Comparison of the *narE* sequences obtained by PCR and sequencing from a collection of clinical isolates of *N. gonorrhoeae* and the *narE* sequence (NG0563) of strain FA1090 showed 99% nucleotide sequence identity between them. Despite the presence of the tetra nucleotide duplication, -35 and -10 boxes and a ribosome binding site were found upstream from an alternative start codon (GTG). Alignment between meningococcal NarE and the predicted protein encoded by NGO563 showed that this putative protein possesses a deletion in the N-terminal (1-50 aa) as a consequence of the nucleotide duplication, but conserves critical features for ADP-ribosylation such as a binding site of NAD^+ (YISTT domain and H57 residue), a classical E-x-E catalytic site previously described for bacterial ADPRTs (EKEV domain) and the Fe/Zn-S binding site (C18 and C79 residues). In addition, molecular modelling of the putative gonococcal NarE showed that the structural perturbation caused by terminal truncation was compensated and the spatial structure for the NAD^+ pocket and its binding site, the catalytic site and the Fe/Zn-S center were not affected.
Conclusions: Our results suggest that the *narE* gene of *N. gonorrhoeae* may be expressed and that the gonococcal protein may also display ADPRT activity similar to meningococcal NarE. Further studies are in progress to determine the role of NarE in gonococcal pathogenesis. This work was supported by Wellcome Trust 090301 (UK) and FONDECYT 1120712 (Chile).

P 067

HexR controls glucose-responsive genes and glucose metabolism in *Neisseria meningitidis*

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Background: *Neisseria meningitidis* (Nm) colonizes the nasopharynx and can cause septicemia and meningitis. Nm, an obligate human pathogen, has no other known reservoir outside of the human host. Nm uses a restricted range of carbon sources including glucose and lactate, the availability of which varies in diverse host niches, such as nasopharynx, blood and cerebrospinal fluid. The efficient metabolism of a carbon source might be a signal for proper fitness, or indicate the bacteria what host-related tissue it has infected and which virulence genes should be turn on or off.

Results: To investigate Nm regulatory responses to sugar availability we performed transcriptome analysis of bacterium grown *in vitro* in defined media in the presence or absence of glucose and lactate. Approximately 5 % of the global gene expression responded to glucose availability whereas only 1% responded to lactate and 0.5% responded to both. Genes of the Entner-Doudoroff and pentose phosphate pathways both involved in the glucose catabolism are upregulated in the presence of glucose. On the other hand genes related to the lactate catabolism are found repressed in presence of glucose, indicating a hierarchy on carbon sources utilization. We found two transcriptional regulators that responded to glucose, namely *gdhR* which was downregulated and NMB1389, a *hexR*-like regulator, which was upregulated. Deletion analysis of both of these regulators showed that the *hexR* (NMB1389) gene was responsable for much of the glucose-responsive regulation and co-ordinates the central metabolism of Nm in response to the presence of glucose.

Conclusions: The expression of genes found regulated by the carbon sources include those of metabolic pathways, transport and binding proteins and putative virulence factors. From the microarray analysis, HexR functions as a repressor of glucose-responsive genes and we were able to identify two main classes of differentially regulated genes: those HexR-repressed which are induced by glucose and those HexR-repressed that are co-repressed by glucose. Purification of the HexR protein will allow the members of the HexR regulon to be identified and to determine whether HexR may differentially regulate distinct classes of genes in response to intermediate products of the glycolysis pathway.

P 068

A random mutagenesis screen for loss of nitrite reductase (AniA) activity identifies a potential copper chaperon required for AniA activity

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Question: In the absence of oxygen Neisseria gonorrhoeae is able to use nitrite as a terminal electron acceptor to grow anaerobically. In the first step of this pathway, an outer membrane nitrite reductase, AniA, converts nitrite (NO2-) to nitric oxide (NO.). Nitric oxide is then converted to nitrous oxide (N2O) via nitric oxide reductase (NorB). The AniA active site contains both Cu(I) and Cu(II) centres which have an important role for electron transfer in catalysis. The aim of this study is to identify the accessory proteins required for AniA activity (i.e. with roles in for example, electron transport, or copper loading).
Methods: In the presence of nitrite in growth media a norB mutant strain of N. gonorrhoeae accumulates nitric oxide leading to bacterial death. This lethal phenotype of the NorB mutant can be overcome by loss of AniA function. This observation allowed development of a genetic screen in which cells could only survive if they lost AniA activity. Using random deletion mutagenesis (van der Ley et al., 1996) in the selection condition of 2mM nitrite enabled identification of genes required for AniA function.
Results: Using the genetic screen method we established, over 150,000 colonies were screened and 197 colonies showed positive growth in the nitrite selection media. Two types of nitrite positive mutants were found. The majority of the mutants had deletion inaniA. The second type of mutants had deletion in a putative gene of AniA Copper Chaperone (accA). In the absence of accA, AniA activity was lost, although western blot analysis indicated that AniA was expressed at wild type levels. Nitrite reductase activity could be restored by supplementing growth media with excess copper, suggesting AniA was an apo enzyme in the mutant strain. Analysis showed that this potential copper chaperone is present in all published Neisseria genomes and is predicted to be a periplasmic protein with a Cu(I) centre.
Conclusions: Our study has identified a putative copper chaperone that is essential for AniA activity, and this may help in our understanding of the molecular mechanisms of copper homeostasis in Neisseria.
Reference: van der Ley, P., Kramer, M., Steeghs, L., Kuipers, B., Andersen, S.R., Jennings, M.P., Moxon, E.R., and Poolman, J.T. (1996). Identification of a locus involved in meningococcal lipopolysaccharide biosynthesis by deletion mutagenesis. Mol Microbiol19, 1117-1125.

P 069

The MisR/MisS Two-Component Regulatory System Influences Expression of *lptA* and Modulates Phosphoethanolamine Decoration of Lipid A in *Neisseria gonorrhoeae*

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Question: Cationic antimicrobial peptides (CAMPs), a vital component of the human innate host defense, are constitutively produced by neutrophils and can be inducibly produced by epithelial cells. Curiously, numerous studies have shown that while many gonococci are killed by neutrophils, a small subset of the infecting population can survive and perhaps even replicate within neutrophil phagolysosomes. We hypothesized that sub-lethal levels of CAMPs at epithelial surfaces might trigger changes in the expression of resistance genes and prepare gonococci for life inside phagolysosomes. Due to its well-established importance for CAMP resistance in meningococci, we asked if the MisR/MisS two component regulatory system (TCS) could impact gonococcal resistance to CAMPs. **Methods:** Strain susceptibility to polymyxin B was evaluated using minimal inhibitory concentration testing on solid GC agar. Expression of the *lptA* gene was assessed by beta-galactosidase assays performed on gonococci grown in liquid GC broth. Lipid A isoform abundance was determined using MALDI-TOF mass spectrometry on purified preparations of gonococcal lipid A. **Results:** We found that a loss-of-function mutation in *misR*, which encodes the response regulator of the TCS, decreased gonococcal resistance to polymyxin B (a model CAMP). Additionally, we found that the *misR* mutation decreased expression of *lptA*, which encodes a lipid A phosphoethanolamine (PEA) transferase. PEA modification of lipid A mediated by LptA was previously shown to be a key resistance factor in meningococci against a human CAMP termed LL-37. Because MisR activated expression of *lptA*, we asked if loss of MisR impacted PEA modification of lipid A. We found that the *misR* mutant of strain FA19 produced a unique lipid A profile differing from that produced by parent strain FA19. Interestingly, the proportion of PEA-substituted lipid A seemed to decrease in the *misR* mutant, while the proportion of a species with an observed m/z ratio of 1861.55 Daltons was greatly increased. Studies are now underway to determine the chemical composition of the 1861.55 Dalton lipid A species. **Conclusions:** We conclude that MisR transcriptionally controls PEA modification of lipid A in gonococci and that this mechanism is important for gonococcal resistance to CAMPs.

P 070

Establishment of a quantitative proteomic method for comparison of Neisseria meningitidis strains

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N. meningitidisis carried asymptomatically in the nasopharynx by 8-25% of the population. Meningococci also lead to epidemic outbreaks of disease, causing morbidity with significant economic burden, and mortality rates of >10%. Developing a quantitative proteomic method would facilitate comparison of closely related strains that cause invasive disease with those that are only found in carriage.

Previous work to compare meningococcal proteomes by Vipond et al.[1] and Tsolakos et al.[2] focused on gel based methods. 2D DIGE is used to quantify relative protein expression following separation according to molecular weight and isoelectric charge. This method is suitable for comparison of populations of the same strain as it relies on co-migration of identical proteins for comparison, but sequence variation between homologous proteins and subsequent spot shift hinders analysis of distinct strains.

Non-gel based Mass Spectrometry (MS) provides an alternative by quantifying conserved peptides despite the variation in proteome. MS-based quantitative comparison employs different stable isotope labelling to create a specific mass tag that can be detected and quantified by MS. Stable Isotope Labelling by Amino acid in cell Culture (SILAC) uses metabolic incorporation of native (light) or heavy $^{13}C_6$-lysine/$^{15}N_4$-arginine. Quantitative difference is detected at the peptide MS level and sequence identified by MS/MS. Minimal growth media is supplemented with essential heavy or light amino acids enabling cells to achieve equal and complete incorporation. Human cells achieve complete incorporation of the heavy amino acids after 5 cell doublings[3]. However, growth of the meningococcal strain was slow in minimal media and yielded little protein. MS analysis revealed both heavy and light lysine and arginine residues amongst the identified peptides, indicating incomplete incorporation. Therefore, it was concluded that the SILAC approach requires further optimisation for it to be feasible.

Chemical tags are an alternative to metabolically incorporated labels. Tandem Mass Tagging (TMT) utilises sets of tags that share chemical structure and mass so co-migrate during separation. The tags covalently bind to amino groups of peptides which are generated following tryptic digestion[4]. MS/MS is used to identify tagged peptides based on reporter ion tag sequence, generating relative quantitation. The unique tag enables multiple strains to be analysed within one biological sample. As TMT avoids culture in defined media, the method can potentially be used for the quantitative assessment of neisserial proteins.

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[2]Tsolakos N, Lie K, Bolstad K, Maslen S, Kristiansen PA, Hoiby EA, Wallington A, Vipond C, Skehel M, Tang CM, Feavers I, Wedege E, Wheeler J. Characterization of meningococcal serogroup B outer membrane vesicle vaccines from strain 44/76 after growth in different media. Vaccine 2010; 28:3211-3218.

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[4]van Ulsen P, Kuhn K, Prinz T, Legner H, Schmid P, Baumann C and Tommassen J. Identification of proteins of Neisseria meningitidis induced under iron-limiting conditions using the isobaric tandem mass tag (TMT) labelling approach. Proteomics 2009; 9: 1771-1781.

P 071

Lack of Lipid A Pyrophosphorylation and of a Functional *lptA* Gene Reduce Inflammation by *Neisseria* Commensals

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The interaction of the immune system with Neisserial commensals remains poorly understood. Herein we report that inflammatory signaling in THP-1 monocytic cells was much greater with pathogenic than with commensal *Neisseria*. Mass spectrometry of lipid A and intact lipooligosaccharide (LOS) revealed that this difference was characterized by lack of lipid A pyrophosphorylation and phosphoethanolaminylation in four commensal species. Greater sensitivity to polymyxin B was consistent with the absence of phosphoethanolamine moieties. Southern hybridizations and bioinformatics analyses of genomic sequences from all eight commensal *Neisseria* species confirmed that the *lptA* gene was absent in 15 of 17 strains examined. A meningococcal *lptA* deletion mutant had reduced inflammatory potential, further illustrating the importance of lipid A pyrophosphorylation and phosphoethanolaminylation in bioactivity. Unlike the other commensals, whole bacteria of two *N. lactamica* commensal strains had low inflammatory potential, whereas the lipid A had high level pyrophosphorylation and phosphoethanolaminylation and induced high level inflammatory signaling, supporting previous studies indicating that this species uses mechanisms other than altering lipid A to support its commensalism. Overall, our results indicate that lack of pyrophosphorylation and phosphoethanolaminylation of lipid A contributes to the immune privilege of most commensal *Neisseria* by reducing the inflammatory potential of the LOS.

P 072

Proteomic analysis of surface protein antigens from serogroup B *Neisseria meningitidis* following proteinase K treatment

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Aim: Serogroup B strains (menB) are responsible for the majority of incidents of meningococcal septicemia and meningitis in many countries where polysaccharide vaccines against other serogroups have been introduced. Several surface-expressed proteins have been implicated in protective immune responses against menB and attracted attention as potential vaccine candidates. To identify new surface antigens, we characterized the menB surface proteome using a combination of protease treatment and comparative proteomics.
Methods: Live meningococci were treated with proteinase K at various concentrations and their proteome compared to that of untreated bacteria by differential two-dimensional (2-D) gel electrophoresis. Surface proteins cleaved by proteinase K were detected by the reduction or loss of the intact protein species in 2-D gels from treated bacteria and identified following in-gel trypsin digestion of the respective spots and liquid chromatography-tandem mass spectrometry. The proteins identified were further assessed for immunogenicity in 2-D immunoblots using murine immune sera raised against the homologous strain.
Results: Fourteen proteins with a predicted surface localization were identified including ten previously characterized surface antigens. PorA, OpcA and fHbp were completely digested by proteinase K whilst the remaining proteins showed partial digestion reflecting differences in proteins accessibility and/or expression on the surface. 2-D immunoblots confirmed the immunogenic properties of the known surface antigens and further identified the thiol-disulfide interchange protein DsbA2, the amino acid ABC transporter NMB1612 and the type IV pilus biogenesis protein PilP as novel potential surface immunogens.
Conclusions: The approach used in this study could prove an effective tool to characterize the antigenic proteome of pathogenic bacteria and assess the presence and accessibility of antigens on the bacterial surface with implications for vaccine development. Meningococcal proteins DsbA2, NMB1612 and PilP may warrant investigation as potential vaccine candidates.

P 073

The Meningitis Research Foundation Meningococcal Genome Library

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We are creating an open-access on-line library of the whole genome sequences of every invasive Meningococcal isolate from England, Wales and Northern Ireland from the epidemiological year 2010/11. DNA from 514 isolates received by the Health Protection Agency Meningococcal Reference Unit (MRU) has been sent to the Sanger Institute for sequencing using Illumina technology. In Oxford, short-reads are submitted to an automated assembly and core-genome annotation pipeline. In brief, reads are assembled *de-novo* using VELVET and uploaded to PubMLST for reference-based annotation; genomes and their associated epidemiological data (including year of isolation and serogroup) are available to the public at all stages of annotation via Meningitis.org, PubMLST.org and Neisseria.org/MRF. A gene discovery pipeline is being implemented for the annotation of regions not in the PubMLST database. These are largely members of the accessory genome such as mobile elements and pseudogenes.

At the time of writing a pilot study of 12 isolates from 2010/11 had been completed. As anticipated from the results of published studies of current UK meningococcal epidemiology, all 12 genomes were capsular group B (MenB). Of five ST-269 clonal complex (cc) isolates, two were ST-1161 and shared identical typing-antigens, and three had unique strain designations. There were two *fHbp* alleles and a single *nhba* allele associated with cc269 in this dataset. A further five isolates belonged to cc41/44; two were ST-1194 but all isolates had unique combinations of typing-antigens. Four of the five cc41/44 isolates had the same *fHbp* allele and there were three *nhba* alleles associated with this clonal complex. There was a single cc461 isolate and an ST-1831 isolate that did not belong to a defined clonal complex. This comprehensive population-level dataset will support the research and pharmaceutical communities in all fields of meningococcal research, such as in the design of interventions, in the monitoring of drug resistance, and in the understanding of pathogenicity and virulence determinants. The provision of fully annotated genomes affords an investigation into the feasibility of whole genome use in routine disease surveillance, and supplies baseline MenB population data prior to the introduction of new vaccines that aim to target MenB.

P 074

Validation of high throughput draft genome sequence production for a diverse collection of *N. meningitidis* isolates

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The production of finished genomes is technologically intensive and in many cases requires a large investment of time and money to complete and therefore makes high throughput finishing out of the question for large bacterial isolate collections. There are two basic standards in which genomes are now produced (1) the gap-closed or finished genome, and (2) the incomplete or draft genome; the latter of which now accounts for the majority of bacterial genomes found in many public databases. This study investigated the use of high throughput genome sequence production for a large collection of diverse *Neisseria meningitidis* genomes assembled *de novo* from short read sequencing files. The study examined the repeatability, robustness and reliability for producing high quality genomes sequences and used the extensive functionality of the Bacterial Isolate Genome Sequence Database (BIGSdb) to annotate and analyse the genomes. To date we are unaware of any published data investigating the validation of high throughput draft genome sequence production in this manner.

Results: One-hundred-twenty *N. meningitidis* genomic DNA extracts were multiplexed and sequenced using the Illumina GAII platform. Extractions included 10 replicates and 1 triplicate preparation. The short reads were assembled using a Velvet scripted optimization program. The majority of assemblies (65) reached 95% of the expected N. meningitidis genome size, 55 achieved 90% expected size, and 1 assembly was less than 90% (1,975,180bp). Assemblies were scanned and over 1400 defined loci were assigned for each genome using BIGSdb. All replicate assemblies were concordant with each other in size and genome coverage. Assembled genomes were interrogated using 108 coding sequences (CDS) and 25 typing loci to assess genome coverage and sequence identity. There were 106 incomplete loci within 34 assemblies, occurring across 56 CDS. Of the typing loci, 24 assemblies had a single allele conflict and 3 assemblies had 2, 3 and 4 conflicts. All 33 typing loci were retested: 2 sequencing results were inconclusive by Sanger sequencing, 4 were concordant with the original Sanger derived sequence, and 27 were concordant with Illumina derived sequence. Error rate testing using the finished genomes of FAM18 and Z2491 were estimated for insertion/deletions and nucleotide changes.

Conclusions: The use of a high throughput method for producing whole genome sequences, in both scale and efficiency, produces quality draft sequences that provide a very high degree of data applicable for both comparative and evolutionary studies. Draft genome sequencing of Neisseria strains has the potential to create a catalogue of variation that can be used to address longstanding questions that have been until now economically impractical to address and provide the basis for population-scale sequence analysis.

P 075

Forty years of evolution of a meningococcal clone analysed by whole genome sequencing

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Background: The hypervirulent sequence type (ST)-32 complex (earlier designated electrophoretic type (ET)-5 complex), which was first identified in Norway in 1969, has been responsible for outbreaks and epidemics worldwide. The history and evolution of the clonal complex was studied using whole genome sequencing.
Methods: A total of 44 isolates belonging to the ST-32 clonal complex were selected using the strain collection from the WHO Collaborating Centre for Reference and Research on Meningococci in Oslo. The strains, isolated between 1969 and 2008, were from all continents and included representatives from the major outbreaks. Whole genome sequencing was performed using Illumina technology and genomes were assembled de novousing Velvet. The resultant contigs were then uploaded to a local installation of Bacterial Isolate Genome Sequence Database ($BIGS_{DB}$), screened and tagged with approximately 1200 Neisseria loci. The $BIGS_{DB}$ Genome Comparator tool was used to compare whole genome sequence data to the reference MC58 genome (Tettelin et al., 2000).
Results: Of the >2100 genes of *N. meningitidis* the full sequence was obtained for 1723 in each of the 44 isolates. Of these, 1466 were variable. Split-tree analyses of all variable loci revealed a complex structure with three major groups of genomes. One group included strains from Asian origin and Australia; another group consisted mainly of isolates from Norway covering a 40-year period and from USA; the third comprised strains from Spain, Cuba, Brazil and Africa. The strain MC58 isolated in UK in 1983 clustered separately with a strain from Canada obtained in 1978. Strong association was seen between the clusters defined by whole genome sequencing and variation in the major outer membrane proteins PorA and FetA. Ribosomal multilocus sequence typing (rMLST) distinguished two large clusters that appeared unrelated to other evolutionary characteristics of the ST-32 complex
Conclusions: This analysis demonstrated the feasibility of whole genome sequencing for evolutionary analyses of relatively large number of meningococcal strains belonging to a hypervirulent clonal complex. Our study provides additional evidence of a likely Asian origin of the ST-32 complex.

P 076

Temporally paired isolates of *Neisseria meningitidis* compared by whole genome sequencing

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Background: From 1999 to 2001 the UK undertook a large scale study of carried meningococci in young adults aged 15-19. Participants were swabbed annually over a period of 2-3 years. Within the Oxford region 160 participants were culture positive for *Neisseria meningitidis* 2-3 years of the three study period.
Methods: A subset of 50 paired isolates from the carriage study, which were previously characterized by multilocus sequence typing (MLST), serogroup and capsular region genotype, were selected based on 0, 1, 2, 3 or 4 allelic changes in their MLST profiles between each year. Whole genome sequencing was performed using the Illumina GAII platform and generated short reads were assembledde novousing the Velvet scripted optimization program. The contigs were uploaded to the Bacterial Isolate Genome Sequence Database (BIGSdb) and scanned for over 1400 *Neisseria* loci defined in the database. The BIGSdb Genome Comparator tool was used to compare each set of paired genomes, genomes with the same sequence type (ST) and STs belonging to the same clonal complex, to investigate how similar assigned MLST sequence types are at the genomic level.
Results: Of the 100 isolates, 91 belonged to 16 defined clonal complexes; 39 of 50 pairs shared the same MLST sequence type, 8 pairs differed at 1 of the 7 MLST loci, 2 pair differed at 2 loci, and 1 pair was different at 4 of 7 (the point at which any 2 MSLT sequence types (ST) are considered not to be clonally related). There were no paired genomes with an MLST profile that differed at 3 of the 7 MLST loci in the data set. After filtering loci, genomes were interrogated for 1328 defined loci contained within the BIGSdb. Genome wide allelic variation for matching STs and one MLST allele change in an assigned complex were very similar and ranged from 19 to 69 loci per paired set of genomes; paired genomes with STs not associated with a clonal complex ranged from 27-187 variable loci. Paired genomes with 0, 1 or 2 MLST loci differences had an average of 1258 matching loci per paired genome. In contrast the paired sample with 4 of 7 different MLST loci had 1112 variable loci and 106 matching loci.
Conclusions: Whole genome sequence comparisons of paired temporal samples of the same or similar MLST profile not only adds support to the hypothesis that certain complexes and sequence types are persistent over time and space but may also change the way in which we think about and define or characterize 'carriage of the same organism'.

P 077

Population scale bioinformatic analysis and validation by deep-DNA sequencing indicate phase variation in 115 to 277 Neisseria meningitidis genes

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TheNeisseria meningitidis (Nm) chromosome shows a high abundance of simple sequence DNA repeats (SSRs) that accumulate stochastic, reversible mutations at high frequency. This mechanism is reflected in an extensive phenotypic diversity that facilitatesNmadaptation to dynamic environmental changes. To date, phase-variable phenotypes mediated by SSRs variation have been experimentally confirmed for 24Nmgenes.

Here we present a population-scale bioinformatic analysis of 20Nmgenomes, that allowed us to classify 277 genes as strong (52), moderate (60) and weak (165) candidates for phase variation. Deep-coverage next generation DNA sequencing data generated for 5 of the genomes, from single colonies grown overnight under non-selective conditions, confirmed the presence of high-frequency, stochastic variations in 93%, 71% and 27% of the strong, moderate and weak candidates, respectively, for a total of 115 experimentally confirmed phase-variable genes. A significant fraction of the non-confirmed candidates was predicted to show phase variation in other strains, or under different experimental conditions.

Sequence context analysis confirmed previous observations of a predominant presence of variable SSRs in genes affecting the external cell phenotype or involved in regulatory functions. However, an unexpectedly broad spectrum of other metabolic functions was also revealed to be impacted, and most variable SSRs were predicted to induce phenotype changes by "tuning" gene expression rather than mediating on/off translational switching.

Phylogenomic analysis revealed that only half of the strong candidate phase-variable genes are either specific to theNmspecies or have undergone a significant increase in SSR length following speciation, suggesting that other species, particularly within theNeisseriagenus, may share a propensity to phase-variation comparable toNm.

Overall, our results indicate that stochastic, high frequency variation of SSRs has a potential impact on Nm metabolism and phenotypic diversification significantly broader, and qualitatively different, than previously expected.

P 078

Clonal complex association of *Neisseria meningitidis* capsule synthesis genes

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Background: A prerequisite for meningococcal virulence is the expression of an extracellular polysaccharide capsule of which twelve have been defined based on biochemical differences. Of these, six serogroups (A, B, C, W, X and Y) cause the majority of meningococcal disease. The diversity of capsule genes encoding enzymes for the biosynthesis of the capsular polysaccharides and those implicated in the translocation of the high molecular weight polysaccharides to the capsule surface was analysed in Illumina-derived whole genome sequence data belonging to a globally acquired collection of N. meningitidis isolates spanning several decades.

Methods: A total of 35 serogroup A isolates, 47 serogroup B isolates, 17 serogroup C isolates as well as single serogroup Z, E, W and Y isolates were screened for each capsule gene giving rise to a list of exact or partial matches detailing percent sequence identity score, alignment length and E-values for each capsule gene searched. New alleles arising from partial matches and consisting of at least 70% sequence identity were manually checked for correct start and stop codons and aligned with known alleles before assignment. Sequence ambiguities, premature stop codons, truncated sequences or frame shifts were also identified where necessary.

Results: Allele sequences from individual genes and/or the entire capsule loci were exported from BIGSDB in the extended Multi-FASTA alignment format (xmfa) and used for phylogenetic analyses. Allelic profiles of capsule genes among all isolates were extracted allowing associations between clonal complexes and capsule genes to be extrapolated.

Conclusion: Association of capsule genes with clonal complexes was apparent, particularly among isolates belonging to the hyper invasive clonal complexes including ST-1, ST-4, ST-5, ST-11, ST-32 and ST-41/44, indicative of persistence of discrete sets of capsule genes in association with clonal complexes, despite evidence for widespread genetic exchange among meningococci. The maintenance of this association provides an explanation for the low frequency of capsule switching and/or replacement reported among meningococci following the extensive use of meningococcal vaccines in immunisation campaigns compared to other bacteraemic pathogens such as Streptococcus pneumoniae.

P 080

Restriction and Sequence Alterations Affect DNA Uptake Sequence-Dependent Transformation in *Neisseria meningitidis*

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Transformation is a complex process that involves several interactions from the binding and uptake of naked DNA to homologous recombination. Some actions affect transformation favourably whereas others act to limit it. Here, meticulous manipulation of a single type of transforming DNA, allowed for quantifying the impact of three different mediators of meningococcal transformation:*Nla*IV restriction, homologous recombination and the DNA Uptake Sequence (DUS). In the wildtype, an inverse relationship between the transformation frequency and the number of *Nla*IV restriction sites in DNA was observed when the transforming DNA harboured a heterologous region for selection (*ermC*) but not when the transforming DNA was homologous with only a single nucleotide heterology. The influence of homologous sequence in transforming DNA was further studied using plasmids with a small interruption or larger deletions in the recombinogenic region and these alterations were found to impair transformation frequency. In contrast, a particularly potent positive driver of transformation inNeisseria sp.are short DUS in the transforming DNA that contribute by a yet undefined mechanism. Increasing the number of DUS in the transforming DNA was here shown to exert a positive effect on transformation. Furthermore, an influence of variable placement of DUS relative to the homologous region in the donor DNA was documented for the first time. No effect of altering the orientation of DUS was observed. These observations suggest that DUS is important at an early stage in the recognition of DNA, but does not exclude the existence of more than one level of DUS specificity in the sequence of events that constitute transformation. New knowledge on the positive and negative drivers of transformation may in a larger perspective illuminate both the mechanisms and the evolutionary role(s) of one of the most conserved mechanisms in nature: homologous recombination.

P 081

Functional Deconstruction and New Dialects of the DNA Uptake Sequence in Neisseriaceae

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Transformation is regulated by a DNA repeat in the human pathogen Neisseria meningitidis and a few other bacteria. Their respective genomes harbour thousands of short DNA Uptake Sequences (DUS) 5'-AT-GCCGTCTGAA-3' whose presence is required in the transforming DNA to limit uptake and recombination to exclusively involve homologous DNA. This study discovered new dialects of DUS in the genomes of the Neisseriaceae family members. In total, eight DUS dialects are described that display considerable variation between species but are maintained within each species. We show that all the new DUS dialects share the same 5'-CTG-3' core and correlate to the genome-based phylogeny of the family Neisseriaceae.

Competence for transformation was investigated in Eikenella corrodens, Kingella denitrificans, Neisseria elongata and N. meningitidis. Without exception, each bacterial species was shown to preferentially transform with its own DUS compared to other DUS dialects. Experimentally meningococcal DUS was deconstructed by single nucleotide transversions showing the discrete impact of the individual bases constituting DUS on the transformation process. Altering the conserved DUS core 5'-CTG-3' practically abolished transformation. Transversion in the remaining nucleotides did have a less radical impact but still effectively lowered the transformation frequency. Despite sharing the same 5'-CTG-3' DUS core, the differences between dialects make up an very effective transformation barrier between these species and thereby preventing horizontal gene transfer, an ultimate cause of speciation.

P 082

Assessing *Neisseria* core proteomes provide insights into phylogeny and pathogenesis factors of this group of microorganisms

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Introduction: Currently, there exists no broad-spectrum vaccine for prevention of meningococcal disease by serogroup B *Neisseria meningitidis* (MenB). As with many other infection diseases, the development of a vaccine is hampered by the genetic variability of the microorganism causing it [1]. The recent availability in online databases of multiple genomes from distinct strains of MenB allows for the incorporation of information on gene variability into the algorithms that search for novel antigens and drug targets. In this context, we present an algorithm that determines the set of equivalent proteins of a group of proteomes, i.e. their core proteome [2], as well as the set of equivalent proteins of a subgroup of proteomes that are absent in a second subgroup.

Methods: After selecting 17 MenB strains with full genomic information, we have used the UCSC Blat software [3] to perform an all-vs.-all proteome comparison. With this data, for each protein in a proteome we have determined its best match in every other proteome. Using a full-reciprocity criterion [2,4] we have used this information to assign all protein equivalences between the proteomes, delivering the core proteome for the 17 strains. From this data we have also calculated the percentage of common proteins between strains in a pairwise manner and used it to obtain a similarity matrix that is given as input for the construction of a phylogenetic tree. Finally, we have introduced three strains of *Neisseria lactamica* (non-pathogenic set) in the calculation to identify the fraction of the core proteome of MenB that is absent in *N. lactamica*, thus pointing at potential MenB virulence factors.

Results: We have identified 1278 proteins present in all 17 MenB proteomes, and determined unambiguously the equivalences for 87.53% of all proteins of these proteomes. After introducing N. lactamica in the calculation, we have found 59 proteins present in the core proteome of MenB that do not have any equivalent in the N. lactamica strains. Additionally, we have used all this information to create a phylogenetic tree (Figure1) that significantly correlated with a tree constructed using DNA information from MLST genes [5].

Discussion: We have developed a method and a tool for the identification of the set of proteins that define a common core of a set of proteomes. Phylogenetic trees created from this data are in full agreement with previous genetic knowledge of the species, indicating that the core proteomes generated may have evolutive significance. For MenB, the resulting set of conserved proteins could be explored for broad-spectrum vaccines or antibiotics. Additionally, using a combination of pathogenic and non-pathogenic related organisms we have found a list of conserved proteins only present in the pathogenic species that could be related to virulence.

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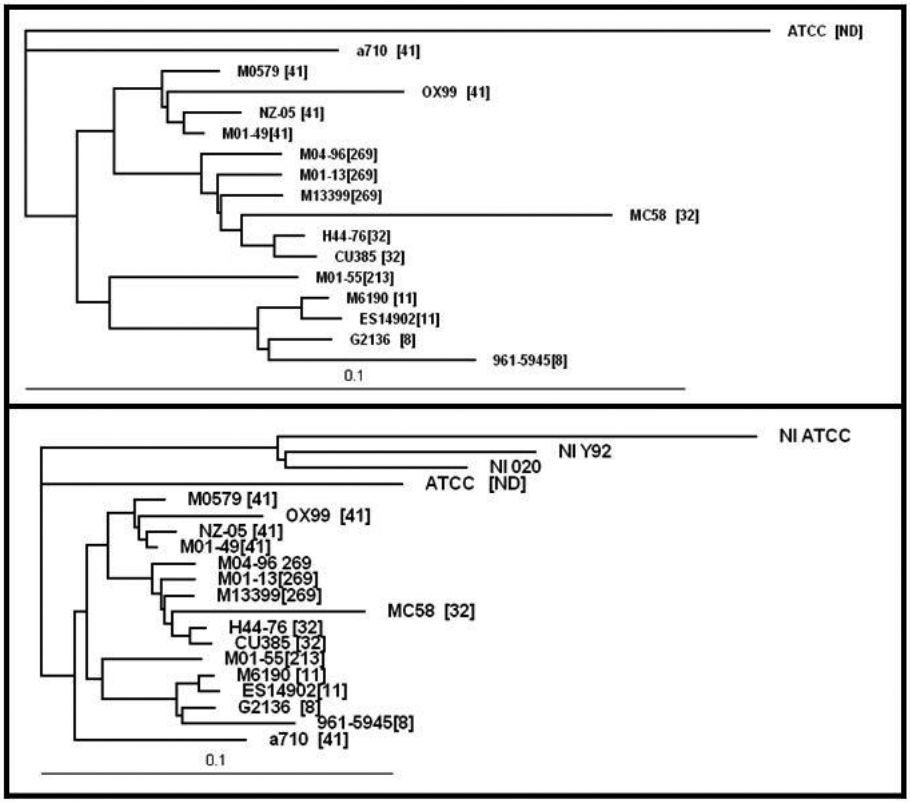
Figure1: Phylogenetic trees constructed with the data obtained through the core proteome calculation. Top panel:Using 17 *N. meningitidis* serogroup B species, notice that all but alpha710 appear grouped according their ST (last number in the species name).Bottom panel:Same tree adding three species of *N. lactamica*(Nl ATCC, Nl Y92, Nl 020) as out-group.

figure 1



P 083

A comparative-genomics algorithm to identify the source of protein variability in the core proteome of *Neisseria meningitidis* serogroup B

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Most cases of invasive meningococcal disease in developed countries have been caused by *Neisseria meningitidis* serogroup B (MenB). The prospect for a universal anti-MenB vaccine has been hampered in part due to the degree of genotypic and phenotypic variation that occurs between the different circulating strains. The aim of this study was to analyze variation within the proteins conforming the full MenB core proteome and to investigate the genetic source of the detected variability. The study used this information to characterize the relative contribution of recombination compared with point mutation in the diversification of MenB proteins. The evolutionary inferences were also associated with subcellular localization of each protein group. To construct a core proteome (set of "ortholog" proteins common to all strains) we selected 17 MenB strains with complete genome information and showing wide genetic diversity by means of their sequence types. This panel of strains represents the most important hyper-virulent lineages circulating around the world. For DNA-based analyses the core genome was obtained converting the core proteome into the corresponding DNA sequences. A computational pipeline was developed and implemented to combine results from conventional protein analysis tools (PSORTb, SignalP, BLAST) with genetic algorithms found in the PHYLIP and HyPhy packages. The method can detect the source of variability in MenB core genes searching for evidences of recombination (GARD[1]) and positive selection (PAML[2]). To avoid false positives, the results from the recombination test were taken into consideration to estimate positive selection. Our analysis of protein othologs across the selected genomes identified 1278 ORFs present in all 17 MenB strains. From this set of ortholog proteins, 95% of the ORFs showed some level of variation (at least one variable site), and 20% of these variable proteins appeared to evolve under positive selective pressure. Interestingly, among positively selected proteins, there are membrane associated as well as cytoplasmic proteins. Positive selection was strongly associated with recombination. Specifically, 223 of the 242 genes under positive selection also showed evidences of recombination. Recombination was detected in the 59.1% of the ortholog groups. Our method gives an estimate of the ratio of recombinational events to mutational events of aproximately 3.12 for MenB core genome.
In pathogens like *N. meningitidis* that use antigenic variability to escape the immune response, conserved antigens have been selected by evolution to be poorly immunogenic. On the other hand, antigen conservation would facilitate the development of a universal vaccine. Proteins identified in this study, belonging to the MenB core proteome but showing at the same time variability and even positive selective pressure, are thus pinpointed as potentially immunogenic or involved in mechanisms of antimicrobial resistance. These proteins can be further evaluated as prophylactic candidates, possibly within a multi-variant formulation taking account of the detected variability.

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P 084

Genome-wide assessment of within-host evolution in Neisseria meningitid is during invasive disease

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The hypothesis of within-host evolution explains meningococcal pathogenicity as an inadvertent consequence of short-sighted within-host evolution due to increased mutation rates associated with rapid phase shifting at simple-sequence repeat contingency loci which has primarily evolved as an adaptive mechanism for the colonization of diverse hosts [1]. In addition, a recent case report of an accidental laboratory infection with a serogroup A hypermutator strain identified short insertions and deletions in the genome of the blood isolate compared to the initial isolate the patient was working with and which also affected several phase variable genes [2]. To put this hypothesis to a systematic experimental test, we sequenced the genomes of throat-blood isolate pairs from four different patients comprising one sequence type (ST)-42 serogroup B pair, two ST-11 serogroup C pairs and one ST-23 serogroup Y strain pair. Whole-genome sequences were obtained for of all throat isolates at almost 40-fold coverage using Roche GS FLX Titanium Series Chemistry and annotated using the GenDB platform [3]. Potential contigency loci were computationally identified using the criteria given by Saunders et al. [4]. The genomes of the corresponding blood isolates were re-sequenced at over 1000-fold coverage on a Illumina Genome Analyzer IIx and mapped onto the reference genomes of the throat isolates by combining the results from the three different mapping programs BWA [5], Bowtie2 [6] and Novoalign (Novocraft Technologies). All sequence differences between the genomes of the throat and blood isolates were verified using standard Sanger sequencing. In addition, all strain pairs were also subjected to ex vivo growth/survival assays in human whole-blood. First analyses identified recombinational events in the genomes of two throat/blood isolate pairs that had likely occurred during or after the invasive process. However, so far we failed to identify differences between throat/blood isolate pairs at contingency loci in this sample, and we could also not observe different growth/survival rates of throat/blood isolate pairs in human blood. Therefore, our data do currently not support the within-host evolution hypothesis of meningococcal virulence.

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P 085

Distribution of rare Neisseria meningitidis serogroups in European carriage isolates

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Background: Based on biochemical differences, twelve capsule polysaccharides have been defined and of these, six serogroups (A, B, C, W, X and Y) cause the majority of disease with the remaining serogroups E, H, I, K, L and Z predominantly associated with carriage. The latter are not routinely screened for in reference laboratories due to a lack of suitable assays. This study describes a molecular sequence based technique enabling the detection of all serogroups.

Methods: Within the capsule locus, there are four genes,ctrA-ctrD, which encode proteins involved in capsule transport. Comparison ofctrAnucleotide sequences obtained from whole genome sequences revealed that the 5'end of the gene was serogroup specific from which a multiplex PCR amplifying all meningococcal serogroups was designed. In addition, the complete carriage collection obtained from the Czech Republic in 1993 was whole genome sequenced from which genogrouping was possible.

Results: Over 1000 non-serogroupable isolates in the UK Carriage isolate collection were genogrouped using thectrA-assay. Among these, serogroup E, Z, L, IK and Z isolates were found. This was also found among carriage isolates in the Czech Republic.

Conclusions: This study reveals for the first time the distribution of the rarer serogroups in N. meningitidis isolates obtained from carriage in the U.K and the Czech Republic. In addition, an association between sequence type and serogroup was apparent. Whilst these serogroups have been predominantly associated with carriage, surveillance of these may become more of an issue should capsule replacement occur in the future.

P 086

From genes to genomes: current status of the *Neisseria* reference libraries hosted on PubMLST.org

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The PubMLST *Neisseria* database has hosted allelic diversity data for multilocus sequence typing (MLST) and major antigens for the past decade and currently has records for approximately 20,000 isolates sampled from over 100 countries. In anticipation of the increased availability of whole genome sequence data, the PubMLST database began hosting genomic data in 2009.

The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates using the BIGSdb platform. Loci have been defined within the database for most of the core genome in a manner analogous to MLST so that sequence diversity is now indexed at >1200 loci with each unique gene sequence assigned an allele number.

The platform facilitates many applications including:

1) Annotation: Genomes consisting of multiple contigs assembled from short read data can be uploaded to the database and their allelic diversity will be automatically annotated.

2) Functional studies: Loci have been grouped in to schemes for genes encoding enzymes from pathways of central metabolism, enabling analysis of sequence diversity to be related to function.

3) Epidemiology: Typing and other epidemiological markers can be extracted from genome data automatically enabling comparisons. The built-in Genome Comparator tool facilitates rapid gene-by-gene comparison of hosted genomes. This can be performed using either the database defined loci or an annotated reference genome as the source of comparison sequences. Outputs include tables of variable loci, a distance matrix of allelic differences and a Neighbor-Net graph, providing a graphical representation of relationships among isolates. This can be informative for outbreak investigation and for forensic analysis of transmission.

In conclusion, the *Neisseria* PubMLST database, and the underlying BIGSdb platform, is well positioned to facilitate the analysis of whole genome data for clinical and epidemiological purposes, providing an accessible means to readily extract, organise and compare relevant information from sequence data.

P 087

Movement of Correia Repeat Enclosed Elements during laboratory culture

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The Correia Repeat Enclosed Element, an IS-like element, has been predicted to be mobile within the gonococcal genome. Although there is evidence of ancestral movement of these elements, no previous study has provided evidence for current mobilisation. Previous studies have compared the genomic locations of Correia Repeat Enclosed Elements in the *Neisseria* spp., demonstrating that otherwise identical regions in the same species have either a Correia Repeat Enclosed Element or the target TA insertion site. In this study we report for the first time movement of Correia Repeat Enclosed Elements, through inversion of the element at its chromosomal location. Analysis of Ion Torrent generated genome sequence data from *Neisseria gonorrhoeae* strain NCCP11945 passaged for 10 weeks in the laboratory under standard conditions and stress conditions revealed a total of 47 inversions: 26 were exclusively seen in the stressed sample; 9 in the control sample; and the remaining 12 were seen in both samples. The sequence read data provided evidence for their mobility during laboratory culture, through inversion rather than translocation. These inversions have the capability to alter gene expression in *N. gonorrhoeae* through the previously determined activities of the sequence features of these elements.

P 088

Sequence features contributing to chromosomal rearrangements in *Neisseria gonorrhoeae*

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Through whole genome sequence alignments, breakpoints in chromosomal synteny can be identified and the sequence features associated with these determined. Alignments of the genome sequences of *Neisseria gonorrhoeae* strain FA1090, *N. gonorrhoeae* strain NCCP11945, and *N. gonorrhoeae* strain TCDC-NG08107 reveal chromosomal rearrangements that have occurred. Based on these alignments and dot plot pair-wise comparisons, the overall chromosomal arrangement of strain NCCP11945 and TCDC-NG08107 are very similar, with no large inversions or translocations. The insertion of the Gonococcal Genetic Island in strain NCCP11945 is the most prominent distinguishing feature differentiating these strains. When strain NCCP11945 is compared to strain FA1090, however, 14 breakpoints in chromosomal synteny are identified between these gonococcal strains. The majority of these, 11 of 14, are associated with a prophage, IS elements, or repeat enclosed elements which appear to have played a role in the rearrangements observed. Additional rearrangements of small regions of the genome are associated with pilin genes. Although horizontal transfer has played a role in gene acquisition, it is these sequence features mediating chromosomal rearrangements which appear to be a driving force in the evolution of diversity within the species.

P 089

Roles for DNA repeats in *Neisseria gonorrhoeae*, including protein sequence variation.

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There are many types of repeated DNA sequences in the genomes of the *Neisseria* spp., from homopolymeric tracts to tandem repeats of hundreds of bases to sequences scattered hundreds of times across the genome. These each play differing roles in the biology of these bacteria, including mediating antigenic variation, phase variation, and differential expression of genes. Here we present analysis of the repetitive sequences in the *Neisseria gonorrhoeae* strain NCCP11945 genome sequence and comparisons to other data. Evidence is presented for 34 phase variable genes in this strain and for 48 phase variable genes in this species, including a new class of phase variation that causes amino acid changes at the *C*-terminus of the protein, never before described in the *Neisseria* spp. Strain NCCP11945 also contains 29 coding repeat containing genes, one more than the repertoire identified in *N. gonorrhoeae* strain FA1090. Areas of the genome lacking common neisserial repeat elements were investigated as regions of potential horizontal transfer. In addition, inverted repeats of the neisserial uptake sequence predicted to act as transcriptional terminators were identified for approximately one-seventh of the annotated CDSs in the strain NCCP11945 genome. The various repetitive sequence elements are important to the biology, adaptation, and evolution of *N. gonorrhoeae*.

P 090

Diversity of the *opcA* gene in *Neisseria* species – a genomic investigation.

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Background and Methods: OpcA is one of the major *Neisseria meningitidis* (Nm) outermembrane proteins. It is known to be involved in adhesion and invasion of epithelial and endothelial cells, to be immunogenic and to induce and serum bactericidal antibodies. Absence of the gene has been demonstrated previously in ST-11 and ST-8 complex strains. Expression of the protein is phase variable, being regulated at the transcriptional level by a variable poly-cytidine tract in the promoter region. This study investigated the diversity of the *opcA* gene and also the organisation of the locus in a large collection of meningococcal genomes and other *Neisseria* species. Analysis of selection was also carried out using a Bayesian algorithm in the Omegamap program. The *Neisseria* BIGSdb database was interrogated and whole *Neisseria* genomes scanned for presence of the gene.
Results: At the time of writing, there were 45 alleles in 364 *Neisseria* species genomes that were tagged for the gene. These included ten orthologous alleles: eight in *Neisseria gonorrhoeae* (Ng) and two in *Neisseria* commensal species – *N. polysaccharea* (Np) and 'N. bergeri' (formerly assigned *N. polysaccharea*). There was 51% nucleotide sequence identity amongst all the alleles; 57% between Nm and Ng; 96% amongst all Nm alleles and 90% between Np and Ng alleles. Of the 83 ST-11 and eight ST-8 complex genomes scanned, none appeared to contain the gene. This was the case also for the nine ST-213 complex and nine capsule null-related ST-53 complex isolate genomes. Clonal complex was predictive for *opcA* allele. All ST-269 complex (n-16) and all but one ST-32 complex (n=48) genomes were *opcA-1*. All but one isolate of the serogroup A-related ST-1, 4 and 5 complex genomes (n=45) had the *opcA-3* allele. Analysis of selection on the gene indicated a low level of positive selection (d_N/d_S 1-1.34) in an area corresponding to loop 5 of the protein.
Conclusions: The *opcA* gene was found in two disease-related and members of one commensal *Neisseria* species. Within each species, the gene had exceptionally low sequence diversity. Like other meningococcal genes it was structured in its relationship to clonal complex and serogroup. Its absence in ST-11 and ST-8 complexes and presence in some members of a commensal species indicates it is not essential for virulence. As a putative vaccine antigen, these data together shed more light on its potential candidacy as a vaccine component.

P 091

Variation in *pilS* Cassette Number in *Neisseria meningitidis*

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Question: *Neisseria meningitidis* expresses cell surface type 4 pili, which are involved in motility, host cell adhesion, DNA uptake, and virulence. The antigenic major pilin subunit, PilE, varies in part due to homologous recombination with *pilS* cassettes. These cassettes are similar in sequence to *pilE*, but lack N-terminal coding sequence and are silent (not expressed). The invasive serogroup B MC58 strain contains 8 *pilS* cassettes. However, the variability in number of *pilS* cassettes is not well documented in other serogroups or in carriage isolates. The number of *pilS* cassettes affects pilus antigenic variation and may impact meningococcal invasiveness and pathogenicity. We investigated *pilS* cassette content from available whole genome sequences and a collection of serogroup Y carriage isolates.

Methods: Ten serogroup Y clonal complex (CC) 23 meningococcal genomes were analyzed. In addition, 22 publicly available meningococcal genomes, including two serogroup A, fifteen serogroup B, three serogroup C, one serogroup X, and one capsule null strain, were obtained from the NCBI GenBank sequence repository and were surveyed for *pilS* cassette number. Genomes were visualized using the Sybil comparative genomics browser. Primers were designed for locations in conserved genes flanking the *pilE*/*pilS* region to estimate, based on PCR amplicon size, the number of *pilS* cassettes in a collection of 40 additional serogroup Y carriage strains.

Results: The number of *pilS* cassettes ranged from one to ten among the 32 whole genomes examined. While there was some correlation between number of *pilS* cassettes and clonal complex, there was considerable variability within clonal complexes. Sequenced whole genomes of serogroup Y CC 23 *N. meningitidis* strains were as varied in the number of silent cassettes as the collection as a whole, containing one to nine *pilS* cassettes. Among whole genomes surveyed, a carriage strain from the US, a carriage strain from the UK, and an invasive strain from the UK, all serogroup Y ST-23, had only one *pilS* cassette. In two other serogroup Y strains, a transposase was inserted between the first and second *pilS* cassettes. An additional serogroup Y strain contained multiple *pilS* paralogs in three separate regions distant from *pilE* and associated in all cases with transposases. PCR estimation of cassette number confirmed variability in *pilS* number within serogroup Y carriage strains.

Conclusion: We found wide variation in the number of silent *pilS* cassettes among *N. meningitidis* strains, both within the same clonal complex and sequence type and among diverse clonal complexes. The presence of a transposase adjacent to *pilS* within the CC23 lineage suggests that transposon mediated recombination may be involved in the variability of *pilS* cassette numbers. A decrease in the number of *pilS* cassettes may impact meningococcal virulence by reducing the potential for antigenic variation.

P 092

Phenotypic diversity of commensal isolates of *Neisseria* from healthy children and adults, and cystic fibrosis and sepsis patients

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The respiratory microbiome is normally colonized by several Neisserial species. One species in particular, *Neisseria meningitidis,* is associated with invasive disease in children and adults, with an average of 235 cases being reported annually in Canada [1]. While the commensal Neisseria have been identified as infectious agents in sporadic case studies [2], they are widely accepted to be largely harmless, and potentially helpful members of the respiratory microbiome [3]. One major disadvantage in the identification and cultivation of Neisserial species from clinical samples is that laboratory culture methods often suppress the growth of commensal Neisseria and as a result, their true contribution to health and disease may be underestimated. Recently, our laboratory has successfully identified a number of Neisserial species from various disease states that are commonly missed in routine laboratory culture [4].

Furthermore, given the propensity for horizontal gene transfer amongst the Nesseriaceae, it may be that phenotypic distinctions lose their ability to predict behaviour of individual isolates, and instead the acquisition of a pathogenicity factor in a normally commensal bacterium can give it infectious potential. In our laboratory, the presence of a number of pathogen-associated phenotypes in commensal Neisserial species isolated from cystic fibrosis (CF) and sepsis patients were analysed and compared to samples from healthy children and adults, in order to determine whether disease associated isolates of *Neisseria* have specific phenotypes that contribute to the development of disease.

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P 093

Conservation and evolution of conserved meningococcal pilins in a genomic context

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Type four pili are polymers consisting predominantly of PilE, the major Pilin protein, and play a key role in the initial adhesion of Neisseria to epithelial cells. Until now Neisseria pilin has been regarded as a paradigm of antigenic variation. Extensive sequence variation of pilin occurs both *in vitro* and *in vivo* during human infection and arises through recombination of the expression locus *pilE* with *pilS* cassettes. Pilin variation has been shown to influence bacterial interactions with cells and is also a proposed mechanism of immune escape. We recently found that within certain isolates of *N. meningitidis*, the *pilE* sequence is highly conserved and does not show the characteristic sequence variation (Cehovin *et al*, 2010). In this work we used the genome sequences of Neisseria isolates available in the Integrated Microbial Genomes (http://img.jgi.doe.gov/) and Bacterial Isolate Genome Sequence (BIGSdb, University of Oxford) databases to explore the genomic organization and conservation of *pilE* and *pilS* loci. We show that meningococcal strains belonging to a limited number of clonal complexes associated with epidemic disease (i.e.the hyperinvasive lineages- ST-1, ST-5, ST-8 and ST-11) i) harbour class II *pilE* genes which are highly conserved in terms of sequence and genomic location, ii) have a reduced repertoire of conserved *pilS* cassettes and iii) lack the guanine quartet (G4) sequence required for pilin variation. Through analysis of available genomes of commensal species (56 genomes in total), we provide evidence that the *pilE* genes in these lineages appear to have been acquired from commensal Neisseria species by horizontal transfer on three distinct occasions.

In summary, we have used available genome databases to define a conserved pilin gene arrangement specific to hyperinvasive lineages. We propose that the pilin encoded by these isolates may result in Type four pili with specific properties that contribute to the high transmissibility and low colonisation associated with these lineages.

P 094

Evidence of the Population Genetic Relationships of *Neisseria meningitidis* Isolates Circulating in the Republic of Belarus with those from Western and Eastern European Countries

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Introduction: Introduction. Modern molecular epidemiological surveillance systems, including the accumulation of multilocus sequence typing (MLST) data on N. meningitidis (NM) isolates, provides new possibilities for exploring novel models for pathogen population structure. Fixation index (Fst) is a population genetic parameter that measures variation in allele frequencies among population subdivisions and varies from 0 to 1. The epidemiological meaning of high Fst values is the absence of pathogen exchange between separate populations, with low Fst values indicating unbounded circulation of NM and potential genetic material exchange.

Aim: Our aim was to investigate the population genetic relationships of the NM isolates circulating in Belarus with those from Western and Eastern European countries.

Materials and methods: Twenty-six N. meningitidis strains isolated from patients in different regions of Belarus were studied. We exported allele frequencies of MLST-typed isolates from 14 European countries. The total number of studied isolates was 11460. Fixation index Fst was estimated in Arlequin 3.1 as pairwise comparisons of the Belarusian pathogen population and populations from individual European countries. The credibility of inferred Fst values was estimated using the t-test.

Results: Studied NM strains from Belarus have more novel STs (88.46%) than previously described STs. Sequence types with clonal complex designation (16 isolates) were more widespread than non-clonal STs. There were 7 isolates from ST-41/44/Lineage 3 and 5 isolates from ST-103 complex. Clonal complexes ST-11/ET-37, ST-174, ST-18 and ST-53 were represented by one strain only. Populations from European countries can be divided into three groups according to Fst values inferred by each MLST gene. A group of countries for which NM populations were similar to those in Belarus includes the UK, Germany, The Netherlands and Finland. Populations of NM in these countries have the lowest values of fixation index of every MLST gene notwithstanding their geographic distance from Belarus (Figure 1). The second group of countries (Russia, Poland, Italy and Spain) had pathogen populations that were the most dissimilar to the Belarusian population. Significance of the Fst differences between NM populations in these countries and Belarus was high ($p<0,001$). NM populations from other countries (Ireland, Czech Republic, Slovenia, etc) were found to be in the middle of fixation index range. Most of the Russian NM isolates were collected in Moscow (73.68%) , which is very close to Belarus. Therefore high Fst value between NM populations from Russia and Belarus can't be explained by spatial distance. This difference of genetic exchange in bacterial populations from Belarus and both distant and close countries seems to be associated with varying level of adaptation in different pathogen variants. Some European STs are possibly better adapted and these STs began to colonize people living on new territories. This hypothesis is supported by the presence in Belarus of NM isolates belonging to European hyperinvasive ST-41/44/ Lineage 3 clonal complex instead of the Russian ST-1 complex/subgroup I/II complex.

Conclusions: The N. meningitidis population circulating in Belarus is characterized by a higher level of genetic exchange with populations from Germany, UK, The Netherlands and Finland in comparison to neighboring countries such as Russia and Poland. Inferred patterns of genetic exchange indicate higher-level adaptation of European isolates compare to those isolates from Russian.

Figure 1
Map of Fst values of pairwise comparisons of aroE gene in Belarus and European countries. The multiplier for Fst values is 100 000.

P 096

Conservation of three Meningococcal antigens in the genus Neisseria

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The genus *Neisseria* is a large group of gram-negative bacteria that colonize the mucosae of many animals. Beside several species that are only rarely associated to disease, this genus also contains two major human pathogens, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, that generally asymptomatically colonize humans, but in a small percentage of the cases cause systemic disease. The taxonomy of the genus *Neisseria* based on sequence analysis is difficult because these species are less distinct than what is normally accepted for species definition. In other cases, the different ecology makes genetic exchange less frequent, like, for instance, the relatively low level of genetic transfer found between *N. meningitidis* and *N. gonorrhoeae*. This blurred border between distinct species is due to the high level of recombination between these organisms, that often coexist in the same environment for relatively long periods of time. The recent development of different recombinant vaccines against *Neisseria meningitidis* of serogroup B (MenB) has raised the question of the impact of these on other neisserial species. To understand the possible effect of a vaccination, we studied the level of molecular conservation and the evolutionary mechanisms driving the diversification of the major vaccine antigens like fHbp, NHBA and NadA, in different neisserial species, including *N. meningitidis*, *N. gonorrhoeae*, *N. lactamica*, *N. polysaccharea* and *N. cinerea*.

P 097

Emerging invasive Neisseria meningitidis serogroup Y in Italy, 2000 to 2011

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The aim of the present study was to investigate the clinical features of patients and the microbiological characteristics of invasive serogroup Y meningococci emerging in Italy from 2000 through 2011.
A total of 60 Invasive Meningococcal Disease (IMD) cases due to serogroup Y have been documented in the country; 54 isolates were sent to National Reference Laboratory (NRL) at the ISS. All the strains were serogrouped,serotyped and the antibiomicrobials susceptibility for penicillin,ciprofloxacin,ceftr iaxone and rifampin were determined by Etest method. MLST and porA VR1 and VR2 typing,were determined using the Neisseria website (http://pubmlst.org/neisseria/).
Before 2005, serogroup Y ranged from 1 to 4%, then, in 2006, was 7% and increased up to 16% in 2011. Patients infected by serogroup Y meningococci were mainly females (34) and 19 males; the average age was 30 years, older than that found due to other serogroups. Meningitis was the most frequent clinical picture reported (55,6%). All the isolates were fully susceptible to the drugs tested except for the 28% showing a decreased susceptibility to penicillin with a MIC range of 0,094-0,19μg/mL. Nine different phenotypes were identified and the prevalent was the Y:14:NST. A total of 11 sequence types (STs) were identified, of which ST-23 and ST-3171 as the predominant. The ST-23 clonal complex (cc)accounted for the 88%; the ST-167cc for the 7,2%; ST-41/44 cc and ST-174 cc for the 2,4%. Sequencing of the porA gene revealed a total of 8 PorA types, of which the most frequently detected was : P1.5-2, 10-2.
The emergence of serogroup Y meningococci has been here described. Based on phenotypic and genotypic characteristics, the Italian serogroup Y meningococci showed similar molecular traits. An ongoing surveillance will permit to monitor carefully the spread of this serogroup in the different regions of the country.

P 098

Molecular profiles of serogroup B and C Italian meningococci after the introduction of MenC vaccination

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In Italy, incidence rate for invasive meningococcal diseases is quite low and remained stable over the last years (0,3x100,000 inhabitants). However, after the introduction of Men C vaccination epidemiological changes have been observed. In order to explore in deep the phenomenon, as National Reference Laboratory for meningococci, we investigated the clinical features of patients with IMD from 2000 through 2011 and the molecular characteristics of meningococci collected during the last three years (2009-2011). From 2000 through 2011, a total of 2647 IMD cases have been reported; serogroup B accounted for the 74% in 2011 and serogroup C for the 18%. From 2009 through 2011, 280 meningococci have been sent to NRL; of which 277 have been completely characterized for MLST, PorAVR1, PorAVR2 and FetA, using conventional methods.

A significant decrease of serogroup C meningococci has been observed after the introduction of MenC vaccination (2005) in different Italian Regions. The prevalence of serogroup C was 36,4% in 2005 and 12,2% in 2011. In particular, IMD incidence among infants and children aged 0 to 4 years decreased from an incidence of 1,3 per 100,000 inhabitants in 2005 vs.0,18 in2011. Twelve Clonal Complexes (CCs) within serogroup B isolates have been identified. The ST-41/44 cc was the most common (45,4%). This clonal complex remained the predominant among serogroup B from 2009 through 2011. Two ccs, ST-198 and ST-167, were firstly associated to serogroup B in Italyin 2011. Serogroup C isolates belonged to 5 main ccs. The ST-11 cc was the most important (56%). The ST-8 cc (18,4%) is one of the most frequently identified until 2009, and then it decreased in favor of others clonal complexes as the ST-334 cc, accounting for 40% in 2011. Within serogroup B/ST-41/44 cc the predominant PorA-VR variants were: VR1 7-2 and VR2 4, accounting for the 90%. Within serogroup C/ST-11, the VR1 5 and the VR2 2, were the predominant in 2009 (58,1% and 53.1%, respectively); in 2011 serogroup C/ST-334 increased and was characterized by the presence of VR1 7-4 and of VR2 14-6, accounting for, respectively, the 40% in 2011. We found 26 FetA variants within serogroup B and 9 within serogroup C. F3-9, firstly identified among serogroup C in 2009 (6,3%), now is the most important and accounting for the 40%. The common finetype among serogroup B was B:7-2,4:F1-5 (27.4%) and among serogroup C, C:5-1,10-8:F3-6 (33.3 %).

We believe that the introduction of routine immunization against serogroup C for infants and school-aged children may have contributed to the considerable shift in meningococcal serogroup and to the molecular changes among serogroup C. A careful meningococcal surveillance is required, especially after the introduction of MenC vaccination and before the new MenB vaccines.

P 099

Continuing increase in invasive meningococcoal capsular group Y disease in England and Wales, 2007–11

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Background: Invasive meningococcal capsular group Y (MenY) disease is rare, but its incidence has recently been reported to be increasing in a number of European countries, including the United Kingdom.
Methods: The Health Protection Agency conducts enhanced surveillance of invasive meningococcal disease in England and Wales. This study describes the epidemiology and outcome of invasive, laboratory-confirmed MenY disease in England and Wales between January 2007 and December 2011.
Results: There were 306 MenY cases over the 5-year period (average annual incidence, 1.1 per million population). The number of MenY cases increased from 36 (0.7/million) in 2007 to 95 (1.8 per million) in 2011. One third of cases occurred in older adults aged ≥65 years (n=113, 37%) and only 10% (n=32) occurred in children aged <5 years. Diagnosis was confirmed by culture in 193 (63%), PCR only in 63 (21%) and by both methods in 50 (16%) cases. Most isolates were obtained from blood (84%), followed by cerebrospinal fluid (14%) and rarely from other sites (2%). The overall case fatality ratio (CFR) was 20% (62/306 cases) and was highest in older adults aged ≥85 years (21/35 cases, 60%) followed by 65-84 year-olds (22/78, cases, 28%) and 45-64 year-olds (8/57 cases, 14%) compared to 8% (11/135) in <45 year-olds.
Conclusions: Although invasive MenY disease remains uncommon, its incidence continues to increase across all age groups inEnglandandWalesand is associated with significant case fatality particularly among older adults.

P 100

Molecular and epidemiological characteristics of *Neisseria meningitidis* in Scotland, 2009–2012

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The SHLMPRL receives *Neisseria meningitidis* isolates from regional microbiology laboratories throughout Scotland for serogrouping, biochemical confirmation, antimicrobial susceptibility testing, MLST and PorA VR sequencing. In line with EMGM recommendations, the laboratory has recently started FetA VR sequencing on all meningococcal isolates.

From 2009 to April 2012, 215 meningococcal isolates were received mainly from sporadic cases of invasive meningococcal disease (IMD). The majority were blood isolates (n=105, 48.8%) but also included throat (n=40, 18.6%), eye (n=19, 8.8%), sputum (n=18, 8.4%) and CSF (n=16, 7.4%) isolates. These were characterised as serogroup B (n=138, 64.2%), Y (n=13, 6.0%) or W135 (n=10, 4.7%). A single isolate of each of the serogroups X, Z and 29E was also identified. Fifty isolates (23.3%) were non-serogroupable with around half possessing the capsule null locus. MLST characterised these isolates into 128 STs, 107 of which could be assigned to 24 distinct clonal complexes (CCs). The most common STs were, ST-53 (n=17), ST-213 (n=14), ST-41 (n=7), ST-1161 (n=6), ST-1157 (n=6) whilst the most common CCs were, ST-41/44 (n=44), ST-269 (n=34), ST-53 (n=19) and ST-213 (n=18). Notified cases of meningococcal disease accounted for 90.9%, 88.2%, 31.6% and 66.7% respectively of these CCs. 10.7% of isolates could not be assigned to a currently defined clonal complex. Meningococci were highly diverse with respect to PorA and FetA VR and based on EUCAST clinical breakpoints, all isolates were fully susceptible to chloramphenicol, cefotaxime, and rifampicin. Reduced susceptibility to penicillin was observed in 35.3% (n=76) of the meningococcal isolates and one sputum isolate had reduced susceptibility to ciprofloxacin.

In summary, meningococcal disease in Scotland is composed primarily of sporadic cases with the vast majority due to serogroup B, most commonly of the ST-41/44 or ST-269 clonal complexes. Serogroup C conjugate vaccination continues to prove successful as no cases of IMD due to serogroup C has been reported in Scotland since 2007.

P 101

Association between incidence of meningococcal disease in young children and population prevalence of smoking in Norway, Sweden, Denmark and The Netherlands between 1975 and 2009

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Objective: To investigate the relationship between the incidence of invasive meningococcal disease (IMD) among children under 5 years old and population prevalence of smoking.
Method: A retrospective, longitudinal, observational study was performed using data from Norway, Sweden, Denmark and The Netherlands for the period 1975 to 2009.
Results: In Norway there was a significant positive relationship between the incidence of IMD in children under 5 years of age and the annual prevalence of daily smokers aged 25 to 49 years, both unadjusted (RR = 1.04 - 1.06, p <0.001) and after adjustment for time of year (quarter), incidence of influenza-like illness and household crowding (RR = 1.05 - 1.07, p <0.001). Depending on age group, the risk of IMD increased by 5.2 - 6.9% per 1% increase in smoking prevalence among individuals aged 25 to 49 years in adjusted analyses. Using limited datasets from the other three countries, unadjusted analyses showed positive associations between IMD in children <5 years related to older smokers in Sweden and the Netherlands, and negative associations between IMD in children <5 years related to younger smokers in Sweden. However, there were no demonstrable associations between incidence of IMD and prevalence of smoking, after adjustment for the same confounding variables.
Conclusions: The reduced incidence of IMD in Norway between 1975 and 2009 may partly be explained by the reduced prevalence of smoking during this period. High quality surveillance data are required to confirm this in other countries, and the effect of dominant genotypes should be further elucidated. Strong efforts to reduce smoking in the whole population including targeted campaigns to reduce smoking among adults may have a role to play in the prevention of IMD in children.

P 102

Epidemiology of invasive Meningococcal disease in the Czech Republic

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Background: Nation-wide enhanced surveillance of invasive meningococcal disease (IMD) was implemented by the National Reference Laboratory for Meningococcal Infections (NRL) in 1993 when a clone of N. meningitidis C, ET-15/37, ST-11, occurred and caused increased IMD morbidity and case fatality rates. Since then, valid and comparable data have been available.
Material and methods: The case definition is consistent with the ECDC guidelines. Culture and PCR are used for confirmation of cases. Notification is compulsory and Neisseria meningitidis isolates from IMD cases are referred to the NRL to be characterized by serogrouping, PorA and FetA sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).
Results: After the prevalence of serogroup C (ET-15/37, ST-11) in the period 1994-1999, serogroup B has been prevalent since 2000 and the morbidity has a decreasing trend. In 2011, the IMD incidence decreased to 0.6/100 000 and the overall case fatality rate was 12.3 %. The disease was caused mainly by serogroup B meningococci (69.2 %) while IMD cases due to serogroup C decreased to 6.2 %, i.e. the lowest incidence rate since1993. In2011, serogroup Y caused 3.2 % of IMD cases and the same percentage was reported for serogroup W135. In 2011, no death was caused by serogroup C, for the first time since 1993. Serogroup B accounted for most deaths, with a case fatality rate of 11.1 %. One death was caused by serogroup W135. The highest age-specific morbidity rates were observed in the lowest age groups, i.e. 0-11 months and 1-4 years (10.2/100 000 and 2.8/100 000, respectively), and were associated with high prevalence of serogroup B. The third highest age-specific morbidity rate was reported in the adolescent age group, i.e. 15-19 years (2.2/100 000), with serogroup B accounting for the highest morbidity in this age group as well (1.5/100 000).
Conclusions: The incidence of IMD has a downward trend in the Czech Republic since 2000. The proportion of serogroup C cases decreased significantly while serogroup B cases show an upward trend. Serogroup Y and W135 cases increased slightly, but some were fatal.

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P 103

Molecular methods in the surveillance of invasive Meningococcal disease in the Czech Republic

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Background: The National Reference Laboratory for Meningococcal Infections (NRL) has been conducting enhanced surveillance of invasive meningococcal disease (IMD) in the Czech Republic (CR) since 1993.

Materials and methods: Neisseria meningitidis isolates from IMD cases are referred to the NRL to be characterized by serogrouping, PorA and FetA sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/). Non-culture PCR detection of N. meningitidis, H. influenzae and S. pneumoniae and non-culture PCR typing of N. meningitidis directly from clinical specimens (http://emgm.eu/emert/) enabled diagnosis in culture-negative IMD cases and deaths.

Results: In CR, 65 IMD cases (0.6/100,000 population) were reported in 2011. Most IMD cases were caused by serogroup B (69.2 %), followed by serogroups C (6.2 %), Y (3.2 %), W135 (3.2 %), and X (1.3 %). The percentage of laboratory confirmed IMD cases increased from 89.6 % in 2010 to 93.8 %. In 2011, 50.8 % of IMD cases were confirmed by PCR, with PCR being the only method to reveal positivity in 12.3 % of IMD cases. It is highly desirable that the PCR diagnosis should be continued until serogroup identification. The proportion of IMD cases caused by unidetified serogroups dropped from 23.9 % in 2010 to 16.9 % in 2011. Since 1993, the NRL has been performing multilocus sequence typing (MLST) of all IMD isolates. High IMD morbidity and mortality in the mid 1990s was caused by N. meningitidis C hypervirulent clone cc11. The following clonal complexes were most frequently associated with IMD in 2011: cc41/44 (17.9 %), cc269 (10.3 %), cc18 (10.2 %), and cc32 (10.2 %), all of serogroup B. Only 5.1 % of isolated from IMD were assigned to serogroup C clonal complex cc11. Molecular methods allow the investigation of epidemiological relationships. In 2011, no secondary case of IMD was identified based on the analysis of epidemiological data and isolate genotypes.

Conclusions: Molecular methods are essential in IMD surveillance. In 2011, 93.8 % of IMD cases were laboratory confirmed in the CR. The non-culture PCR method confirmed 50.8 % of IMD cases and was the only one to detect positivity in 12.3 % of IMD cases.

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P 104

2011 Update of the Vaccination Strategy in the Czech Republic

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The vaccination strategy against meningococcal disease is regularly updated in the Czech Republic (CR) to reflect changes in the epidemiological situation and availability of meningococcal vaccines. The National Reference Laboratory for Meningococcal Infections (NRL) analyses the surveillance data, including molecular characterisation of isolates and produces recommendations for vaccination against meningococcal disease for use by the National Immunisation Committee. A MenC conjugated vaccine was available in the CR since 2001, a tetravalent conjugate vaccine A,C,Y,W135 for the age above 11 years was launched in October 2010 and a new A,C,Y,W135 conjugate vaccine for all ages including small infants will be available in autumn 2012. A MenB vaccine other than porin-based is required. The involvement of serogroup Y in IMD cases has increased in the last years, causing the highest serogroup-specific case fatality rate. In 2011, no death was due to serogroup C while serogroup W135 caused a fatal IMD case. The vaccination strategy against meningococcal infection is based in the CR on building long-lasting individual protection and not population immunity. The following vaccination guidelines have recently been submitted by the NRL to the National Immunisation Committee: vaccination of infants aged 2-6 years with tetravalent conjugate vaccine A,C,Y,W135; booster vaccination (and/or primary vaccination) of pre-adolescents aged 11-14 years with tetravalent conjugate vaccine A,C,Y,W135; the 7-10-year booster interval should be shortened when epidemiologically or clinically indicated. The guidelines will be discussed by the National Immunisation Committee when a new tetravalent conjugate vaccine A,C,Y,W135 for infants is available in the CR. The registration of a new MenB vaccine is expected and the vaccination strategy is discussed. The MenB vaccine is planned to be incorporated into the infant immunization schedule at the age of 2-6 months, as the highest age specific incidence of IMD is caused by serogroup B in under-one-year-olds. The work was supported in part by grant NT11424-4/10 of the Internal Agency of the Ministry of Health of the Czech Republic.

P 105

Typing of Romanian *Neisseria meningitidis strains at Institut für Med. Mikrobiologie und Hygiene, Graz, Austria, as part of ECDC/IBD – labnet activities*

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Background: IBD labnet – ECDC funded laboratory network for the surveillance of invasive bacterial diseases (2008).
Aim: to study Romanian meningococci circulating strains, in the IBD-labnet training program, supported by ECDC.
Methods: 14 *N. meningitidis* strains coming from CSF, were collected at the National Reference Laboratory from Cantacuzino Institute and analysed at the National Reference Laboratory for *N. meningitidis* – AGES in Graz.
The strains were tested by phenotypical and RT-PCR method for species identification, serogrouping was performed by agglutination with Remel sera (for culture strains) and RT-PCR, and susceptibility to antibiotics (penicillin, ceftriaxone, rifampicin, ciprofloxacin) by E test. Por A was also performed for 11 strains and fet A for 7 strains.
Results: three non-culture strains analysed by RT-PCR revealed one group B and two group nonB-non C. The other 11 strains tested, revealed 45.45 % group B and 54.54 % group C. The susceptibility testing results were analyzed according to EUCAST v.1.3.
Two strains were penicillin resistant and nine strains showed penicillin intermediate resistance.
Concerning Por A, three strains belonging to group B, revealed P. 22,14-26,36. The other meningococcal strains showed the following aspects: P. 7,10-8,36-2 (2 strains), P.5-2,2-2,36-2 (1 strain), P.5-1,10-8,36-2 (2 strains), P.12,2-2,36-2 (1 strain) and P.7,16,35 (2 strains).
Conclusions: the study was limited by possible underestimation of the incidence of invasive meningococcal disease in Romania and susceptibility to penicillin in meningococci should be monitored in the future.

P 106

The current status of invasive meningococcal disease in Poland

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The aim of the study was to characterise invasive meningococcal disease (IMD) in Poland in 2011.
Material and methods: In Poland, epidemiological follow-up of IMD is based on mandatory notification of cases to the National Institute of Public Health-National Institute of Hygiene and on voluntary laboratory based surveillance conducted by the National Reference Centre for Bacterial Meningitis (NRCBM). The study encompassed all invasive isolates of*Neisseria meningitidis* received by the NRCBM in 2011. The isolates were identified, serotyped, characterised by susceptibility testing and MLST. A PCR technique was used for identification of the etiological agent directly from clinical materials in the case of a negative culture.
Results: In 2011, the NRCBM identified 303 of laboratory confirmed IMD cases (0.76/100.000). The incidence in patients under 1 and 5 years of age was 14.63 and 7.53, respectively. There were 213 invasive meningococcal isolates and 90 PCR-positive reactions with primers specific for*Neisseria meningitidis*. A serogroup was defined for 271 (89.4%) cases. Majority of IMD infections were caused by meningococci of serogroup B (MenB, n=170; 62,7%), followed by serogroup C (MenC, n=97; 35.8%), Y and W-135 (n=2, 0.7% each). Decreased susceptibility to penicillin (MIC ≥ 0.12mg/L) characterised 22.2% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 198 meningococci analyzed by MLST, 90 STs were found, although 68 of them were represented only by one isolate. More than 79.3% of isolates belonged to 11 known clonal complexes (cc). Among MenB isolates 66 STs and 14 cc were found; the most common were representatives of ST-32/ET-5cc (34.7%), ST-41/44cc (17.4%), ST-18cc (14.9%), ST-213cc and ST-269cc (5.0% each). MenC group was less heterogeneous with 24 STs and 10 cc identified. The most frequent were isolates of ST-103cc (37.8%), ST-41/44cc (17.6%) and ST-11cc (9.5%).
Conclusions: Poland, where population-based MenC vaccination was not introduced so far, belongs to countries with a low IMD incidence rate. In 2011, the number of IMD cases increased in Poland in comparison with previous year (n=227) as well as percentage of MenB isolates (62.7 vs 51.8%). Clonal complexes of ST-32/ET-5cc, ST-41/44cc and ST-103cc are well established in our country.

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Meningococci of clonal complex 103 in Poland

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Aim: The purpose of the study was to characterizeNeisseria meningitidis isolates, belonging to clonal complex 103, responsible for invasive meningococcal disease (IMD) in Poland.
Material and methods:The study encompassed all invasive meningococcal isolates collected between 1997 and 2011. Meningococci were identified, serotyped and characterized by MLST,porA andfetA typing. Minimal inhibitory concentrations were assessed by Etests and M.I.C.Evaluators.
Results: Until 2001 no cases caused by meningococci belonging to 103cc had been registered, but afterwards there were 126 cases of IMD, including 6 fatal cases among adults (4.8%). All isolates belong to serogroup C. Between 2002 and 2008 meningococci of 103cc represented a small proportion of all MenC isolates in Poland, but this has changed significantly over the following years and amounted up to 36.2% in 2009, 48.6% in 2010 and 36% in 2011. All isolates were susceptible to 3rd generation cephalosporins, chloramphenicol, ciprofloxacin and rifampicin, while 3.2% were nonsusceptible to penicillin with MIC 0.12mg/L. There was a very high level of resistance to trimethoprim-sulfamethoxazole (83.2%). MLST analysis showed that all isolates but three represent sequence type ST-5133. Interestingly, since 2004 there were 23 isolates which differ by one or 2 alleles from ST-5133 but not belonging to 103cc. Analysis ofporArevealed that the most commonvariant was 18-1/3 (75.4%) and 5-1/2-2 (9.5%), but there were also single isolates with other variants such as: 7/16, 7/30, 18-1/9 and 18-1/2-3. Three isolates did not haveporAgene. Sequencing of fetA indicated that all isolates of ST-103cc, except two, had F3-9 variant.
Conclusions: Isolates of ST-103cc, present in Poland since 2002, are currently the most common among MenC meningococci. The case fatality ratio connected with this clonal complex is lower than the one observed for ST-11cc, which was widely represented in Poland between 2006 and 2008. Molecular analysis ofporAandfetAshows that they constitute a homogenous group. Antimicrobial susceptibility tests indicate that they are susceptible to antibiotics used in IMD therapy.

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Investigation of meningococcal disease outbreaks in Polish voivodeships in 2006-2008

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Background: *N. meningitidis* infection is characterized by high fatality rate, substantial sequelae and has a potential to cause epidemics. Invasive Meningococcal Disease (IMD) occurs endemically in Poland. Annually 200-350 cases are notified to the Polish surveillance system (incidence rate of 0.54-1.03/100 000 inhabitants). The sanitary and epidemiological stations are statutorily obliged to undertake containment strategies in case of outbreak to prevent the spread of the disease.

Aim: To quantify the activities undertaken by sanitary and epidemiological stations within the meningococcal outbreaks in three voivodeships in Poland in 2006-2008 with a focus on administration of chemoprophylaxis, collection of throat swabs and vaccination.

Methods: A retrospective analysis of an existing database of thestructured data collected by the voivodeship sanitary and epidemiological stations. The data on management of the outbreaks was supplemented by surveillance data and information on tests results from National Reference Centre for Bacterial Meningitis.

Results: Of the total of 39 IMD cases notified, 28 (71.8%) were diagnosed as septicaemia, 8(20.5%) as meningitis accompanied by septicaemia and 3 (7.7%) as meningitis. The age distribution of the analyzed cases ranged between 2 to 36 years with the predominance of teenagers(51.2%). The complications appeared in 43.6% (17/39). Four deaths (10.3%) were reported. The laboratory confirmation revealed that the serogroup C meningococci was indentified in 21 cases (53.8%). Out of 1367 close contacts, i.e.: family members, medical personnel, colleagues to the cases, 1 288 (94.2%) were administered chemoprophylaxis, from 191(13.9%) throat swabs were collected and 107 (7.8%) were vaccinated. In the course of vaccination campaign 39 464 (circa 95%) persons from the population at risk of developing meningococcal disease were vaccinated. These public health measures to prevent the spread of the disease were associated with a workload of 2 416 work hours.

Conclusion: In addition to the treatment of IMD, the resources associated with the containment of *N. meningitidis* outbreak in Poland are important. Measures should be undertaken to prevent such outbreaks. A routine vaccination programme could reduce this burden.

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Costs associated with meningococcal disease outbreaks in Poland in 2006–2008

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Background:Invasive meningococcal disease (IMD) has a considerable economic impact on the health care system. Some of these costs relate to outbreak management that requires coordinated efforts from various institutions. The percentage of serogroup C meningococci has increased over recent years which will lead to additional costs for managing associated outbreak. Implementation of vaccination could reduce this burden.

Aims: To assess the costs of the activities (acute IMD care and containment strategies of close contacts and population at risk of developing meningococcal disease) undertaken to control the outbreak in 2006-2008 in three voivodeships in Poland.

Methods:A retrospective analysis of an existing database of structured data collected by the voivodeship sanitary and epidemiological stations. The data was supplemented by surveillance data and information on hospitalization and diagnostics costs from institutions involved in management of meningococcal cases.

Results:Seven outbreaks of invasive meningococcal disease were notified to the Polish surveillance system involving in total 39 IMD cases over the study period. The total cost associated with these outbreaks was PLN 2 597 021,06 (PLN 66 590,3 per notified case). The vaccination campaign accounted for 75.1 % (PLN 1 948 766,2) of the total cost. The costs of hospitalization and laboratory diagnostics accounted for 21.5% (PLN 559 494,0) and 2.2% (PLN 57 304,0), respectively. The costs of the chemoprophylaxis, throat swabs collection, vaccination of the close contacts and rehabilitation were relatively low, with a respective share of 0.7% (PLN 17 702,2), 0.2% (PLN 6 174,0), 0.2% (PLN 5 691,2) and 0.1% (PLN 1 890,00).

* Euro = PLN 3,78 (2007)

Conclusion: The cost burden associated with IMD outbreak management in Poland is relatively high. The implementation of the routine vaccination could contribute to the decrease in the number of meningococcal cases and associated costs.

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Epidemiological Surveillance for Invasive Meningococcal Disease – Russian Federation, 2010

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Question: Incidence of the invasive meningococcal disease (IMD) in the Russian Federation shows at present, since 2004, a tendency for a slight decrease. A wide spectrum of epidemiological parameters was used to describe the current characteristics of IMD in Russia.
Methods: The study includes the data of two systems of epidemiological surveillance: the official one (Federal State Statistical Monitoring) and non-official one (personalized register of IMD cases in the Russian Federation by the Reference Centre for Monitoring of Bacterial Meningitis) for 2010.
Results: Incidence of IMD decreased from 2.02 per 100,000 people in 2004 to 0.98 per 100,000 people in 2010. The overall number of IMD cases in 2010 was 1428, of which only 619 were confirmed in laboratory (43.3%). Serogroup landscape of meningococci isolated from lab-confirmed cases was diverse: 27% were meningococci of serogroup A, 32% were of serogroup B, 20% were of serogroup C, 2% were of other serogroups, and the serogroup was not determined in 19% of cases. The proportion of children <14 y.o. was 69.6% (994 cases), of which the percentage of children <5 y.o. was 55.8% (555 cases) and that of children <1 y.o. was 24.2% (241 cases). Of the overall number of the cases, the highest percentage corresponded to children, which did not attend day-care centers (50.6%). Proportion of males was somewhat higher than females (58%). The number of fatal cases was 223 with case-fatality ratio (CFR) of 15.6%. The highest CFRs were observed in children <1 y.o. and in elderly persons ≥65 y.o. (20.5% and 31.7% respectively). The lowest CFR was observed in the age category of 10-14 years (6%). Phenotypic and genotypic analysis was carried out for some strains of meningococci isolated from cerebrospinal fluid of patients with IMD. Of the four strains of serogroup A meningococci belonging to the ST-1 clone complex/subgroup I/II, three were attributed to sequence type 75 and one - to sequence type 3349. All strains were phenotypically similar and were characterized as 5-2 on PorA VR1, as 10 on PorA VR2, and as F3-5 on FetA VR. Vaccination inRussiais carried out by polysaccharide vaccines (multivalent meningococcal conjugate vaccines [MCVs] is not licensed for use in Russian Federation yet) according to epidemiologic indications in IMD focal outbreaks or in areas with increased/increasing incidence of IMD among the risk cohorts.
Conclusion: The incidence of meningococcal disease in the Russian Federation is quite low although, taking into account the size of population in Russia, the number of cases registered annually is high. In 2010, menigococci of serogroups A, B, and C were identified in roughly equal proportions. Highest incidence is observed in young children and infants. Most often, fatal cases also occurred in young children and infants as well as in elderly persons. The data on phenotypic and genotypic structure of the strains of meningococci of serogroup A are similar to the structure of the strains circulating in the interepidemic period. Availability of MCVs in the near future in Russia will help to control IMD in the high-risk groups identified by established epidemiologic surveillance systems.

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Molecular epidemiology of *Neisseria meningitidis* in the United States before and after quadrivalent meningococcal conjugate vaccine introduction

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Background: *Neisseria meningitidis* (Nm) serogroups B, C and Y are the major causes of meningococcal disease in the US. A quadrivalent meningococcal conjugate vaccine (MenACWY) was licensed in 2005 in the US to protect against serogroups A, C, W135 and Y disease and is recommended for adolescents aged 11-18 years. Coverage with MenACWY among 13-17 year olds was 11.7% in 2006 and 62.7% in 2010. To monitor changes in the molecular epidemiology of Nm in the US, we compared the molecular features of Nm stains before and after vaccine introduction.
Methods: Nm strains from 2006-2010 (n= 610) were collected through population-based Active Bacteria Core surveillance and characterized by multilocus sequence typing (MLST) and genotyping outer membrane proteins PorA and FetA. Molecular features of these strains were compared to previously-published data on 1175 strains from 2000-2005 to determine changes that occurred between the two periods using Monte Carlo methods. All analyses were done using SAS version 9.2 (The SAS Institute, Cary, NC).
Results: A total of 311 sequence types (231 in 2000-05 vs 125 in 2006-10), 27 assigned clonal complexes (cc) (25 in 2000-05 vs 21 in 2006-10), 142 PorA types (108 in 2000-05 vs 66 in 2006-10), and 51 FetA types (45 in 2000-05 vs 33 in 2006-10) were identified. The majority of the common molecular types that appeared in ≥ 5 strains from 2006-10 also predominated in 2000-5, with particular molecular types associated with specific serogroups. New molecular types were detected in 2006-10. Significant changes were observed in the distribution of various PorA, FetA or PorA:FetA types among vaccine serogroups between the two periods ($p<0.001$), but not among other serogroups. The distribution of strain genotypes (cc:PorA:FetA) showed significant changes ($p<0.001$) among both vaccine serogroups and serogroup B. The proportion of strains that had undergone capsular switching was 5.5% and 7.5% among all age groups ($p= 0.26$), and 6.5% and 6.8% ($p= 0.96$) among adolescents aged 11-20 years, before and after vaccine introduction, respectively.
Conclusions: Consistent with previous observations, the population structure of US meningococcal strains is dynamic with some changes occurring over time, but the basic structure maintained. Changes in the distribution of strain genotypes among serogroups between the two eras are likely driven by horizontal gene transfer events, not selected by vaccine, as these changes were present in both vaccine and non-vaccine serogroups. Lack of significant change in the proportion of capsular switching strains in 2006-10 suggests that capsule switching is a natural event in meningococcal population that is not driven by vaccine selection. The impact of vaccine on molecular epidemiology of Nm in the US needs to be reevaluated after sustained high MenACWY coverage among adolescents.

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Geotemporal Analysis of *Neisseria meningitidis* clones in the United States: 2000–2005

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Background: Historically the detection of meningococcal outbreaks has relied on serogrouping and traditional epidemiologic methods. Advances in molecular epidemiology have improved the ability to distinguish unique *Neisseria meningitidis* strains, enabling the classification of isolates into clones. Around 98% of meningococcal cases in the United States are believed to be sporadic.
Methods: Meningococcal isolates from nine Active Bacterial Core surveillance (ABCs) sites (approximately 38 million persons under surveillance) from January 1, 2000 through December 31, 2005 were classified according to serogroup, multilocus sequence typing, and outer membrane protein (OMP) (porA,porB, andfetA) genotyping. A clone was defined as isolates that were indistinguishable according to this characterization. Census tract-level population data for ABCs surveillance sites were obtained from the U.S. Census Bureau Census 2000 Summary File (SF1) 100-Percent Data Set. Case data were aggregated to the census tract level and all non-singleton clones were assessed for non-random spatial and temporal clustering using retrospective space-time analyses with a discrete Poisson probability model.
Results: 1,063 cases were geocoded and had isolates available for analysis. 438 unique clones were identified, 78 of which had ≥2 isolates. 702 cases were attributable to non-singleton clones, accounting for 66.0% of all geocoded cases. 34 statistically significant clusters comprised of 112 cases (10.5% of all geocoded cases) were identified. Clusters had the following attributes: included 2 to 11 cases; 2 days to 41 months duration; radius of 0 to 141 km; and attack rate of 0.4 to 57.8 cases per 100,000 population. Serogroups represented among the clusters were: B (n=12 clusters, 45 cases), C (n=13 clusters, 32 cases), and Y (n=9 clusters, 35 cases); 22 clusters (64.7%) were caused by serogroups represented in meningococcal vaccines. Scans of contiguous surveillance areas detected a cluster spanning the Georgia-Tennessee border comprised of more cases (n=7) than the separate clusters of that clone identified independently in each state. None of the clusters met the CDC definition of an outbreak.
Conclusions: Around 10% of meningococcal disease cases in the U.S. could be assigned to a cluster. Molecular characterization of isolates, combined with geotemporal analysis, is a useful tool for understanding the spread of virulent meningococcal clones and patterns of transmission in populations.

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Population-based, Geographic and Molecular Assessment of *Neisseria meningitidis* Over Two Decades (1989-2010) in Metropolitan Atlanta, Georgia

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Question: To evaluate the incidence, geographic and temporal trends and the spread of invasive clonal complexes (CCs) of *N. meningitidis* in Atlanta between 1989 and 2010.
Methods: Active population-based laboratory surveillance was conducted in Atlanta (8 counties 1989-96, expanded to 20 counties 1997-2010) through the CDC-funded ABCs Program. Molecular typing was performed using multi-locus sequence typing (MLST) of meningococcal isolates to identify CCs. Cases were geocoded using ArcMap10 to geographically and temporally assess invasive meningococcal disease.
Results: A total of 468 "sporadic" cases of invasive meningococcal disease were detected between 1989 and 2010; 232 cases (50%) occurred in adults 18 years of age or older. Reflecting the U.S. national trend, the incidence decreased from 1.25/100,000 population in 1989 to 0.78 in 1999 to just below 0.1 in 2010 (χ^2 for linear trend, $p = 0.007$). Serogroup C accounted for a majority of the cases in the first decade of the surveillance. The incidence of serogroup C meningococcal disease significantly diminished from 0.81 in 1989 to 0.20 in 1999 to 0.02 in 2009 (χ^2 for linear trend, $p < 0.001$). Incidence rates of serogroup B varied between 0.1 and 0.38/100,000 in the first decade of the study, but declined after 2001. Serogroup Y disease emerged in the mid-1990s, peaked in incidence in 1997 (0.6/100,000), and continued to cause disease at lower rates since then. MLST typing has to date identified CCs of 258 invasive meningococcal isolates. Almost all serogroup C and Y disease was caused by isolates in CC ST-11 and CC ST-23, 94% and 93% respectively, suggesting that closely related strains were circulating in the community and causing sporadic disease. Serogroup B disease strains were more heterogeneous, but three distinct genetic lineages, ST-41/44 (37%), ST-32 (20%) and ST-162 (11%) were most common. Geocoding demonstrated that geographically clustered cases, most without recognized epidemiologic association, appeared over months to years, then became broadly distributed in the surveillance area. Serogroup Y ST-23 cases, for example, emerged during 1993-1997, and were initially clustered in one county. From 1997-2001, ST-23 cases predominated in two adjacent counties. After 2001, this clustering effect, seen in the first decade of surveillance, disappeared. During 1989-1997, two adjacent surveillance counties averaged more than 1.5 meningococcal cases/100,000 annually, representing over half of all Atlanta cases; strains were mostly ST-11, serogroup C. In contrast, the distribution of ST-41/44 and other predominantly serogroup B CCs were geographically dispersed throughout each time interval and persisted in causing invasive meningococcal disease over 20+ years in the population.
Conclusions: Active surveillance for serogroup-specific invasive disease combined with sequence typing and geocoding provides a useful method for closely monitoring trends in meningococcal disease over time. A detailed understanding of meningococcal disease in a community will affect recommendations for future vaccine or other prevention strategies.

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Molecular epidemiology of serogroup C meningococcal disease outbreaks caused by three different clonal complexes of *Neisseria meningitidis*, Rio de Janeiro State, Brazil

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Background: This study reports molecular surveillance of clusters of meningococcal disease in the Rio de Janeiro State, Brazil.
Methods: The genetic background of *Neisseria meningitidis* associated with case clusters or outbreaks were analysed by MLST and sequencing of OMP genes: *porA*, *porB*, and *fetA*.
Results: Since 2000, the number of cases of serogroup C disease steadily increased, and now it is responsible for more than 90% of all confirmed cases. Between 2003 and 2012, 4 outbreaks involving 46 cases and 4 case clusters involving 17 cases were identified; all caused by serogroup C *N. meningitidis*. ST-3780 (cc103), ST-7708 (cc103), ST-11 (cc11), and ST-639 (cc32) were responsible for the outbreaks, whereas ST-7696 (cc32), ST-3779 (cc103), and ST-not yet assigned were responsible for the case clusters. The overall case fatality rates were 22% and 29%, respectively. The average age of patients was 12-years old (range, 3 months to 42 years). At admission, 76% presented with a non-blanching rash. Immunization was implemented for two outbreaks, once in 2003 and again in 2008.
Conclusions: The use of molecular approaches identified three hypervirulent clones exhibiting serogroup C capsule causing outbreaks and clusters of meningococcal disease in the same region. All outbreaks and clusters were potentially vaccine preventable with monovalent serogroup C meningococcal conjugate vaccine.
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Emergence of Meningococcal Disease Serogroup C in Salvador, Brazil

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Neisseria meningitidis has become a leading cause of bacterial meningitis after dramatic reductions in the incidence of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b infections by the use of conjugate vaccines. Recent estimates indicate that there are more than 500,000 cases of meningococcal meningitis each year worldwide, with a lethality rate of 10%. In Brazil, serogroup B was associated with the majority of meningococcal disease during the 1980s, with a peak in 1996. Since 2001, however, the number and proportion of cases due to serogroup C are increasing markedly, followed by a reduction in the cases due to serogroup B. The aim of this study was to describe the incidence of meningococcal meningitis in Salvador, Brazil, analyzing the clinical and epidemiological characteristics of the cases, and to determine the laboratorial characteristics and the clonal distribution of the isolates. Therefore, a prospective cohort was conducted during the period of 2001-2011 in a reference hospital for the treatment of infectious diseases in Salvador, Brazil. Clonal diversity was studied by PFGE and MLST. In this period, 375 patients with meningococcal meningitis were included in the study, from whom 183 cases were due to serogroup B and 181 for serogroup C. Patients infected with serogroup B were younger than those infected with serogroup C ($p<0.0001$). There was no significant difference in terms of intensive care unit admissions, lethality rate and cerebrospinal fluid's profile comparing the two serogroups. From 2001 to 2006, serogroup B was the most frequent isolated in the population, with an incidence rate varying from 0.25 to 1.27 cases per 100,000 inhabitants. From 2006 on, serogroup C incidence exceeded that of serogroup B, which was responsible for zero cases in 2010, while serogroup C had an incidence of 1.02 cases/100,000 inhabitants in the same period. The isolate C:23:P1.14-6, which was a circulating isolate since 2001, was responsible for the major proportion of cases in this period. PFGE patterns have confirmed that the emergent clones of serogroup C were related to those circulating previously. In conclusion, meningococcal meningitis is still a life-threatening disease responsible for high incidence and lethality rate in the population studied. In the past 5 years, the molecular epidemiology of this disease has changed in Salvador, Brazil, where serogroup C is now responsible for the majority of cases and the most frequent isolate is present since 2001.

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Invasive *Neisseria meningitidis* strain expressing capsular polysaccharides W135 and Y in Brazil

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Background: *Neisseria meningitis* expressing dual capsules has rarely been described. We present a description of a *N. meningitidis* expressing two capsule polysaccharides isolated in 2008 from a patient from the interior of Rio de Janeiro State, Brazil.
Methods: The bacterium was isolated from the CSF of a 3-year old girl. Serogroup was determined by slide agglutination with specific rabbit antisera (BD Difco, Maryland, USA) and serogroup-specific PCR (genogroup). Serotype and serosubtype were determined by immunoblot at the National Meningitis Reference Centre (IAL-SP). MLST and sequencing of outer membrane protein genes: *porB*, *porA*, *fetA*, and *fHbp* and DNA sequencing of the *siaD* gene were performed.
Results: Although the isolate was identified as serogroup Y by genogrouping it agglutinated with both anti-W135 and anti-Y antisera. Subcapsular antigens were characterized as 17,7:P1.5 by monoclonal antibodies. The genotype was defined as 3-100:P1.5-1,10-80:F1-7:ST-7694 (cc175); fHbp in variant 3/subfamily A (peptide ID 162). Sequence of *siaD* gene encountered a point mutation resulting in a change at amino acid position 310, which encoded for serine instead of glycine ($siaD_{y}$) or proline ($siaDw_{135}$) that leads of this dual antigenic specificity.
Conclusions: The isolate described here has common subcapsular antigens of serogroup Y isolates in Rio de Janeiro and belongs to a cc that has been typically associated with serogroup Y in other countries of Latin America. It was found when serogroups W135 and Y emerged causing substantial number of clinical cases in this continent. The monitoring of such isolates has implications for routine surveillance.
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Cross-sectional survey of *Neisseria meningitidis* carriage among the population aged 1-45 years, Shanxi province, China

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Background and aims: Since 1985 and the introduction of the meningococcal polysaccharide A vaccine, the reported incidence of meningococcal disease in China has decreased to a rate of 0.2 per 100,000 since year 2004. Knowledge of the current *Neisseria meningitis* (*Nm*) carriage status and associated risk factors is important to optimize effective immunization programs.

The aims of this study were to describe the carriage rates and spectrum of the isolated *Nm* strains and the associated risk factors among the target population in one province of China.

Methods: This was a cross-sectional survey, including 2000 healthy participants, randomly selected and allocated to one of four predefined age-groups in two counties (Xiangfen/Fenyang) of the Shanxi province. The participants were 1-45 years old and systematic swab and blood collection was performed in October-November 2009.

The analyses were descriptive and the carriage rate calculated with the 95% confidence intervals using the exact binomial distribution for proportions. The univariate analyses of the associated factors with the carriage rate were calculated using chi-squared tests.

Results: Of the 2000 participants, 282 *Nm* carriers were identified corresponding to a carriage rate of 14.10% (CI 95%: 1.60-15.70) without a county effect.

The carriage rate was only significantly associated with the age (10-24 years age-group/other age-groups: 18.30%/5.16%), sex (male/female: 15.95%/12.33%), epidemic meningitis vaccine exposure (exposed/unexposed: 6.58%/16.78%), exposure to antibiotic treatment within the last 30 days (Yes/No: 14.73%/9.26%) and living conditions of the participants (living in institutions/at home: 20.69%/8.34%).

Among the 282 *Nm* strains, 146 (51.7%) were non-groupable, other 136 were identified with different sero-group (74 group B, 21 group C, 12 group Y, 7 group A, 5 group X, 4 group W135, 1 group H). The group-B strain was the most prevalent in the two counties and among all age groups.

Conclusions: The results of this study confirm that carriage of meningococci is most prevalent in adolescents and young adults and that living conditions can affect the carriage rates. Furthermore, previous meningococcal vaccination and exposure to antibiotics may decrease carriage for a period of time after administration. Although serogroup B was the most prevalent strain in the population, the presence of other virulent strains such as serogroup A, C, W135, Y and X underline the potential of these strains to cause disease and highlights the need of primary prevention with serogroup B and other multivalent meningococcal vaccines.

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A meningococcal carrier survey, in Italy

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Background: Meningococcus colonizes the upper respiratory tract, mainly without causing disease. It spreads directly from host to host. In Europe and in other countries, it has been estimated approximately 25% of the adolescents are healthy carriers [1].
M&M - 157 subjects, age 14-22, underwent 4 rounds of throat swabbing. Swabs were taken at time points spaced one month, over a three month time period. Swabs were plated onto selective medium, to discriminate Neisseria meningitidis carrier strains from other commensal species. In parallel, RT-PCR of samples extracted from each swab were performed, also for meningococcal species identification. Colonies grown on selective medium were plated on enriched medium, and DNA was extracted. Molecular methods were used for species confirmation, and for assessment of capsule group. Subtype, cc and ST of all strains were determined. Also, sequence diversities and MATS of the 4CMenB components were assessed in all strains (results in Simmini et al., poster no 271)
esults: 32/157 subjects were found positive for meningococcus at one or more swab sessions. The carriage incidence, calculated as average of four swab sessions, was 11%. In case meningococcal isolation occurred in the same subject at successive swabs, the carried strain features were compared, to distinguish between the persistence of the same carrier strains and colonisation by new strains. All subjects carrying meningococcus at multiple swabs were carrying the same strain isolated at the first respective positive swab. Three subjects had been carrying the same respective strains over the whole survey time period. Ability of strains to spread through the community was analysed. Two strains were isolated in more than one subject. In both cases, individuals colonised by the same strain were close contacts.
Discussion and Conclusions: Serological and genetic features of all carrier strains isolated during this study were assessed. Higher diversity of carrier strains, once compared to pathogenic strains was confirmed in terms of capsular group, cc, ST and PorA subtype. Also, carriage persistence and different strain spreading capabilities were compared. Three strains demonstrated carriage persistence for three months, the longest possible in this study.
[1] Claus H, Maiden MC, Wilson DJ, McCarthy ND, Jolley KA,Urwin R, et al. Genetic analysis of meningococci carried by children and young adults. J Infect Dis 2005; 191 April (8): 1263-71.

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Oropharyngeal carriage of *Neisseria meningitidis* in adolescents and young adults in the United Kingdom: results from a longitudinal swabbing study

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Question: Data on the epidemiology of oropharyngeal carriage of *Neisseria meningitidis* are important in informing vaccine strategy, since vaccines which impact on colonisation may induce herd immunity. We therefore sought to describe the current epidemiology of oropharyngeal carriage of *N. meningitidis* in adolescents and young adults in England in 2011-2012. **Methods:** Oropharyngeal swabs were taken on 2 or 3 occasions over a 4 to 6 month period from students at secondary (high) school and the first year of university. Students enrolled in the penultimate year of secondary school had swabs taken at an additional visit approximately one year after enrolment. Two swabs were taken at each visit. One swab was immediately plated onto Thayer-Martin improved medium, and colonies suspected to be *N. meningitidis* were characterised further. The *N. meningitidis* isolates were assessed by slide agglutination and PCR to determine serogroup and genogroup respectively. The other swab was screened by PCR to determine if *N. meningitidis* could be detected without culture. **Results:** Swabs were obtained from 977 participants from March to May 2011, 877 in June/July (59 of whom were newly recruited), 918 in September/October and 166 in February/March 2012. Participants were aged between 10 and 25 years at enrolment from 13 schools and 5 universities in London, Southampton, Oxford and Bristol. At the first visit *N. meningitidis* was isolated from 137 (14.0%) swabs, from which 70 (7.2%) had a serogroup successfully identified by agglutination from 68 individuals. Amongst these, serogroup Y was most common (22 isolates, 2.3%), followed by serogroup B (15, 1.5%) and serogroup C (9, 0.9%). Carriage rates of *N. meningitidis* were 17/369 (4.6%) in 10-14 year olds, 20/182 (11.0%) in 15-16 year olds, 49/254 (19.3%) in 17-18 year olds and 51/172 (29.7%) in 19-25 year olds. Carriage rates were 40/283 (14.1%) in the final 2 years of school and 68/219 (31.1%) at university. Serogroup Y was identified in 11 (5.0%) of the swabs from university students, while 9 (4.1%) were serogroup B. A trend to increased rates of carriage with increased age was seen for most serogroups, for example serogroup B increased from 4/551 (0.7%) in 10-16 year olds to 4/254 (1.6%) in 17-18 year olds and 7/172 (4.1%) in 19-25 year olds. Carriage of serogroup C was seen in 5/254 (1.57%) of 17 to 18 year olds. No consistent changes in carriage rates were observed across successive visits. **Discussion:** The increase in meningococcal carriage rates observed during late adolescence in this study suggest that, if immunisation was to have an impact on the oropharyngeal carriage of meningococci, vaccines should be administered prior to the rise in colonisation, i.e. in the early teenage years. Consistent with other recent UK studies serogroups B and Y continue to be the most commonly carried serogroups in the age groups studied.

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Quality Improvements for the non-culture (PCR) Confirmation of Meningococcal disease in England

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Since 1997, the Health Protection Agency (HPA) Meningococcal Reference Unit (MRU) has used real-time Taqman™ *ctrA* PCR to confirm meningococcal disease direct from clinical samples in a duplex assay with *ply* (pneumolysin) to detect *Streptococcus pneumonia*: Such that 55% of current meningococcal cases are PCR only confirmed. Confirmation of group B (>85% of cases in the UK) required a subsequent $siaD_B$ PCR assay. In order to improve the quality of the service a four-component, freeze-dried, multiplex assay was introduced from November 2011.

To the duplex assay were added the $siaD_B$ and an in-house internal control (IC) in a stable, freeze-dried, PCR reaction mix supplied as either 8 well strips or 96-well plates; reducing operator input. Since 2003, the ctrA assay included an additional reverse primer (5'-TTGCCGCGGATTGGCCACCA-3') to that published (Corless *et al*., 2001) to detect the small number of clinical samples negative with the original assay. To assess sample inhibition and reagent function an in-house full process IC assay was developed using plant sequences (hydroxypyruvate reductase from *Cucurbita* cv. *Kurokawa amakuri*). All clinical samples were processed using Qiagen MDx™ (for nucleic acid extraction) and Universal™ liquid handling workstation. 5510 clinical samples (representing 4807 patients) were tested with the quadraplex assay to yield 339 *ctrA* positive samples (277 patients) of which 289 samples (237 patients) were confirmed as group B. Turnaround time improved to 1.2 days, including group B confirmation. Twelve inhibitory samples were observed of which 4 were blood culture bottle fluids. To date, no clinical samples have proven $siaD_B$ positive but *ctrA* negative. In addition, no meningococci have been isolated from matchedctrA negative clinical samples (same site and time).

The introduction of the quadraplex PCR assay has improved quality; the reassurance provided by the IC detection, the simultaneous confirmation of group B, fewer manipulations and overall reduction in turnaround times for the majority of meningococcal positive samples. The multiplex approach for screening and group B identification also provides a mechanism for identifying sequence variants in the *ctrA* gene and illustrates the potential for the emergence of variants that may present problems in PCR based assays, highlighting the need for continued surveillance of both invasive disease and carrier isolates.

Corless CE,*et al*. 2001. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicaemia using real-time PCR. J. Clin. Microbiol. 39:1553-1558.

P 122

Serogrouping *Neisseria meningitidis* carriage isolates by slide agglutination and genotyping by TaqMan real-time PCR: a head-to-head comparison

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Background: *Neisseria meningitidis* (Nm) is the leading cause of bacterial meningitis in infants, adolescents and young adults. Although this organism is a common commensal and the throat is its only known reservoir, the relationship between carriage and invasive disease is not well understood. Nm serogrouping of invasive disease isolates by slide-agglutination serogrouping (SASG) assay, using well characterized, commercial antibody reagents against surface polysaccharides, identifies and distinguishes the five major disease causing serotypes (A,B,C,W and Y). The utility of this methodology has not been demonstrated with carriage isolates. Moreover, the assay has not been standardized: antibodies used in different labs are from different sources, and interpretation of the assay results is highly subjective. TaqMan real-time PCR is well suited to provide genogroup information that is more readily interpretable. In addition, it has the potential to be applied to both cultured isolates and directly to throat swabs without culture to provide an unbiased assessment of Nm carriage.

Methods: Invasive disease isolates are described in Murphy et al. 2009 (1). Carriage isolates were collected from healthy 9th grade and university age volunteers in a year-long epidemiology study to investigate prevalence ofN. meningitidis in these age groups as measured by carriage in the throat. Isolates were serogrouped using three methods (SASG (DIFCO), Flow cytometry (FACS) (2) and Dot Blot (3)) and were genogrouped by TaqMan. Concordance between methods was determined using a concordance rate for matching assay results for each isolate; the denominator was the total number of samples tested for the paired methods. The 95% confidence interval on the concordance rate was calculated using the Clopper-Pearson's exact method. Within each diagnostic method, Fisher's exact test was used to compare the proportion of strains not groupable between the isolates and the invasive strains.

Results & Discussion: 100% of the invasive isolates were genogrouped by TaqMan. The FACS and Dot Blot Assays identified serogroups for 98% of the isolates where as SASG recognized 81%. Concordance analysis confirmed that TaqMan, FACs and Dot Blot approaches had the highest level of pairwise concordance (>94.9% (88.6, 98.3)) compared with concordance between SASG and the other 3 methods(<78.8% (69.4,86.4). In contrast, fewer carriage isolates were typable by TaqMan (62%), and of these <39% were serogroupable by any method. This was due to the reduced ability of the strains to express capsule compared to invasive isolates ($p<0.0001$ by Fisher's exact test) and the accuracy of the serogrouping methods, as demonstrated by the low concordance to the Taqman assay (< 18.3% (11, 27.6)). This analysis demonstrates that carriage strains are less likely to harbor intact capsular polysaccharide operons than invasive disease isolates, and those carriage strains which are genogroupable have a reduced capacity to express capsule. These observations support the perspective that capsular polysaccharides play a critical role in virulence and highlight the inherent difficulties encountered when serogrouping carriage isolates.

1) Murphy, E, et al. 2009. JID 200:379 -389. 2) McNeil, LK. et al. 2009. Vaccine 27:3417-3421. 3) Longworth, E. et al. 2002. FEMS Immuno & Med Micro. 32:119-123

P 123

Optimal molecular typing methods for surveillance of invasive *Neisseria meningitidis*

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Question: Invasive meningococcal disease occurs mainly endemic, but also as less frequent disease clusters among teenagers and young adults. For surveillance of circulating invasive strains, designation is recommended by serogroup, PorA variable regions, FetA variable region, multilocus sequence typing (MLST) sequence type (ST) and clonal complex (cc). Further genotypic characterisation may be achieved by sequencing other genes encoding membrane bound proteins; *porB*, the *penA* gene involved in penicillin resistance and the *fHbp* gene encoding a novel vaccine component. For outbreak investigations, other molecular methods have also been proposed, such as highly variable multiple-locus variable number tandem repeat analysis (HV-MLVA).

The aim of this study was to describe the current epidemiology of invasive *Neisseria meningitidis* isolates in Sweden and to find an optimal molecular typing scheme with appropriate resolution power for both surveillance and outbreak investigations.

Methods: All invasive *N. meningitidis* isolates in Sweden from 2010 to 2011 (n=118) were included. Thirteen isolates were spatiotemporally associated in six different clusters. All isolates were characterised by MLST and sequencing of the *fetA, fHbp, penA, porA* and *porB* genes, and typed with MLVA using four highly variable loci.

Results: Serogroup Y corresponded to 39% and 52% of all invasive isolates in Sweden in 2010 and 2011 respectively, of these, 58% and 35% of the isolates were clonal (sulfadiazine resistant, with genosubtype P1.5-2,10-1,36-2, ST23, cc23, *porB* allele 3-36, *fetA* allele F4-1, *fHbp* allele 25 and *penA* allele 22). However, a sulfadiazine susceptible variant of the clone seems to be emerging. The above recommended strain designation had a lower discrimination index (94%) than described in previous studies. Replacing the *fetA* gene with *fHbp* only reduces the discriminative ability by 1% but *fHbp* typing may be more informative in the current vaccine era. HV-MLVA detected two small clusters with strong spatiotemporal connections and identical genetic profiles and separated one small spatiotemporal cluster where the strains belonged to the Y clone, and no connection between cases could be found.

Conclusions: Our study supports the current recommended typing scheme except for the *fetA* typing which could be replaced by *fHbp*. Furthermore, HV-MLVA should be used as a first hand rapid method with high resolution for outbreak identification.

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Genetic diversity of *Neisseria gonorrhoeae* is a continuing challenge for molecular detection of gonorrhoeae

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Identification of genetic targets specific to *Neisseria gonorrhoeae* for use in molecular methods of detection has been a challenge due to the close relatedness of the genome to that of other *Neisseria* spp., particularly *Neisseria meningitidis* and the propensity of *N. gonorrhoeae* to acquire DNA from other organisms. Considerable advances have been made in recent years and both commercial and in house assays have become more robust and are in routine use in many laboratories, exhibiting increased sensitivity over more conventional methods.

The *porA* pseudogene in *N. gonorrhoeae*, originally thought to be found as an expressed *porA* gene only in *N. meningitidis*, has inactivating deletions in both the promoter and the hypothetical PorA coding region and hence is not expressed. It is also sufficiently different from the *porA* gene in *N. meningitidis* to be useful for diagnostic purposes and the in house method, originally described by Whiley et al, (2005), is used both in situations where commercial assays are not appropriate or affordable and as a confirmatory assay. However, a clinical gonococcal isolate with a *Neisseria meningitidis porA* sequence which gave false negative results in this assay was reported from Australia in early 2011, with subsequent reports from Scotland and Sweden.

In this report we describe two gonococcal isolates that were received in December 2011, by the reference service at the Health Protection Agency, London, originally from urethral swabs from two male patients, not known to be sexual contacts. Both isolates were confirmed, biochemically and immunologically as *N. gonorrhoeae*, but repeatedly gave negative results with two different in house real-time PCR assays for the *porA* pseudogene. Further characterisation of these isolates identified the presence of a meningococcal *porA* sequence and that they reacted with meningococcal PorA antibody. Additionally they belong to serovar Bropyst, and to NG-MAST sequence type (ST) 5967 and MLST ST1901. These isolates are similar to the original description from Australia and one of the isolates from Scotland but different from the second isolate from Scotland and that from Sweden.

Gonococcal isolates that give false negative results with the *porA* pseudogene PCR have now been identified in four countries, three of which are in Europe and do not appear to be clonal. This report highlights the genetic diversity of *N. gonorrhoeae*, which remains a challenge for molecular detection.

Whiley DM, Sloots TP. Pathology 2005:37:364-70

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Presence of *porA* and examination of PorA expression in invasive *Neisseria meningitidis* isolates

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Introduction: Differences in the outer membrane protein, PorA, divides *Neisseria meningitidis* (Nm) into different subtypes that can be characterised both phenotypically and genotypically. PorA is considered one of the most immunogenic proteins expressed by Nm and is therefore of interest in a vaccine perspective. Based on existing and future PorA-containing vaccines, there is a general interest to investigate, not only, the prevalence of different subtypes but also the expression as well as the amount of expression of PorA in clinically relevant isolates. Previous studies have shown that the PorA protein is expressed in most isolates but the amount of expression varies. This variation of expression alternatively lack of PorA can be explained by several mechanisms like, "slipped-strand mispairing" during the replication in the promoter or in the coding sequence, point mutations or insertion of IS elements in the coding sequence or the deletion of the *porA* gene.

Aims: Assessing the presence of *porA* and examine the PorA protein expression in invasive Nm. Survey the function of the monoclonal antibody 9-1-P1.C by comparing the co-agglutination results with DNA sequencing and mRNA detection.

Material and methods: A total of 571 cases of invasive meningococcal disease occurred in Sweden during 2002-2011, 471 of these were culture confirmed in our laboratory. In the present study 29 Nm isolates were included, comprising all identified Nm isolates where the *porA* gene was not completely genosubtypable (using VR1, 2, and 3), alternatively the expression of PorA was not detected using the monoclonal antibody 9-1-P1.C from Zollinger/NIBSC.

In addition to genosubtyping and co-agglutination, the entire *porA* gene including the promoter region will be presented and expression and the amount of expression (mRNA) will be reviled using RT-PCR.

Results and discussion: Among the 29 isolates, 1 was negative and 3 were not fully genosubtypable and 27 were not detected by the monoclonal antibody. Experiment are ongoing were the entire gene is amplified and sequenced and by using a RT-PCR method the amount of mRNA is detected and the expression of PorA is quantified in pathogenic Nm.

Lessons about the expressed PorA protein itself, not only the detection of the gene via genosubtyping, is important in order to determine the protective efficacy of existing and future vaccines currently under development aiming to cover all the commonly occurring groups that cause meningococcal disease worldwide.

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Diversity and Temporal Trends after 5 years PorA Molecular Typing in Austria

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Introduction: The National Reference Centre for Meningococci (NRCM) identifies the serogroup and serosubtype for all strains submitted. In the year 2007 molecular typing of the PorA variants was implemented. For the first time all PorA variants(VR1,2,3) of the strains could be detected. After 5 years molecular typing it is feasible to assess the diversity and distribution of PorA variants and detect possible trends in Austria.

Methods: In the time period 2007-2011, 246 invasive Meningococcal disease (IMD) strains and 200 carriage strains were referred to the NRCM. Further 54 cases of IMD were diagnosed with PCR (1) at the NRCM. The PorA variants 1, 2 and 3 were sequence typed (1) from all 446 strains and 54 nonculture confirmed cases. These 500porAgenotypes are the basis of this retrospective study.

Results: The 500 genotypes, invasive and carriage, subdivide into 116 different PorA variant combinations. Serogroup B was the most prevalent serogroup during all 5 years. The most frequent PorA combination for the 244 serogroup B was P1.7, 16, 35 with 13.99%. Serogroup C was diagnosed 123 times, 56.1% were P1.5, 2, 36-2. Polyagglutinable (PA) strains were recovered 69 times. Here the most frequent combination was P1.5, 2, 36-2 with 13.04%. Thirty percent of the 30 serogroup Y were P1.5-2, 10-28, 36-2. Serogroup W135 was isolated in only 12 cases over the 5 year period. Twenty-five per cent were P1.18-1, 3, 35-1.

The PorA combination P1.5, 2, 36-2 was also the most frequent in all age groups. The second most common combination in the age group <4 years was P1.7, 16, 35. In the age group 15-19 years P1.7-2, 4, 37 was the second most frequent combination. The age groups <1 and 1-4 years show minimal distribution in the frequency of PorA combinations. In the age group 15-19, there is a much greater diversity and along with P1.5, 2, 36-2 other PorA combinations are more prominent.

In the 5 year period, VR1 saw an increase in the P1.5 family from 18.7% to 27%. The P1.7 group decreased from 36.6% to 19.8%. The VR2 P1.2 increased from 11.2% to 18.9%. No significant decreases in VR2 were registered. In VR3 the greatest increase was seen from 29.97% to 46.23% in the P1.36 group. The greatest decrease was by P1.35 (28.04%-11.3%) and P1.37 (15.9%-6.6%)

Conclusion: The results show a great diversity in the PorA variant combinations. The highest average percentage of 17.5% lies by P1.5, 2, 36-2. Of the other variant combinations, 80% are represented < 1.0%. As expected, the combination P1.5, 2, 36-2 was also found most often in the risk age groups. Here the greater diversity is found in the 15-19 age group. The trend analysis of the single PorA variants shows temporal trends in the VR 1 and 3.

1) Molling P et al., Direct and rapid identification and genogrouping of Meningococci and porA amplification by LightCycler PCR. J Clin Microbiol. 2002; 40(12):4531-5.

P 127

Extra Genital Gonorrhea Testing in a multi Ethnic Study Clinic

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Background: Extragenital infections with gonorrhea (GC) can be missed if screened only by routine methods of urethral or urine specimens. The reported prevalence of pharyngeal GC in men who have sex with men has varied from 5.3-9.2% and there is limited data in women. The objective of this study was to determine the rates of pharyngeal and rectal GC infection in MSM and women in the Miami Dade County Health Department (MDHD) STD clinic.

Methods: Routine screening for pharyngeal GC infections in MSM was implemented in the MDHD STD clinic in October 2011 using APTIMA Combo 2 Assay®. Rectal GC screening has been performed in men and women who report receptive anal intercourse since July 2009. Validation studies were performed in the Florida Department of Health Laboratories prior to implementation of these tests. Retrospective review of medical records of individuals tested for rectal and pharyngeal GC from January 1st, 2010 until December 31st, 2012.

Results: A total of 3,044 tests were performed during the study period and 344 were positive (11.3%). Most of these tests were done in men: 2,672 with a positivity rate of 12%. The highest positivity rate was found in pharyngeal testing in women (10.6%), followed by rectal GC in men (12.5%). The lowest positivity rate was found in rectal specimens from women (4.5%).

Conclusions: The prevalence of rectal and pharyngeal gonorrhea in women and men who have sex with men in the MDHD STD clinic is higher than what has been previously reported in other centers. These findings emphasize the importance of extragenital testing of exposed anatomical sites in men and women.

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Neisseria Gonorrhoeae and VPH more than a medical problem

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Statement of purpose: Preventing cervical cancer in primary prevention through talks involving everyone in their responsibility of their decision making and in base of diagnosingNeisseria Gonorrhoeae can be one useful tool.

Statement of material and methods: Review of bibliography in a worldwide basis and our experience.

Statement of results: Neisseria Gonorrhoeae is responsible for the sexually transmitted disease(STD) gonorrhea. Patients positiveNeisseria Gonorrhoeae should also be tested for other STD. Half of the women who get cervical cancer are between 30 and 55 years of age. Changes could be also found in the cervix when they are still precancerous even at childhood. Genital human papillomavirus (HPV) is the most common sexually transmitted disease (STD) in the world. Adults and adolescents are infected. HPV is the single most significant risk factor for development of cervical cancer. About 30 types in the HPV family are spread through unprotected sexual intercourse. Other HPV types can cause cervical cancer and other less common cancers of the vulva, vagina, anus, and penis. Women who have had a large number of sexual partners are more likely to develop cervical cancer. Even if they have not had many sexual partners, if their male sex partner has had many previous partners, their risk for cervical cancer could be higher due to the increased risk of having contracted HPV. Still cervical cancer is the second-most common cause of death from cancer in women across the world. Widespread use of HPV vaccines are expected to have a huge impact in resources-of poor countries. In those areas, cervical cancer is often the most common cause of death from cancer in women.

Statement of conclusions: STD transmission can be reduced by the usage of latex barriers, such as condoms or dental dams, during intercourse, oral and anal sex but that are straitened in the case of HPV, and by limiting sexual partners. Hybrid capture assays, which permit simultaneous detection of HPV and other STD with high sensitivity, may be a useful diagnostic method because one bigger social problem can be found.So, we are before certain big public health, social and resource problems and it is necessary to give a social and educational turn in base of a schedule to make publicly known by mean of informal speech devoted to the welfare of the community, addressed people in schools, universities and other facilities.

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Men with Gonococcal Urethrits are Co-Infected with Other STIs: Influence on Transmission of Gonococcal Infection to Female Sex-Partners

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Introduction: *Neisseria gonorrhoeae* infection is often accompanied by other sexually transmitted infections. We hypothesized that the presence of other STIs in men with gonococcal urethrits may facilitate transmission of gonorrhea to female sex-partners.

Methods: 396 men with symptomatic urethral discharge presenting for diagnosis/ management to the Nanjing (Jiangsu Province, P.R. China) STD Clinic were enrolled between 04/12/2011 and 04/10/2012, in a study to define/characterize the presence of (other) microbial agents known to cause symptomatic urethritis that may accompany gonorrhea. Gonococcal-infected enrollees were also invited to participate in a study of gonococcal transmission to their female contacts. Based on an algorithm that required gonococcal-infected men to have had two or more sex-partners within 30 days of presentation and the female partner to have named the index male as her only sex-partner in the 2 weeks prior to or 2 weeks after the onset of his symptoms, one-way exposure and possible transmission of gonorrhea from men to their female sex-partner was identified and the influence of other STIs in men on gonococcal transmission to women was examined.

Results: The age distribution in the 396 men (overall, 16 to 76 years) was remarkable for the predominance of mid-age men; 54.3% (215/396) were between 35 and 60 years old. The majority were married 274/396 (69.2%). 96.5% (357/370) had completed at least junior high school. A quarter (24.8%) of the men reported a previous episode of gonorrhea (90/363). Gram's stain of urethral exudates showed ≥5 PMNs/oil immersion field in 377/396 (95.2%) specimens; 231/396 (58.3%) were Gram's stain positive for intracellular gram-negative diplococci (GNIDs), including 66 exudates taken from 139 men who were known to have self-administered antibiotics, most commonly cephalosporins, macrolides, quinolones and amoxicillin in the previous 30 days. Five specimens that were negative for GNIDs grew *Neisseria gonorrhoeae.* More than 50% (123/236) of men with gonococcal urethritis were co-infected with other STIs. Among men with gonorrhea, co-infections with *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum* and *Trichomonas* vaginalis occurred in 20.76% (49/236), 11.44% (27/236), 15.26% (29/190 and 26.27% (62/236), respectively. 49.0% (194/396) of men had had two or more female sexual partners in the past 30 days. 106 men had eligible partners but only 78 (19.7%) were local and potentially identifiable. Men who infected their partners were slightly younger (33 years of age vs. 37) and fewer had other STIs: 47.1% (8/17) for those who transmitted and 71.4% (5/7) for those who did not transmit gonococcal infection (p=0.386).

Conclusions: More than half of men with gonorrhea had STI co-infections. However, other factors alone or in combination with additional STIs may have influenced transmission of gonorrhea from men to their female partners. Recent use of antibiotics was noted in more than one-third of male subjects. This includes 22% of men whose Gram stain showed GNIDs, emphasizing the potential high rate of infection with antibiotic resistant *N. gonorrhoeae* in this population.

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Confirmation of APTIMA COMBO 2® Positive Neisseria gonorrhoeae Results using Manual and Automated Platforms in a Geographically Diverse Population

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Background: To review the performance of the Gen-Probe APTIMA Combo 2® [AC2] and APTIMA GC [AGC] assays for the detection and confirmation of Neisseria gonorrhoeae [GC] in a routine diagnostic laboratory from a geographically diverse population. The assay methods include both the manual DTS and automated PANTHER versions of the assays.
Methods: Retrospective diagnostic results for GC detection by Gen-Probe APTIMA assays from a diverse population were reviewed for the period December 2007 to May 2012 inclusive. A total of 270,197 test results, primarily from regions of Western Australia and the Northern Territory [NT] of Australia were available for analysis. Results for both AC2 and AGC were available for 6263 samples. The APTIMA assays were performed according to the manufacturer's instructions. Samples received before October 2011 were processed using the DTS version of the AC2 assay and the DTS analyte specific reagents for AGC. After this date, all samples were processed using the automated PANTHER instrument.
Results: 6263 results were initially reactive (positive or equivocal) by the AC2 assay. A major error (discordant result) defined as AC2 positive - AGC negative occurred for 42 samples (0.67% of initial reactives). 6 of these patients were positive at another sample site on the same day. Using this extended definition of infected status, the percent of major errors reduces to 0.57%. Additionally, minor errors defined as AC2 positive - AGC equivocal, AC2 equivocal - AGC positive and AC2 equivocal - AGC negative occurred for 30 (0.48% of initial reactives), 13 (0.21% of initial reactives) and 38 (0.61% of initial reactives) respectively for a combined total of 1.3% minor errors. 5 AC2 positive - AGC equivocal results and 3 AC2 equivocal - AGC negative results were positive at another sample site on the same day. Using this extended definition of infected status, the combined total percent of minor errors reduces to 1.17%. Using the definition of infected status defined previously, major errors occurred most frequently with samples from female patients (25/36), from urine samples (24/36), from the NT (29/36) and from the year 2008 (14/36). 4756 unknown or non-approved Gen-Probe sample types were tested. Of 208 initially positive results, only 2 discordant results were found; 1 oropharyngeal swab and 1 unknown site. The oropharyngeal sample was repeatedly positive by AC2 and negative by AGC and was tested with the DTS versions of the assays. This patient was a 17 year old male and was positive in urine and urethral samples on the same day. 30 samples (0.48%) were equivocal by both assays. One of these samples was positive at another site on the same day.
Conclusions: For samples where both assays were used, initially positive or equivocal by the AC2 assay, the initial overall agreement between the AC2 and AGC assays was 98.04%.The resolution of discordant samples by the consideration of other test results on the same day increases this figure to 98.26%. Initial AC2 results for the detection of GC can be interpreted with high confidence.

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Carriage of *Neisseria meningitidis* serogroups and genogroups in a population of English university students prior to the Novartis V72_29 meningococcal carriage study

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Background: Where broadly implemented, meningococcal serogroup C conjugate vaccines have provided both direct individual protection and indirect protection via impact on oropharyngeal carriage. With multivalent (ACWY) conjugate vaccines now available, and a serogroup B vaccine under regulatory review, we are investigating the effect of these vaccines on carriage in university students in England - (NCT01214850).
Methods: In this phase III study, students in 10 universities across England were enrolled from September to December 2010 to provide oropharyngeal swab samples before and at 5 subsequent timepoints up to 12 months after vaccination with either one dose of licensed quadrivalent meningococcal conjugate vaccine followed by saline placebo (Menveo™; n = 956), or two doses of either an investigational meningococcal serogroup B (4CMenB: n = 932) or licensed Japanese Encephalitis vaccine (Ixiaro™; n = 948). Swabs were plated directly and phenotypic and genotypic strain characterization was performed on isolates.
Results: In all, 2968 students, mean age 19.9 years, provided 2836 evaluable samples at baseline from 947 (33%) of which *Neisseria* species were cultured. The majority (98%; n=930) were identified as *N. meningitidis.* This high carriage rate was principally due to high prevalence of group B (10%; n=269) and Y (7%; n=196) with lower prevalence of group W (2%; n=48) and C (0.3%; n=9). Among these, the percentage of strains which were serogroupable was highest for groups B (71%), Y (70%) and W (68%) and lowest for group C (22%). None of the group C isolates had a PorA/B profile consistent with the cc11 strain responsible for the previous UK outbreak. The most common group B ccs were cc41/44 (25%), cc213 (24%), cc269 (9%), cc162 (5%) and cc35 (4%).
Conclusion: High rates of carriage were observed among university students prior to vaccination at study entry with group B and Y contributing the majority of serogroupable strains. Clonal complex patterns among group B strains are relatively unchanged from the previous large UK carriage studies performed from 1999-2001. Post-vaccination carriage analysis is ongoing to include timepoints as late as one year after study entry.

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Epidemiology of serogroup W135 meningococcal disease in Brazil

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Meningococcal disease (MD) is an important cause of morbidity and mortality and a leading cause of bacterial meningitis and septicemia in children and young adults worldwide. In Brazil, in the last decade, serogroup B and C have been the most common disease-causing serogroups, however, since 2000 there has been a significant increase from 2.0% to 5.8% (p=<0.0001) of cases of MD caused by serogroup W135. From 2000 to 2011, 38,671 cases of MD were reported in Brazil, and the incidence rate was 1.6 per 100,000 inhabitants. Only 35.2% (n=13,635 cases) were laboratory confirmed. Of those, MenW135 were responsible for 5.8% (559 cases) showing the case fatality rate persistently high, varying between 13.0% and 32.5%, depending on the age and of the clinical presentation. 35.2% of MenW135 MD cases were under 5 years of age. A total of 303 MenW135 invasive strains were received by Brazilian National Reference Laboratory. The two most common antigenic combinations were W135:2a:P1.2 (96/303, 32.0%) and W135:2a:P.5,2 (49/303, 16.2%) belongs mainly to the ST-11complex/ET37 complex. 14.0% of the MenW135 strains displayed reduced susceptibility to penicillin. Our rates of Men W135 MD cases remain low, but we have observed a slight increase of this serogroup over 12 years of laboratory-based surveillance. Considering the potential to cause invasive disease or outbreaks and the availability of tetravalent polysaccharide conjugate vaccine, the continued surveillance is crucial to guide the MD control and prevention strategies.

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Culture and non-culture analysis of the distribution and phase variation status of haemoglobin receptors among representative MenB cases referred to the Health Protection Agency Meningococcal Reference Unit

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Introduction: Neisseria meningitidisis a leading cause of meningitis and septicaemia. In the host, the essential nutrient iron is sequestered into complexes with various proteins. Meningococci acquire iron from these complexes via several receptor systems. Two such systems, HmbR and HpuAB, are variably distributed among meningococcal lineages. They are subject to phase variation (PV) by virtue of intragenic homopolymeric tracts and display antigenic diversity, suggesting interaction with host immunity. A relatively high prevalence among invasive isolates of at least one of these systems in an 'on' configuration suggests a possible role in disease.

The genomic presence and PV status ofhmbRandhpuAin isolates representative of recent English, Welsh and Northern Irish group B meningococcal epidemiology are reported. To address potential PV switching during passage/analysis of isolates, direct characterisation of PV status within clinical specimens was performed.

Methods: Isolate/specimen pairs (n = 80, received between December 2008 and April 2011) were selected based on MLST and PorA. Characterisation was performed using PCR, sequence analysis and GeneScan fragment analysis.

Results: All isolates possessed alleles for at least one system. Alleles for both systems were present in 37.5% of isolates, whilehmbRorhpuAalone occurred in 57.5% and 5.0% of isolates, respectively. Isolates possessinghmbRalone were prevalent in cc32 (83.3%; 5/6), cc213 (100%; 5/5) and cc41/44 (95.2%; 20/21). cc269 was evenly distributed among isolates possessing both systems (50.0%; 11/22) orhmbRalone (45.5%; 10/22). Predicted PV status was consistent between isolates and their respective specimens for homopolymeric tract lengths up to 12 nt. Approximately 73% of isolates were predicted to have at least one system in the 'on' state.

Discussion: A high proportion of isolates putatively expressed at least one iron acquisition system, thus suggesting a possible role in disease. Alternative systems may be important in the remaining isolates. Discrepant isolate/specimen PV statuses at longer tract lengths are likely to be an artefact of strand slippage during PCR. As such, this comparative analysis shows that clinical isolates subject to limited culture passage can provide an accurate representation of the expression status of phase variable genes during infections thereby underpinning future investigations of the contributions of phase variable genes to meningococcal pathogenesis.

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Meningococcal carriage Survey in a high Endemic Region in Spain

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Introduction: The Region of Cantabria (Spain) present incidence rates for meningococcal disease (MD) much higher than those found globally in Spain. During the epidemic wave of the late 70's, the rate was 38.37 x105 in Cantabria while it was 12.8 inSpain, during 1987 they were 16.03 and 5.47 respectively, and 2.97 vs 1.25 in 2009. The reasons for this difference are poorly understood.

Objectives: The overall objective is to know the meningococcal strains circulating among asymptomatic carriers in Cantabria in terms of prevalence in different age groups as well as their characteristics (serogroup/genogroup, genosubtype, ST). The information will allow us to compare with the data from those strains associated with clinical cases on the region to determine how virulent clones are circulating and how frequent other clones only occasionally isolated in clinical cases are isolated in general population.

Materials and Methods: Two thousand five hundred and twenty seven subjects randomized in a total population of 64,483 between 5 and 20 years old were included on the carriers survey. In the samples the presence *Neisseria meningitidis* was determined, and the isolates were characterized with monoclonal antibodies for the determination of serogroup, and using molecular techniques for the genosubtype and clonal complex (CC) determination. In addition, those strains isolated from patients with MD in Cantabria from 2005 to 2009 (79 strains) have been also characterized.

Results and Conclusions: Neisseria meningitidis has been isolated in 278 (11.0%) over all. The highest frequency was foud among those between 17-19 years with 31.3%. So far we are presenting the data about full characterization of 75 strains isolated from carriers and the comparison with data obtained from 79 invasive strains.

Most clinical isolates (58.75%) are associated with two clonal lines, the ST-11/ET-37 CC (30%) and ST-32/ET-5 CC (28.75%). Ten additional CCs appeared in a low frequence. Noteworthy is the high presence of the ST-11 CC, associated with hypervirulence, whose high presence has not been observed so markedly in other Spanish regions. By contrast, there is a low presence of both clonal lineages (two isolates ST-32/ET-5 CC, and none of ST-11/ET-37 CC) among carriers, indicating a very low level of circulation among general population. Although the strains isolated from carriers were more heterogeneous (17 CC were found), 26.67% of these isolates belonged to ST-53 CC, absent among clinical isolates. These preliminary data indicate the existence of large differences between both population of isolates, associated and non associated with clinical cases in Cantabria.

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Prevalence of *N. meningitidis* carriers in two prisons in Mexico during the epidemiological emergency of a meningitis outbreak in Mexico City (2010)

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Background: In response to a increase in the number of cases of invasive meningococcal disease, in Mexico city in 2010, which was associated to prisoners; and as a part of the implementation of a health drive on jails a survey prospective was carried out to determine the prevalence carriers of *N. meningitidis* (Nm),in this population.

Methods: The size was distributed in the two jails, in proportion to the existing population; North (n=165) and east (n=296) randomly selected. Pharyngeal swabs were obtained, identification of *Nm* by culture and latex test. Serogroup was confirmed by application of a multiplex-PCR test. Susceptibility to penicillin, ciprofloxacin, and cefotaxime was determined following the the CLSI recomendations. Genetic diversity was determined by ERIC-PCR and Pulsed Field Electrophoresis (PFGE). Multilocus sequence typing (MLST) was performed in the serogroup C isolates.

Results: 461 individuals were included, finding an overall prevalence carriers of 10.45%, (48/461), with small variations from east prison (9.45%) to north prison (12.1%). A total of 48 strains of Nmwere isolated, being the serogroup distribution: B (15), C (18) Y (9) and NG (6). All strains were susceptibleto Ciprofloxacillin and Cefotaxime and more than 90% showed some level of resistance to penicillin. Eighteen positive cultures identified as serogroup C showed a similar pattern by PFGE and ERIC-PCR, 27.7% (5/18) belonged to the ST11/ET37 CC.

Conclusions:The prevalence of carriers in the study was significant, particularly by the presence of serogroup C isolates related to invasive disease, identified as ST11/ET37 CC resistant to penicillin, that has been associated with the outbreak in Mexico City.

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Phenotypic and genotypic characterization of *Neisseria meningitidis* strains isolated from patients and healthy carriers in Mexico.

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Background: Although in México invasive meningococcal disease (IMD) is notifiable, the reports about meningococcal disease have been scarce. In addition there is not data related to the presence of genotypes associated with "hyperinvasive lineages" on the country. An increase in the number of IMD cases was notified in 2010 in Mexico city, which was associated with two different prisons

Aim: To analyze the presence of hypervirulent clones in healthy carriers prisoners and their relationship with isolates from IMD patients.

Methods:We analyzed, isolates of *N. meningitidis* from prisoners in two jails of Mexico City, characterizing both, oropharyngeal and patients isolates. The serogroup was confirmed by a Multiplex-PCR test. Genetic diversity was determined by Pulsed Field Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST).

Results:Fifty seven isolates of *N. meningitidis* were included,being 44 isolated from carriers[B (13), C (17) Y (8) and NG (6)]and 13 from patients [C (7), W_{135} (4) and Y (2)]. In the serogroup C isolates, the PFGE showed just one closely related restriction profile, in 4 isolates from carriers as well as in 7 strains from patients, belonging all of them to the ST11/ET37 CC. The presence of other hypervirulent clones like ST41/44 CC and ST23 CC were detected among strains isolated from carriers.

Conclusions: This is the first report in the country linking the presence of hypervirulent clones in healthy carriers and patients. The presence of hypervirulent CCs among asymptomatic carriers suggest that the actual number of IMD cases might be highest than the cases recovered by the national notification system. A continuous improvement of the surveillance strengthening the laboratory field will offer a more accurate picture of the situation of IMD in Mexico.

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Is serogroup A meningococcal polysaccharide a thymus dependent antigen?

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Backraund: Meningococcal serogroup A (MenA) capsular polysaccharide is structurally distinct from that of the other serogroups including MenC. While MenC polysaccharide clearly behaves as a T independent antigen with poor immunogenicity in young children, inducing hyporesponsiveness after multiple doses and showing an inability to prime for memory responses, the nature of the immune response to MenA is less clear. In contrast to other meningococcal polysaccharides, MenA antibody responses have been demonstrated in early infancy, a feature more typical of T-dependent antigens.
Objective: To compare MenA and MenC specific B cell and functional antibody responses to a quadrivalent meningococcal conjugate vaccine and a quadrivalent plain polysaccharide vaccine.
Methods: An open-label parallel group randomised clinical trial was conducted involving 150 healthy adult volunteers aged 18-70 between June 2009 and October 2010 in Oxford, UK. Participants were randomised to receive either 2 doses of a quadrivalent conjugate MenACWY vaccine given 28 days apart (Group 1, n=75), or one dose of a quadrivalent MenACWY polysaccharide vaccine followed by one dose of a conjugate MenACWY vaccine 28 days later (Group 2, n=75). Between-group comparisons were made of MenA and MenC responses, as assessed by serum bactericidal assays (SBA) and antigen specific plasma cell and memory B cell counts performed at baseline, 7 and 28 days after each vaccination.
Results: Participants in Group 2 who had received a prior polysaccharide vaccine had lower SBA geometric mean titres 28 days after conjugate vaccination (15.93 for MenA and 39.30 for MenC) than those in Group 1 who received a single dose of conjugate alone (40.72 and 107.85 respectively). Participants in Group 2 also had lower plasma cell geometric mean counts 7 days after a single conjugate vaccination (1.41 for MenA and 1.32 for MenC) than those in Group 1 (3.97 and 5.21 respectively).There were no differences in the behaviour of the MenA and MenC components of each vaccine. Adverse events were similar in each group.
Conclusions: Despite previous studies suggesting that MenA might be a T-dependent antigen, these data provide the first clear evidence that MenA plain polysaccharide vaccine behaves in the same way as MenC polysaccharide, i.e. as a T-independent antigen. These data provide further immunological support for using MenA conjugate vaccines in place of plain polysaccharides.
Clinicaltrials.gov identifier: NCT00901940
Sponsor: University of Oxford

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Sequelae following invasive meningococcal disease pre and post introduction of meningococcal C vaccine

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Aim: The burden of disease from sequelae following invasive meningococcal disease (IMD) is substantial. Conjugate meningococcal C vaccines were introduced into the Australian National Immunisation Schedule in 2003. The aim of this study was to assess the burden of disease from sequelae following IMD pre and post introduction of meningococcal C vaccine in South Australian children.
Methods: Clinical details of sequelae and immunisation history were collected from medical records of all children admitted to the Women's and Children's Hospital, Adelaide with IMD between 2000 and 2010.
Results: Of 109 children hospitalised with IMD, 52.3% were female and 12% were Aboriginal or of Torres Strait Islander origin. Almost half (48.7%) were transferred from another hospital with the commonest presenting features including fever (91.7%) and rash (76.2%). The majority of cases were caused by serogroup B (67.9%) with 8.3% caused by serogroup C and 21.1% of unknown serogroup. There were 55 children admitted in the pre-vaccine era (2000-2003) and 54 in the post vaccine era (2004-2010). Sequelae occurred in 40.4% (n=44) of children including limb amputation, skin scarring, seizures, renal failure, neurological (cerebral infarct, cranial nerve palsy) hearing loss, reactive arthritis and other major/minor sequelae. In the pre-vaccine era 41.8% of children had sequelae on discharge compared to 38.8% post meningococcal C vaccine introduction. Two cases due to serogroup C occurred in previously vaccinated children. Two deaths occurred; serogroup B (n=1), serogroup C (n=1).
Conclusions: A high proportion of children admitted with IMD develop incapacitating sequelae. A similar proportion of children developed sequelae in the pre and post vaccine era. Aboriginal children are at increased risk of hospitalisation from invasive meningococcal disease.

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Impact of conjugate meningococcal serogroup C vaccination on invasive meningococcal disease in Germany, 2002 – 2010

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In Germany conjugate meningococcal group C (MenC) vaccination in the 2nd year of life in 2006. A catch-up campaign was not undertaken, but vaccination of all persons<18 years was recommended on an individual basis. We analysed serogroup (Sg) and finetype specific incidence of invasive meningococcal disease (IMD) from 2002-2010 to evaluate possible effects of MenC vaccination. Presentation at IPNC will include 2011 data.

Sg- and age-specific IMD incidences were calculated using statutory surveillance data matched to results of molecular genetic finetyping (Sg, PorAVR1, PorAVR2, FetA) performed by the national reference laboratory. Vaccine uptake was estimated from school entry surveys and prescription monitoring. An index of discrimination was calculated to quantify finetype diversity.

MenC vaccination coverage was 69.8% in 4-6-year old children at school entry in 2010 (n=544,557). Limited unpublished data suggest higher and lower vaccination uptake in younger and older children, respectively. From 2002-2010, IMD incidence of the two predominant Sg B and C decreased significantly from 0.63 to 0.32/100,000 inhabitants and 0.26 to 0.10, respectively, in persons <25 years. Slopes of the SgC and SgB incidence curves were similar before/after MenC vaccination implementation. Incidence of SgC IMD (Incidence rate ratio (IRR): 0.81; 95%CI: 0.77-0.86, p<0.0001) declined more steeply than SgB IMD (IRR: 0.91; 95%CI: 0.88-0.95, p<0.0001) in 1-5 year-olds (p for difference=0.0001), but not other ages. The decline in SgC incidence was steeper in the 8 federal states with higher (IRR: 0.88, 95%CI: 0.84-0.91, p<0.0001) than in the 8 states with lower vaccination uptake (IRR=0.82, 95%CI: 0.79-0.86, p<0.0001; p for difference=0.01), based on rates of prescribed MenC vaccine doses, which peaked in 2007. Declining SgC incidence was associated with a decrease in less common SgC finetypes, leading to a significant reduction in finetype diversity. SgB finetype diversity remained stable.

The impact of MenC vaccination in Germany is weaker than in countries that implemented catch-up campaigns, although interpretation is complicated by already low and decreasing incidence before vaccination. Our results suggest that suboptimal vaccination coverage may lead to reduced transmission of rarer finetypes, which presumably have lower reproductive numbers. More effective use of vaccination resources in Germany might be achieved by rigorously targeting adolescents in addition to 1-year-olds.

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Meningococcal Serogroup C disease and vaccine failures in England and Wales

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Background: EnglandandWalesintroduced meningococcal Serogroup C conjugate (MCC) vaccine in November 1999 as a 2, 3 and 4 month routine schedule and as a catch-up campaign for all up to 18 years. Three licensed MCC vaccines have been used interchangeably in the infant programme since May 2001. The schedule became 2 doses in infancy with a 12 month MCC/Hib combined booster in September 2006.
Methods: Since 1984, all microbiology laboratories have been encouraged to submit cultures ofN meningitidis for characterisation to the Health Protection Agency (HPA) Meningococcal Reference Unit (MRU). Since 1996, the MRU has provided a national meningococcal PCR diagnostic service.
The HPA Immunisation Department has followed up all cases of laboratory-confirmed invasive serogroup C meningococcal (MenC) disease with local Health Protection Units and General Practitioners to establish MCC vaccine history, birth place and recent travel. MCC vaccine failure constituted MenC disease with onset >10 days after the last dose of MCC vaccine scheduled for that age.
Vaccine uptake was collected using an established routine surveillance system for routine childhood immunisations. Vaccine effectiveness was calculated using the screening method.
Results and Conclusions: MenC cases fell from 955 in 1998/99 to an annual average of 24 from 2005/06-2010/11. Vaccine histories were obtained for 629 (99.7%) of 631 vaccine eligible MenC cases between 1/1/00-15/1/12. There were 94 vaccine failures and all 3 licensed vaccines have featured. Forty-four failures had received a primary infant course and 8 additional cases also received a booster at 12 months. Only 21 cases were in individuals who were school aged when they received MCC vaccine. A high proportion of recent cases were born abroad (and thus were not eligible for MCC vaccine) or had recently travelled abroad.
There were 5 deaths in fully immunised cases aged ≥6 months to <20 years: a CFR of 5.4%. The CFR in this age group was 11.3% in 1998/99, before MCC vaccine, and suggests a protective effect against severe disease (RR 0.5,95% CI 0.2 to 1.2). Vaccine effectiveness within 12 months of infant immunisation was estimated at 97% (95% CI,91% to 99%) falling to 67% (95% CI,-66% to 88%) after 1 year ($p<0.01$). Effectiveness in the catch-up cohort aged 3-18 years remained over 90% after 12 years. Even with waning vaccine-induced immunity, MenC disease continues to be extremely rare 12 years after MCC vaccine introduction in England and Wales.

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Determining the appropriate age for a second immunization with meningococcal serogroup C conjugate vaccine; an intervention study among Dutch teenagers

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Introduction: In September 2002 a single Meningococcal serogroup C conjugated (MenCC) vaccination was implemented into the Dutch National Immunization Programme (NIP) for all children aged 14 months accompanied with a mass campaign for 1-18 year olds. MenC-polysaccharide (MenC-PS) specific antibody levels decline rapidly after primary vaccination in young children. Besides young children, adolescents also have an increased risk of developing invasive MenC disease. Therefore a second MenCC vaccination may be needed to protect this age group and attain longer lasting protection in the future, We recently (October 2011) started thesecond immunization MenC studyto establish the most appropriate age for this second MenCC vaccination.

Methods: Three age-groups were recruited consisting of healthy 10 year olds (n=91), 12 year olds (n=91) and 15 year olds (n=86) respectively. All participants received a primary MenCC vaccination with the MenC-PS tetanus toxoid conjugated vaccine (NeisVac C™) at young age (±9 years earlier),and were again vaccinated with the same MenCC at the beginning of the study. Blood and saliva samples were collected prior to (T0) and 1 month (T1) after vaccination and will be collected 1 year after vaccination (T2, October 2012). Serum MenC-PS specific IgG and IgA levels and serum IgG subclasses and avidity were measured using a fluorescent-bead-based multiplex immunoassay (MIA). Analyses for antibody levels in saliva and functional antibody levels in serum (SBA) are currently in progress.

Results: 268 participants were enrolled of which 264 (98.5%) completed both visits at T0 and T1. GMCs of serum MenC-PS specific IgG at baseline (T0) were 0.26 (95%CI 0.22-0.31), 0.32 (95%CI 0.26-0.39) and 0.40 (95%CI 0.31-0.51) for the 10, 12 and 15 year olds respectively. These levels increased enormously to 134.0 (95%CI 117.0-153.4), 193.6 (95%CI 168.6-222.3) and 174.3 (95%CI 147.5-206.0), respectively at T1. IgG1/IgG2 ratios increased between 2.5-3.0 fold, more in an age dependent manner, while the avidity ratio increased a 1.5 fold independent of age. Serum IgA GMCs also increased enormously around a 200-fold.

Conclusion: 9 years after primary vaccination, MenC-PS specific antibody levels increased spectacularly 1 month after a second MenCC vaccination. The high IgG levels after vaccination were mostly caused by a rise in IgG1, although the role of IgG2 seems to increase with age. SBA data will follow shortly to confirm this secondary response.

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Immunogenicity of a reduced conjugate meningococcal serogroup C vaccination schedule in infancy

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Background and Aims: The immune response induced by an infant reduced dose serogroup C meningococcal (MenC) conjugate vaccination schedule was investigated in a multicentre open label randomised controlled trial carried out in the UK and Malta.

Methods: 509 healthy infants were randomised to one of 4 groups to receive a dose of either MenC-CRM_{197} or MenC-TT, both at 3 months of age; or two doses of MenC-CRM_{197} at 3 and 4 months, or no primary MenC conjugate vaccine doses. Participants were concurrently immunised with DTaP-IPV-Hib at 2, 3 and 4 months and PCV13 at 2 and 4 months. Venepuncture was performed at 5 months of age. The proportion of individuals achieving MenC serum bactericidal antibody titres, using baby rabbit complement (rSBA), ≥1:8 and ≥1:128 was compared in each group by computing the differences between groups. Differences were considered significant if the 95% confidence interval (CI) did not include zero.

Results: In this interim analysis the two-dose MenC-CRM_{197} priming schedule resulted in significantly higher percentage of participants with rSBA titres ≥1:8 and ≥1:128 at 5 months of age than did a single-dose of MenC-CRM_{197} (Differences: 15.3% [95% CI 9.5-24%]) and 50% [95% CI 40.1-60%], respectively). One dose of MenC-TT resulted in a higher percentage of vaccinees with rSBA ≥1:8 and ≥1:128 than did one MenC-CRM_{197} dose (Differences: 8.3% [95% CI 2.2-20%] and 29.7% [95% CI 15.3-42%], respectively). There were no significant differences in the percentage of participants with rSBA≥1:8 after two MenC-CRM_{197} doses when compared with those receiving a single MenC-TT dose (Difference: 7% [95% CI 0-14.4%]) although the percentage of vaccinees with rSBA≥1:128 after two MenC-CRM_{197} doses was higher (Difference: 20.3% [95% CI 12-31.2%]). At 5 months of age MenC rSBA GMTs were higher after immunisation with MenC-CRM_{197} at 3 and 4 months of age (618) than MenC-CRM_{197}at 3 months (53;p

Conclusion: Although higher bactericidal antibody levels are induced after a two dose MenC-CRM_{197} primary schedule, a high proportion of infants achieved rSBA ≥1:8 following a single dose of MenC-CRM_{197} or MenC-TT vaccine at 3 months. The MenC-TT vaccine resulted in a significantly higher proportion of infants with rSBA≥1:8 and ≥1:128 than one MenC-CRM_{197} dose. The impact of "priming" responses on persistence in later infancy and response to a toddler booster will provide important information to underpin future schedules.

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Phase II clinical study of a Brazilian meningococcal C conjugate vaccine

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Introduction: Development of a Brazilian meningococcus C conjugate vaccine is strategically important, considering large epidemics and several outbreaks seen in different regions of the country in the last decades in a wide age-range, making the potential target population for this vaccine in Brazil at about 80,000,000 children, adolescents and young adults.

Objectives: Immunogenicity and safety of meningococcal C conjugate vaccine, produced by Bio-Manguinhos/Fiocruz (BM),Brazil, by reductive amination using hydrazide-activated tetanus toxoid as carrier protein.

Methods: Phase II randomized, single-blinded study, in 360 healthy male and female children from 1to 9 years of age, never vaccinated against meningococcus C: 240 received 0.5 mL of the candidate vaccine and 120 received a reference vaccine, Neisvac-C®. BM candidate vaccine: meningococcal C polysaccharide 10 μg (strain 2135), conjugated to tetanus toxoid 10-30 μg, aluminium hydroxide 0.35 mg (Al^{+3})/ dose. Diary cards were filled out by parents/tutors during the first 3 days after vaccination and adverse events solicited and non-solicited were recorded during the first 30 days after vaccination.

Results: Immunogenicity: Seroconversion (4-fold increase in titer): 226/240 (94.2%; 95% CI90.4; 96.8) for BM vaccine, 118/120 (98.3%; 95% CI 94.1; 99.8) for Neisvac-C®. The difference of seroconversions vaccine in test/reference vaccine was -4.1% (95% CI-8.5%; 0.3%). Bactericidal geometric mean titers (GMT) for C polysaccharide (95% CI), BM vaccine, pre: 2.1 (2.0; 2.1); post: 230.0 (187.1; 282.9); Neisvac-C®, pre: 2.07 (2.0; 2.1); post: 1036.0 (832.1; 1289.7). Post GMT ratio vaccine for test/reference vaccine: 0.22 (95% CI: 0.16; 0.30). Differences on immunogenicity were higher for children less than 5 years of age.

Safety: Both vaccines were well tolerated and adverse events were mild or moderate.

Conclusion: BM vaccine immunogenicity and safety are promising, however its immunogenicity was inferior to Neisvac-C®. Studies to evaluate duration of immunity and memory, as well as to improve its immunogenicity, are in progress, before starting the Phase III studies.

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Scale up of Brazilian meningococcal C conjugate vaccine production for Phase III clinical trials

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Neisseria meningitidis is one of the most important pathogens as causes of meningitis and other clinical manifestations worldwide. Several outbreaks caused by group C has occurred in different regions of Brazil since 2001. Currently, this group account for more than 70% of all meningococcal diseases in the country, becoming a serious public health problem. The target population to be immunized with this vaccine is about 80,000,000 of children, adolescents and young adults. Bio-Manguinhos (Fiocruz) has developed all steps to produce, purify and control an effective Brazilian group C protein-polysaccharide conjugate vaccine (MenCPS-TT) by modified reductive amination, using hidrazide-activated tetanus toxoid as carrier protein. Different lots of the freeze-dried vaccine produced in a pilot scale and purified by tangential flow ultrafiltration (Centramate System - Pall BioPharmaceuticals) in the downstream procedure, were studied in Phase I and II trials. The experimental vaccine showed satisfactory results of safety and immunogenicity in adults and children from 1 to 9 years old, respectively. Before starting the Phase III trials the production of vaccine has been scaled up to obtain industrial lots under reproducible conditions. The preliminary results of quality control assays showed the efficiency of scaled up purification process used (Centrasette - Pall BioPharmaceuticals) in the removal of reagents, by-products and unconjugated polysaccharide in the steps for production of oxidized polysaccharide, activated-protein and conjugate bulks. The vaccine lots showed sugar free contents around 10% as measured by capillary zone electrophoresis. The immune response in mice is under investigation in order to evaluate if the scaled up lots have induced antibodies with high bactericidal activity comparable to that one observed with lots obtained in a smaller scale.

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Identification of key parameters that impact the sensitivity of the MenC-rSBA assay to natural antibodies

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Question: To assess functional antibody responses after *Neisseria meningitidis* serogroup C (MenC) vaccination, a serum bactericidal activity assay is commonly employed that uses rabbit complement as the exogenous complement source (rSBA assay). We have compared the rSBA assay performed at GlaxoSmithKline (GSK) Biologicals with the assay performed at the UK Health Protection Agency (HPA) and found a higher sensitivity to natural immunity with GSK's assay. This higher sensitivity was antibody-mediated. A gap analysis between HPA and GSK assays has identified several differences that may help explain the different sensitivity to natural immunity. In this work the key parameters impacting assay sensitivity have been elucidated.
Methods: For the identification of assay parameters that may affect the sensitivity of the MenC-rSBA assay, an experimental plan was designed, which evaluated 16 conditions with different combinations of the assay parameters; these included two references, one with all HPA assay parameters and one with all GSK assay parameters. All conditions were assayed with rSBA, and the agreement of the responses with the HPA reference assay was analysed. Parameters that affected the agreement of an assay with the HPA reference assay were identified and ranked using graphical and statistical tools, such as Pareto chart and Analysis of variance (ANOVA).
Results: Three parameters were found to contribute to a good agreement between GSK's and HPA's MenC-rSBA assay: sequential addition of the working seed and complement ($p = 0.0238$), shaking ($p = 0.0429$) and production of working seed ($p = 0.1077$).
Conclusions: Different technical parameters used in GSK's rSBA assay against meningococcal serogroups A, C, W-135, and Y render the assay more sensitive to naturally acquired antibodies, explaining the low concordance between GSK's and HPA's rSBA assay for pre-vaccination functional antibody titres.
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Development and validation of a method for free polysaccharide determination in a Brazilian group C meningococcal conjugate vaccine using capillary electrophoresis technique

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Neisseria meningitidis group C is an encapsulated bacterium that causes several diseases and is associated with high mortality rates becoming a serious public health problem. Bio-Manguinhos is developing a conjugate vaccine constituted by covalent attachment of capsular polysaccharide to hydrazide-activated tetanus toxoid through reductive amination, which is currently being evaluated in Phase II trials in children between 1-9 years. Free components quantification was necessary as a vaccine process control assay and intended to prevent exacerbated adverse reactions occurrence and/or vaccine immunogenicity reduction. The World Health Organization recommends a free protein maximum level in the conjugate vaccine, but does not set a limit for the free polysaccharide content. Thus, the aim of this study was to develop and validate a quality control method appropriate to separate and quantify free polysaccharide present in the conjugate vaccine against N. meningitidis group C, using capillary electrophoresis technique. To unbound polysaccharide separation free capillary zone electrophoresis was used and were tested some tetraborate buffer concentrations, pH and temperature conditions to obtain the best separation resolution. It was possible to determine the free polysaccharide content and validate the proposed method, which was linear in 0.047 to 0.164 mg/mL range, showed a matrix effect, 0.0154 mg/mL of detection limit and 0.0454 mg/mL of quantification limit. Repeatability and intermediate precision of the method was also evaluated. The robustness results showed that the analysis temperature needs to be controlled for reliable results. The methodology developed and validated will be used to evaluate the conjugate batch that will be submitted to Phase III clinical studies and in the routine quality control of the conjugate vaccine.

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Bridging of two serum bactericidal antibody assays using rabbit complement performed at the Health Protection Agency and at GlaxoSmithKline Biologicals

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Question: In the United Kingdom, three monovalent meningococcal serogroup C (MenC) vaccines have been licensed based on functional antibody responses measured at the Health Protection Agency (HPA) by a serum bactericidal antibody assay using rabbit complement as an exogenous complement source (rSBA). During post-licensure surveillance, an HPA rSBA-MenC titre of 8 was confirmed as the antibody threshold that best correlates with vaccine effectiveness, and this threshold has since been extended to rSBA assays for meningococcal serogroups A, W-135, and Y. The rSBA-MenC assay performed at HPA has been defined as the reference assay. HPA is also known to have expertise with rSBA testing for serogroups A, W-135, and Y, but no reference laboratory exists for these serogroups. rSBA assays have also been performed at GlaxoSmithKline (GSK) Biologicals' laboratories to evaluate the immunogenicity of meningococcal vaccines, hence a technical bridge between HPA and GSK rSBA assays was performed.
Methods: We compared functional antibody responses to serogroups A, C, W-135, and Y measured by either HPA or GSK rSBA assays on the same set of serum samples taken at pre-vaccination and one month post-vaccination in various clinical studies. Concordance and Deming regression analyses were performed. Immunoglobulin (Ig) G and IgM levels were correlated with rSBA levels. Some pre-vaccination samples were depleted of IgG and/or IgM and tested by rSBA assays to better understand the contribution of each isotype to rSBA activity.
Results: The functional antibody titres measured by GSK and HPA rSBA assays for the four serogroups on post-vaccination samples showed a good level of concordance (agreement >80%) and correlation (r value >0.7). The post-vaccination rSBA activity measured by both GSK and HPA assays was mediated by specific antibodies, mainly IgG, induced by the vaccine. In contrast, in pre-vaccination samples, a significant number of samples were positive with GSK rSBA assays, but negative with HPA rSBA assays. Inhibition experiments using homologous meningococcal polysaccharides as competitors confirmed the specificity of the GSK rSBA assays. Depletion experiments demonstrated that removal of IgM induced a decrease in GSK rSBA titres, suggesting that the pre-vaccination functional antibody titres measured by GSK rSBA assays were mainly mediated by pre-existing IgM. Moreover, correlation analyses between IgM levels and rSBA titres suggested that GSK rSBA assays might be more sensitive to IgM compared to HPA rSBA assays.
Conclusions: GSK and HPA rSBA assays for meningococcal serogroups A, C, W-135, and Y have a comparable sensitivity to vaccine-induced antibodies, but the GSK rSBA assays were more sensitive to naturally acquired antibodies than the HPA rSBA assays.
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Comparison of the safety and immunogenicity of an investigational quadrivalent meningococcal ACWY-tetanus toxoid conjugate vaccine and a marketed quadrivalent meningococcal ACWY-diphtheria toxoid vaccine in healthy individuals 10-25 years of age

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Question: Universal immunization of preadolescents, adolescents and young adults against invasive meningococcal disease with a quadrivalent meningococcal ACWY (MenACWY) conjugate vaccine is recommended in a number of countries. We compared the safety and immunogenicity of an investigational tetanus toxoid-conjugated MenACWY vaccine (MenACWY-TT) with a marketed diphtheria toxoid-conjugated MenACWY vaccine (MenACWY-DT).
Methods: In a randomized, controlled, observer blinded, multicenter trial (NCT01165242), 1016 participants 10-25 years of age not previously immunized with a meningococcal vaccine were randomly allocated in a 1:1:1 ratio to receive a single dose of one of two lots (A or B) of MenACWY-TT or a single lot of MenACWY-DT. The primary outcome was the non-inferiority of the vaccine response after lot A MenACWY-TT compared to MenACWY-DT for all 4 serogroups. Vaccine response was defined as a post-vaccination serum bactericidal antibody (hSBA) titer, performed in the presence of human complement, against each of the 4 serogroups (A, C, W, Y) of at least 1:8 in persons initially seronegative (<1:4) or as a 4-fold increase in titer pre- to post-vaccination in persons initially seropositive (≥1:4). Adverse events (AEs) following immunization were measured 4 (solicited AEs) and 31 days (unsolicited AEs) post-immunization.
Results: The mean age of participants was 16.3 years (range 10-25 years; female:male ratio=1.05); 993 (97.7%) participants completed the study. The non-inferiority of the lot A MenACWY-TT in terms of the percentage of participants with hSBA vaccine response against each of the serogroups was demonstrated since for each serogroup separately, the lower limit of the two-sided 95% confidence interval for the difference between groups (MenACWY-TT response minus Men ACWY-DT response) was ≥ the pre-defined clinical limit of -10%. Vaccine response ranged from 51-82.5% for the four serogroups after MenACWY-TT (both lots) compared to 39-76.3% for the four serogroups after MenACWY-DT. Exploratory analyses showed significantly higher geometric mean antibody titers against serogroups W and Y after MenACWY-TT (both lots); the proportion of participants with hSBA titers ≥1:8 against serogroups W and Y were also significantly higher in MenACWY-TT recipients (lots A and B) compared to MenACWY-DT recipients (91.3% and 89.7% vs. 83.2% for serogroup W; and 98.1% and 98.4% vs. 94.1% for serogroup Y). Pain was the most common injection-site event reported by 50.8-55.4% across the three groups. Fatigue and headache were the most common systemic solicited adverse event, reported by 27.3-29.2% and 25.5-26.4% of participants respectively.
Conclusion: MenACWY-TT vaccine was well tolerated and elicited an immune response that was non-inferior to that of a marketed MenACWY-DT vaccine.

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Tetravalent polysaccharide-prothein formulation, from capsular polysaccharides of groups A, C, Y, and W 135 N. Meningitidis to diphtheria toxoid

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Introduction: N. meningitidis is a leading cause of bacterial meningitis and sepsis throughout the world. There are thirteen different serogroups of this bacterium, which have been identified on the basis of their capsular polysaccharides (PsC). Six of these serogroups (A, B, C, W135, Y and X) are the main cause of meningococcal diseases nowadays. Conventional vaccines based on meningococcal PsC elicit an immune response in children and adults; however their efficacy in infants and young children is limited, due to the Timo independent nature of the PsC. These T-independent antigens could become T-dependent through conjugation to a carrier protein. The conjugate vaccines demonstrated to be very effective but also very expensive. Currently, the Center of Biomolecular Chemistry and the Finlay Institute have a join research project with the aim of developing conjugated vaccines against serogroups A, C, W135 and Y of N. meningitidis. The aim of this work is preparation of tetravalent polysaccharide-protein formulation, from capsular polysaccharides of groups A, C, Y and W135 N. meningitidis to Diphtheria Toxoid.
Materials and Methods: General working methodology was as follows: i. Fragmentation of PsC, ii. De-OAcetilation iii. Activation, iv. Conjugation v. Formulation and Immunization in experimental animals vi. Immunological response evaluation. For preparation of monovalent conjugates, we used acid and basic buffers; periodate oxidation and reductive amination. The final products of these reactions were characterized by physicochemical techniques. Monovalent and Tetravalent formulations were prepared and immunized in Balb/C mice. The immunological response was evaluated by immunoenzymatic and serum bactericidal assays. **Results:** There was a high recovering in fragmentation and activation reactions, with structural identity conservation. The conjugates have a broad and controlled carbohydrate to protein ratio and low free protein. The IgG antibody and bactericidal titers elicited by monovalent and tetravalent formulations were high against four PsC.
Conclusions: 1. Four conjugates presented low free protein and carbohydrate structure conservation. 2. Bactericidal and antibody titers elicited in experimental animals, by the monovalent and multivalent formulation were high against PsC. 3. General work methodology for the N. meningitidis A, C,Y and W135 conjugate is ready to development scale.

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Development of a serogroup X meningococcal conjugate vaccine

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Neisseria meningitidisis one of the major causes of bacterial meningitis worldwide with a high associated mortality. In Africa, group A has been the major epidemic disease-causing meningococcal serogroup prior to the introduction of the glycoconjugate vaccine MenAfriVac™.[1] Recently, meningococcus serogroup X (MenX) has received increasing epidemiological attention because of outbreaks of meningitis caused by this serogroup in the African Meningitis Belt.[2, 3]

The increasing endemic level of MenX disease and the absence of a vaccine against this serogroup argue for increased research to develop a vaccine. Given the success of other meningococcal glycoconjugate vaccines, the MenX capsular polysaccharide (MenX CPS) represents a rational target antigen for vaccine design. MenX CPS has been purified with high purity from bacterial culture of a suitable strain. Different glycoconjugates were then synthesized using CRM_{197} as carrier and tested in mice. Post immunization sera were analyzed by ELISA for specific anti-MenX CPS IgG. Functionality of the antibodies elicited against the MenX CPS was assessed in a serum bactericidal assay using rabbit complement (rSBA).

All conjugates induced good levels of anti-MenX CPS IgG titers. Moreover, antibodies showed strong bactericidal activity against Meningococcal X strain.

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Structural characterization and stability of meningococcal serogroup X in aqueous solution

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Serogroup X meningococcal (MenX) disease has recently received increased attention because of outbreaks recorded in the African Meningitis Belt, with increased endemic levels of MenX disease over the past two years.
The structure of serogroup A, C, W and Y meningococcal polysaccharides has been already fully elucidated by NMR. MenX capsular polysaccharide (MenX CPS) structure is also documented but few other characterization data have been published.
^{1}H NMR, ^{31}P NMR and HPLC have been applied for complete characterization of MenX CPS and to evaluate its stability in aqueous solution as compared to MenA capsular polysaccharide (MenA CPS). The stability study demonstrated that MenX CPS is less susceptible to hydrolytic degradation than MenA CPS. The different stereochemistry of the N-acetyl group at position C_2 of mannosamine (MenA CPS) and glucosamine (MenX CPS) respectively might play a fundamental role in this susceptibility to polysaccharide chain degradation.

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Impact of Quadrivalent Meningococcal Conjugate Vaccine (MenACWY) Coverage on Disease Incidence in the United States, 2004–2010

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Background: In 2005, quadrivalent meningococcal conjugate vaccine (MenACWY) was routinely recommended for 11-18 year-olds. U.S. vaccination coverage in 13-17 year olds reached 62.7% in 2010 but varied substantially by state (26-90%). Measuring impact of vaccination is challenging in the setting of historically low meningococcal disease incidence.

Methods: To assess direct and population effect of variability in increasing vaccine coverage, we used Pearson's correlation coefficient to compare the slope of vaccine coverage over time with the slope of meningococcal disease incidence by state among 13-17 year olds, <5 year olds, and ≥25 year olds. We used National Immunization Survey-Teen state-specific coverage estimates for 13-17 year-olds during 2008-2010. States were grouped into quintiles based on 2010 coverage data. Vaccine coverage was assumed to be 0% in 2004 (the year before MenACWY licensure). We included all cases of Neisseria meningitidis reported through the National Notifiable Diseases Surveillance System during 2004-2010. US census data were used to calculate state-specific disease incidence.

Results: Meningococcal disease incidence declined from 0.56 to 0.15 cases per 100,000 during 2004-2010. For 13-17 year olds, state-specific analysis revealed a correlation of -0.39 between the positive coverage slope and negative disease incidence slope ($p<0.01$). In the state-grouped analysis, there was a stronger correlation of -0.95 ($p=0.01$). There was no correlation between adolescent coverage and disease incidence for < 5 year olds and ≥25 year olds.

Conclusion: This ecologic analysis suggests that states with more rapid uptake of MenACWY vaccine have achieved greater declines in meningococcal disease in adolescents. There was no evidence of herd immunity. Increasing uptake in low coverage states will be important to achieve maximum benefit of the adolescent MenACWY vaccine.

P 153

Cross-bactericidal antibodies against MenY S1975 strain are induced by vaccination with *PedvaxHIB*™

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Question: A novel, combined *Haemophilus influenzae* type b (Hib) and *Neisseria meningitidis* serogroup C (MenC) and Y (MenY)-tetanus toxoid (TT) conjugate vaccine (Hib-MenCY-TT) has been evaluated in Phase II and Phase III studies. Several studies used *PedvaxHIB*™ (Merck) as the control vaccine for the fourth dose of Hib vaccine. *PedvaxHIB*™ is a conjugate vaccine made of purified Hib-PRP linked to outer membrane protein (OMP) complex from MenB strain B11. In two independent studies (NCT00134719 and NCT00289783), induction of bactericidal antibodies (as measured by SBA with human complement (hSBA)) against strain MenY S1975 was observed in subjects who received a single dose of *PedvaxHib*™. Therefore, we investigated whether the anti-MenY response was mediated by bactericidal antibodies specific to OMPs shared between MenB B11 and MenY S1975 strains. MenB B11 strain and MenY S1975 strain both belong to serotype 2a and serosubtype P1.5,2.

Methods: An inhibition assay has been developed to investigate whether antibodies generated in response to vaccination with OMP from the *PedvaxHIB*™ vaccine could cross-react with OMPs from the MenY S1975 strain. Serum samples from the Hib-MenCY-TT and *PedvaxHib*™ groups were absorbed either with MenY purified polysaccharide, MenY S1975 inactivated strain or MenB B16B6 inactivated strain, and then tested in MenY hSBA. The MenB strain B16B6 was used instead of the B11 strain (not commercially available), because it belongs to the same serotype and serosubtype.

Results: The inhibition analysis showed that samples positive by MenY hSBA after *PedvaxHib*™ vaccination could be divided into three categories: samples that showed bactericidal activity inhibition (I) by both MenY and MenB strains but *not* by PS-Y; (II) by both MenY and MenB strains *and* by PS-Y; (III) by PS-Y and the MenY strain but not the MenB strain. In both studies, more than 80% of post-*PedvaxHIB*™ immunisation samples that were negative in MenY ELISA and positive in MenY hSBA, were inhibited by the MenB strain.

Conclusions: We have shown that the majority of serum samples that were positive by MenY hSBA following *PedvaxHIB*™ vaccination could be inhibited by the MenB strain B16B6 but not by the MenY polysaccharide, indicating that the observed response against MenY S1975 strain was due to cross-reactive anti-OMP antibodies.

Funding by GSK Biologicals

PedvaxHIB is a trade mark of Merck

P 154

Immunogenicity of a bivalent A+W-135 meningococcal outer membrane vesicle vaccine compared with meningococcal conjugate and polysaccharide vaccines

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Objectives: Serogroups A and W-135 meningococci are the main causes of meningococcal disease in sub-Saharan Africa (the meningitis belt). The outbreaks are clonal and express the same surface proteins PorA and PorB over time. In such a setting, an outer membrane vesicle (OMV) vaccine could be effective. NIPH and FI are now collaborating to produce an A+W-135 OMV vaccine, and it is desirable to compare its immunogenicity with available commercial vaccines.
Methods: The vaccine is produced from representative epidemic serogroup A and W-135 strains from Africa. The immunogenicity of the A+W-135 OMV vaccine was compared in mice with the commercially available serogroup A or ACYW-135 conjugate vaccines (MenAfriVac, Serum Institute of India and Menveo, Novartis, respectively) and an ACYW-135 polysaccharide vaccine (Mencevax, GlaxoSmithKline). IgG antibody responses were measured by ELISA and functional activities were assessed by bactericidal (SBA) and opsonphagocytic (OPA) assays. Two different doses of the vaccines were tested ($1/10^{th}$ and $1/50^{th}$ of a human dose).
Results: High SBA titers against both serogroup A and W-135 strains were observed in mice after one or two doses of the bivalent OMV vaccine. The serogroup A conjugate vaccine (MenAfriVac) also induced SBA titers after one and two doses dose against serogroup A. The SBA titers induced by the OMV vaccine were higher than those obtained with MenAfriVac and Menveo. No SBA response was detected after one dose of the ACYW-135 conjugate or plain polysaccharide vaccine. Corresponding results were observed in OPA. Antibody analysis showed that high levels of IgG antibodies against OMV from both serogroups were detected in mice immunized with the bivalent OMV vaccine. The A-conjugate vaccine induced anti-A polysaccharide IgG, while the ACYW-135 conjugate vaccine was found to induce IgG antibodies against both A and W-135 polysaccharides. No anti-polysaccharide A or W-135 IgG antibodies were detected in mice receiving the plain polysaccharide vaccine or the OMVs.
Conclusion: This study shows that the bivalent A+W-135 OMV vaccine may induce comparable or higher levels of functional antibodies than current conjugate vaccines, and could be a future alternative or supplement to conjugate and polysaccharide vaccines in the meningitis belt. A phase I clinical trial with this vaccine is planned in Cuba in the end of 2012.

P 155

Impaired TACI expression is responsible for the unresponsiveness of x-linked immunodeficient mice to polysaccharide vaccines

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Antibodies against capsular polysaccharides of meningococcal bacteria are extremely efficient in killing meningococcal bacteria. However, because polysaccharides are T cell independent type II antigens they are weakly immunogenic and they do not induce antibodies in infants, the age group most vulnerable to meningococcal infections. Previously, we had shown that severely reduced expression of TACI in newborn mice is responsible for the unresponsiveness of newborns to polysaccharide antigens. In this study, we investigated the signals required for the developmental regulation of TACI expression. We showed that microbial colonization does not play a role in upregulating TACI after birth. We next measured TACI levels in x-linked immunodeficient (xid) mice that are deficient in B cell receptor signaling and respond poorly to polysaccharide vaccines. Results showed that xid mice express significantly reduced levels of TACI as compared to wild type mice (Figure 1A). More importantly, xid mice B cells did not secrete immunoglobulins and did not proliferate after stimulation with BAFF or APRIL, the two ligands of TACI. Analysis of signaling cascade induced by BAFF and APRIL showed that while canonical NFκB pathway was blocked (Figure 1A), non-canonical NFκB pathway was intact in XID mice B cells. These data suggested that reduced TACI expression leads to impaired signaling because TACI is known to be responsible for the induction of canonical NFκB pathway. Since xid mice are known to respond poorly to polysaccharide vaccines, it is highly likely that reduced TACI expression plays an important role in this outcome. The dependence of BAFF and APRIL induced Ig secretion on the expression levels of TACI was further demonstrated in experiments where xid mice B cells were pre-stimulated with the toll-like receptor 9 ligand, CpG. Incubation of xid mice B cells with CpG increased the expression of TACI on B cells (Figure 1B) and rendered them susceptible to BAFF or APRIL induced immunoglobulin secretion. Moreover, BAFF and APRIL induced canonical NFκB pathway activation was restored in CpG pre-stimulated xid mice B cells (Figure 1B). Finally, immunization of xid mice with a prototype T cell independent type II antigen, NP-Ficoll led to the generation of antibodies against NP (Figure 2). These results suggest that B cell receptor stimulation is responsible for the gradual increase of TACI expression after birth.

figure 1

Figure 1

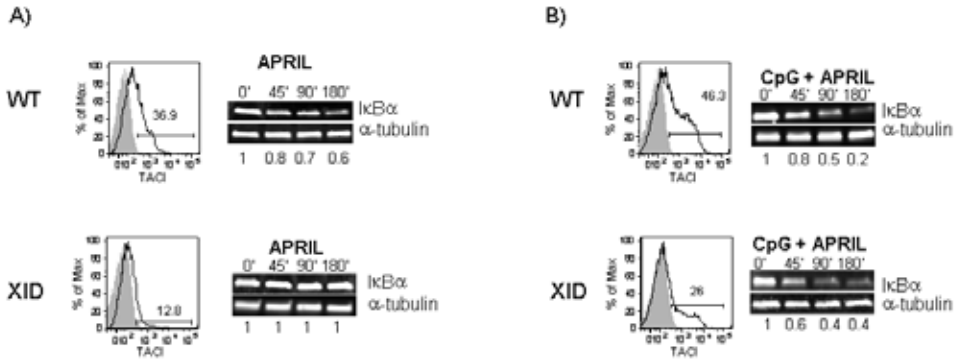

Figure 1. TACI expression and signaling in XID mice B cells. (A) Expression levels of TACI were measured on purified B cells from XID or wild type (WT) mice in flow cytometry assay. Same cells were also exposed to APRIL stimulation for indicated durations. Lyzed cells were subjected to western blot analysis using antibodies against IκBα molecule. Alpa-tubulin antibodies were used as loading control. Degradation of IκBα molecule is a direct result of canonical NFκB pathway activation. (B) Expression levels of TACI were measured on purified B cells from XID or WT mice after pre-stimulation with CpG for 24-hours. Same cells were also exposed to APRIL stimulation for indicated durations. Lyzed cells were subjected to western blot analysis using antibodies against IκBα molecule. Alpa-tubulin antibodies were used as loading control.

figure 2

Figure 2

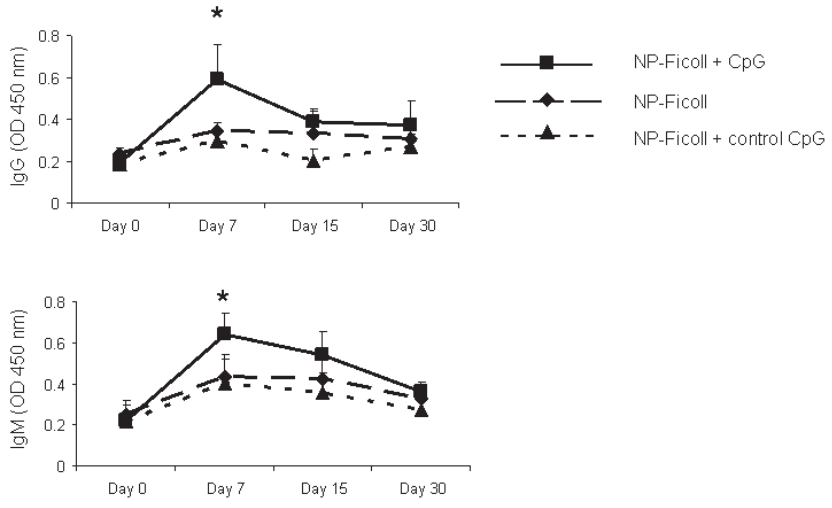

Figure 2. Immunization of xid mice with NP-Ficoll. Groups of five xid mice were immunized with indicated vaccines. Serum anti-NP IgG and IgM antibody levels were measured in ELISA. Non-stimulatory CpG containing GpC sequence was used as control. NP-Ficoll and CpG were given at 40 μg/mouse dose.

P 156

Characterization of a novel human/mouse chimeric *CEACAM1* transgenic mouse as candidate host permitting neisserial infection

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Neisserial outer membrane Opa proteins adhesins recognize the N-terminal domain of human carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family members to help facilitate colonization and infection *in vitro*. However, a detailed understanding of the importance of this interaction as well as that of other bacterial adhesins is limited due to the lack of animal models that reflect the intimate host-pathogen interactions that typify these human-restricted bacteria. To overcome this limitation, our group has previously established a novel CEACAM-humanized mouse model in which full-length human *CEACAM* genes were integrated into the mouse genome. Indeed, depending upon CEACAM expression, these mice could successfully be colonized by both *N. gonorrhoeae* and *N. meningitidis*.
While mice do not express CEACAM3, CEACAM5 or CEACAM6, but do express an ortholog of CEACAM1 that is not recognized by the neisserial Opa proteins. To account for the fact that simple overexpression of CEACAM1 can cause suppression of immune cell activation and that the cytoplasmic tail of human CEACAM1 is not conserved with the murine sequence, we undertook to generate a new *CEACAM1* targeted mouse in which exon 2 of mouse *Ceacam1*, which encodes for the N-terminal domain, is replaced with its human counterpart. This knock-in approach generated a chimeric CEACAM1 protein with the Opa-binding N-terminal domain of human origin, while the promoter and remaining portions of the protein are native to the wild type mouse.
Herein we describe our genetic, CEACAM1 protein and phenotypic analysis of the chimeric *CEACAM1* mouse. Gross tissue expression of the human/mouse-chimeric CEACAM1 generally reflected that of murine CEACAM1 in chimeric mice via Western blotting and immunohistochemistry-based analysis of diverse tissues and organs. Flow cytometry of murine immune cells was used to compare chimeric CEACAM1 and murine CEACAM1 expression levels and regulation in response to activation. Cell:cell adhesion studies using immortalized cell lines derived from mouse embryonic fibroblast cells (MEFs) generated from the transgenic mice are used to investigate the *trans*-intercellular binding function of the chimeric CEACAM1. Finally, a mouse intranasal colonization/infection studies were used to compare the susceptibility of the full length humanized versus human/mouse transgenic CEACAM1 mouse to *N. meningitidis* infection.

P 157

Development of an *in vitro* 3D model to simulate the human blood-cerebrospinal fluid (B-CSF) barrier

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Background: *Neisseria meningitidis* is a human-specific pathogen with the capacity to cause sepsis and meningitis. Since this microorganism is strictly human specific the analysis of the pathogenesis requires development and use of transgenic mice.
The aim of this study was to construct an endothelial cell barrier in order to develop an *in vitro* 3D model of a human blood-cerebrospinal fluid (B-CSF) barrier using human brain microvascular endothelial cells (HBMEC) or primary endothelial cells, isolated from human skin. The subarachnoideal space was constituted by a biological vascularized scaffold of collagen I/III (BioVaSc).
Methods and Results: The BioVaSc was generated from a decellularized porcine small bowl segment with preserved tubular structures of the capillary network within the collagen matrix. For generating an *in vitro* B-CSF barrier the BioVaSc was than reseeded with HBMEC or primary endothelial cells from human skin. Various concentrations of HBMEC or primary endothelial cells were applied and cultivated for 2, 5 and 7 days under static and dynamic conditions. The static culture tests revealed an optimal cell concentration of 2 x 10^5 cells / cm^2 BioVaSc and an optimal cultivation period of 2-5 days. These conditions revealed a tight cell monolayer. Immunohistological staining was performed to characterize the cells for the expression of the endothelial markers CD31 and vWF. Whereas HBMEC tend to loose expression of CD31 and vWF during cultivation on the BioVaSc, primary cells still expressed both markers on this scaffold. Further staining against ZO-1 proved the development of tight junctions and the tightness of the endothelial barrier.
Conclusion: In the present work it is shown that the BioVaSc is a suitable basis for the development of a tight endothelial barrier with the use of primary endothelial cells. Work in progress will integrate meningeoma cells into the scaffold to generate an *in vitro* organoid B-CSF barrier system.

P 158

Identification of genes required for host colonization in *Neisseria meningitidis*

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Question: Our objective was to establish an epithelial cell culture model in a continuous flow system in order to identify genes required for meningococcal colonization of epithelia.
Methods: Our laboratory constructed an ordered library of 4548 signature-tagged mutants (STM) among which 3625 transposon insertions have been mapped. We selected and transferred 1600 of these mutations in the capsulated strain 8013 (serogroup C). The 1600 mutants were analyzed for their inability to survivein a colonization model which consists of T84 epithelial cell line cultured in IBIDI* microslides under continuous flow system.
Results: We screened 96 pools of approximately 20 mutants in our colonization model. We identified 55 candidates. Mutants displaying a significant growth defect in cell culture medium or early adhesion defect were discarded.
To confirm and evaluate the colonization defect we conducted individual testing of all the attenuated mutants. We introduced the mutations in a GFP expressing strain and quantified biofilm developement onto epithelial cells using a Confocal Laser Scanning Microscope and the COMSTAT computer program. Six mutants exhibited a significant colonization defect compared to parental strain. The interrupted genes are mainly involved in adaptation to a low-oxygen environment. This finding is consistent with previous studies showing an important role of denitrification during biofilm formation by *Neisseria* species.
Since the initial screen is a competition assay, we hypothesized that some of the mutants identified in the screen could have a defect only in competition with a wild type strain. Mutation in two groups of genes belonging to a two-partner system secretion (TPS) locus and to a multiple adhesin family (*maf*) locus were defective when competing with the parental wild type strain onto an epithelial cell monolayers.

P 159

Effective plasmid DNA and small interfering RNA delivery to diseased human brain microvascular endothelial cells

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Genetic interference assays in cell culture models make a significant contribution to the enlightenment of meningococcal pathogenesis. Expression of exogenous DNA or siRNA *in vitro* is significantly affected by the particular delivery system utilized.

In this study, we evaluated the transfection efficiency of plasmid DNA and siRNA into the `hard-to-transfect' human brain microvascular endothelial cells (HBMEC) and meningioma cells, which constitute the blood-cerebrospinal fluid barrier, a target of *Neisseria meningitidis*. Chemical transfection methods and various lipofection reagents including Lipofectamin™, FuGene™, or jetPrime®, as well as physical transfection methods and electroporation techniques were applied. To monitor the transfection efficiencies, HBMEC and meningioma cells were transfected with the reporter plasmid pTagGFP2-actin vector and efficiency of transfection was estimated by fluorescence microscopy and flow cytometry.

We established protocols based on electroporation using Cell Line Nucleofector® Kit V with the Amaxa® Nucleofector® II system from Lonza and the Neon® Transfection system from Invitrogen resulting in up to 41% and 82% GFP-positive HBMEC, respectively. Optimal transfection solutions, pulse programs and length were evaluated. We furthermore demonstrated that lipofection is an efficient method to transfect meningioma cells with a transfection efficiency of about 81%.

Finally, we applied the successful electroporation protocols to deliver synthetic siRNA to HBMEC and analyzed the role of the actin-binding protein cortactin in *N. meningitidis* pathogenesis.

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Pool screen of a gonococcal high density transposon library to identify new virulence factors

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Neisseria gonorrhoeae is a gram-negative human-specific pathogen that causes the sexually transmitted disease, gonorrhea. In 1-3% cases, the bacteria spread within the host and cause disseminated infections. Till now the factors involve in this process and the molecular mechanisms are still unknown. Here, we use transposon mutagenesis combined with deep sequencing approach to identify and characterize further the factors required for *Neisseria* disseminated infection.
We established a protocol to perform Tn5 transposon mutagenesis in *Neisseria* strain MS11 and successfully constructed a high-density transposon library harboring more than 100,000 mutants. Insertions are equally distributed throughout the genome with an average insertion of one transposon every 22 bp. With this mutant library, we can identify essential genes for *Neisseria* and screen for diverse phenotypes. We present a screen for gonococcal invasion factors by infecting cells under low-phosphate conditions mimicking systemic bloodstream infection. Genes of invasion factors are identified by high-throughput sequencing the transposon-chromosome junctions of input and output libraries. Furthermore, selected candidates are validated by defined deletions and their role in invasion further validated. We will discuss the usefulness of this approach to identify novel gonococcal pathogenicity factors.

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Creation of a complete collection of mutants in *N. meningitidis*

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It is widely believed that the thousands of available genome sequences will be instrumental in a better and eventually comprehensive understanding of cellular life and the rational design of therapies against pathogens infecting mankind, livestock or crops. However, the limited understanding of gene function remains an important bottleneck. Of the numerous global ("omics") approaches designed to generate functional information, arguably the most direct way to gene function is to search for specific phenotypic alterations by screening an archived collection of defined mutants with mutations in each non-essential gene. Unfortunately, because the creation of such toolboxes is difficult, they are rare and currently available only in a handful of bacterial species: *E. coli*, *A. baylii* and *S. sanguinis*. Our aim is to produce such a collection of mutants in the meningococcus. Building up on the previously described NeMeSys database which led to the assembly of a library of mutants in approx. 900 genes, we are performing a systematic targeted mutagenesis of the remaining 1,000 genes using a splicing PCR method to produce gene deletions that are transformed directly into the meningococcus. This effort, which is well under way, will lead to the identification of the minimal genome of *N. meningitidis* and the design of an archived complete library of mutants in an important human pathogen. This toolbox, which will be freely available to the *Neisseria* scientific community, is expected to have an important impact (i) by facilitating, speeding up, and improving cost-effectiveness of future research, and (ii) by promoting both gene by gene and high-throughput functional studies.

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The European response plan to the threat of multi-drug resistant *Neisseria gonorrhoeae*

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The threat of multi-drug resistant *Neisseria gonorrhoeae* (MDR-NG) is now upon us. Antimicrobial surveillance data generated by the European gonococcal antimicrobial surveillance programme (Euro-GASP) has shown that strains exhibiting decreased susceptibility to cefixime have increased from 5% in 2009 to 9% in 2010, and have spread from 10 to 17 countries in just one year. While interim data from Euro-GASP 2011 has shown a small decrease to 8%, isolates with higher cefixime MICs (0.5 mg/L) and isolates with decreased susceptibility to ceftriaxone have been detected for the first time in the same survey. All the above mentioned isolates additionally display resistance to ciprofloxacin. Gonorrhoea treatment failures with cefixime have started to emerge in Europe and have been officially reported so far from Austria, France, Norway, and the United Kingdom, and one case of treatment failure with ceftriaxone has been documented in Sweden. A response plan has been created by the European Centre for Disease Control and Prevention (ECDC) to control and manage the threat of MDR-NG in Europe. The plan aims to; i) strengthen the surveillance of gonococcal antimicrobial susceptibility; ii) maintain and develop capacity for culture and susceptibility testing; iii) establish a strategy to timely detect treatment failure; iv) outline recommended public health actions, and v) increase awareness. This European public health response plan is urgently required, as alternative therapies are not available and the loss of cefixime and ceftriaxone as treatment options for gonorrhoea would have a significant negative impact on public health.

P 163

Increasing prevalence of penicillin nonsusceptible *Neisseria meningitidis* isolates in Belgium between 2000 and 2011

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Neisseria meningitidis colonizes the human nasopharynx and invasive strains can cross the epithelial barrier to cause meningitidis and/or septicemia. Immediate treatment with effective antibiotics is critical for a successful outcome.

This study was conducted to evaluate the evolution of the antimicrobial susceptibility of *Neisseria meningitidis* causing invasive diseases in Belgium in the period of January 2000 to December 2011. A total of 2,045 cases of *N. meningitidis* from invasive infections were analyzed by antimicrobial susceptibility testing at the Belgian Meningococcal Reference Centre. The majority of strains were susceptible to antibiotics that are currently used for the treatment and prophylaxis of meningococcal disease, but the prevalence of clinical isolates with reduced susceptibility to penicillin increased over the years. The systematic shift of the curves toward higher penicillin MICs in the susceptible population indicated that this population became less sensitive to penicillin in this period. A 402-bp DNA fragment in the 3′ end of *penA* was sequenced for the 303 nonsusceptible meningococcal strains isolated between 2000 and 2011 to examine the genetic diversity and evolution of their *penA* gene. In conclusion, the data obtained in our study support the statement that the position of penicillin G as a first choice in the treatment of invasive meningococcal diseases in Belgium should be reexamined. Despite an important number of isolates displaying a reduced susceptibility to penicillin, at present the expanded-spectrum cephalosporins, such as ceftriaxone, are not affected. The follow-up of the evolutionary changes in antimicrobial resistance has also proved to be essential for the recommendation of an appropriate antimicrobial treatment for invasive meningococcal diseases.

P 164

Recent trends in gonococcal resistance against third-generation cephalosporins and introduction of a new *penA* mosaic type in the Netherlands

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Introduction: Decreasing antimicrobial susceptibility to third-generation cephalosporins among *Neisseria gonorrhoeae* (NG) has been described. In a earlier study, in 64% of 74 strains with decreased susceptibility to cefotaxime isolated in 2006-08, a *penA* mosaic gene, always type XXXIV, had been found. Aim of the study was to monitor susceptibility trends and to investigate further dissemination of certain clonally related NG strains with decreased susceptibility to third-generation cephalosporins in patients visiting the STD outpatient department in Amsterdam.

Methods: The yearly number of NG isolates obtained by culture in our laboratory varied between 865 and 1135. Resistance monitoring was done for cefotaxime from 2007-2011 and for cefixime and ceftriaxone from 2010-2011. Susceptibility testing of gonococcal isolates was done by E-tests on GC-agar plates. The presence of a *penA* mosaic gene was determined with a PCR. Strain typing was done by NG-MLVA and by sequencing of the penA gene.

Results: The percentage of isolates with diminished susceptibility to cefotaxime (MIC > 0.12, EUCAST breakpoint) was 7.9% in 2007 and increased to 12.6%, 11.8% and 13.7% in 2008 2010, respectively, whereas only 7.9% was found in 2011. We detected only 3 strains in 2010 and 1 in 2011 with an MIC of 0.125 against cefixime, which is around the EUCAST breakpoint (0.12) and none with a higher MIC. One isolate with an MIC of 0.19 against ceftriaxone (above EUCAST breakpoint) was found in 2010, and none in 2011. Using an arbitrary breakpoint of ≥ 0.032, decreased susceptibility to cefixime and ceftriaxone fell from 17.6% to 11.2% and from 11.3% to 5.1%, respectively, between 2010 and 2011.

In the period 2009-2010, 123 of 133 tested NG isolates (93%) with a cefotaxime MIC > 0.125 had a *penA* mosaic gene, as well as 30/33 isolates (91%) with a cefotaxime MIC ≤ 0.125, but a cefixime MIC of ≥0.064 or a ceftriaxone MIC ≥ 0.047. All *penA* mosaic positive NG isolates were assigned to the same NG-MLVA cluster, except for 10 isolates. Of 8 of these, as well as of 6 isolates belonging to the cluster, the *penA* gene was sequenced. Ten out of these 14 strains were again type XXXIV, which was the same sequence type as had been found in 2006-08. Remarkably, 4 isolates from 3 patients were assigned to a different cluster containing isolates that had PBP2 pattern X, which had first been identified in the WHO Western Pacific Region. Notably, two of these patients were women, whereas the overwhelming majority of PBP2 type XXXIV isolates had been cultured from MSM.

Conclusions: Diminished susceptibility to cefalosporins among NG strains is widespread. A decline was found in 2011. One cluster with isolates with reduced cephalosporin susceptibility has been rapidly expanding, mainly among MSM, reflecting probable clonal spread, whereas a second type was more recently introduced.

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Neisseria gonorrhoeae: –first nationwide study on the spread of antibiotic resistance in Germany

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Introduction: Neisseria gonorrhoeae is one of the most frequent causes of sexual transmitted diseases worldwide. Drug resistance in gonococci has become an increasing public health problem. Data on the spread of antibiotic resistance among gonococcal isolates in Germany, however, is scarce.
Methods: In a surveillance study conducted by the Paul-Ehrlich-Society between October 2010 and December 2011, 23 laboratories all over Germany were asked to send N. gonorrhoeae isolates to the reference laboratory in Frankfurt am Main. Species verification was performed biochemically using ApiNH as well as with mass spectrometry using VITEK-MS. Antimicrobial susceptibility testing was performed using the Etest method according to the manufacturer's instructions. Clinical breakpoints set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for interpretation of susceptibility results. For molecular epidemiological examination, strains were genotyped by means of N. gonorrhoeae multi-antigen sequence typing (NG-MAST). Therefore a two-digit allelic profile of a strain was generated by sequencing internal regions of the genes encoding two variable outer membrane proteins, *Por* and *TbpB*.
Results: A total of 213 gonococcal isolates were analyzed in this nation wide study. Applying EUCAST breakpoints, resistance was found to be high to ciprofloxacin (76%) and tetracycline (41%). Penicillin non-susceptibility was determined for 86% of isolates. Azithromycin resistance was 6%, while all strains were susceptible to spectinomycin, cefixime, and ceftriaxone. Molecular typing of gonococcal isolates revealed a great heterogeneity of sequence types (ST), but ST1407 predominated.
Conclusions: This is the first German multi-centre surveillance study on antibiotic susceptibility of N. gonorrhoeae with implications for antibiotic choice for treatment of gonorrhea. The World Health Organization supports the concept that an efficacious treatment of gonorrhea results in at least 95% of infections being cured. Accordingly, as spectinomycin is not available on the German market, only third generation cephalosporins such as cefixime and ceftriaxone should be used empirically for treatment of gonorrhea in Germany.

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Antibiotic susceptibility profile of Neisseria meningitidis Croatian invasive isolates – "five-year" journey

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Introduction: Invasive meningococcal disease (IMD) in Croatia is mainly sporadic, commonly caused by Neisseria meningitidis serogroup B. Rapid and accurate laboratory PCR based *in house* method used in routine work improved confirmation of clinical diagnosis more than fifty percent in last 7 years. However, antibiotic susceptibility testing is done only from culture isolates. Resistance to some antimicrobial agents has long been recognized in some countries. We are still not facing with the increased resistance of *N. meningitdis*. Despite that it is important to pay attention on possible changes in its antibiotic susceptibility profile which is the basis for therapy of IMD as well as for prophylaxis of cases contacts.
Materials and methods: We have analysed the data of IMD isolates of hospitalized patients in the University Hospital for Infectious Diseases "Dr Fran Mihaljević" from 2008 to may 2012. The incidence of isolates confirmed by culture and/or PCR were recorded as well as serogroup distribution and antibiotic MIC (minimal inhibitory concentration) profile of cultivated *N. meningitidis* for penicillin, ceftriaxone, ciprofloxacin and rifampin. All isolates were tested on Mueller-Hinton agar with defibrinated sheep (CLSI) or horse blood (EUCAST) using E-tests. For susceptibility results CLSI (2008-2010) and EUCAST (2011- may 2012) interpretation criteria were used.
Results: During analyzed period a total of 126 *N. meningitidis* from patients with IMD were isolated. Even 53,2 % (67/126) were detected only by PCR and 24,6 % (31/126) by both methods, cultivation and PCR. All these case were sporadic. Serogroup B was predominant 108/126 (85,7 %) while 13/126 cases were group C (10,3 %). Serogroup W135 was recorded in one case. First case of serogroup Y was detected in 2009 and total of 4 cases of serogroup Y were recorded (3,2%). *N. meningitdis* was isolated from blood of 58/126 (50%) patients, from CSF of 37/126 (27,7%) and from blood and CSF of 33/126 (22,3%) patients. Elevated penicillin MIC (≥0.094 μg/ml) was recorded in 15 isolates, 2 in 2008 (0.94 and 0.125 μg/ml), 2 in 2009 (0.125 μg/ml), even 7 in 2010 (ranged from 0.094 to 0,25 μg/ml), 3 in 2011 (0.94 to 0.5 μg/ml) and 1 in 2012 (0.19 μg/ml). Only one isolate was resistant (0.5 μg/ml) while other isolates were of intermediated susceptibility. All isolates were susceptible for ceftriaxone, ciprofloxacin and rifampin with very low MIC values (median <0.002 μg/ml, 0.003 and 0.008, respectively).
Conclusion: Although in Croatia *N. meningitidis* serogroup B is still highly predominant it has to be noted emergence of serogroup Y. Resistance to agents of choice for therapy (e.g,ceftriaxone) and prophylaxis (ciprofloxacin, ceftriaxone, rifampin) of IMD has fortunately not yet developed in Croatia. However, it looks that median values of MICs are increasing and that would be of interest to foresee involvement of molecular methods in antibiotic resistance surveillance.

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Antibiotic-resistant of *Neisseria gonorrhoeae* in Italy from 2003–2011–characterization of multidrug-resistant isolates

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Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* has continued to increase and multi drug resistant (MDR) isolates have been reported worldwide. *N. gonorrhoeae* resistant to cephalosporins, the currently class of drug recommended for treatment, and treatment failures have been also recently documented, contributing to consider gonorrhoea as a possible untreatable infection. A study on antimicrobial susceptibility among Italian gonococci has been established from 2003 and coordinated at the Istituto Superiore di Sanità (ISS). The main aims of this study are: (a) to evaluate the antimicrobials susceptibility on circulating gonococci; (b) to analyze the molecular features of those resulting resistant to different class of drugs.
In the period 2003-2011, 1241 viable *N. gonorrhoeae* isolates, collected from 6 University Centres and STI Clinics in different Regions, have been sent to ISS. Susceptibility against ciprofloxacin, penicillin, cefixime, azithromycin, ceftriaxone, spectinomycin, tetracycline was assessed by Etest and by agar dilution method following standard procedures. Clinical breakpoints were those indicated by the EUCAST (Version 2.0, 2012). *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) were performed on 81 isolates with MDR phenotype. Ciprofloxacin resistance has been increased from 38% in 2003 to 59% in 2011 (63% in 2010), whereas the penicillin resistance decreased from 76% in 2003 to 6% in 2011. Gonococci resistant to cefixime (MIC>0.125mg/L) emerged in 2008 and then has been increased (around 10%); the rate of azithromycin resistance decreased from 14% in 2007 to 6% in 2011. No resistance or decreased susceptibility to ceftriaxone or spectinomycin was found. A total of 81 isolates were MDR, representing the 7.2% of the total analyzed (1106) in the period 2003-2011. Atotal of 11 different profiles of resistance were observed, of which the two main interesting groups of MDR isolates were: 37 resistant to ciprofloxacin, penicillin and tetracycline and 1 isolate resistant to ciprofloxacin, penicillin, tetracycline, azithromycin and cefixime. NG-MAST analysis identified 40 sequence types (ST). The two most common STs were the ST661 (10%) isolated mainly in 2003 and the ST1407 (17%) associated to isolates collected during the last years and resistant to cefixime. Despite Italy is a country with a low incidence of gonorrhoea infections, the number of gonococci resistant to one or more antimicrobials is increasing. During the time, we have been observed a change in the pattern of antimicrobial susceptibility among Italian gonococci and a genetic variability of MDR isolates due to the disappearance of ST661 and the paralleled increased number of isolates belonging to ST1407 resistant to cefixime.

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Multi-Antigen Sequence Types (NG-MAST) and Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* isolated in Canada, 2010

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Question: *Neisseria gonorrhoeae* have developed resistance to many antibiotics and have decreasing susceptibilities to the 3rd generation cephalosporins, the current recommended treatment. Surveillance data of *N. gonorrhoeae* multi-antigen sequence types (NG-MAST) and antimicrobial susceptibilities of Canadian *N. gonorrhoeae* isolated in 2010 are reported.
Method: A total of 1233 *N. gonorrhoeae* isolates were collected by Canadian provincial public health laboratories in 2010 and submitted to the National Microbiology Laboratory for testing. NG-MAST sequence types (1) and minimum inhibitory concentration (MICs) by agar dilution were determined for each isolate. MIC interpretations were based on the criteria of the Clinical Laboratory Standards Institute (2).
Results: In 2010, the most common sequence types (STs) found in Canada were ST1407, ST3150 and ST3158 at 13.3%, 11.3% and 9.0% respectively. These STs were comprised of multi-drug resistant profiles including chromosomal multi-resistant *Neisseria gonorrhoeae* (CMRNG, with resistance to penicillin, tetracycline and erythromycin) and Probable CMRNG along with ciprofloxacin resistance (CipR) and/or azithromycin resistance (AziR). Isolates within types ST1407, ST3150 and ST3158 were identified as either CMRNG or Probable CMRNG at 95.1%, 89.3% and 93.7%, respectively. Within the ST1407 group, 95.1% and 95.1% isolates are approaching non-susceptibility to cefixime and ceftriaxone (MIC>=0.063 mg/L), respectively. Within the ST3158 group, 98.2% and 91.9% of isolates are approaching non-susceptibility to cefixime and ceftriaxone, respectively.
Conclusions: Monitoring of antibiotic susceptibilities of *N. gonorrhoeae* isolates in Canada is important to ensure that treatment guidelines address the decreasing susceptibilities to the prescribed antibiotics for gonorrhea infections. Canada's 2010 most prevalent NG-MAST type, ST1407 is of particular interest since it has been associated with cefixime treatment failures in Norway, France and Austria (3), reported in the USA (4), Australia (5), Sweden (6) and the United Kingdom (7) and has recently been associated with high level ceftriaxone resistance (3).
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Standards Institute. Wayne, PA, 2011. 3. Unemo M, Golparian D, Nicholas R, et al. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: Novel *penA* mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother 2012; 56(3):1273-1280. 4. Pandori M, Barry PM, Wu A, et al. Mosaic penicillin-binding protein 2 in *Neisseria gonorrhoeae* isolates collected in 2008 in San Francisco, California. Antimicrob Agents Chemother. 2009;53:4032-4034. 5. Tapsall JW, Ray S, Limnios A. Characteristics and population dynamics of mosaic *penA* allele-containing *Neisseria gonorrhoeae* isolates collected in Sydney,Australia, in 2007-2008. Antimicrob Agents Chemother 2010;54:554-556. 6. Golparian D, Hellmark B, Fredlund H, Unemo M. Emergence, spread and characteristics of *Neisseria gonorrhoeae* isolates with in vitro decreased susceptibility and resistance to extended-spectrum cephalosporins in Sweden. Sex Transm Infect 2010;86:454-460. 7. Ison CA, Hussey J, Sankar KN, Evans J, Alexander S. Gonorrhoea treatment failures to cefixime and azithromycin in England, 2010. Euro Surveill. 2011;16(14):pii=19833. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19833

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Biochemical analysis of Penicillin-Binding Protein 2 containing Ala501 mutations that confer decreased susceptibility/resistance to expanded-spectrum cephalosporins

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Decreased susceptibility to the expanded-spectrum cephalosporins ceftriaxone and cefixime in *Neisseria gonorrhoeae* has increased dramatically over the past decade, and recently resistance to the last remaining recommended treatment option, ceftriaxone, was described, raising fears that gonorrhea may become untreatable under certain circumstances and in some settings. The major reason for decreased susceptibility has been the emergence of mosaic alleles of *penA* encoding penicillin-binding protein 2 (PBP 2), the major target for these antibiotics, with up to 70 mutations relative to wild-type. Less prevalent in most settings has been the emergence of non-mosaic alleles of *penA* containing A501V or A501T mutations just past the KTG active site motif that confer decreased susceptibility to ceftriaxone and cefixime. We have shown that A501 mutations, when introduced into mosaic *penA* alleles, confer full resistance to expanded-spectrum cephalosporins (Tomberg J *et al.* Biochemistry. 2010), and recently, a novel mosaic *penA* allele containing an A501P mutation was described from a patient who had treatment failure with cefixime (Unemo M *et al.* Antimicrob Ag Chemother. 2012). To understand the role of Ala501 mutations in mosaic alleles, we transformed FA19 with the mosaic *penA* allele from 35/02 harboring a randomized codon at position 501 and selected for increased cefixime resistance. From this screen, we identified only three amino acid alterations (A501V, A501T, and A501R) that resulted in increased cefixime resistance, indicating that only a small subset of mutations are capable of conferring resistance. Surprisingly, an A501P mutation was not selected, even though sequencing of the library suggested equal randomization of all three positions of the codon. MIC analyses showed that mutation of Ala501 to Val or Thr conferred a ~2.5-fold increase in resistance, whereas mutation to Arg or Pro (introduced by site-directed mutagenesis) increased resistance nearly 5-fold. PBP 2-6140CT (PBP 2 containing 4 C-terminal mutations) harboring either an A501V or A501T mutation was crystallized and revealed a major increase in ordering and some reorganization of the β3-β4 hairpin that is immediately adjacent to the active site. Modeling of β-lactams into the crystal structure indicates that the mutations likely introduce a steric clash with the R1 substituent of the expanded-spectrum cephalosporins, which presumably explains the decreased susceptibility/resistance caused by these mutations.

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Distinct Regulatory Mutations Impacting Antimicrobial Resistance in Gonococci – Influence of Growth Conditions

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Question: The MtrCDE efflux pump of Neisseria gonorrhoeae confers resistance to a diverse array of antibiotics and host antimicrobial compounds. The mtrCDE operon lies 250 base pairs downstream and is transcribed divergently from the gene encoding the pump operon repressor, mtrR. A number of mutations in the mtr locus have been identified that increase resistance of gonococci to MtrCDE substrates. Mutations in the coding region of mtrR often disrupt the helix-turn-helix DNA binding domain, decreasing affinity of MtrR for its binding site at the mtrCDE promoter, and generally confer low levels of resistance. In contrast, two mutations in the intergenic region between mtrR and mtrCDE have been characterized that confer high levels of resistance. One, a point mutation in the inverted repeat of the mtrR promoter, decreases the spacing between the -10 and -35 promoter elements, diminishing transcription of mtrR and allowing increased transcription from the overlapping mtrCDE promoter. The other, a C to T transition mutation 120 base pairs upstream of the mtrC start codon (mtr_{120}), generates a second promoter for mtrCDE. Interestingly, in a recent examination of 121 clinical isolates, the single base pair deletion was found in 86 strains, while the mtr_{120} mutation was found in only one. The goal of this study is to determine the cause for the discrepancy in the frequency of these mutations.
Methods: The effect of mtr mutations on growth was assessed by growth in liquid cultures, either singly or in competitive co-culture. The minimal inhibitory concentrations of pump substrates conferred by each mutation were compared using two-fold agar dilution assays. The rate of mutation to mtr_{120} or the single base pair deletion was determined by selecting spontaneous mutants of wild-type strain FA19 resistant to high levels of MtrCDE substrates from cells grown overnight on plates or from cells grown in biofilms. Mutations were confirmed by sequencing.
Results: Neither mutation confers a growth defect or competitive advantage during growth in broth cultures. Additionally, these mutations independently confer similar levels of resistance to pump substrates, which appear to be the maximum achievable levels, as resistance is not increased in a double mutant bearing both mutations. Surprisingly, selection for spontaneous high-level resistant mutants of strain FA19 from planktonic cells yielded only the mtr_{120} mutation, whereas selection from cells grown in biofilms yielded primarily the single base pair deletion.
Conclusions: Our findings suggest that the growth environment, and so perhaps type of gonococcal infection, may play an important role in the selection of specific mtr mutants. Further studies utilizing in vivo infection models will better elucidate the role of infection site in selection and maintenance of resistance mutations.

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Inactivation of the MtrCDE, MacAB, and NorM efflux pumps in *Neisseria gonorrhoeae* strains with clinical resistance to extended-spectrum cephalosporins make them susceptible to several antimicrobials

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Neisseria gonorrhoeae has developed resistance to all antimicrobials introduced for gonorrhoea treatment. Recently, the first gonococcus (H041) displaying extensive drug-resistance (XDR) including high-level resistance to ceftriaxone was found in Japan. Ceftriaxone is the last remaining option for first-line, empiric treatment and the new "superbug" *N. gonorrhoeae* may in the near future cause untreatable gonorrhoea. For future treatment, to avoid rapid development of resistance, new thinking may be crucial.
In this study, the MtrCDE, MacAB, and NorM efflux pumps were genetically-inactivated in a total of 21 *N. gonorrhoeae* strains, including the first high-level ceftriaxone-resistant strain (H041) and additional strains causing treatment failure with cefixime. The minimum inhibitory concentrations (MICs) of ceftriaxone, cefixime, penicillin G, ampicillin, ertapenem, azithromycin, ciprofloxacin, kanamycin, gentamicin and spectinomycin were determined before and after the inactivation of the efflux pumps using Etest.
Inactivation of the MtrCDE, MacAB and NorM efflux pumps in H041 resulted in increased susceptibility to ceftriaxone, i.e. MIC decreased from 4 mg/L to 0.5-0.75 mg/L, 1 mg/L, and 2 mg/L, respectively. Strains causing treatment failure with cefixime were affected in similar way. In general, by genetic inactivation of the efflux pumps the MIC decreased for most of the antimicrobials examined.
In conclusion, genetic inactivation of especially the MtrCDE efflux pump can make antimicrobial resistant *N. gonorrhoeae* strains fully susceptible. Accordingly, inhibitors of especially the MtrCDE efflux pump, in particular administered in combination with appropriate antimicrobials, might be a future treatment option for gonorrhoea. Inhibition of specific gonococcal efflux pumps could be an important and novel approach to improve the efficacy of antimicrobials no longer in use due to resistance.

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Co-administration of the cyanobacterial lipopolysaccharide antagonist CyP with antibiotic inhibits cytokine production by an *in vitro* meningitis model infected with *Neisseria meningitidis*

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Question: in this study, the objective was to determine the anti-inflammatory properties of CyP, a lipopolysaccharide (LPS)-antagonist from the cyanobacterium *Oscillatoria planktothrix*, used in combination with antibiotic chemotherapy during infection of an *in vitro* meningitis model with *Neisseria meningitidis* (meningococcus).

Methods: Monocultures of human meningioma cells and meningioma-primary human macrophage co-cultures were infected with *Neisseria meningitidis* (10^2-10^8 cfu/monolayer) or treated with isolated outer membranes (OM) or purified LPS (0.1-100ng/monolayer) frommeningococci. CyP (1-20μg/monolayer) was added at intervals from t=0-4h, with and without benzylpenicillin (1-20 μg/monolayer). The antagonistic effect of CyP and its adjunctive properties to benzylpenicillin administration was determined by measuring cytokine levels in culture supernatants after 24h.

Results: CyP significantly inhibited ($p<0.05$) the secretion of IL-6, IL-8, MCP-1 and RANTES (overall reduction levels from 50->95%) by meningioma cell lines and meningioma-macrophage co-cultures, challenged with either live meningococci or bacterial components. Inhibition was effective when CyP was added within 2h of challenge ($p<0.05$) and still pronounced by 4h. In the co-culture model, CyP alone partially inhibited IL-1β secretion but did not prevent TNF-α secretion. By contrast, penicillin alone inhibited IL-1β and TNF-α, but did not reduce MCP-1 and RANTES secretion. However, co-administration of CyP and penicillin in both models had an additive effect and restored the overall inhibitory profile.

Conclusions.CyP inhibits cytokine production in an *in vitro* meningitis model and augments the anti-inflammatory response when combined with benzylpenicillin. Administration of a LPS-antagonist with antibiotic merits consideration in the emergency treatment of patients presenting with meningococcal infection.

Reference: Oliver R, Staples KJ, Heckels J, Rossetti C, Molteni M, Christodoulides M. Coadministration of the cyanobacterial lipopolysaccharide antagonist CyP with antibiotic inhibits cytokine production by an in vitro meningitis model infected with *Neisseria meningitidis*. J Antimicrob Chemother. (2012);67(5):1145-54.

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Fluoroquinolone resistance-conferring *gyrA*$_{91/95}$ mutations provide enhanced *in vivo* fitness to Neisseria gonorrhoeae and alter the transcription 77 genes

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Question: We recently reported that fluoroquinolone-resistance conferring gyrA$_{91/95}$ mutations result in increased the fitness of Neisseria gonorrhoeae (Gc) strain FA19 in an experimental genital tract infection model. Here we asked whether these mutations would alter the in vivo fitness of other Gc strains and whether comparison of transcriptional profiles from wt and gyrA$_{91/95}$ mtuant Gc would provide a clue as to the basis of this fitness advantage.

Methods: gyrA$_{91/95}$ double point mutations were introduced into the ciprofloxacin sensitive (Cip^S) strains MS11 and FA1090. Susceptibility of wt and mutant strains to Cip was determined by agar dilution. The in vitro and in vivo fitness of each gyrA$_{91/95}$ mutant relative to its parent strain was assessed by broth culture and competitive infection of female BALB/c mice, respectively. RNA was extracted from wt strains and their respective gyrA$_{91/95}$ mutants after culture on solid agar for 18 hrs and total RNA sequencing was performed. Data were analyzed using the CLC Bio software suite. All sequences were mapped to the annotated FA1090 genome, and read counts for each mapped gene were normalized to the total number of reads per sample using the Reads Per Kilobase perMillion reads mapped.

Results: As expected, gyrA$_{91/95}$ mutants of strains FA1090 and MS11 showed intermediate Cip resistance (Cip^I). No difference in the relative fitness of mutants and parent strains was observed in vitro, but gyrA$_{91/95}$ mutants exhibited a 10- to >10-fold increase in fitness relative to the Cip^S parent strain during murine infection. Interestingly, the gyrA$_{91/95}$ allele conferred a greater fitness advantage to MS11 and FA1090 compared to FA19. Analysis of differential transcriptional reads between the three pairs of gyrA$_{91/95}$ mutant and parent strains identified several up-regulated genes implicated in biological pathways critical for bacterial survival in a resource-limited environment. In total, 77 transcript reads for annotated genes that are implicated in biological processes displayed at least a 2-fold increase between at least two of the three gyrA$_{91/95}$ mutant and isogenic parent pairs. Additionally, expression ofgyrA was decreased in each mutant relative to the parent strain.

Conclusions: We recently hypothesized that gyrA$_{91/95}$ mutations, the first step in Cip resistance, may create reservoir for subsequent acquisition of full Cip resistance by increasing microbial fitness. Here we showed gyrA$_{91/95}$ mutations confer increased fitness to two other strains and used RNA sequencing as an unbiased means of capturing global changes in RNA transcript levels directed by Cip^I gyrA$_{91/95}$ mutations. Thus far, the data are consistent with the hypothesis that transcriptional changes might provide enhanced survival in the context of the host infection. This work is the first step towards identifying the reason for enhanced in vivo fitness of gyrA$_{91/95}$ (Cip^I) mutants.

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LpxC inhibitors as novel therapeutics for treatment of antibiotic-resistant *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae infections are currently treated with the expanded-spectrum cephalosporins ceftriaxone and cefixime. However, strains resistant to these drugs have emerged in the last two years, suggesting that the cephalosporins may eventually be rendered ineffective against gonococcal infections. Few other antibiotics have proven activity against *N. gonorrhoeae* and there are no new candidates for gonococcal treatment on the horizon. To this end, we are investigating inhibitors of LpxC (UDP-3-O(acyl) N-acetylglucosamine deacetylase), the zinc-dependent enzyme that catalyzes the first committed step in lipid A biosynthesis, as a novel class of antibiotics against *N. gonorrhoeae*. Despite evidence that lipid A is dispensable in the closely related species *N. meningitidis*, we found that it is essential in *N. gonorrhoeae*, suggesting that LpxC represents a good target for anti-gonococcal antibiotics. We have evaluated a panel of diacetylene LpxC inhibitors for activity against *N. gonorrhoeae*; the best characterized inhibitor, LPC-067, has an MIC of 0.1 μg/ml against the standard laboratory strain, FA19. LpxC inhibitors are bactericidal and lead to a nearly complete loss of viability after 2 hours of exposure. Replacement of the gonococcal LpxC with the *E. coli* LpxC orthologue, which is inhibited at low concentrations of LPC-067, decreases the MIC of LPC-067 to 0.001 μg/ml, indicating that the target of LPC-067 is indeed LpxC. LPC-067 inhibits the growth of 35/02, a penicillin-resistant and cephalosporin-intermediate resistant strain, with an MIC of 1 μg/ml, suggesting that these inhibitors will also be effective for treating emerging cephalosporin-resistant strains of *N. gonorrhoeae*. Newly synthesized compounds are even more effective than LPC-067, with MICs less than 0.005 μg/ml against FA19 and less than 0.05 μg/ml against 35/02. Thus, LpxC inhibitors are a promising class of compounds for the development of new antibiotics to treat gonococcal infections.

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Novel inhibitors of *N. gonorrhoeae* penicillin-binding protein 2 identified by high-throughput screening

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Penicillin-binding proteins (PBPs) are transpeptidases required for cell-wall synthesis in bacteria and are best known as the proven targets for β-lactam antibiotics. Unfortunately, their value as clinical targets is being challenged by mutations that lower susceptibility of PBPs for inhibition by β-lactams, whilst retaining their essential transpeptidase function. The outcome is increasing prevalence of *N. gonorrhoeae* strains that exhibit resistance to third-generation cephalosporins and fewer options in the clinic to treat gonorrhea. To address this challenge, new antimicrobials are needed to treat resistant *N. gonorrhoeae*. We have developed a high-throughput assay that uses fluorescence polarization (FP) to distinguish the fluorescent penicillin, Bocillin-FL, in free or PBP-bound form. This assay was used to screen a 50,000 compound library for potential inhibitors of *N. gonorrhoeae* PBP 2, and 32 compounds were identified that exhibited >50% inhibition of Bocillin-FL binding to PBP 2, including a cephalosporin that provided validation of the assay. After elimination of compounds that failed to exhibit concentration-dependent inhibition, the antimicrobial activity of the remaining 24 was tested. Of these, 7 showed antimicrobial activity against susceptible and penicillin- or cephalosporin-resistant strains of *N. gonorrhoeae*. In molecular docking simulations, two of these inhibitors docked successfully into the active site of the enzyme and each mediate interactions with the active-site serine nucleophile. This study demonstrates the validity of a FP-based assay to find novel inhibitors of PBPs and paves the way for more comprehensive high-throughput screening against highly resistant strains of *N. gonorrhoeae*. It has also provides a set of lead compounds for optimization toward novel anti-gonococcal agents.

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Increase of African-type penicillinase-producing N. gonorrhoeae strains isolated in Guangzhou, China, 2001–2011

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Background: The continuing spread of drug-resistant gonococci has posed a challenge for successful treatment worldwide. Recently third-generation cephalosporins-resistant strains were isolated in Japan. Guangdong Province in South China has one of the highest gonococcal resistance rates inChinaand a large number of international migrants. We investigated the in vitro antimicrobial susceptibility and genotypes of N. gonorrhoeae strains isolated inGuangzhou, the capital city, from 2001 to 2011.
Methods: MICs to penicillin, ceftriaxone, tetracycline, ciprofloxacin, and spectinomycin were determined by agar plate dilution and susceptibilities were interpreted according to WHO standards. β-lactamase production was determined by paper acidometric testing. The resistant plasmids were determined for-penicillinase-producing N. gonorrhoeae (PPNG) and high-level tetracycline resistant N. gonorrhoeae (TRNG) by PCR and the isolates were genotyped.
Results: Of1250 consecutive gonococci isolated from 2001-11, no ceftriaxone and spectinomycin resistant strains were found, but the prevalence of strains less susceptible to ceftriaxone rose from 17% to 46.5%. The MIC_{90} for ceftriaxone showed intermediate sensitivity (0.06-0.125μ/mL) and spectinomycin near the resistant level (16-32μ/mL). The resistance to penicillin, tetracycline and ciprofloxacin were from 85%, 85% and 78% in 2001 to 81.9%, 100% and 98.4% in 2009, respectively. A total of 349 (27.9%) PPNG and 539 (43.1%) TRNG strains were detected. 232 (18.6%) strains were both PPNG and TRNG. PPNG rose from 17.0% to 32.3% and TRNG rapidly increased from 26.0% to 41.7%. Genotyping TEM-1 gene showed 331 (94.8%) PPNGs carring the Asia-type β-lactamase plasmids in 2001-11. The Africa-type PPNG emerged in 2008 and has increased from 1(1.3%) to 8(6.2%) in 2008-11. Genotyping of tetM gene showed that all 539 TRNGs were Dutch variants.
Conclusion: Gonorrhea resistance continues to be a major public health problem inGuangzhou. The emergence and increase of an African gonorrhea resistance variant may be related to the large African diaspora inGuangzhou, migration of Chinese toAfrica, or other migration patterns. More research is needed to determine what practices, systems, and behaviors contribute to escalating resistance patterns.

figure 1

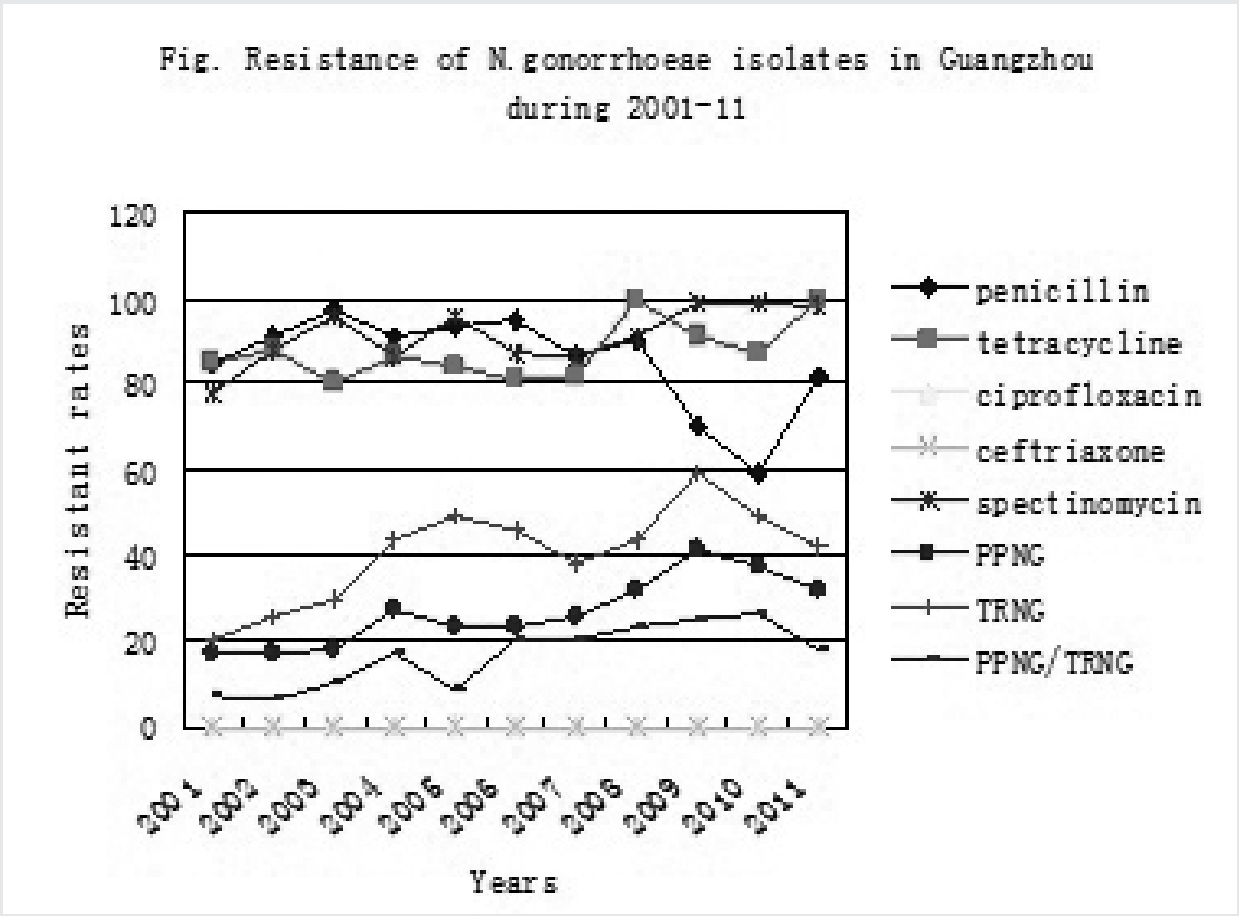

figure 2

Tab. The prevalence of PPNG and TRNG plasmid types in Guangzhou in 2001-11 (%).

Year	PPNG			TRNG	
	Asia-type	Africa-type	Others*	Dutch-type	America-type
2001	100	0	0	100	0
2002	100	0	0	100	0
2003	100	0	0	100	0
2004	100	0	0	100	0
2005	100	0	0	100	0
2006	100	0	0	100	0
2007	100	0	0	100	0
2008	98.7	1.3	0	100	0
2009	98.7	1.3	0	100	0
2010	94.9	5.1	0	100	0
2011	93.8	6.2	0	100	0

* Toronto- , New Zealand-, and Nîmes-types.

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Study on the genetic polymorphisms in the related resistant genes of Neisseria gonorrhoeae with reduced susceptibility to ceftriaxone in China

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QuestionIncreasing antimicrobial resistance in Neisseria gonorrhoeae (NG) is a major challenge for prevention and control of gonorrhea. The recommended first-line treatment for gonorrhea in China is ceftriaxone or spectinomycin. In recent years, the emergence and spread of NG strains with reduced susceptibility to ceftriaxone (Cef^{i}) has been reported in China. It's essential to monitor the susceptibility to ceftriaxone of NG strains and launch research on the resistance mechanism of NG strains resistant or non-susceptible to ceftriaxone. Our aim was to investigate the correlation of different polymorphisms in the penA, porB, and mtrR genes of Cef^{i} isolates, defined as having an MIC value equal to or greater than that of strain WHO K or WHO L, which are WHO recognized as Cef^{i} strains.
Methods Seventeen Cef^{i} isolates and 4 susceptible NG isolates were characterized by NG-MAST method and sequencing of penA, porB, and mtrR alleles.
ResultsNone of the 21 NG isolates contained a penA mosaic allele, but a 501 substitution (A501T) in conjunction with a 542 substitution (G542S) were present in PBP2 (coded by penA gene) of 8 Cef^{i} isolates, and a 551 substitution (P551S or P551L) in conjunction with a 501 substitution (A501V) were present in PBP2 of 6 Cef^{i} isolates. A 218 substitution (I218M) in conjunction with a 257 substitution (M257T) and a 259 substitution (G259V) were present in PorB1b (coded by porB1b gene) of 4 Cef^{i} isolates, and another 257 substitution (M257R) were present in PorB1b of 6 Cef^{i} isolates. All 17 Cef^{i} isolates were assigned 13 NG-MAST sequence types, of which 3 were novel.
Conclusionsmutations in penA and porB genes may play a role for the reduced susceptibility to ceftriaxone in NG.

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Structure and functional mechanism of the human antimicrobial peptide channels

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Multicellular organisms fend off fungal and bacterial infections by peptide-derived broad-spectrum antibiotics. The human body provides an extensive surface for the growth of microbes and their suppression requires the secretion of efficient antimicrobial peptides (AMPs). As AMPs directly compromise the integrity of microbial cell membranes and thereby evade many pathways of resistance development, AMPs are viewed as a foundation for the next generation of antibiotics. However, despite the identification of a large number of AMPs, the mechanistic and structural basis of the antibiotic function of AMPs has remained speculative. Here, we present the oligomeric channel structure and antibiotic mechanism of dermcidin (DCD) and LL-37, two major human AMPs on skin and epidermal surface. The X-ray crystal structure, functional data and atomistic simulations of DCD in membranes reveal that its active state is a transmembrane barrel-stave channel, constructed from a hexamer of anti-parallel, elongated helices, stabilised by zinc ions. Electrophysiological measurements of the channel in planar lipid bilayers show that DCD forms highly conductive pores that are regulated by zinc. Molecular dynamics simulations in lipid bilayers provide detailed insight into the permeation mechanism for ions and water, and demonstrate an adjustment of the pore to various membrane types. Our study provides a comprehensive mechanism for the membrane-disruptive action of a mammalian AMP at the atomistic level. Natural antibiotics such as Dermcidin and LL-37 are less prone to causing resistance as they target microbial membranes instead of key factors of the host cell cycle (e.g. ribosomes). Therefore our results will form a basis for the structure-based, rational design of new AMP-derived antibiotic agents. As DCD is active against multi-resistantS. aureusandC. albicansstrains, rationally enhanced DCD variants have the potential to treat bacterial infections that are no longer susceptible to traditional antibiotic agents.

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The only mutation in ribosomal protein S5 confers the resistance of Neisseria gonorrhoeae to spectinomycin

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Spectinomycin (SPC) remains a useful reserve option for gonococcal therapy. The emergence of multidrug-resistantNeisseria gonorrhoeae (NG) strains with decreased susceptibility to cefixime makes it the only medicine still effective against gonorrhoea infection in analogous cases. However, adoption of SPC as the routinely used drug of choice was soon followed by reports of SPC resistance.The only previously reported mechanism of SPC resistance in NGwas 16S rRNA substitutions. Here we report a first case of SPC resistance in NG due to isolated mutation in ribosomal protein S5 (RPS5) without corresponding changes in 16S rRNA.

Two isogenic strains grown on GC-agar plate with 64 mg/ml of SPC were occasionally selected. They derived from clinical e03.04 NG isolate initially identified as susceptible to SPC by routine bacteriological testing. Both selected strains carried identical Thr24Pro mutation in the RPS5 which has not been found in e03.04 NG. The working hypothesis was that this certain mutation confers SPC resistance. To prove this we performed the spot transformation of other susceptible to SPC NG strain m07.05 by the amplified fragment of NG genomic DNA (3163 bp) contains mutant RPS5 gene with DUS incorporated during PCR (primers: GGATGCCGTCTGAAttaccaaagaaactaccgtgg and GGATGCCGTCTGAAaaagcaactgaggaagagcg). All individual transformants (n = 42) grown on GC-agar with 64 mg/ml of SPC carried the same Thr24Pro mutation in the RPS5.

Hereby we have reported for the first time an alternative way to known 16S rRNA C1192U mutation for SPC resistance formation of NG.In conclusion it should be mentioned that previously no RPS5 mutations associated to SPC resistance, neither with nor without changes in 16S rRNA genes were found inNeisseriagenus.

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Molecular epidemiological typing within the European gonococcal antimicrobial resistance surveillance programme (EURO-GASP) reveals predominance of a multi-drug resistant clone

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Background: Treatment of *Neisseria gonorrhoeae* infection is compromised by antimicrobial resistance, and decreased susceptibility and resistance to the third generation cephalosporins (cefixime and ceftriaxone) currently recommended for empirical treatment is emerging in Europe. In the absence of alternative therapies, there is an urgent need to develop strategies to limit dissemination of resistant gonococcal strains and enhanced surveillance of gonorrhoea is central to this. This study explored the molecular epidemiology of *N. gonorrhoeae* in Europe in relation to antimicrobial resistance and patient characteristics.
Methods: Gonococcal isolates (n=966) collected from 21 countries for the 2010 EURO-GASP were typed by *Neisseria gonorrhoeae* Multi Antigen Sequence typing (NG-MAST), which sequences variable regions of *por* (490 bp) and *tbpB* (390 bp). For countries submitting low numbers, additional isolates from 2009 (n=100) were included. Associations between sequence type (ST) and i) antimicrobial resistance and ii) patient characteristics were explored by univariate and where applicable, multivariate analysis.
Results: A total of 406 STs were identified, 125 of which occurred in ≥two isolates. Seven major Genogroups (Gs) of closely related STs (varying by <1% at one locus, while the other locus is identical) were defined. G1407, observed in 20/21 countries and predominant in 13/21 countries, accounted for 23% of all isolates and was associated with decreased susceptibility to cefixime, ciprofloxacin resistance, and raised Minimum Inhibitory Concentrations (MICs) for ceftriaxone and azithromycin. While men who have sex with men (MSM) had significantly higher odds of infection with this genogroup, G1407 was widespread in the heterosexual population also. G225, associated with ciprofloxacin resistance, was also prevalent (10% overall) being observed in 19/21 countries. None of the other genogroups were associated with antimicrobial resistance but associations with heterosexual patients (G2, G25, G387) or MSM (G2992) were identified.
Conclusions: The predominance of the multi-drug resistant clone G1407 in Europe is worrying given the recent reports of recommended third generation cephalosporins failing to treat infections with this clone. Identifying associations between ST and antimicrobial resistance aid understanding of the dissemination of resistance within a population and could facilitate development of targeted intervention strategies.

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Modelling epidemics in the African meningitis belt: Insights from stochastic metapopulation models

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The epidemiology of *Neisseria meningitidis* in sub-Saharan Africa is defined by periodic but irregular epidemics usually caused by serogroup A disease. Although a new group A conjugate vaccine is being introduced into the region, long-term control strategies have not yet been determined and other serogroups may still cause epidemics. Our understanding of the epidemiology of *N. meningitidis* infection in this region is incomplete and mathematical models can provide a useful framework for investigating hypotheses about the drivers of epidemics. Previous simple deterministic mathematical models have suggested that the timing of such epidemics may depend heavily on population immunity and need not require inter-annual variation in climatic effects. But it is very important to understand whether such predictions are robust to the choice of model used. Here we present a stochastic metapopulation model in which different communities are epidemiologically linked by individuals carrying infection from one community to another. Seasonality is incorporated by sinusoidal increases in either the force of infection or the probability of progressing to invasive disease given carriage or both. We compare the qualitative and quantitative behaviour of this model to that of simple deterministic models and explore whether population immunity is still the key factor in determining epidemic timing. We then examine how synchronisation of outbreaks in different communities affects rates of carriage and disease, and discuss the relationship between hyperendemicity, local outbreaks and "epidemic waves".

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Impact of the First National Meningococcal A Conjugate Vaccine Implementation, Burkina Faso 2011–2012

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Background: Serogroup A Neisseria meningitidis causes major epidemics of meningitis in sub-Saharan Africa. An affordable, highly immunogenic serogroup A meningococcal conjugate vaccine (PsA-TT) was licensed in 2009. In 2010, Burkina Faso became the first country to implement a national preventive campaign, vaccinating 11·4 million persons aged 1-29 years.

Methods: We examined national population-based meningitis surveillance data—cases and deaths aggregated at the district-level (1997-2011) and enhanced case-based (2007-2011)—from Burkina Faso (available 2012 data will be used to update analysis prior to presenation). We assessed changes in mortality rates and incidence of suspected meningitis, bacterial meningitis by age, and serogroup-specific meningococcal disease, and estimated the risk during the first year after PsA-TT implementation.

Results: During the 14-year period prior to PsA-TT introduction, Burkina Faso recorded 148,603 cases and 17,965 deaths of suspected meningitis, and 174 district-level epidemics. After vaccine introduction, there was a 71% decline in risk of meningitis (hazard ratio [HR] 0·29, p<0·0001) and a 64% decline in risk of fatal meningitis (HR 0·36, p<0·0001). We observed a statistically significant decline in risk of probable meningococcal meningitis across the age group targeted for vaccination (62%, p<0·0001), as well as among children aged less than 1 year (54%, p=0·02) and persons aged 30 years and older (55%, p=0·003) who were vaccine-ineligible. Zero cases of serogroup A meningococcal meningitis occurred among vaccinated individuals, and local epidemics were eliminated. The incidence of laboratory-confirmed serogroup A N. meningitidis dropped significantly to 0·01 per 100,000 persons per year representing a 99·8% reduction in the risk of meningococcal A meningitis.

Conclusions: Early evidence suggests the conjugate A vaccine has dramatically reduced the rate of meningitis in persons in the target age-group, and in the general population due to high coverage and community immunity. These data suggest that fully implementing PsA-TT vaccine could end epidemic meningitis of serogroup A in sub-Saharan Africa.

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Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity

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Background: The conjugate vaccine against serogroup A *Neisseria meningitidis* (NmA), MenAfriVac, which was first introduced in mass vaccination campaigns of 1-29-year-olds in Burkina Faso in 2010, may reduce meningococcal carriage and confer herd immunity.
Methods: We conducted a repeated cross-sectional study of meningococcal carriage in a representative portion of the 1-29-year-old population in three districts in Burkina Faso before and up to 13 months after mass vaccination, yielding a total of 45,847 tonsillo-pharyngeal samples. One district was vaccinated in September 2010, while the other two were vaccinated during a country-wide mass vaccination in December 2010.
Results: Based on an analysis of 20,326 samples, overall meningococcal carriage prevalence was 3.98% before vaccination and NmA prevalence was 0.39% (Kristiansen et al. *Clin Vaccine Immunol* 2011). In October-November 2010, NmA carriage in the unvaccinated districts was comparable to baseline level but absent in the vaccinated district. Serogroup X *N. meningitidis* (NmX) dominated in both vaccinated and unvaccinated districts. Four additional sampling campaigns were performed every three months in 2011 in the three districts. Based on 22,093 samples, overall post-vaccination meningococcal carriage prevalence was 6.95%, with NmX dominating but declining for each campaign (8.66-1.97%). No NmA was identified post-vaccination. Overall vaccination coverage in the population sampled was 89.7%, declining over time in the 1-year-old group (87.1-26.5%), as non-vaccinated infants reached 1 year. Thus, NmA carriage was eliminated in both the vaccinated and unvaccinated population from 3 weeks up to 13 months after mass vaccination.
Conclusions: MenAfriVac has the ability to eliminate NmA carriage and confer herd immunity by preventing NmA colonisation and transmission.

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Increase of salivary antibodies targeting the serogroup A capsule of *N. meningitidis*, after vaccination with MenAfriVac

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Question: Conjugate vaccines may stimulate the immune system so that serum antibodies diffuse onto oral mucosa and prevent colonization. Can immunization with the serogroup A meningococcal conjugate vaccine, MenAfriVac, increase the antibody concentration in saliva? The study was done in Burkina Faso, the first country to fully implement MenAfriVac in December 2010.

Methods: We conducted a longitudinal study with 200 school children aged 10-14 years living in Ouagadougou, the capital of Burkina Faso. Saliva samples were collected using OraSure collection device®, before vaccination with MenAfriVac and 8 weeks after. We determined the concentrations of specific IgG (IgG binding to serogroup A polysaccharide) and total IgG using an in-house ELISA test. We also report the specific IgA and total IgA concentrations measured in a bead-based multiplex assay (Bio-Plex) for a subset of 81 samples. Day-to-day variation was estimated by measuring specific and total IgG in 19 individuals sampled twice before and twice after vaccination.

Results: A total of 199 participants, evenly distributed in each age group, were included in the analysis, of which 53.3% were male. All the participants reported to have been vaccinated with MenAfriVac, 66% presented a vaccination card.

Specific IgG and IgA concentrations were significantly higher after vaccination ($P<0.001$, paired t-test), while total IgG and IgA concentrations remained stable. Day-to-day variation within the same individual was non-significant.

The proportion of individuals with a detectable specific IgG concentration in saliva increased from 22% (mean, 25 ng/ml) before vaccination to 63% (mean, 126 ng/ml) after vaccination. Specific IgA concentrations increased after vaccination for 76.3% of the participants, of which 44.3% had a 2-fold increase or higher.

Conclusions: Immunization with MenAfriVac contributed to a significant increase of salivary IgG and IgA antibodies targeting the serogroup A polysaccharide capsule of *N. meningitidis*. The results are coherent with studies on other conjugate vaccines and can explain why serogroup A carriage was eradicated after MenAfriVac vaccination in Burkina Faso.

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A meningococcal A conjugate vaccine trial in infancy – a step towards routine immunization in the African meningitis belt

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Question: Meningococcal A epidemics remain a major plague in countries in the African meningitis belt. An affordable MenA conjugate vaccine was developed through the Meningitis Vaccine Project, and introduced at public health scale in 2010-11, using single dose mass campaigns among 1 to 29 year-olds in countries of the meningitis belt, with success. While roll-out in countries is ongoing, strategies for protection of new birth cohorts are being evaluated.
Methods: We conducted a dose ranging study of the newly developed MenA conjugate vaccine in infants to evaluate the safety and immunogenicity of three different doses administered in a two dose schedule at 14 weeks and 9 months, or in one dose schedules at 9 or 12 months concomitantly with the EPI vaccines. Starting in 2008, 1198 infants were recruited in the Kassena Nankana districts of Northern Ghana and followed up till 2012.
Results: Results confirmed noninferiority of the alternate dosages - 5μg and 2.5μg of polysaccharide A (PsA) - to the licensed dosage - 10μg of PsA. No significant interferences with co-administered EPI vaccines were found. The proportions of subjects with seroconversion post-9-month vaccination were high in all MenA vaccine groups, but the magnitude of the responses was higher in subjects previously primed with MenA vaccine (2 doses regimensvs.1 dose regimen), nonetheless administration of a single dose at age 9 months induced a high immune response. No significant safety concerns were identified. The majority of adverse events were due to infections consistent with background morbidity in the district.
Conclusions: Sustainable protection from MenA disease among new birth cohorts could be achieved through immunization starting in late infancy at 9 months, with an eventual booster in the second year of life. A potentially powerful strategy for sub-Saharan countries, that could allow paired administration of the MenA and measles vaccines.

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A period of low meningococcal meningitis incidence in northern Togo in the absence of MenAfriVac

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Background: Northern Togo lies within the African meningitis belt and experiences hyper-endemic or epidemic meningitis yearly during the dry season. Historically,Neisseria meningitidis serogroup A has been the primary pathogen causing large epidemics but serogroups W135 and X also have shown epidemic potential in the region in recent years [1, 2].
Methods: From May 2010 to April 2011, we enrolled all patients presenting with suspected meningitis to five hospitals and health centers in Tône District, Togo. Cerebro-spinal fluid (CSF) was collected and analyzed by culture and PCR. Togo had not introduced pneumococcal or meningococcal conjugate vaccines during the study period.
Results: We enrolled 128 patients with suspected meningitis, of which 69% had purulent CSF. 31% were under age 5 years, 24% 5-14, and 45% at least 15 years of age. Among the 60 cases with a confirmed bacterial etiology (50%), pneumococcus was identified in 46 (77%),Haemophilus influenzae in 7 (12%: 6 type b and 1 non-b) and Nm in 6 (10%), of which 5 were serogroup W135 and 1 was serogroup X. Suspected meningitis incidence was highest between December and March (>50 per 100,000) and peaked in infants (150/100,000) and again between ages 30 and 49 (57/100,000). Meningococcal cases occurred primarily in children 1 to 9 years old (5 of 6 cases).
Conclusions: During the study period, meningococcal meningitis occurred infrequently, affected children almost exclusively, and involved only serogroup W135 even in the absence of NmA conjugate vaccine use. Pneumococcal meningitis occurred 10 times more frequently and across all ages. The absence of serogroup A meningococcal meningitis contrasts with most previous seasons and highlights the importance of long-term surveillance to monitor temporal changes in disease epidemiology and to assess the impact of conjugate vaccines.

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Low carriage of serogroup A across the Meningitis Belt

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Question: To define pattern of meningococcal carriage across African meningitis belt and to measure the impact of serogroup A conjugate vaccine (MenfriVac™) on carriage.
Methods: The African Meningococcal Carriage Consortium (MenAfriCar) conducted cross-sectional studies in the rainy seasons of 2010 and 2011 to measure age-specific carriage prevalence in urban and rural areas of 7 countries (Senegal, Mali, Ghana, Niger, Nigeria, Chad, Ethiopia). The 2010 surveys in Mali and Niger were conducted shortly before mass vaccination with MenAfriVac™ in December that year. Following a population census, households were randomly selected within defined urban and rural sites. An age stratified sample of 2,000 subjects (5,000 pre-vaccination in Mali and Niger) in each country was targeted for collection of posterior pharyngeal swabs. Risk factor questionnaires were collected. Microbiological tests were performed on site to identify meningococci and also serogroup by slide agglutination. DNA samples from oxidase positive, Gram negative diplococci were sent to the University of Oxford for molecular characterisation.
Results: In the 2010 survey over 18,000 swabs were obtained, 91% of the target. Overall meningococcal carriage prevalence was close to 3% (547/18173). The age group with the highest carriage were 5-14 year olds. Carriage of serogroup A was very low, with no isolates found in six countries and a prevalence of 0.34% (7/2023) in Chad. W135 was the predominant serogroup (n=88, 0.5% carriage prevalence) followed by X (n=10)and Y (n=7). However, the majority of meningococci identified were capsule null (n=287 to date). Similar results were obtained in the second survey one year later. Some variation was seen between years for example the prevalence of X changed from 0.05% (2/4219) to 1.96% (37/1886) in Niger, and the prevalence of Y from 0% (0/2012) to 1.0% (19/1915) in Ethiopia. The pattern of A carriage was very similar with a prevalence of 0.36% in Chad, but absent or very low in the other countries.
Conclusions: The almost complete absence of serogroup A carriage was notable , as was the predominance of capsule null meningococci . The lack of serogroup A in Mali and Niger made it impossible to measure vaccine effectiveness against carriage. Further work is ongoing to measure carriage in the dry season of 2012, vaccine effectiveness in Chad, baseline population immunity against serogroup A, transmission of meningococci within households and serological correlates of protection against carriage.

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Longitudinal Study of *Neisseria meningitidis* Carriage Among Households in Bamako, Mali

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Objectives: Despite knowledge thatNeisseria meningitiscarriers are the primary source of transmission, many questions remain about the epidemiology of carriage in the African meningitis belt. We aimed to assess the prevalence of carriage by age in Bamako, Mali, at multiple points before and after mass-vaccination and to identify changes in carrier status by following households longitudinally.
Methods: In May 2010, we conducted a cross-sectional, age-stratified baseline survey of 400 residents of 116 randomly selected households. Subsequently, 20 of these households (with 202 residents) were visited every four weeks from July to November 2010 (6 visits) and from September to November 2011 (3 visits). At each of the 10 visits, participants provided a pharyngeal swab and answers to a questionnaire. Carriers were identified from bacteriological and biochemical tests and were confirmed with molecular assays. We calculated the overall and age-specific prevalence of carriage at each visit.
Results: Forty (2.2%) of the 1,831 swabs collected during the entire study were positive for N. meningitidis of any serogroup. Three serogroup W135 and two serogroup Y meningococci were identified. In the cross-sectional survey, the overall prevalence was 4.5% and carriage was highest in those aged 15-29 years (9.1% (95% CI: 3.7-15.8%) and 5-14 years (7.4% (2.8-13.6%)) relative to the other age groups (30 years 1.0% (0.02-5.3%). At that time, 26.3% reported having received a meningitis vaccine in the past six months. During the first six follow-up visits in the 20 households conducted before the December 2010 MenAfriVac vaccination campaign, carriage ranged from 1.2% to 3.5%. Fifty-five percent of participants reported receiving MenAfriVac during the campaign. Preliminary results indicate that no carriers of any serogroup were identified during the three post-mass vaccination visits in these households, though response rates were low (57.4%-62.4%).
Conclusions: We found a relatively high proportion of children and young adults carrying N. meningitidis in Bamako during the baseline survey, a period of low incidence of invasive disease. Household follow-up identified changes in carriage before and after the MenAfriVac vaccination campaign. Further analyses are needed to confirm these findings, to determine the molecular characteristics of the carried meningococci, and to explore epidemiological variation in carriage over time.

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High throughput molecular characterisation of carriage isolates from the African meningitis belt pre and post vaccination with MenAfriVac

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Background: The African meningococcal carriage consortium (MenAfriCar) is investigating the epidemiology of *Neisseria meningitidis* carriage across the African meningitis belt using both cross-sectional and household studies and a range of analysis tools. Molecular characterisation of isolates collected from seven countries across the belt, prior to and following vaccination with the new meningococcal serogroup A conjugate vaccine (MenAfriVac), is being carried out at the University of Oxford.

Methods: A high throughput pipeline has been developed for the processing of samples from their arrival in Oxford through to the completion of molecular characterisation, at every stage documenting results from these procedures within the Bacterial Isolate Genome Sequence Database (BIGSdb). The presence of a large set of reference sequences within BIGSdb allows quick and reliable designation of alleles to sequences deposited in the database. Following preparation and archiving of the received isolates, speciation assays are carried out in two stages; 1) a fragment of the *rplF* gene is sequenced in all isolates allowing putative *Neisseria* speciation, 2) *rplF* negative isolates undergo *16S* rRNA sequencing to identify other bacterial species. The serogroup of confirmed *N. meningitidis* isolates is determined using custom designed assays for the identification of all 12 serogroups and the *cnl* locus. The isolates first undergo *cnl* sequencing; positive sequences are uploaded to BIGSdb where an allele is assigned, and *cnl* negative isolates are then tested for capsule type. A multiplex PCR and sequencing reaction is performed for 9 variants of the *ctrA* gene identifying serogroups 29E, Z, X, L, I, K, A and H or the presence of a sialic acid capsule. To distinguish the latter (serogroups W, Y, B, C), further PCR and sequencing is carried out on the *siaD* gene of the capsule locus. This new set of serogrouping reactions replaces the PCR-only method initially used to determine serogroups A, W, X and Y on isolates from the first cross-sectional study.

Results: At the time of writing 4581 isolates had been received, archived and can be uniquely identified within BIGSdb based on a BIGS id number. Of these, 1826 had been tested using the *rplF* speciation assay revealing 676 as *N. meningitidis*. Four hundred and fifty nine of the isolates have been found to be capsule null, 333 being *N. meningitidis*.

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Carriage and immunity against meningococcal meningitis in the African meningitis belt – summary interpretation of three population-based studies in Burkina Faso, 2003 – 2008

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Introduction: Duration of antibody persistence and indirect protection are expected to be major determinants of the impact of a serogroup A (NmA) conjugate vaccine in the African meningitis belt. However, little is known about carriage, natural immunity and correlates of protection, specifically in this region. **Methods:** We provide a summary interpretation of data from three population-based carriage and seroprevalence studies that have been conducted in Bobo-Dioulasso, Burkina Faso, in 2003, 2006 and 2008. The studies were implemented with similar methods, including culture and PCR analyses of oropharyngeal swabs, and measurement of serum bactericidal antibody (SBA) titers against serogroups A and W135. They covered different situations with regard to meningococcal epidemiology, serogroup predominance and population vaccination status. Meningitis incidence from concomitant PCR-based surveillance was also considered. Results: NmA carriage was only identified in an epidemic situation, where it was strongly associated with serogroup A SBA titres ≥8. The age-specific seroprevalence of serogroup A SBA titres ≥8 was similar in all surveys, with the exception of substantially lower prevalence in February 2003, an extraordinary period marked by absence of NmA from invasive disease. The pattern of age-specific seroprevalence was similar to that of NmA carriage prevalence (epidemic situation) and of NmA meningitis incidence in all periods. SBA titers were 40-fold lower against the NmA 3125 strain compared to the F8238 reference strain, with comparable age-specific pattern. **Conclusions:** The seroprevelance patterns suggest that NmA probably circulated intensively during our studies and induced natural antibody, but since colonization was only identified during an epidemic, methods for NmA detection on mucosal surfaces need to be further investigated. Seroprevalence as currently measured is directly related to disease incidence (not indirectly, as the Goldschneider paradigm suggests) and probably also to the risk of carriage acquisition. A possible explanation is that carriage induces short-lived antibody with low protective function. In addition, hypothetical direct invasion from the pharynx to the meninges may diminish the effective protection provided by serum antibodies. The specific meningococcal pathophysiology in the meningitis belt and mechanisms of mucosal immunity need to be better understood, including correlates of protection against carriage.
SBA measured against the reference strain may capture mainly natural immunity against subcapsular antibody. Current methods therefore may be less appropriate for studying the persistence of vaccine-induced capsular antibody and should be improved.

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Household crowding and social mixing patterns in seven countries in the African meningitis belt

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Question: How do household crowding and social mixing patterns vary across the African Meningitis Belt? Do these factors impact on the spread of infectious diseases in the area?
Methods: In 2010, the African Meningococcal Carriage Consortium (www.menafricar.org) conducted cross-sectional surveys in urban and rural areas of seven countries. Details of household crowding and social mixing patterns were recorded and their association with self-reported recent respiratory symptoms were analysed by univariate and multivariate regression models.
Results: Country-average household crowding ranged from 1.8 to 2.9 people per room between Ghana and Ethiopia respectively. Social mixing patterns varied three-fold, between 0.5 and 1.5 meetings attended in the last week. Those who attended 3 or more meetings a week (frequent mixers) were more likely to be older, male (OR=1.27, $p<0.001$) and live in urban areas (OR=1.45, $p<0.001$). Frequent mixers and young children, but not residents of crowded households, reported more respiratory symptoms (OR=2.2, $p<0.001$ and OR=3.2, $p<0.001$ respectively).
Conclusions: There are substantial variations in household crowding and social mixing patterns across the African Meningitis Belt. This study finds a clear association between age, increased social mixing and respiratory symptoms. It lays the foundation for developing an age-structured heterogeneous mixing pattern to model the spread of respiratory disease, and potentially the meningococcus, through the African Meningitis Belt.

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High carriage rates of commensal *Neisseria* species in Malawi including a previously un-named species

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Background: Meningitis rates are high in Malawi but meningococcal meningitis is comparatively rare. We performed a carriage study to investigate if carriage of *Neisseria spp* might be conferring protection. **Methods**: 790 healthy individuals aged 0 to 92 years were recruited from Ndirande township, Blantyre, grouped by age and weighted to younger age groups. An oropharyngeal swab was plated directly onto selective media. Up to four colonies with morphology typical of *Neisseria spp* were subcultured and identified using biochemistry, MLST, and rMLST. Genome sequencing was undertaken on 79 isolates. **Results:** 311 *Neisseria spp* were isolated from 301 individuals. Overall carriage of *Neisseria spp* was 38.1%. This rose from 10.3% in infants less than 6 months of age, to 66.1% in those aged 18-24 months, and remained over 62% up to 10 years of age, and above 22% up to 60 years. *N. meningitidis* was carried in 5.1% of the population and in 9.4% of those aged 3-60 years (maximum carriage 11.4% of 5-10 year olds). The most common isolate was *N. lactamica* which was carried in 192 (24.3%) of those sampled in all age groups, peaking at 55% of children aged 18-24 months. The second most common isolate was an un-named commensal *Neisseria species* which had been previously cultured in Europe but not at these rates (proposed name *N. bergeri*). Identification was confirmed by genome sequencing. It accounted for 58/311 (18.6%) of all *Neisseria spp* isolated. It was cultured in 4.4% of children <6 months of age and was isolated only in children less than 15 years. Carriage rates were 18.8% and 18.6% in children aged 3-5 years and 5-10 years respectively. *N. lactamica* and *N. bergeri* accounted for 80% of all isolates. We isolated *N. polysaccharea* from 15 individuals (5% of isolates) and carriage peaked at 9.2% of 2-3 year olds. **Conclusion:** Carriage rates of commensal *Neisseria spp* were high in this Malawian population, and may account for the relatively low rates of meningococcal disease seen. The finding of high carriage rates of a previously un-named commensal *Neisseria* species in younger age groups is unexpected. The role of this species in the development of natural immunity deserves further work.

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Epidemiological changes in meningococcal meningitis strains in Niger from 2008 – 2011: attempt at an explanation and perspectives for a vaccination strategy

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Objectives: The epidemiology of bacterial meningitis in the African 'meningitis belt' appears to change periodically. In order to design an effective vaccination strategy and preparedness, we examined the epidemiological and microbiological patterns of bacterial meningitis, and more particularly meningococcal meningitis, in Niger over the period 2008–2011. This time period includes the mass vaccination campaigns with the newly developed meningococcal A conjugate vaccine (MenAfriVac™).
Methods: Cerebrospinal fluid samples were collected from different health structures throughout Niger and were analysed by culture and seroagglutination and/or speciation polymerase chain reaction (PCR), followed by genogrouping PCR and multi-locus sequence typing (MLST) for cases of Neisseria meningitidis meningitis.
Results: N. meningitidis serogroup A cases were prevalent in 2008 and 2009 [98.6 and 97.5% of all N. meningitidis cases, respectively]. The prevalence regressed in 2010 [26.4%], with the emergence of serogroup W135 cases [72.2%], and finally disappeared in 2011. All N. meningitidis serogroup A strains analysed (N=49) by MLST over the 2008-2011 period belonged to ST-7, except three isolates displaying ST-2859. All N. meningitidis serogroup W135 strains analysed (N=30) by MLST in 2010 and 2011 were ST-11 whereas most of the serogroup W135 strains analysed before 2008 belonged to ST-2881.The vaccination response to the epidemics, the mass vaccination campaigns with MenAfriVac™, the geographical distribution of N. meningitidis serogroups A and W135 cases are also described.
Conclusions: The decline of serogroup A cases is correlated with vaccination data allowing speculation on the evolution of sequence type lineages and natural immunization of the population. The current availability of polysaccharide vaccines, particularly those including serogroup W135, should be addressed in a very next future.

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Pattern and burden of meningitis caused by Neisseria meningitidis serogroup X in the African Meningitis Belt

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Introduction: Neisseria meningitidis is a major cause of bacterial meningitis in Africa with high associated mortality. Meningococcal group X is the most recent serogroup to have shown epidemic potential in the African Meningitis Belt. Little is currently known about the epidemiology of serogroup X meningitis in Africa and how it will be affected by the introduction of the MenAfriVac meningococcal group A glycoconjugate vaccine.

Methods: We analysed data from published studies and the World Health Organisation, describing cases of serogroup X meningitis in sub-Saharan Africa, and compared them to data available on meningitis caused by other aetiologies.

Results: Data reporting of meningococcal group X meningitis has been sporadic and incomplete. Cases have been reported in six countries in the African Meningitis Belt in two geographical regions: one in West Africa and one in East Africa. Niger and Burkina Faso have been most affected. From 1995 to 2005, reported cases of group X meningitis followed a similar pattern to that for all-cause meningitis. Large outbreaks occurred with ST 181 group X meningococci in Niger in 2006, with 4465 reported meningitis cases. 600/1139 (53%) CSFs in which meningococci were identified contained group X bacteria, with group A meningococci accounting for most of the remainder of cases. Since 2006, serogroup X meningitis incidence has followed an inverse pattern to both serogroup A and all-cause meningitis. Meningitis due to group X generally occurs at a younger age than for group A and is highest in children aged between five and nine years. Mortality is generally higher than that associated with group A meningitis and, in contrast with group A meningococcus, rates of carriage are usually much higher than rates of invasive disease. Mass vaccination with MenAfriVac in Burkina Faso, Niger and Mali at the end of 2010, has been followed by a dramatic fall in incidence of meningitis due to group A meningococcus, while at the same time meningitis outbreaks due to meningococcal group X and W135 have occurred.

Conclusion: These findings support concerns about the continued epidemic potential of meningococcal group X and the fluidity of group X epidemiology, particularly in the face of the MenAfriVac mass vaccination. To assess the future epidemic potential of serogroup X meningitis in Africa, more systematic and comprehensive surveillance is required. Vaccination and other ways of reducing the threat of group X and other non-meningococcal A serogroups need to be found.

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Natural immunity against serogroup X *N. meningitidis* in Togo following a serogroup X meningococcal disease outbreak

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Objective: Serogroup X *N. meningitidis* has recently emerged as a cause of localized disease outbreaks in sub-Saharan Africa. Epidemics or outbreaks have particularly been noted from 2006-2008 in Niger, Uganda, Kenya and Togo. There is no vaccine against serogroup X meningococci, and it is unknown whether the serogroup X polysaccharide is immunogenic in humans or whether antibodies directed against might be the basis for protective immunity with a vaccine.
Methods: Sera from 300 healthy individuals (100 aged 3-5 years, 100 aged 13-19 years and 100 aged 20-25 years) from a community affected by a serogroup X outbreak in Togo in 2007 were recruited for blood sampling. These were compared with sera from 300 healthy individuals with a similar age distribution obtained from the Health Protection Agency (HPA) seroepidemiology collection. Serum bactericidal antibody (SBA) was measured using serogroup X strain BF2/97 with rabbit complement. Antibodies against serogroup X polysaccharide and outer membrane vesicles (OMVs) were quantified by ELISA.
Results: The proportion of Togolese individuals with an SBA titre of ≥1:8 against a serogroup X strain was 28% among those aged 3-5 years, 35% among those aged 13-19 years and 32% among those aged 20-25 years. The corresponding proportion with an SBA titre of ≥1:128 were 26%, 22% and 21%, in the respective age groups. For the U.K. population overall, the proportion with an SBA titre of ≥1:8 was 14%, and 6% had an SBA titre of ≥1:128. The geometric mean concentration (GMC) for serum IgG against serogroup X polysaccharide in the Togolese population was 75 AU/mL, 237 AU/mL and 294 AU/mL in the three age groups respectively and 115 AU/ml overall in the UK. Antibody concentrations against serogroup X OMVs in Togo were 673, 1230 and 1140 AU/ml in the 3 age groups, respectively.
Conclusion: These data suggest that exposure to serogroup X meningococci, presumably through naso-pharyngeal colonisation, has occurred in Togo. This presumably indicates transmission in the population. Despite the increase in overall antibody concentration with age, the similarity in SBA titre in all age strata suggests that this exposure does not guarantee induction of functional antibodies. Further studies in individuals with proven exposure to serogroup X antigens are warranted.

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Carriage of *Neisseria lactamica* in Burkina Faso in the 1-29 year old: epidemiology and molecular characterization

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Background: *Neisseria lactamica* is a true commensal bacterium sharing the same ecological niche as the pathogenic *Neisseria meningitidis*, which is responsible for outbreaks and large epidemics, especially in sub-Saharan Africa. To better understand the epidemiology of *N. lactamica* and its relationship to *N. meningitidis*, we studied *N. lactamica* carriage in 1-29 year old people in Burkina Faso.
Methods: A repeated cross-sectional carriage study was conducted in three districts of Burkina Faso between 2009 and 2011. Oroharyngeal swabs were collected from 1-29-year-old residents of the districts and the presence of *N. lactamica* was evaluated. Molecular characterization was done in a subset of isolates. Odds ratios (OR) were calculated by logistic regression using survey methods to account for the cluster sampling design.
Results: *N. lactamica* carriage was detected in 18.1% of 45,847 oropharyngeal samples. Carriage was highest among 2-year-olds (40.0%) and decreased with age. Carriage was higher for males (19.0%) than females (17.5%) (OR, 1.11; 95% CI, 1.04-1.18) while among the 18-29-year-olds, carriage in women (9.1%) was significantly higher than in men (3.7%) (OR, 2.58; 95% CI, 2.01-3.31). There was no significant seasonal variation of *N. lactamica* carriage and no significant change in carriage rates after introduction of a serogroup A meningococcal conjugate vaccine, MenAfriVac. Multilocus sequence typing was performed on a selection of 142 isolates. A total of 62 different genotypes, of which 56 were new, were identified. The clonal complexes dominating in Burkina Faso were essentially those dominating in the UK (Bennet et al. *Infect Immun* 2005).
Conclusions: *N. lactamica* carriage in Burkina Faso showed little geographic and seasonal variation in comparison with that seen for carriage of *N. meningitidis*, and did not significantly change after the MenAfriVac vaccine introduction. Age distribution of *N. lactamica* carriers was similar to that observed in other countries. The genetic diversity of *N. lactamica* isolates was high but the isolates were genetically similar to those circulating in industrialized countries.

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Evaluation of the IgG subclass antibody response to a meningococcal serogroup A conjugate vaccine in African toddlers

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Epidemics of serogroup ANeisseria meningitidis are a major unresolved public health problem in sub-SaharanAfrica. A newly licensed serogroup A meningococcal conjugate vaccine, MenAfriVac (PsA-TT) was previously studied in a Phase II clinical study to evaluate the safety and immunogenicity in children 12-23 months of age (Sowet al.NEJM 2011).

For the primary phase, subjects were randomised to receive PsA-TT, Meningococcal serogroup A, C, Y, W135 polysaccharide vaccine (PsACWY), or Haemophilus influenzaetype b conjugate vaccine (Hib-TT). Ten months following primary vaccination the 3 groups were further randomised to receive either PsA-TT, 1/5th dose of PsACWY, or Hib-TT. Blood samples were taken prior to and 1 month post primary vaccination, prior to and 7 and 28 days following booster vaccination. The IgG subclass response was characterised using a serogroup A-specific IgG subclass specific ELISA.

For IgG1, priming with PsA-TT gave significantly higher geometric mean concentrations (GMCs) (21.37 μg/mL, 95% CI 18.46-24.74) than PsACWY (GMC 1.93 μg/mL, 95% CI 1.58-2.38) with respective fold rises from baseline of 47 and 3 (P<0.001). Ten months post-vaccination IgG GMCs had declined to 1.28 μg/mL and 0.81 μg/mL for the PsA-TT and PsACWY groups, respectively. Both groups had significantly higher GMCs than the Hib-TT group (0.41 μg/mL, P<0.001). One month post booster, participants primed and boosted with PsA-TT showed a marked increase in the secondary IgG1 antibody response as compared to other cohorts neither primed nor boosted with PsA-TT (P<0.001 to <0.01).

For IgG2, priming with PsA-TT or PsACWY gave GMCs 5.99 μg/mL (95% CI 5.07-7.08) and 0.98 μg/mL (95% CI 0.77-1.25). Ten months post-vaccination, GMCs were 0.72 μg/mL and 0.38 μg/mL, respectively (P<0.01). Groups primed with PsA-TT and boosted with PsA-TT, PsACWY or Hib-TT gave GMCs of 35.88 μg/mL, 13.22 μg/mL and 0.57 μg/mL, respectively. One month post-booster these were 33.97 μg/mL, 15.10 μg/mL and 0.49 μg/mL, respectively. Groups primed with PsACWY and boosted with PsA-TT, PsACWY or Hib-TT gave GMCs of 16.18 μg/mL, 2.33 μg/mL and 0.31 μg/mL, respectively. One month post booster GMCs were 27.01 μg/mL, 2.64 μg/mL and 0.19 μg/mL, respectively.

PsA-TT showed overall superiority to the PsACWY by greater IgG1 and IgG2 GMCs and prolonged antibody persistence. The predominant IgG subclass induced by either PsA-TT and PsACYW in these young children is IgG1.

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The global meningococcal initiative – efforts to prevent and control meningococcal disease in India

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Background: The Global Meningococcal Initiative (GMI) is led by international experts in meningococcal immunology, epidemiology, vaccinology, and public health. The goal is to prevent meningococcal disease (MD) through education, research, and vaccination.
Methods: In January 2012, the GMI met with Indian experts to review India's MD burden and to explore MD prevention/control strategies.
Results:Neisseria meningitidis is the third most common cause of sporadic bacterial meningitis in children <5 years, with a higher incidence in temperate northern versus tropical southern regions. Actual incidence is not reliably known, due to sub-optimal surveillance and insufficient microbiological support for diagnosis. Approximately every 20 years, India experiences large MD outbreaks that are confined to the northern part of the country and are caused almost exclusively by serogroup A. The latest outbreaks, beginning in 2005, have occurred in Delhi, Meghalaya, and Tripura. Outbreaks responses were ad hoc: mandatory case reporting by hospitals in Delhi, temporary strengthening of laboratory diagnostics, chemoprophylaxis of close contacts/high-risk groups, and limited reactive polysaccharide vaccination. Although an Indian facility is manufacturing a serogroup A conjugate vaccine for use in sub-Saharan Africa, it is not presently used in India. The GMI recommends replacement of polysaccharide vaccines with conjugate vaccines, if possible. Although routine immunization is endorsed in some settings, costs and data limitations make such an intervention not presently feasible in India. To improve understanding of the true burden of MD, the GMI recommends—in addition to the need for routine disease surveillance—(1) soliciting existing reference centers to generate diagnostic data using real-time polymerase chain reaction and latex agglutination tests to complement traditional microbiological methods and (2) initiating carriage and seroepidemiological studies. Unless robust data are available, it will not be possible to prioritize MD for routine vaccination on the basis of epidemiological evidence.
Conclusions: MD burden in India is underestimated, and reliable surveillance data are needed. Control efforts should focus on expanding surveillance and educating physicians and officers of the National Regulatory Authority on the importance of MD as a cause of death or disability. Conjugate vaccines should be used for outbreak control and immunization of high-risk persons.

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Unencapsulated meningococci change Opc expression, LPS immunotype and pilin antigen upon serum exposure

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Neisseria meningitidis employs extracellular polysaccharides structures and outer membrane proteins to cope with human serum complement attack. We modified a medium throughput colorimetric serum bactericidal assay (1) to screen for factors influencing serum resistance in a genetically engineered sequence type (ST)-41/44 clonal complex (cc) strain lacking lipopolysaccharide (LPS) sialylation, polysaccharide capsule, and the factor H binding protein (fHbp. After serum killing of >99.9% of an original bacterial suspension and consecutive plating, 1000 surviving clones were screened for enhanced serum resistance using the colorimetric assay. 35 clones belonging to three mutant classes showed enhanced serum resistance.
Class I (n=31): Enhanced expression of Opc due to phase variation. Opc expression augmented vitronectin binding and reduced membrane attack complex insertion as shown recently (2).
Class II (n=2): LPS immunotype switch from immunotype L3 to L8/L1 due tolgtAandlgtCphase variation. Isogenic mutant analysis demonstrated that in ST-41/44 cc strains immunotype L8/L1 was indeed more serum resistant than immunotype L3. Not surprisingly, immunotypes L8 and L1 were frequently observed in ST-41/44 cc isolates from both carriage and disease.
Class III (n=2): PilE allelic exchange driven by gene conversion. The clones showed enhanced autoaggregation. We hypothesize that this caused increased serum resistance by establishing a physical barrier to complement activation. Sequence analysis of single colonies derived from the clones revealed the mutation of a residue in the C-terminal hypervariable region D of PilE previously associated with increased pilus bundle formation (3).
The study highlights the ability of meningococci to adapt to environmental stress by phase variation and intrachromosomal recombination affecting subcapsular antigens.

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P 200

Factor H dependent regulation of the alternative pathway of complement by meningococci that lack fHbp, NspA, capsule and lipooligosaccharide sialic acid

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Introduction: The alternative pathway (AP) of complement forms a key arm of innate immune defenses against *Neisseria meningitidis*. Meningococci use several, often redundant, mechanisms to inhibit the human AP. Bacterial factors known to inhibit the human AP include expression of groups B or C capsular polysaccharide, lipooligosaccharide (LOS) sialic acid, factor H-binding protein (fHbp) and Neisserial surface protein A (NspA). In light of recent findings that disease-causing isolates may lack fHbp and the ability of strains that lack both fHbp and NspA to cause bacteremia in infant rats, we sought to identify additional mechanisms that meningococci may use to restrict AP activation.
Methods: Seven diverse strains of *N. meningitidis* that lacked capsule, LOS sialic acid, fHbp and NspA were constructed (quadruple mutants). C3 deposition through the AP was measured by FACS using human, mouse, rhesus macaque and baby rabbit serum that were treated with Mg/EGTA or depleted of complement C2. C3a generation was measured by ELISA.
Results: Four of seven quadruple mutants inhibited the human AP ($P<0.05$). None of these strains regulated adult rhesus or mouse AP. Significant inhibition of C3 deposition ($P<0.05$) was also observed when pure AP components (C3, fB, fD and fH) were used. The addition of purified human fH to mouse complement resulted in regulation of the mouse AP on all 4 isolates suggesting a human fH-dependent mechanism. Consistent with this, an AP regulating strain generated less C3a over time than a strain that did not regulate the AP. Of the 4 strains that regulated the human AP, all expressed PorB2 and 3 were from the hypervirulent ST-11 clonal lineage. A construct containing fH domains 6 and 7 fused to murine Fc bound to an AP regulating strain in a PorB2-dependent manner and enhanced C3 deposition in human sera.
Interestingly, the quadruple mutants decreased AP-mediated C3 deposition, independent of human fH, when baby rabbit complement was used (rabbit fH has not been shown to bind to meningococci); additional AP inhibition occurred when purified human fH was added.
Conclusions: Collectively, these data suggest a novel fH-dependent mechanism of AP inhibition by select strains of *N. meningitidis*. AP regulation by baby rabbit complement, independent of the addition of human fH was also noted. Identifying the molecular basis for these novel AP evasion mechanisms will increase our understanding of complement evasion by meningococcal isolates that lack fHbp, as could occur under immune pressure exerted by fHbp-based vaccines.

P 201

Complement and antibody mediated killing of Opa-negative *Neisseria gonorrhoeae* by human neutrophils

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Introduction: Neutrophils are a primary mediator of the human innate immune responses to bacterial infections. *Neisseria gonorrhoeae* infection promotes an inflammatory response characterized by the recruitment of neutrophils to the site of infection. *N. gonorrhoeae* can interact with neutrophils either directly (non-opsonic interactions) or indirectly through opsonins on its surface. Opsonin receptors include the Fc receptor (engages IgG) and complement receptor 3 (CR3), which engages iC3b primarily. Non-opsonic interactions are mediated by interactions of gonococcal opacity protein (Opa) with carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs).
Methods: We examined the role of complement and natural Ab in gonococcal phagocytosis by human neutrophils. To eliminate Opa-CEACAM interactions we used an Opa-negative mutant of strain FA1090.
Results: Human neutrophils efficiently killed gonococci in the presence of normal human serum (14% ± 12 survival compared with survival in neutrophils without serum). To dissect the relative roles of the Fc receptor and CR3 in gonococcal phagocytosis, we used IgG-depleted and C3-depleted human sera. Survival of gonococci in the presence of neutrophils in IgG-depleted serum or in serum lacking functional C3, was 62% ± 14 and 18% ± 13, respectively, relative to survival of bacteria incubated with neutrophils but in the absence of serum altogether. Depleting both IgG and C3 together from human serum abrogated killing. Increased association of gonococci with neutrophils in the presence of normal human serum was confirmed by FACS analysis and was accompanied by increased CD66b expression (a marker of neutrophil activation). As reported previously, OpaB expressing bacteria were efficiently engulfed and killed by neutrophils in an Opa-CEACAM dependent manner and there was minimal difference in survival in the absence or presence of normal human serum (88% ± 12 survival compared with survival in neutrophils without serum).
Conclusions: These results indicate that both complement and antibody are critical for efficient phagocytosis of *N. gonorrhoeae* in the absence of involvement of CEACAM receptor(s). This study elucidates the mechanisms of opsonic interactions of gonococci with neutrophils and will lead to a better understanding of pathogenesis and protective immune responses that will inform vaccine design.

P 202

Gonococcal susceptibility and resistance to the non-oxidative components of primary human neutrophils

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Infection with *Neisseria gonorrhoeae* (Gc) is marked by a robust inflammatory response, highlighted by the influx of polymorphonuclear leukocytes (neutrophils or PMNs) to the site of infection. PMNs kill invading bacteria through oxidative (reactive oxygen species, ROS) and non-oxidative (proteases, antimicrobial peptides) processes, but viable Gc can be recovered from PMN-rich gonorrheal secretions and from PMNs infected with Gc *ex vivo*. We hypothesize that Gc expresses gene products that help confer resistance to PMNs. Since only non-oxidative components are mobilized in PMNs challenged with Gc, we propose to identify Gc gene products with differential sensitivity to a non-oxidative antimicrobial extract prepared from primary human PMNs.

We have refined a protocol established by Rest (1) for extracting proteins from PMN granules. PMNs collected from blood of healthy human donors are sheared with a ball-bearing homogenizer, and the granules are collected by high-speed centrifugation. The granules are resuspended in pH 4 acetate buffer, and the resulting human neutrophil granule extract (HNGE) contains the bulk of PMN's antimicrobial proteins. To assess anti-gonococcal activity of the HNGE, piliated, opacity protein-negative bacteria of strain FA1090 were exposed to the extract, and survival expressed as the number of Gc surviving after 45 minutes divided by the number of Gc in the inoculum. Our results show that the HNGE has an LD_{50} of 1.0-1.5μg/ml. A Gc mutant (FA1090*Δngo1686*) known to exhibit increased sensitivity to PMNs was found to be more sensitive to the HNGE. In contrast, a mutant in *ngo319* was not, although the corresponding mutant in *N. meninigitidis* was reported to be more sensitive to the cathelicidin LL-37, one of the components of the HNGE (2).

To identify the Gc gene products that modulate bacterial survival after HNGE exposure, we are taking two approaches with a *Tn5*-based transposon mutant library. First, we are selecting Gc mutants that are resistant to high concentrations of HNGE. Second, we are using Illumina sequencing to identify all transposon mutants differentially represented in the Gc population after exposure to HNGE. We will make defined mutations in open reading frames of interest, and the resulting mutants tested for their susceptibility to the HNGE and to intact primary human PMNs. We anticipate these approaches will reveal known and novel Gc gene products important for surviving the innate immune response in acute gonorrhea.

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P 203

Role of NG0969 (*nuc*) in *Neisseria gonorrhoeae* resistance to neutrophil clearance

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Symptomatic infection by *N. gonorrhoeae* results in a highly inflammatory environment caused by a potent infiltration of neutrophils, or polymorphonuclear leukocytes (PMNs). PMNs possess a diverse antimicrobial arsenal with both oxidative (reactive oxygen species) and non-oxidative (proteases, antimicrobial peptides) components. These components can be directed to the phagolysosome to kill microbes intracellularly, or released to the outside of the cell by exocytosis. Another approach used by PMNs to trap and kill microbes extracellularly is the release of neutrophil extracellular traps (NETs). NETs are web-like structures comprised of chromatin and decorated with some PMN antimicrobial proteins and are released from PMNs undergoing a unique form of cell death. In spite of these robust activities, viable bacteria can be recovered from PMNs in gonorrheal disease exudates. We seek to define the bacterial virulence factors that contribute to gonococcal survival after exposure to PMNs. The NG0969 open reading frame encodes a thermonuclease (*nuc*) that contributes to gonococcal biofilm architecture and remodeling (Steichen, et al. 2011). To examine the potential role of nuc in *N. gonorrhoeae* resistance to PMNs, we generated a *nuc* insertion-deletion mutant in a constitutively piliated, opacity-protein negative derivative of strain FA1090. The Δ*nuc* mutant displayed no growth defect in rich media when compared to the parental bacteria. However, when exposed to adherent, chemokine-primed PMNs from healthy human donors, the Δ*nuc* mutant consistently survived less well than the wild-type parent. To understand the mechanism underlying *nuc* sensitivity to PMNs, we used immunofluorescence microscopy to examine PMNs infected with parental or *nuc* mutant bacteria. We find that like other bacterial species and the chemical agonist PMA, *N. gonorrhoeae* can induce the formation of NET structures *in vitro*, which may be influenced by *nuc*. Together, these studies point towards a role of *nuc* in gonococcal resistance to PMN clearance.

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Impact of Neisseria meningitidis outer membrane vesicles (OMV) on immune response by polymorphonuclear neutrophils (PMN)

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Outer membrane vesicles are released from the outer membrane of pathogenic Neisseria and play a potential role in invasive meningococcal disease (IMD). OMV of *Pseudomonas aeruginosa* are viewed as long distance delivery tools for bacterial pathogenicity factors (1). Because meningococcal OMV can be purified from plasma of IMD patients, we asked whether they impact polymorphonuclear neutrophils (PMN), which are part of first line defence against IMD. By flow cytometry and fluorescence microscopy we detected fusion of *Neisseria meningitidis* (*Nm*) OMV to PMN, both in whole blood assays and in isolated cell fractions, but not to T lymphocytes. As result of fusion of OMV, phagocytosis and ROS release by PMN was impaired. Live imaging and chemotactic assays revealed that PMN motility was also affected by OMV. Consequently, PMN mediated killing of *Nm* was impaired in the presence of meningococcal OMV. As a possible causal effector protein we identified NarE, a ribosyl transferase (2) that proved to be present in OMVs and whose knock-out reduced the above mentioned OMV effects on neutrophils. Especially G-protein mediated stimulation of neutrophils appeared to be affected by the presence of NarE containing OMV. Due to the possible effect of NarE on neutrophils we extended recently published investigations (2) on the presence and expression of NarE in meningococcal clonal complexes. The narE gene and NarE protein expression could be detected in ST-32 cc, ST-41/44 cc, ST-845 cc, and partially in ST1-5 cc but not in ST-269 cc, ST-11 cc, and ST-8 cc. Taken together, our data provide evidence that *Nm* is able to modulate neutrophil response by release of OMV which fuse to the cells and affect G-protein mediated signal transduction by toxin delivery of NarE. OMV mediated NarE delivery may support invasive infection of many hypervirulent clonal complexes.

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P 205

Surface components involved in adherence to the Neutrophil uropod

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Neutrophils are important components of the human innate immune system and are rapidly recruited at the site of bacterial infection. Neisseria have been shown to possess numerous strategies to evade neutrophil mediated killing. We reveal a novel type IV pilus-mediated adherence of pathogenic Neisseria to the uropod (trailing edge) of polarized PMNs. The direct pilus-uropod interaction was visualized by scanning electron microscopy and total internal reflection fluorescence microscopy. We showed that the role of the pilus subunit PilC in adhesion differed between N. meningitidis and N. gonorrhoeae. Adhesion to the PMN uropod depended on both PilC variants, while N. gonorrhoeae adhesion did not. Bacterial adhesion elicited accumulation of the complement regulator CD46, but not I-domain-containing integrins, beneath the adherent bacterial microcolony. Electrographs and live-cell imaging of PMNs suggested that bacterial adherence to the uropod is followed by internalization into PMNs via the uropod. We also present data showing that pathogenic Neisseria can hitchhike on PMNs to hide from their phagocytic activity as well as to facilitate the spread of the pathogen through the epithelial cell layer. Recent studies focuses on the components responsible for the specific adherence. We are also evaluating the effects of transport of bacteria by PMNs.

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On the Cutting Edge – Pathogenic and Protective Neutrophil Responses During *Neisseria gonorrhoeae* Infection

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The hallmark of gonorrhea is a neutrophil-dominated cervical or urethral exudate; this hyper-inflammatory response is understood to cause both acute symptoms and the lasting sequelae that result from infection by *Neisseria gonorrhoeae*. The gonococci colonize host tissues by binding epithelial-expressed carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs). Evolutionarily, humans have adapted by acquiring a decoy receptor to capture bacteria that target CEACAMs during infection. This molecular mimic, CEACAM3, is exclusively expressed by human neutrophils and drives a potent opsonin-independent phagocytic engulfment of the bacteria with a concomitant oxidative burst and degranulation response. In this study, we describe an *in vivo* model of *N. gonorrhoeae* infection using transgenic mice that express a spectrum of CEACAMs normally expressed only by human epithelia and neutrophils. While human CEACAM5 facilitates increased colonization of the female urogenital tract, neutrophil-expressed CEACAM3 reduces the burden of infection and increases bacterial clearance. Moreover, biochemical, genetic and genome-wide microarray profiling of gonococcal-infected neutrophils reveals that CEACAM3 engagement triggers a NF-κB-driven signaling cascade that results in a robust pro-inflammatory cytokine, leading to a CEACAM3-dependent acceleration in the accumulation of neutrophils into gonococcal-infected tissues. Taken together, these studies suggest that CEACAM3 elicits a vigorous innate response that represents the sharp edge between protective or pathogenic outcomes of *N. gonorrhoeae* infection.

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Innate recognition by granulocytes differs between *Neisseria gonorrhoeae* strains causing local or disseminating infections

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Background: Several members of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family serve as cellular receptors for *Neisseria gonorrhoeae*. More specifically, the aminoterminal immunoglobulin variable (Ig_V)-like domain of epithelial CEACAMs (CEACAM1, CEA, CEACAM6) is recognized by CEACAM-binding neisserial colony opacity (Opa_{CEA}) proteins and this interaction promotes bacterial colonization of the mucosa. In contrast, recognition by CEACAM3, a CEACAM family member exclusively expressed by human granulocytes, results in efficient uptake and destruction of Opa_{CEA}-expressing bacteria. In principle, CEACAM3-mediated uptake by innate immune cells should limit the spread of gonococci. However, some gonococcal strains can cause disseminating infections and it is currently unknown, how these strains escape detection by granulocyte CEACAM3.
Results: We cloned the ten opagene loci from *N. gonorrhoeae* strain VP1, which was isolated from a patient with disseminated gonococcal disease. All Opa proteins were constitutively expressed in *Escherichia coli* and their CEACAM-binding capacity was profiled against the eleven Opa proteins of the non-disseminating strain MS11. Pull-down analysis with soluble CEACAM Ig_V-like domains revealed that the vast majority of Opa proteins from both strains associate with epithelial CEACAMs. In line with this, bacteria expressing the CEACAM1-binding Opa_{65} protein of strain VP1 were able to associate and invade CEACAM1-expressing cells and to trigger increased matrix adhesion of the infected epithelial cells. In sharp contrast to strain MS11, not a single Opa protein of strain VP1 interacted with the human granulocyte receptor CEACAM3. Accordingly, bacteria expressing VP1 Opa_{65} were not taken up by primary human granulocytes and failed to trigger an oxidative burst.
Conclusions: Our results suggest that the Opa protein repertoire of the disseminating gonococcal strain VP1 is able to engage epithelial CEACAMs, which could promote successful mucosal colonization. At the same time, strain VP1 lacks Opa proteins, which are recognized by granulocyte CEACAM3. The failure of CEACAM3-mediated innate immune detection might be linked to VP1's ability to cause disseminated infections.

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Upward and Onward – Dissecting the relative contribution of human CEACAMs within upper and lower female urogenital tract mucosa

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Neisseria gonorrhoeae is exclusively adapted to humans through its ability to interact with a range of human specific factors. Although an infection model for prolonged colonization of wild type mice has been established, it cannot account for the exquisite specificity of gonococcal Opa protein adhesins for cell surface receptors of human origin. In this study we compare infection of wild type and transgenic mice expressing various combinations of the human carcinoembryonic antigen-related cell adhesion molecules CEACAM1, CEACAM3, CEACAM5 and CEACAM6, which are differentially expressed on a variety of cell types encountered by the bacteria during infection. Along the mucosal lining of the female genital tract, the epithelial-expressed CEACAMs form an expression gradient, with CEACAM5 and CEACAM6 being predominantly expressed within the lower genital tract while CEACAM1 is localized in the upper portion. Presence of human CEACAM5 and CEACAM6 enabled significantly higher levels of gonococcal colonization and tissue penetration during intra-vaginal infections; studies are currently underway to consider the relative contribution of CEACAM1 during ascending infection. Conversely, expression of the decoy phagocytic receptor, CEACAM3, on neutrophils correlated with a substantial increase in their migration to the site of gonococcal inoculation, an increased proinflammatory cytokine response, and more effective bacterial clearance. When considered in the context of the differential specificity of gonococcal Opa proteins for each human CEACAM, our results suggest that the expression of Opa variants with the ability to engage different CEACAMs may control progression from primary colonization, to ascending infection, to gonococcal-induced inflammation and disease.

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OxyR Regulates *minD*, a Cell Division Gene that Contributes to Virulence in *Neisseria gonorrhoeae*

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Objective: To ascertain the role of *oxyR* (encoding redox-responsive transcriptional regulator) in cell division and the virulence of *Neisseria gonorrhoeae*.
Materials and Methods: An isogenic *oxyR* mutant of *N. gonorrhoea* (Ng) CH811 was constructed by insertional inactivation (Ng KB1). Differential Interference Contrast (DIC) light microscopy and Transmission Electron Microscopy (TEM) were performed on the mutant and the wild-type. In Ng CH811 and Ng KB1 with/without exposure to 10 mM H_2O_2, the expression level of *minD*, a cell division gene, was estimated by qRT-PCR and western blot analysis. Ng CH811 and the *oxyR* mutant were both transformed with pLES94 (control) and pIB1 (pLES94 with a 748 bp amplicon containing the putative promoter region of $minD_{Ng}$ in fusion with lacZ), and then analysed for ß-galactosidase activity to study *oxyR*-mediated regulation of the *minD* promoter (*minD*p). Adherence/invasion assays and immunoblot analysis for signalling mediators were done on genitourinary epithelial cells (THUEC and ME180) infected with Ng CH811 and its *oxyR* mutant.
Results: The mutation in *oxyR* (Ng KB1) caused non-midline formation of the cell division septum, anomalous DNA segregation, and increased aggregation of bacterial cells as determined by TEM and DIC light microscopy. Compared to Ng CH811 in stationary phase, Ng KB1 displayed increased expression of *minD*, a cell division gene implicated in Ng virulence. Furthermore, the exposure to H_2O_2 (oxidative stress) induced upregulation of MinD in wild-type Ngcultures, but did not alter MinD levels in the *oxyR* mutant. As compared to NgCH811 (pIB1), the *oxyR* mutant (pIB1) displayed significantly higher β-galactosidase activity, thereby suggesting more *minD* promoter activity in the mutant. *oxyR* negatively regulated the promoter region (*minD*p) upstream of *minD*. The *oxyR* mutant of *N. gonorrhoeae* was deficient in invading into the human genitourinary epithelial cells and subsequent triggering of MAP kinase.
Conclusions: Gonococcal cell division is transcriptionally regulated by OxyR through its repressive effects on the *minD* promoter. The increased expression of *minD* in the presence of H_2O_2 and at stationary phase exemplifies the significance of its regulation by stress-responsive OxyR. Altered levels of *oxyR*-regulated *minD* cause aberrant cytokinesis resulting in abnormal gonococcal morphology. Considering the already established roles of *minD* and gonococcal shape in virulence, the attenuated virulence of the *oxyR* mutant can now be partially explained.

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The lipid-modified azurin from the human pathogen Neisseria gonorrhoeae

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Host immune response to pathogenic microorganisms, frequently employes oxidative stress in the form of reactive oxygen species (ROS). Some of the most common ROS found in biological systems involve the superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radical.[1] Pathogenic bacteria from the species Neisseria gonorrhoeae and Neisseria meningitidis, that cause gonorrhea and meningitis, respectively, have developed numerous defense mechanisms to cope with oxidative stress of the constant exposure to ROS. [2] These mechanisms are essential for cell survival taking since ROS can cause damage to DNA, proteins and cell membranes. One of these mechanisms is based on the family of bacterial cytochrome c peroxidases. These periplasmatic enzymes catalyze the conversion of H_2O_2 to water using haem co-factors [3] andc-type cytochromes or small type copper proteins of the respiratory chain as the electron donor.[1, 3]

In Neisseria genus, cytochrome c peroxidase is thought to also be involved in the protection from oxidative stress. However, its electron donor has not been established. We propose that Laz (lipid-modified azurin) is the physiological electron donor to this enzyme. This is corroborated by the observation that Neisseria gonorrhoeae and Neisseria meningitidis laz mutants were more sensitive to hydrogen peroxide, but not superoxide, than was the wild type organism.[2]Neisseria Laz differs significantly from other azurins in that it contains an N-terminal domain of 39 amino acids that encodes the H.8 epitope (common to pathogenic Neisseria) and they are modified with a lipid-palmityl fatty acid.

In this work, Neisseria gonorrhoeae Laz has been heterologously produced in E.coliand biochemically characterized as a Type 1 copper protein, through visible and EPR spectroscopy. This monomeric electron shuttle protein was structurally characterized using NMR in the reduced state and was shown to belong to the structural family of azurins [4], with a fold belonging to the azurin/plastocyanin family, with eight β-sheets forming a β-sandwich.

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Neutrophil NADPH oxidase inhibition by *N. gonorrhoeae*

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The hallmark of symptomatic gonorrheal disease is a robust influx of neutrophils (polymorphonuclear leucocytes, PMNs) to the site of the infection. Despite this inflammatory response, *N. gonorrhoeae* (Gc) survives clearance by neutrophils, which possess a bactericidal arsenal of proteases, cationic antimicrobial peptides and reactive oxygen species (ROS). In activated PMNs, ROS production is initiated by NADPH-oxidase, a multi-subunit enzyme catalyzing conversion of oxygen into superoxide, the substrate for other ROS including hydrogen peroxide and hypochlorous acid. NADPH oxidase activity is tightly regulated, with subunits located at the membrane (gp91^{phox} and p22^{phox}) and cytoplasm (p47^{phox}, p67^{phox} and p40^{phox}). Upon stimulation and following phosphorylation of the p47^{phox} "organizing subunit", the other cytoplasmic subunits and the GTPase Rac2 are translocated to membranes to assemble an active enzyme. A previous study (1) showed that non-opsonized, Opa-negative, piliated Gc does not induce ROS production in human peripheral blood primary PMNs. Moreover, viable, but not heat inactivated, Gc significantly inhibited the oxidative burst induced by fMLP and *S. aureus*. To further investigate the mechanism of Gc-induced ROS production inhibition, we examined NADPH oxidase assembly in PMNs infected with live Gc or S. aureus, which activates NADPH oxidase. Using cytosol/membrane fractionation of infected PMNs, Gc infection did not increase p47^{phox} and p67^{phox} translocation to membranes over 20 min of infection, in contrast to *S. aureus*, where these proteins translocated to membranes after 10 min of infection. We also failed to detect p47^{phox} localizing to Gc phagosomes in infected PMNs by immunofluorescence, whereas 50% of S. aureus phagosomes were p47^{phox} positive. Phagosomes containing viable or heat-killed Gc both lacked p47^{phox} labeling. The gp91^{phox} and p22^{phox} subunits of NADPH oxidase localized to both Gc and *S. aureus* phagosomes. Our data suggests that Gc interferes with p47^{phox} translocation to PMN membranes, which would lead to NADPH-oxidase assembly failure and may explain the inhibition of ROS production in PMNs infected with Gc.

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Thyroid hormone enhances nitric oxide-mediated bacterial clearance and promotes survival after meningococcal infection

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Question: Euthyroid sick syndrome characterized by reduced levels of thyroid hormones (THs) is observed in patients with meningococcal shock (1,2). It has been found that the level of THs reflects disease severity and is predictive for mortality (3,4). The present study was conducted to investigate the impact of THs on host defense during meningococcal infection.
Methods and Results: We found that supplementation of thyroxine to mice infected with meningococci enhanced bacterial clearance, attenuated the inflammatory responses and promoted survival. In vitro studies with macrophages revealed that THs enhanced bacteria-cell interaction and intracellular killing of meningococci by stimulating inducible nitric oxide synthase (iNOS)-mediated NO production. TH treatment did not activate expression of TH receptors in macrophages. Instead, the observed TH-directed actions were mediated through nongenomic pathways involving the protein kinases PI3K and ERK1/2 and initiated at the membrane receptor integrin αvβ3. Inhibition of nongenomic TH signaling prevented iNOS induction, NO production and subsequent intracellular bacterial killing by macrophages.
Conclusions: These data demonstrate a beneficial role of THs in macrophage-mediated Neisseria meningitidis clearance. TH replacement might be a novel option to control meningococcal septicemia.

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P 213

Nitric oxide metabolism of *Neisseria meningitidis* enhances cGMP activity during infection of human macrophages

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Rationale & Hypothesis (Question): Increased bacterial load in meningococcal sepsis is correlated with severe disease and increased concentrations of circulating nitric oxide (NO) metabolites. As a vasorelaxant, NO binds to the soluble guanylyl cyclase (sGC), found in abundance in vascular smooth muscle and activates cGMP, a critical secondary messenger for vasodilation and platelet inhibition . To avoid killing by NO, meningococci express genes of NO denitrification pathways such as, a nitrite reductase (AniA), a nitric oxide reductase (NorB) and an NO dependent global regulator of denitrifying genes, (NsrR). We previously showed (Laver J, *et al,* FASEB 2010) that NO denitrification mechanisms of *N. meningitidis* reduce abundance of NO species in mammalian cells, so we tested the hypothesis that this activity alters cellular cGMP activity.
Objectives: To examine the effect of bacterial NO metabolism on cGMP produced by Human macrophages (MDM) and endothelial (HMEC-1) cells.
Methods: Human MDMs and HMEC-1 cells were exposed to the NO donor spermine NONOate, together with the phosphodiesterase inhibitor IBMX and a soluble guanyl cyclase sensitiser BAY412272. Cells were infected with wild type *Neisseria meningitidis* (serogroup B strain MC58), a mutant strain for putative Arginine Decarboxylase *(ΔNMB0468)* and an isogenic mutant unable to detoxify NO *(Δ norB).* After two hours of infection, cells were lysed and cGMP was measured by ELISA. A new triple mutant strain *(ΔnorB/ΔAniA/ΔnsrR)* was constructed and characterised for its NO metabolic activity.
Results: In the presence of Spermine NONOate, IBMX and BAY 412272, the level of cGMP was detected at 5.79 pmol/mg (median, IQR 3.5 to 8.382 pmol/mg) protein in human MDMs; without these agonists no cGMP was detected (p N. meningitidis the level of cGMP production (14.26 pmol/mg, IQR 2.934 to 53.68 pmol/mg) was enhanced compared to *ΔnorB*(7.082 mg/ml, IQR 1.620 to 33.09 pmol/mg, p = 0.0693, n=8) and also when compared with *ΔNMB0468* (2.986 pmol/mg, IQR 0.7254 to 4.227, p=0.5605, n=7) infected MDMs. The newly constructed triple mutant *(Δ norB/ ΔAniA/ ΔnsrR)* exhibits reduced metabolism of NO metabolites [NO_X] and nitrites [NO_2^-]; further work is underway to examine its effect on cGMP output.
Conclusion: These preliminary data suggest that NO metabolism by *N. meningitidis* dysregulates cGMP activity in human cells.

figure 1

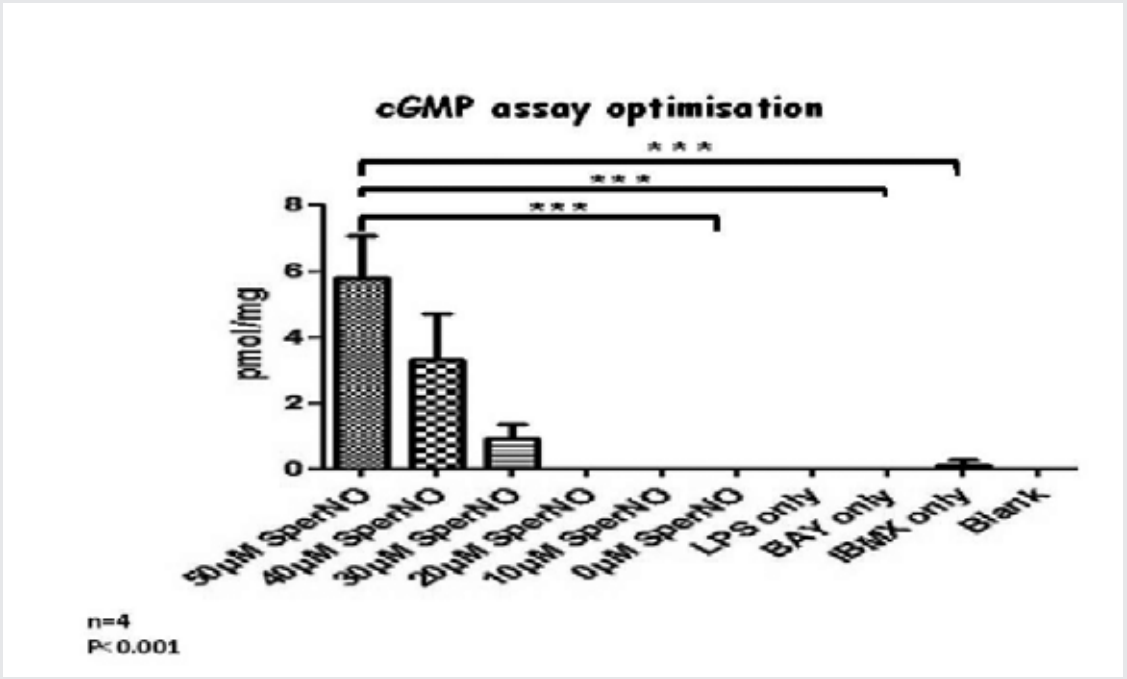
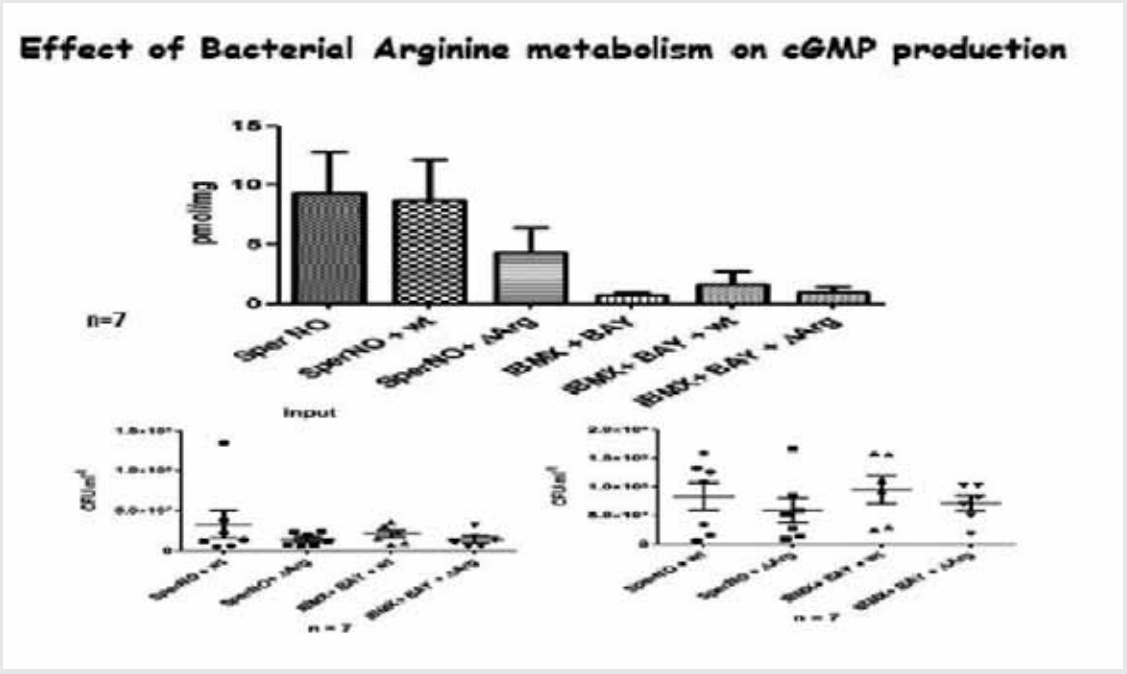


figure 2



P 214

The effect of *Neisseria meningitidis* infection on cellular *S*-nitrosothiol concentrations and the nuclear translocation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

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Question: *S*-nitrosylation, the addition of a nitric oxide (NO) moiety to a thiol group, is an important form of post-translational modification akin to phosphorylation. Many proteins are regulated by *S*-nitrosylation, including members of important signalling cascades such as those involved in various stages of cell death. *S*-nitrosylation of GAPDH has been shown to trigger its binding to, and stabilisation, of the E3 ubiquitin ligase Siah1. This complex then translocates to the nucleus and elicits the proteasome dependant degradation of nuclear proteins. Our previous work demonstrates that infection of activated mammalian macrophages with *Neisseria meningitidis* is capable of depleting endogenously generated *S*-nitrosothiol (SNO) by means of an NO reductase enzyme, NorB. We hypothesise that this depletion may have physiological consequences, resulting from interference in signalling cascades regulated by *S*-nitrosylation, including the nuclear translocation of GAPDH.
Methods: The murine macrophage cell line J774.2 was used due to their high NO output when activated with LPS and IFN-γ. Cells were stimulated for 18 hours before being subsequently infected at an MOI of 10 for a further 4 hours with a variety of mutant strains of *Neisseria meningitidis*. Total SNO present in the lysate was measured using Tri-iodide chemiluminescence. GAPDH translocation was measured using a combination of immunoblotting and flow cytometry techniques following stimulation with LPS and IFN-γ for a period of 2 hours, with subsequent infection at an MOI of 10 with a variety of strains of *N. menigitidis* for a further 13 hours.
Results and Conclusions: We previously showed that *N. meningitidis* phagocytosed by murine macrophages prevents formation of SNO in host cells, and this is dependent on the nitric oxide reductase enzyme, NorB. Here we show that, as expected, in response to LPS and IFN-γ stimulation, GAPDH translocates to the nucleus. However this translocation is prevented by the presence of an infection with *N. meningitidis*, although this is no different when cells are infected with a NorB mutant.

figure 1

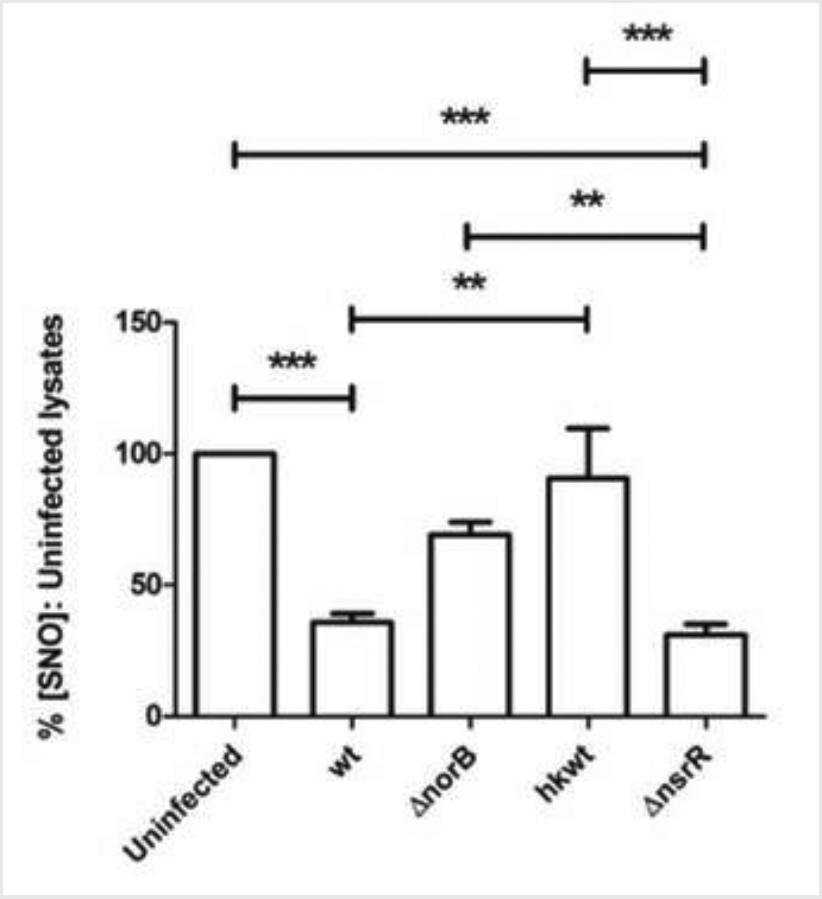
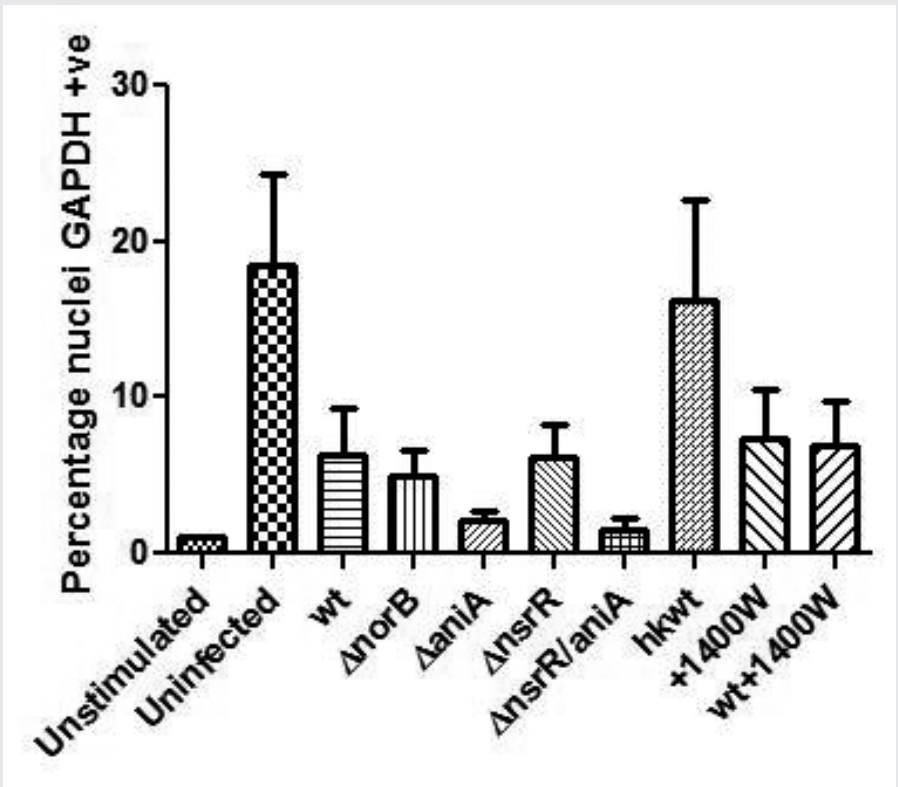


figure 2



P 215

Increased liver nitrite but decreased liver nitric oxide-derived species (NOx) in a murine model of early, fulminant, acute meningococcal septicaemia – the effect of bacterial nitric oxide detoxification

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Rationale & Hypothesis (Question): Meningococcal septicaemia is characterised by high bacterial titres in blood and an increased production of circulating nitric oxide metabolites, including nitrates [NO_3^-], nitrites [NO_2^-] and S-nitrosothiol [SNO]. The formation of SNO, termed S-nitrosylation, is an important form of post-translational protein modification akin to phosphorylation. We demonstrated previously that bacterial NO detoxification reduces the concentration of host-cell SNO. We hypothesise that bacterial NO detoxification during sepsis interferes with host NO homeostasis within the liver, a vital organ in physiological response to infection, and contributes toward pathogenesis.
Objectives: To study the link between bacterial NO detoxification and liver NO metabolites during experimental meningococcal sepsis.
Methods: Female C57 Bl/6 mice were infected by intraperitoneal injection of wild type *Neisseria meningitidis* (serogroup B strain MC58)or an isogenic mutant unable to detoxify NO *(ΔnorB).* Eight hours after infection, livers were extracted and lysates were subjected to ozone-based chemiluminescence to measure multiple NO metabolites such as, nitrates [NO_3^-], nitrites [NO_2^-] andS-nitrosothiol [SNO]. We obtained reproducibly high bacterial titres both in blood and liver lysates.
Results and Conlusions: 1. Increased bacterial burden in liver lysates is positively and significantly correlated with hepatic nitrite concentrations [NO_2^-] (Spearman's rank correlation, r=0.5682, p=0.0020, n=9), negatively and significantly correlated with the hepatic concentration of all NO [NOx] deriviatives (r=-0.4390, p=0.0220, n=9)
2. Production of NO metabolites was not influenced by bacterial NO detoxification machinery (p=0.2704, n=9)

figure 1

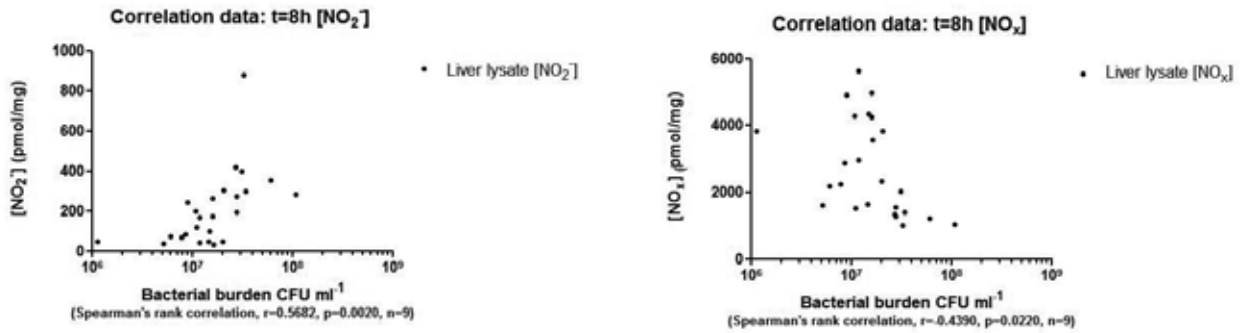
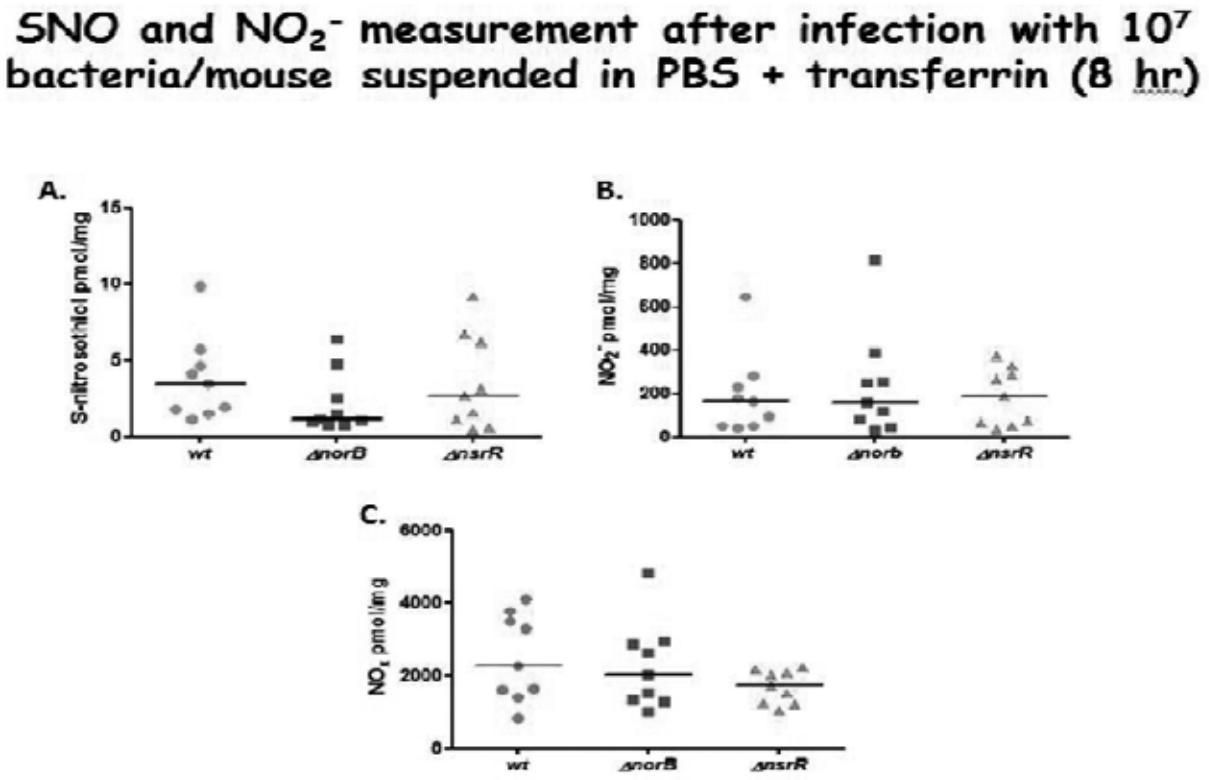


figure 2



P 216

Regulation of NFκB activity via *S*-nitrosylation of the p65 subunit in response to *Neisseria meningitidis* infection

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Question: *S*-nitrosylation, the addition of a nitric oxide (NO) moiety to a thiol group, is an important form of post-translational modification. Many proteins are regulated by *S*-nitrosylation including the transcription factor NFκB. Previous work has shown that both exogenous and endogenous sources of *S*-nitrosothiol (SNO) are capable of regulating NFκB binding to its target sequence via modification of the p65 subunit. We previously showed that NO detoxification mechanisms of *N. meningitidis*act to reduce SNO concentrations in macrophages, so we tested the hypothesis that *N. meningitidis* can modify the inflammatory profile of macrophages via its NO detoxification machinery.
Methods: Murine macrophage cells, J774.2 were used due to their high NO output when activated with LPS and IFN-γ. Initial studies were carried out using the NO donor CysNO, as well as iNOS stimulation with a combination of LPS and IFN-γ to confirm that *S*-nitrosylation of the p65 subunit of NFκB regulates its activity. Both pre-stimulated, SNO-rich J774.2 cells and un-stimulated cells were subsequently infected with WT MC58 *N. meningitidis* along with an array of nitric oxide reductase (NorB) mutants in the presence or absence of the iNOS inhibitor, 1400w. p65 binding activity was measured using a transcription factor ELISA. The nitrosylation status of p65 was determined using *S*-nitrosothiol resin assisted capture (SNO-RAC) followed by immunoblotting.
Results and Conclusions: NFκB was modulated by treatment of lysates with CysNO, due to nitrosylation of the p65 subunit. Cytokine stimulation of iNOS also produced a similar response, with an initial increase in binding activity compared to unstimulated, followed by nitrosylation of p65 within 12 hours, and an iNOS dependant inhibition of p65 binding capacity. Infection with *N. meningitidis* also lead to an initial increase in p65 binding activity, followed by a decrease within 12 hours. This decrease was not modulated by iNOS, however it was associated, paradoxically, with increased nitrosylation of the p65 subunit. Further studies using the donor CysNO showed that following 2 hours of infection, SNO treated whole cells showed a decrease in p65 binding activity compared to untreated, and this reduction was abolished by the addition of the thiol reducing agent dithiothreitol (DTT). This suggests that although p65 is nitrosylated in response to *N. meningitidis*, and that nitrosylation of this subunit is capable of regulating its activity after an infection, it is not the factor responsible for the modulation of pro-inflammatory activity after infection.

P 217

Expression and role of Secretory Leukocyte Protease Inhibitor (SLPI) during *Neisseria gonorrhoeae* infection of female mice

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Questions: Secretory leukocyte protease inhibitor (SLPI) is a small cationic peptide with bactericidal and anti-inflammatory activities that is naturally present in mucosal secretions. We asked whether expression of SLPI is altered during experimental gonococcal infection of female mice and if it is bactericidal against *Neisseria gonorrhoeae*.
Methods: Female BALB/c mice (6-8 weeks old) were treated with water-soluble estradiol to promote long-term susceptibility to *N. gonorrhoeae* and intravaginally inoculated with 10^7 CFU of gonococcal strain FA1090 or PBS. Colonization and PMN influx were followed daily for 10 days by culturing vaginal swab material on GC-VCNTS agar and counting the number of neutrophils per 100 vaginal cells on stained vaginal smears. Vaginal swabs were also collected on days 3, 5 and 7 of infection for RNA extraction and expression of the SLPI gene was measured by reverse transcription followed by RT-PCR. Animals were humanely euthanized on days 3 and 5, and whole genital tracts were processed for immunohistochemical (IHC) staining with anti-SLPI antibody. *In vitro* bactericidal assays were performed by incubating strain FA1090 with different concentrations of human recombinant SLPI and determining the number of viable bacteria recovered after 3 hours incubation.
Results: We found that the expression of SLPI is differentially regulated over the course of experimental murine infection. Transcription of SLPI was upregulated 7-fold in infected mice compared to uninfected mice during the early phase of infection, but then downregulated during the mid- and late phases of infection (5- and 2-fold decreased, respectively). IHC staining of vaginal tissue sections confirmed the RT-PCR results and showed localization of SLPI predominantly in nucleated and squamous epithelial cells lining the mucosa. Human recombinant SLPI was bactericidal against strain FA1090 in a physiologically relevant concentration range, with an ED_{50} of approximately 4 mM.
Conclusions: We conclude that while upregulation of SLPI expression by mucosal epithelial cells occurs early during *N. gonorrhoeae* infection, the gonococcus may have evolved ways to reduce its expression. The downregulation in SLPI expression observed in experimentally infected mice is consistent with lower amounts of SLPI detected in women with gonorrhea. Based on the demonstration that SLPI is bactericidal against N. gonorrhoeae in vitro, we also propose that the gonococcus may have evolved mechanisms for reducing or evading SLPI binding or activity during infection. Future studies will address the mechanisms by which gonococci evade killing by SLPI and the basis of its downregulation during infection.

P 218

Relative importance of LOS sialylation and the MtrC-MtrD-MtrE active efflux pump in gonococcal evasion of host innate defenses

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Questions: Cationic antimicrobial peptides (CAMPs) such as the cathelicidins are found in phagocytic granules and expressed by epithelial cells. *Neisseria gonorrhoeae* (Gc) utilizes several mechanisms for evading CAMPs including the addition of host-derived sialic acid to lipooligosaccharide (LOS), which reduces CAMP binding and efflux of CAMPs through the MtrC-MtrD-MtrE active efflux pump system. Here we asked which of these mechanisms confers the greatest protection from cathelicidins and polymorphonuclear leukocytes (PMNs), and the greatest fitness advantage in a mouse genital tract infection model.
Methods: Single α-2,3 sialyltranferase (*lst*) or *mtrE* mutants and double *lst*, *mtrE* mutants were constructed in Gc strains F62 and MS11. MS11 and F62 undergo similar levels of LOS sialylation when cultured in cytidine monophosphate neuraminic acid (CMP-NANA) but differ in mtrCDE expression as measured by RT-PCR [MS11, increased expression due to an mtrR (repressor) promoter mutation and a recently described -120 mutation (Ohneck et al, 2011); F62, reduced mtrCDE expression and carries an 11 bp deletion in mtrA (activator) (this work)]. Mutants and wild type (wt) bacteria were cultured in CMP-NANA and tested for susceptibility to LL37, murine LL37-related antimicrobial peptide (CRAMP) and PMN killing. Mutants were tested for survival *in vivo* by competitive infection of female BALB/c or CRAMP-deficient BALB/c mice.
Results: *lst* and *mtrE* mutants of strains MS11 and F62 were significantly more susceptible to cathelicidins *in vitro* compared to the wt parent strains as expected. However, *mtrE* mutants and *lst*, *mtrE* double mutants were more susceptible to cathelicidins than *lst* mutants. Mutation of either gene significantly reduced Gc survival within murine PMNs, with *mtrE* mutants more attenuated than *lst* mutants and *lst*, *mtrE* mutants the most attenuated. Both *lst* and *mtrE* mutants were attenuated relative to wt parent bacteria during infection of mice. Interestingly, *lst* mutants but not *mtrE* mutants were significantly less attenuated in mice that lack CRAMP. In competition experiments between *lst* and *mtrE* mutants in normal BALB/c mice, the *lst* mutants out-competed the *mtrE* mutants.
Conclusions: The MtrC-MtrD-MtrE active efflux pump system confers greater protection from cathelicidins and PMN killing and a greater fitness advantage in mice compared to surface sialylation. Loss of both mechanisms renders Gc more susceptible to PMNs than either mechanism alone and CRAMP signficantly challenges nonsialylated Gc during murine infection. The inability to directly demonstrate the importance of CRAMP in killing *mtrE* mutants *in vivo* may be due to the presence of multiple innate effectors (e.g., progesterone) that are expelled by the MtrC-MtrD-MtrE pump. This redundancy in substrates may also be responsible for the higher attenuation of *mtrE* mutants *in vivo*.

P 219

Phosphoethanolamine modification of gonococcal lipid A confers an in vivo survival advantage and modulates induction of proinflammatory cytokines by differential binding to cationic antimicrobial peptides

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Questions: Phosphoethanolamine (PEA) modification of Neisseria gonorrhoeae (Gc) lipid A occurs via the action of the lptA gene and increases resistance to complement and cationic antimicrobial peptides (CAMPs). Here we asked if PEA lipid A modification increases Gc survival in vivo and alters the inflammatory potential of lipid A during infection and in vitro in the presence or absence of CAMPs.
Methods: Experimental infection of female BALB/c mice was used to measure the in vivo survival of wt (FA19), isogenic lptA mutant and complemented lptA mutant (C'lptA) bacteria and the inflammatory response. The inflammatory potential of purified lipooligosaccharide (LOS) and whole Gc was assessed with human and mouse NFκB reporter cell lines in the presence and absence of CAMPs (polymyxin B, CRAMP, LL-37 and BPI). Cytokines and chemokines were measured by multiplex ELISA. Binding of CAMPs to wt and lptA mutant Gc or lipid A was measured by flow cytometry and the limulus amoebocyte lysate (LAL) assay.
Results: In competitive infections, the lptA mutant was 10- and 100-fold attenuated relative to the wt parent bacteria on days 4 and 6 of murine infection, respectively. Complementation increased fitness relative to wt bacteria. In noncompetitive infections with 10^5 bacteria, wt, lptA mutant and C'lptA bacteria colonized mice to similar levels; however, the lptA mutant induced lower levels of TNFα, IL-1β, and MIP1α on day 5 post-inoculation ($p < 0.05$). Together, these results suggest a role for PEA modification in Gc survival and inflammation. However, stimulation of the NFκB pathway by PEA-modified and unmodified LOS occurred at similar levels in mouse embryonic fibroblast (MEF) cells or human embryonic kidney (HEK) cells expressing only the hTLR4-MD2-CD14 receptor complex. Interestingly, in the presence of CAMPs, wt Gc and lipid A were more stimulatory compared to lptA mutant Gc and unmodified lipid A ($p<0.005$ and $p<0.0001$), and the degree of stimulation directly correlated with the levels of proinflammatory cytokines. CAMPs bound preferentially to the lptA mutant versus the wt Gc by flow cytometry and neutralized PEA-unmodified lipid A to a higher extent in the LAL assay. A peptide corresponding to the microbicidal domain of LL37 (residues 17-32) inhibited NFKB signaling induced by both wt and unmodified LOS. However, only full length LL37 enhanced the neutralization of NFKB signaling by unmodified LOS.
Conclusion:PEA modification of Gc lipid A confers a survival advantage in vivo and modulates inflammation. PEA-modified lipid A is more inflammatory during infection and our in vitro data support differential binding of CAMPs to unmodified lipid A as being responsible for the difference in the bioactivity of these two lipid A species during infection. The higher inflammatory potential of PEA-modified wt lipid A could provide a transmission advantage or enhance survival by reducing inhibitory commensal flora.

P 220

Adhesion to Host Cells as Novel Mechanism of Antimicrobial Peptide Resistance

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The obligate human pathogen *Neisseria meningitidis* has evolved numerous strategies to withstand human immune defenses. We have previously shown that the microenvironment provided by host cells plays an important role in meningococcal resistance to the human antimicrobial peptide LL-37 (1). Here, we present evidence that adhesion to pharyngeal epithelial cells protects *N. meningitidis* from physiologically relevant concentrations of LL-37 by inhibiting binding of LL-37 to the bacteria. A similar protective effect of adhesion was observed for other helical peptides, more precisely a cationic model amphipathic peptide (MAP) and the anionic alamethicin. In contrast, adhesion did not protect from the peptide antibiotic polymyxin B or the conventional antibiotics cefotaxime and gentamicin. Live cells were essential for adhesion protection, since bacteria attached to paraformaldehyde-fixed cells were effectively killed. Expression of the polysaccharide capsule was not required for the increased resistance of adhered bacteria. Sequestration of host cell cholesterol on the other hand was able to counteract the protective effect of adhesion and restored LL-37 binding to the bacteria. We suggest that bacteria-induced and cholesterol-dependent host cell responses may contribute to LL-37 resistance.

Reference

(1) Jones A, Geörg M, Maudsdotter L and Jonsson AB (2009). Endotoxin, Capsule and Bacterial Attachment Contribute to *Neisseria meningitidis* Resistance to the Human Antimicrobial Peptide, LL-37. J Bacteriol 191:3861-8.

P 221

Neisseria meningitidis induces the release of the pro-thrombotic von Willebrand factor by the endothelial cells

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The Neisseria meningitidisbacteremia is associated with an outstanding level of endothelial colonization and is very often complicated by thrombosis, the latter being part of thepurpura fulminans,a severe complication of meningococcal septicemia. Our aim was to search for a potential pro-thrombotic endothelial cell response. The endothelial release of the von Willebrand Factor (vWF) into the blood circulation is known to be a major thrombotic event, even though there is no disruption of the endothelium : platelets adhere to vWF and become activated, initiating aggregation and clotting.
Using Human Umbilical Vein Endothelial Cells (HUVEC), we showed thatNmis able to induce a release of the vWF in its largest multimeric forms, the most thrombogenic. Using non-adhering strains, we showed that adhesion strongly facilitates but is not absolutely necessary for the vWF release, suggesting that there is a secreted bacterial factor able to induce the release of the vWF. A filtrated supernatant, obtained from aNmculture in endothelial cells medium, is able to induce the release of the vWF. Heat-killed bacteria or heat inactivated supernatant are unable to induce such a release, proving that it is independent of the Lipooligosaccharide (LOS).
Alltogether these results suggest that Nmsecretes a pro-coagulant factor that is able to induce the release the von Willebrand factor thrombogenic protein by the endothelial cells. In vivo, such an event should induce the adhesion of platelets to the highly colonized endothelium, initiating local thrombosis and, later, capillary disruption and hemorrhage.

P 222

Neisseria meningitidis induces platelet inhibition and increases vascular endothelial permeability via nitric oxide regulated pathways

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Despite extensive availibility and use of antibiotics, infections with *Neisseria meningitidis* still demonstrate a high rate of morbidity and mortality even in developed countries. The fulminant septicemic course with massive bilateral hemorrhage into the adrenal glands, widespread petechial bleeding and purpura of the skin suggest pathophysiological inhibition of platelet function. Our data show that *N. meningitidis* produces the important physiological platelet inhibitor and cardiovascular signalling molecule nitric oxide (NO), also known as endothelium-derived relaxing factor (EDRF). *N. meningitidis*-derived NO inhibited ADP induced platelet aggregation through the activation of soluble guanylyl cyclase (sGC) followed by an increase in platelet cyclic nucleotide levels and subsequent activation of platelet cGMP- and cAMP-dependent protein kinases (PKG and PKA). Furthermore, direct measurement of horseradish peroxidase (HRP) passage through a vascular endothelial cell monolayer revealed that - in the presence of sodium nitrite- *N. meningitidis* significantly increased endothelial monolayer permeability. Immunfluorescence analysis demonstrated NO dependent disturbances in the structure of endothelial adherens junctions after coincubation with *N. meningitidis*. In contrast to platelet inhibition, the NO effects on HBMEC were not mediated by cyclic nucleotides. Our study provides evidence that NO plays an essential role in the pathophysiology of septicemic meningococcal infection.

P 223

Late Repression of NF-κB Activity by Invasive but Not Non-Invasive Meningococcal Isolates Is Required to Display Apoptosis of Epithelial Cells

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Meningococcal invasive isolates of the ST-11 clonal complex are most frequently associated with disease and rarely found in carriers. Unlike carriage isolates, invasive ST-11 isolates induce apoptosis in epithelial cells through the TNF-α /type 1 TNF-α receptor (TNFR1) signaling pathway. This feature dependent on the expression of lipo-oligosaccharides, required alteration of the anti-apoptotic factor NF-κB activity during the late steps of infection that was associated with increased levels of membrane TNFR1and a sustained activation of the apoptotic factor c-Jun N-terminal kinase (JNK). In contrast to ST-11 invasive isolates, infection with carriage isolates lead to prolonged activation of NF-κB that was associated with a transient activation of JNK and increased TACE/ADAM17-mediated shedding of TNFR1 leading to protection against apoptosis. Our data provide insights to understand the meningococcal duality between invasiveness and asymptomatic carriage.

P 224

Substantial local inflammatory response in organs as compared to blood in a porcine model of meningococcal sepsis

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Background: Meningococcal sepsis is regarded as the prototypical fulminant sepsis where massive growth of bacteria in the circulation induces a systemic inflammatory response syndrome. While the circulatory inflammatory responses are profoundly studied, little is known regarding the local inflammation in various organs. In this study we utilized a previously developed porcine model to investigate how different organs are involved in the inflammatory response of meningococcal sepsis.
Methods: Fulminant meningococcal sepsis was simulated by giving anaesthetized pigs exponentially increasing numbers of *N. meningitidis* intravenously. Organ samples obtained at the end of the experiment, immediately after the euthanasia, were homogenized and analyzed for concentration of cytokines and gene transcription by micro array. The relative influence of LPS versus non-LPS molecules were investigated by comparing wild-type *N. meningitides* (H44/76) to an artificially created LPS-deficient mutant *N. meningitides* (44/76lpxA-).
Results: TNF-α, IL-1β, IL-6 and IL-8 were substantially higher in the lungs, liver, spleen and kidney than in plasma. IL-8 was almost totally dependent on the presence of LPS, while the remaining cytokines were increased in response to both bacteria, although most potent in the wild-type bacteria. IL-10 was predominantly increased in the spleen, with no difference between the wild-type and the mutant bacteria. The results from micro array analysis of gene transcription demonstrated exaggerated inflammatory responses in the lung, liver, spleen and kidney being in general partially dependent on LPS. The inflammatory responses in whole blood was very low compared to the organs.
Conclusion: The inflammatory response in this porcine model of meningococcal sepsis predominantly took place locally in organs with only a minor contribution from the circulating leukocytes in whole blood. LPS was the dominant, but not the only inflammatory molecule of the meningococci.

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Neisseria gonorrhoeae triggers the PGE2/IL-23 pathway and promotes IL-17 production by human memory T cells

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PGE2 is a potent modulator of the T helper (Th)17 immune response that plays a critical role in the host defence against bacterial, fungal and viral infections. We recently showed high serum levels of interleukin (IL)-17 in patients with gonococcal infection and we hypothesized that *Neisseria gonorrhoeae* could exploit a PGE2 mediated mechanism to promote IL-17 production. Here we show that *Neisseria gonorrhoeae* induces human dendritic cell (DC) maturation, secretion of prostaglandin E2 and proinflammatory cytokines, including the pro-Th17 IL-23. Blocking PGE2 endogenous synthesis selectively reduces IL-23 production by DC in response to gonococcal stimulation, confirming recent data on PGE2/IL-23 crosstalk. *Neisseria gonorrhoeae* stimulated DC induce a robust IL-17 production by memory CD4+ T cells and this function correlates with PGE2 production. Our findings delineate a previously unknown role for PGE2 in the immune response to *Neisseria gonorrhoeae*, suggesting its contribute via Th17 cell expansion.

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A dual role for the inhibitor of apoptosis protein cIAP2 during *N. gonorrhoeae* infection

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Apoptosis is a tightly regulated programmed-cell death pathway induced to eliminate abnormal or infected cells without eliciting inflammation or damage to surrounding cells. Several bacterial pathogens effectively modulate the host apoptotic and inflammatory responses to infection, consequently contributing to bacterial persistence in the host. The family of inhibitors of apoptosis proteins (IAPs) has been implicated in the establishment of microbial infection in host target cells and in cancer progression due to their dual role in apoptosis and inflammation. It has been reported that survivin, an IAP family member, has both an intracellular and extracellular role in cancer cells by inhibiting apoptosis while promoting proliferative and metastatic potential in neighboring cells when secreted. We have previously established that *Neisseria gonorrhoeae* protects against staurosporine-induced apoptosis in transformed human endocervical epithelial cells (End/E6E7 cells). The ability of *N. gonorrhoeae* to inhibit apoptosis correlated with the upregulation of the cellular inhibitor of apoptosis protein 2 (cIAP2), at both the RNA and protein expression level. In this study we have further characterized the role of cIAP2 in the host inflammatory response to gonococcal infection. Using murine bone marrow derived macrophages (BMDMs) from cIAP2$^{-/-}$ deficient mice, we established a critical role for cIAP2 in the production of IL-1β in response to gonococcal infection. In both HeLa and End/E6E7 cells, we demonstrate that gonococcal infection induced an increase in intracellular cIAP2. Notably, examination of supernatants from infected cells provided the first reported evidence of extracellular cIAP2. Collectively, our studies reveal significant alterations in cIAP2 expression following gonococcal infection, suggesting that changes in expression and localization of cIAP2 in response to *N. gonorrhoeae* may affect both inflammation and apoptosis of infected epithelial cells and potentially, uninfected neighboring cells.

P 227

The role of *Neisseria gonorrhoeae* peptidoglycan in innate immune signaling

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N. gonorrhoeae is a highly adapted human pathogen that has multiple mechanisms of evading host immunity. *N. gonorrhoeae* infection induces a Th17 immune response that does not prevent reinfection. At the same time, *N. gonorrhoeae* also dampens Th1/Th2 responses, which are protective responses in a mouse model of infection. The Th immune responses induced by a pathogen are controlled, in part, through innate immune signaling pathways in antigen presenting cells that recognize Pathogen-Associated Molecular Patterns (PAMPs). Peptidoglycan (PG) is a PAMP recognized by multiple innate immune signaling pathways. *N. gonorrhoeae* sheds 1,6-anhydro PG monomers during growth in a process requiring the action of lytic transglycosylases LtgA and LtgD. The genes encoding LtgA and LtgD are nonessential in *in vitro* growth and redundant in their capacity to promote shedding of 1,6-anhydro PG monomers. We hypothesized that LtgA and LtgD might modulate host inflammatory signaling in response to *N. gonorrhoeae* infection via PG release and breakdown. The ability of *N. gonorrhoeae* strain FA1090 and two isogenic lytic transglycosylase mutant strains, *ΔltgD* and *ΔltgA/ΔltgD*, to stimulate innate immune signaling was studied. *N. gonorrhoeae* lacking LtgA and LtgD (*ΔltgA/ΔltgD*) *induced more inflammatory cytokine production* from bone marrow derived dendritic cells (BMDCs) from C57B/6 mice than isogenic wild-type control bacteria. The enhanced cytokine production phenotype was recapitulated in BMDCs treated with conditioned media from these *N. gonorrhoeae* strains. Conditioned media from the *ΔltgA/ΔltgD* strain also *demonstrated enhanced ability to stimulate two innate immune signaling pathways* in reporter cell lines expressing Nucleotide-binding oligomerization domain containing protein 2 (NOD2) or Toll-like receptor 2 (TLR2). The results suggest that PG processing by *N. gonorrhoeae* lytic transglycosylases LtgA and LtgD reduces the innate immune response to the bacteria, which may influence the adaptive immune responses to this pathogen.

P 228

Innate and adaptive immune responses – Synergistic barriers to nasopharyngeal colonization by *N. meningitidis*

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Our understanding of *N. meningitidis* colonization of the nasopharynx has been limited by the lack of availability ofin vivomodels for this human restricted pathogen. In vitro studies using cell lines have demonstrated that neisserial Opa proteins are specific for human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs). Consistent with CEACAM specificity contributing to host restriction, transgenic mice expressing human CEACAM1 are effectively colonized following nasal administration of *N. meningitidis* whereas wild type (WT) mice are not.

We have now exploited this model to dissect the relative contribution of host innate and adaptive immune processes controlling infection of the nasopharyngeal mucosa. Upon infection, both WT and CEACAM1-humanized mice showed significant infiltrates of meningococci-associated polymorphonuclear cells (PMNs) within the nasal cavity. However, inflammatory cytokines in nasal tissue of CEACAM1-humanized mice were substantially enhanced relative to WT mice, consistent with inflammation being driven by bacterial persistence in these animals rather than simple meningococcal administration. Consistent with a role for neutrophils in controlling ongoing infection,in vivodepletion of neutrophils allowed an increased bacterial burden and prolonged infection in the humanized mice.

While the meningococcal capsule is generally considered to contribute to invasive disease, capsule-deficient meningococci did not persist in the nasopharynx. To explore whether complement-dependent killing may influence colonization, we globally stimulated the complement system prior to intranasal infection and observed that it effectively protected against subsequent colonization. Moreover, there was a synergistic effect of depleting both neutrophils and complement, with colonization greatly enhanced versus either treatment alone.

In considering the role of immunoglobulin in controlling colonization, we observed that repeated intranasal inoculation of WT mice with viable *N. meningitidis* elicited only a weak immunoglobulin response. In clear contrast, persistent infection of the CEACAM1-humanized mice elicited both meningococcal-specific mucosal IgA and protective serum bactericidal antibody titers in the serum that ultimately, upon repeated infection, protected the mice upon re-exposure to the same meningococcal strain.

Combined, these studies reveal a synergistic effect of complement, neutrophils and mucosal IgA in conferring immediate and acquired protection against nasopharyngeal colonization by *N. meningitidis.*

P 229

Neisseria meningitis Porin B enhances innate and adaptive elements of cell-dependent immunity

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Invasive pathogens are recognized by the innate immune system through PAMPs, chief among them the TLR ligands. In the case of N. meningitidis,the outer membrane protein Porin B (PorB) has been extensively studied by our lab. Our lab has identified it as an agonist of TLR2/1 heterodimers, and that PorB induces a number of pro-inflamatory responses in the innate immune system. As a consequence, we have shown it functions as an adjuvant for soluble antigens in vaccine systems. Here we study a number of the modalities of response by the innate and adaptive arms of the immune system to PorB, and the dependence of these responses on TLR2 and the downstream mediator MyD88. We show that PorB drives an increase in the rate of antigen uptake by Antigen Presenting Cells (APCs), as well as an increase in the recruitment of these antigen-carrying cells to secondary lymphoid organs. We provided evidence for these effects bothin vivoandin vitro, using FACS and IF imaging. We also investigate the expansion of antigen-specific T cellsin vivowhen PorB is included in a model vaccine. Eliciting a strong cell-dependent immune response may play an essential role in future efforts to prevent or treat infections by pathogenicNeisseriaspecies. As a well studied and prototypical member of the family of OMP proteins, this characterization of the ability of PorB to support such a response may provide insights for future therapies.

P 230

N. lactamica PorB surface-exposed loops amino acid sequence influences TLR2-dependent cell activation

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The innate immune receptors Toll-like receptors (TLRs) play a major role in activation of host mucosal and systemic defense mechanisms by recognizing a diverse array of conserved pathogen-associated molecular patterns (PAMPs). TLR2, in cooperation with TLR1 and TLR6, is activated by structurally diverse bacterial products, i.e. lipidated factors (lipoproteins and peptidoglycans) and non-lipidated proteins, including bacterial porins. PorB is a pan-Neisserial porin, expressed regardless of organisms' pathogenicity, that induces TLR2-dependent inflammatory responses in airway epithelial cells. However, PorB from *N. lactamica* (*Nlac*, commensal) induces *in vitro* cellular responses of lower magnitude than PorB from *N. meningitidis* (*Nme*, pathogen). These PorB molecules bind to TLR2 *in vitro* with apparent different specificity. The structural and molecular details of PorB/TLR2 interaction are only beginning to be unraveled and might be due to electrostatic attraction. PorB molecules have significant strain-specific sequence variability within surface-exposed regions (loops) putatively involved in TLR2 interaction. We constructed a panel of recombinant PorB loop mutants in which surface-exposed loop amino acids have been switched between *Nlac* PorB and *Nme* PorB. Using these mutants, we have identified residues that influence the TLR2-dependent effect of PorB. Specifically, mutations in loop 5 and loop 7 decreased the activity of PorB. Although these loops are not uniquely responsible for PorB interaction with TLR2 *in vitro* and on the cell surface, signaling events downstream of TLR2 recognition are likely influenced by a hypothetical "TLR2-binding signature" within the sequence of PorB surface-exposed loops among different Neisseriae strains.

P 231

Suppression of T-cell proliferation and activation by *N. meningitidis* – a novel potential mechanism of bacterial pathogenesis.

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Question: Recent work in our lab has demonstrated that polyclonal B-cell activation with TI-II antigen mimics (*e.g* α-δ-dextran) are able to inhibit the TCR-induced proliferation and activation of T-cells. Based on the fact that TI-II antigens are present on whole bacteria, we investigated whether *N. meningitidis* was able to induce B-cell activation but suppress T-cell proliferation and activation. **Methods:** Primary human PBMCs were exposed to fixed *N. meningitidis* or to purified Neisseria outer membrane vesicles (OMVs). A flow cytometry assay was designed to measure stimulatory interactions of B and T-cells. Proliferation was measured using a CFSE dilution assay, while cellular activation was assessed by expression of activation markers. Enrichment experiments by negative selection provided pure primary T and B populations for cell-contact experiments. **Results:** Interestingly, the smallest ratio (1:1) bacteria per cell of fixed wild type meningococcus resulted in profound inhibition of T-cell proliferation and activation. Higher bacterial count (100:1) failed to inhibit T-cell activation and the inhibition on T-cell proliferation was mild. The same experiment performed with a lipooligosaccharides (LOS) deficient mutant showed a similar suppressive pattern, being the smallest inoculum the most effective dose and suggesting that LOS is not the mechanism of inhibition. Purified fresh and heated OMVs of both *N. meningitidis* and *N. lactamica* demonstrated to contain the suppressive factor, suggesting that neither the capsule nor a thermolabile protein are key factors within the mechanism. Latest experiments with *E. coli* and *S. pneumoniae* have clarified that such suppressive effect may be exhibited by bacteria other than *N. meningitidis*, although the resultant inhibition is dose sensitive and varies among organisms. Purification experiments have shown that B-cells are not the cell type responsible of this phenomenon; but it's still unclear if monocytes could be involved. **Conclusions:** Small bacterial inocula of *N. meningitidis* and its OMVs are able to inhibit T-cell proliferation and activation. Furthermore, other microorganisms seem to exhibit the same phenomenon. With current experiments we aim to discover whether the suppressive effect is contact dependent or if a soluble factor produced by PBMCs is necessary.

figure 1

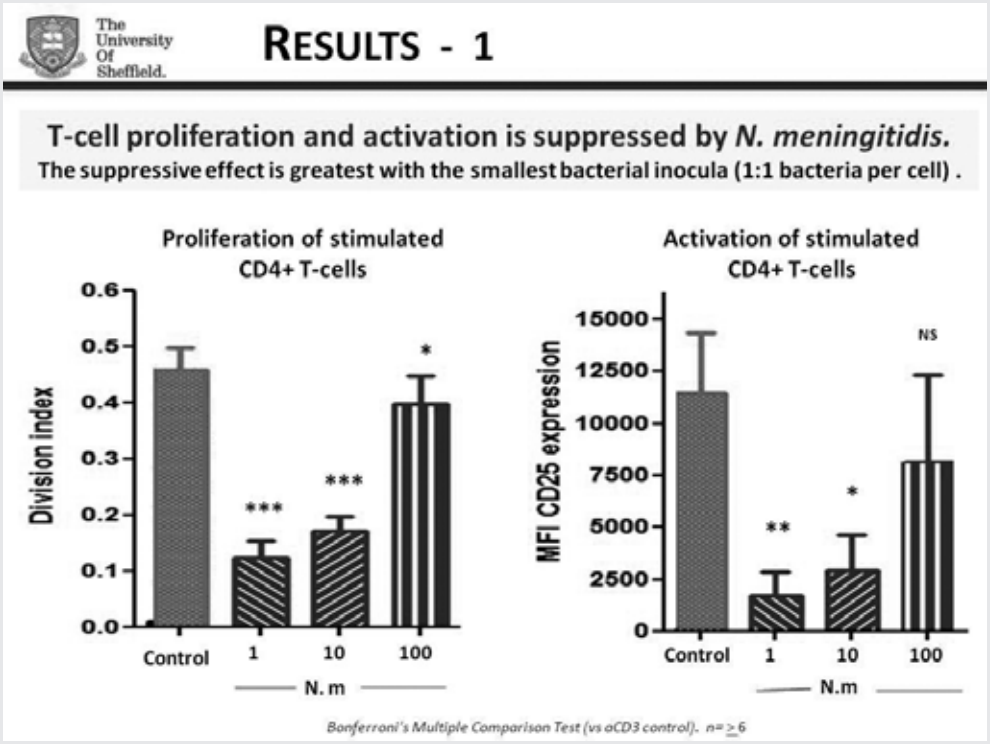
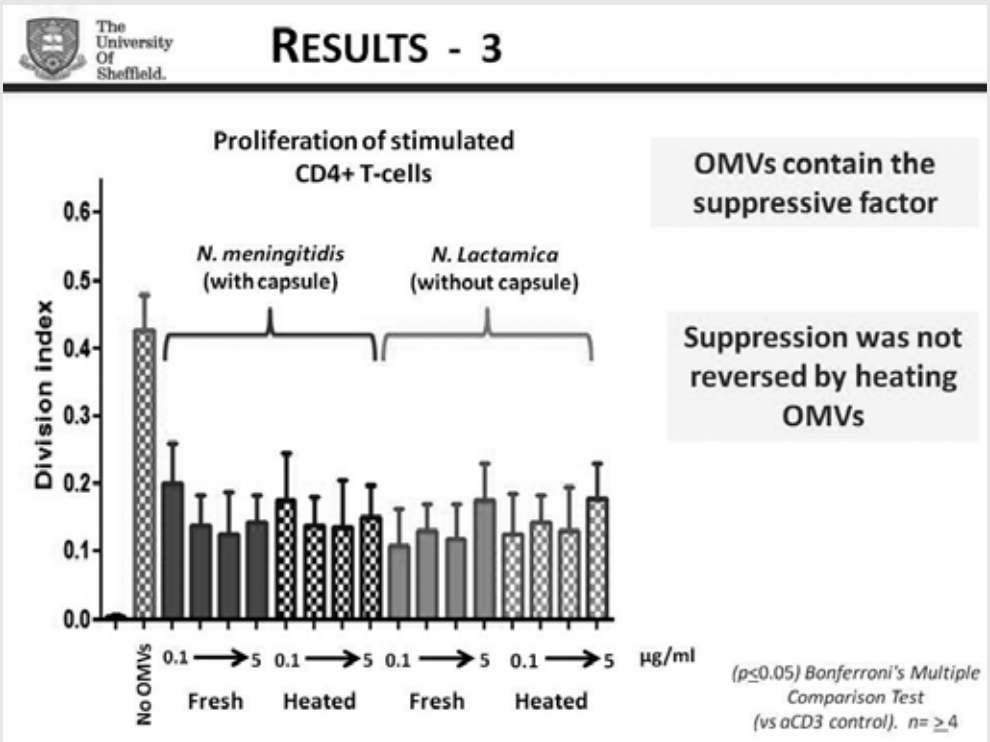


figure 2



P 232

Human innate IgM memory B cell responses to infection with commensal *Neisseria* species are vastly different from infection with pathogenic *Neisseria meningitidis*

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The *Neisseria* are human-restricted organisms, with tropisms for mucosa. Neisseria gonorrhoeae (*Ngo*) colonizes the urogenital tract, and is the cause of gonorrhea, while the commensal *Neisseria* sp., including *Neisseria lactamica* (*Nla*), with *Neisseria meningitidis* (*Nme*) are primarily colonizers of the nasopharynx. *Nme* is an opportunistic pathogen, and can colonize healthy individuals without causing disease, however dissemination away from this site can lead to disease. The factors influencing whether individuals develop disease or not are complex and not well understood. Previously, we have described how *Ngo* infection induced proliferation and differentiation of the human innate IgM memory B cells into antibody secreting cells. The immunologlobulin (Ig) produced was polyreactive and primarily IgM. In humans, IgM memory B cells are responsible for the production of 'innate' Ig. Innate Ig is primarily IgM, low affinity, but broadly reactive. It has been shown to be important during the early stages of infection by preventing the dissemination of pathogens prior to the development of high-affinity clonal Ig responses. Because *Ngo* induced such vigorous responses from the innate B cell subset, we were interested in determining if this effect was common amongst all neisserial species. Unexpectedly, B cell infection with the closely related *Nme* induced poor IgM memory B cell responses, while infection with a number of commensal *Neisseria* sp. resulted in robust innate B cell responses, including the production of high levels of polyreactive IgM. The effects of the commensals on innate B cell responses were, in most cases, stronger than the effects elicited by the gonococci. The *Nla* components inducing the robust IgM memory B cell proliferation were also present within its outer membrane vesicles (OMVs), blebs of membrane that are naturally released during growth, whereas OMVs derived from *Nme* were just as poor as the meningococci in their inability to induce B cell responses. Clear differences between the neisserial species in B cell adhesion is one factor of many contributing to the differences observed in the induction of B cell responses upon infection. This highlights the distinctive ability of the *Nme* to avoid inducing innate B cell responses, and since this is true for a variety of meningococcal strains, it is enticing to hypothesize that this deficit may increase the fitness of this pathogen for asymptomatic carriage and/or disease.

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Differential innate mucosal protection against *N. meningitidis* by commensal Neisseriae

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Our objective was to determine how nasopharyngeal epithelial cells may be differentially protected from *N. meningitidis*-induced damage and infection by the presence of commensal Neisseriae (*N. lactamica* NL4.1, *N. lactamica* Y92-1009, *N. cinerea* 194, *N. polysaccharea* LNP: N462).
Nasopharyngeal epithelial cells (Detroit 562) were challenged with pathogenic *N. meningitidis* serogroup B (NmB, strain MC58), in the presence or absence of commensal Neisseriae. *N. meningitidis* serogroup A (NmA, strain C751) and *S. pneumoniae* serotype 14 (Sp14) were used to assess the specificity of pathogen protection. Damage to the epithelial barrier was determined by measuring pro-inflammatory IL-6 release by ELISA and epithelial cell death using vital dyes, LDH release and caspase 3 activity. The mechanism of cyto-protection was further explored using intrinsic and extrinsic death-inducing agents and finally through Nm adhesion and invasion of host cells, using standard gentamicin protection and viable counts, flow cytometry of antibody labelled pathogen and using bioluminescent constructs of *N. meningitidis*.
Pathogenic Nm strains MC58 and C751 strongly induced inflammatory IL-6, *N. lactamica* Y92 1009 induced moderate levels, whilst *N. lactamica* NL4.1, *N. cinerea* 194 and *N. polysacharea* LNP: N462 induced no significant IL-6 secretion from nasopharyngeal epithelial cells. NmB and Sp14 also caused dose-dependent apoptotic and necrotic cell death, which was significantly reduced by the presence of *N. lactamica* strain NL4.1or *N. polysacharea* LNP: N462 (Fig 1) but not *N. lactamica* Y92-1009. This commensal-specific protection against cell death was not mediated through the TNFα receptor and did not require cell invasion. Protection was, however, found to correlate with significant reductions in NmB adhesion in the presence of *N. lactamica* strain NL4.1 (Fig 2) and *N. polysacharea* LNP: N462 but not *N. lactamica* Y92-1009, heat-killed NL4.1, nor *N. lactamica* OMV. Adhesion by *N. lactamica* strain NL4.1 is 100 fold less than NmB, suggesting an indirect and as yet undefined mechanism of protection.
We conclude that specific strains of live commensalNeisseriae, which naturally colonise the nasopharynx of children and adults, afford host cell innate protection against related Gram-negative and possibly unrelated Gram-positive pathogenic bacteria that naturally colonise the same niche.

figure 1

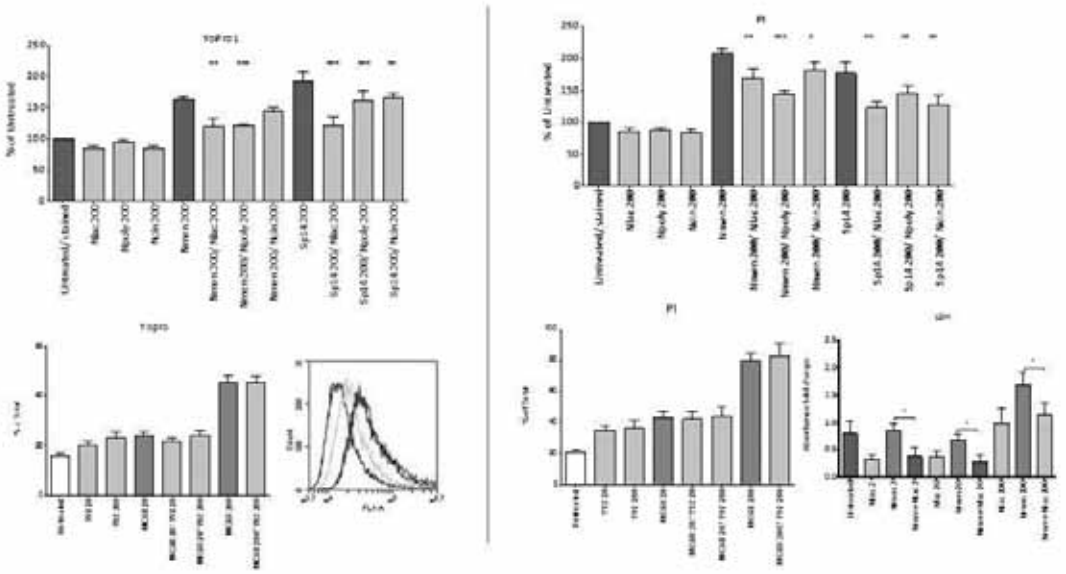

Figure 1. Commensal *N. lactamica strain* NL41, *N. cinerea, N. polysaccharea* but not *N. lactamica* strain Y92-1009 reduce NmB-induced cell death in nasopharyngeal epithelial cells Apoptotic and necrotic cell death was determined after 3h challenge with live bacteria and 21h culture in media containing antibiotics. Fluorescent vital dyes, YoPro1 and PI were used to assess NmB-induced apoptotic and necrotic cell death respectively, in the presence and absence of commensal Neisseriae. Mean ± SEM for n=3 independent experiments each performed in triplicate. *p≤0.05 **p≤0.01 ***p≤0.001
Apoptotic cell death was confirmed using caspase 3 specific antibody staining and flow cytometry (black- unstained control, blue- NmB infected cells, red- NmB infected cells in the presence of N. lactamica Y92-1009, yellow- NmB infected cells in the presence of *N. lactamica* NL4.1). Necrotic cell death was confirmed by measuring cytosolic LDH release in cell cultures.

figure 2

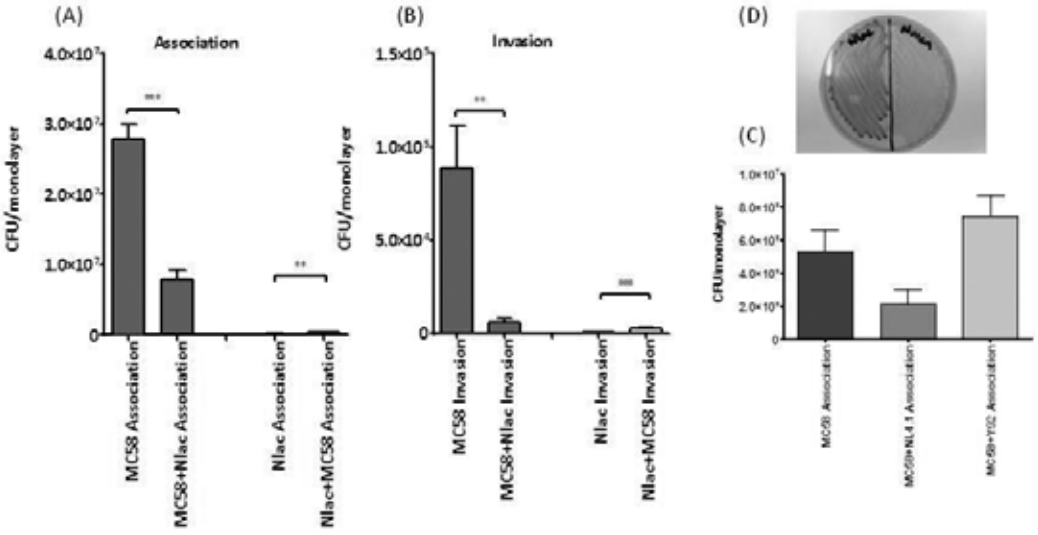

Figure 2. *N. lactamica* strain NL4.1 but not Y92-1009 protects against *N. meningitidis* association of nasopharyngeal epithelial cells. NmB association with nasopharyngeal Detroit 562 epithelial cells was determined in the presence and absence of equal concentrations of *N. lactamica* for 3h. A standard gentamicin protection assay was performed to quantify the levels of (A) NmB association and (B) NmB invasion in the presence of *N. lactamica* strain NL4.1 (MC58 + Nlac) and the level of Nlac association / invasion (Nlac + MC58) in the presence of NmB. (C) NmB association in the presence of *N. lactamica* strain Y92-1009 was also determined and (D) HBHI plates conatining X-gal were used to differentiate N. lactamica from N. meningitidis in co-cultures

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Lactobacilli counteract host cell signalling induced by *Neisseria meningitidis* and thereby provide colonization resistance

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The normal microbiota prevents colonization of pathogenic bacteria and represents an important first line of defence. Different *Lactobacillus* species belong to the normal microbiota of the pharynx and prevent adherence of a wide range of pathogens. However the mechanisms of the adherence inhibition are largely unknown. We investigated the influence of lactobacilli on *Neisseria meningitidis* adherence to host cells. The capacity to interfere with meningococcal adherence differed between *Lactobacillus* strains commonly found in the oral cavity. Inhibitory lactobacilli were found to counteract meningococcal activation of Src, subsequent Ca^{2+} release and upregulation of the calcium responsive gene *EGR1* in host cells. The transcription factor Egr1 is upregulated upon infection with different pathogens, however the importance of this upregulation for bacterial adherence has not been investigated. Interestingly, we found that RNA-silencing of Egr1 reduced meningococcal adherence. Both meningococcal-induced upregulation of *EGR1* and meningococcal adherence was reduced in host cells lacking the surface receptor Integrin beta1. Further, chemical activation of Src restored *EGR1* expression and meningococcal adherence in cells with silenced expression of Integrin beta1. Thus, upregulation of *EGR1* seems to be induced by activation of Integrin beta1 signalling in response to infection with *N. meningitidis*. Furthermore, we found that *Lactobacillus*-inhibition was mediated by peptidoglycan and dependent on TLR2 expression. In addition the influence of lactobacilli on *N. meningitidis* adherence *in vivo* was investigated by intranasal infection of CD46 transgenic mice. Co-infection with inhibitory lactobacilli showed a trend of reduced meningococcal binding, in comparison to infection with *N. meningitidis* alone or co-infection with non-inhibitory lactobacilli. Taken together, these data suggest that certain *Lactobacillus* strains prevent meningococcal adherence by affecting host cell signalling pathways. The composition of theLactobacillusflora of pharynx might be important for how efficiently it provides colonization resistance against *N. meningitidis*.

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The Cell-penetrating Peptide TP10 is Bactericidal to *Neisseria meningitidis* and Prevents Inflammatory Responses upon Infection

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Meningococcal disease is characterized by a fast progression and a high mortality rate. Cell-penetrating peptides (CPPs) are considered promising vehicles for pharmaceutical drug delivery and share structural features with antimicrobial peptides. A screen identified two CPPs, TP10 and MAP, with rapid bactericidal action against *Neisseria meningitidis*. Both peptides were active in human whole blood at micromolar concentrations while hemolysis remained negligible. Fluorescence microscopy of SYTOX Green uptake into live meningococci indicated that TP10 acts by membrane permeabilization. Administration of TP10 in an *in vivo* sepsis model resulted in a significant decrease in bacteremia. Additionally, TP10 was able to suppress cytokine release from infected or LPS-stimulated macrophages, revealing novel immunomodulatory properties. With its endotoxin-neutralizing potential and *in vivo* antimicrobial activity, TP10 is a promising scaffold for the development of peptide antimicrobial agents.

P 236

Differential binding capabilities of MtrA in response to effectors of the MtrCDE efflux pump

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Introduction/Question: MtrA is a member of the AraC family of transcriptional regulators. It has been shown that MtrA is important for transcriptional activation of the RND efflux pump operon termed-mtrCDE; the encoded pump contributed to gonococcal resistance to a wide variety of substrates. A characteristic of this transcriptional regulator is aC-terminal DNA binding domain, of which AraC is the canonical member. Also present is anN-terminal effector binding / dimerization domain which can alter the DNA-binding properties of regulator. MtrA is a global regulator of genes, including activating its own expression and that of two genes, NGO1249 and NG1248, divergently expressed from its own promoter region. In this study, we report that MtrA has a high predicted homology to Rob, anEscherichia coliAraC-family protein involved in activation of antibiotic resistance and organic solvent tolerance. Here we show that the corresponding residues predicted to be important for DNA binding for Rob are the same for MtrA. The ability of effectors to modulate MtrA binding to different target sites showed that Triton X-100, a substrate of the MtrCDE efflux pump, enhances MtrA binding to themtrCDEpromoter region, but has no effect on MtrA binding to its own promoter. This strongly suggests that MtrA can respond to stimuli in by altering its conformation to affect the binding to certain promoters, but not to others.
Methods: DNA binding abilities were of various MtrA mutants was assessed by electrophoretic mobility shift assays (EMSA). The structural homology was done using the Swissprot algorithm and visualised using MacPymol. Mutagenesis of MBP-MtrA was made by splice-overlap PCR to make the following residue changes in the DNA binding region; A227L, R231L and K281L. These were purified and used in EMSA reactions.
Results: All residue mutations were purified with MBP attached to the N-terminus. These mutants ($MtrA_{A227L}$, $MtrA_{R231L}$ and $MtrA_{K281L}$) were severely impaired in their ability to bind to themtrCDE promoter region, demonstrating their importance for DNA binding. This also validated the Swissprot model of MtrA which showed that it is homologous to Rob ofE. coli. The ability of known substrates of the MtrCDE efflux pump to modulate the DNA binding activity of MtrA showed that Triton X-100 could enhance binding of MtrA to themtrCDE promoter region, but not to its own promoter.
Conclusions: The ability ofNeisseria gonorrhoeae to respond to different stimuli is very important given the hostile environment the bacterium inhabits. The ability of MtrA to respond to respond to certain effectors of the MtrCDE efflux pump and increase its affinity for the promoter region allows transient transcriptional upregulation of the efflux pump. However, this response does not affect the binding of MtrA to its own promoter, suggesting a varied response to stimuli has evolved which probably depends on the location and or affinity of MtrA to the binding sites in the promoter region.

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Identification of a *Neisseria* mutant that no longer induces HIV-1 expression in CD4$^+$ T lymphocytes

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A clinical and epidemiological synergy exists between the sexually transmitted pathogens HIV-1 and *Neisseria gonorrhoeae*. *N. gonorrhoeae* has also been shown to stimulate HIV-1 expression in CD4$^+$ T lymphocytes in a dose-dependent manner *in vitro*. This study aims to determine the component of live *N. gonorrhoeae* that induces HIV-1 expression in CD4$^+$ T lymphocytes during co-infection. Induction of HIV-1 expression was evaluated using 1G5 cells, a Jurkat CD4$^+$ T cell line containing a stably integrated luciferase HIV-1 5'-LTR reporter. To determine whether the induction of HIV-1 expression by *N. gonorrhoeae* is due to a general immune response to live bacterial infection or if it the effect is specific to *N. gonorrhoeae*, a panel of bacteria was tested for HIV-1 LRT induction activity, including: the closely related Gram-negative species *Neisseria meningitidis, Moraxella catarrhalis* and *Haemophilus influenzae*; the Gram-positive pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae*; and a laboratory adapted strain of *Escherichia coli*. The *Neisseria spp.*were the only bacteria tested that induced significant expression of the HIV-1 LTR indicating that the HIV-1 inducing effect is specific to *Neisseria spp.* and not due to a previously characterized bacterial innate immune agonist. In order to effectively investigate the components of *Neisseria spp.*that specifically trigger HIV-1 LTR expression during co-infection, a random transposon mutagenesis approach was initiated to obtain a HIV-1 non-inducing mutant. We found *N. meningitidis* to be less fastidious and more amenable to a high-throughput approach than *N. gonorrhoeae*, but induces HIV-1 LTR expression to similar levels. A random transposon library was constructed in *N. meningitidis* B16B6 and 1800 mutants from this library were screened for loss of HIV-1 LTR induction in a high-throughput Jurkat 1G5-based assay. The first round of screening yielded, 96 presumptive mutants, of which 2 were confirmed as non-inducing. The location of the transposon in the *N. meningitidis* genome of these mutants is currently being determined by sequencing out of the transposon with a transposon specific primer. Elucidation of the genes involved in biosynthesis of the specific HIV-1 inducing component of *Neisseria spp.* will provide a new paradigm for the interaction between *N. gonorrhoeae* and the HIV-1 infected host, as well as insight into the epidemiological synergy between two globally important pathogens.

P 238

Neisserial Opa protein engagement of CEACAM1: In vivo consequences and implications for vaccine design

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Neisseria recovered from infected individuals express Opa proteins that specifically adhere to one or more CEACAM family receptors. Of these, CEACAM1 is the most widely expressed, with both constitutive and inducible expression on epithelia, endothelia and leukocytes. Being a co-inhibitory receptor, CEACAM1 has the potential to suppress the activation of a variety of cell types, including those with the potential to contribute to innate and/or adaptive immune responses. Yet, in vivo, Opa adhesin expression may also increase the delivery of intact Neisseria or neisserial-derived outer membrane blebs to CEACAM1-expressing antigen presenting cells, which may enhance their response. The relative contributions of these opposing effects have the potential to either amplify or inhibit innate and/or adaptive immune responses. To account for the fact that Opa proteins bind only human CEACAM1, we performed outer membrane vesicle (OMV)-based vaccine studies with a CEACAM1-humanized transgenic and its wild type parental mouse line. To complement this approach, we created an Opa-deficient mutant of Neisseria meningitidis H44/76 with all four opa alleles disrupted. OMVs were prepared from this, as well as the parental Opa-expressing strain. After two immunizations, the vast majority of immunoglobulin produced was of the IgG class, and there was no obvious effect of Opa protein or CEACAM1 expression on the amplitude of this response. Unexpectedly, all mice (regardless of genotype) that received OMVs with the adjuvant aluminum hydroxide presented with a localized inflammatory response in the form of a nodule at the site of injection, an adverse event that was not apparent in mice who received heat-killed bacteria, OMVs alone or adjuvant alone. The time to resolution for nodules present on the mice that received OMVs containing Opa proteins was prolonged compared to those that received OMVs without Opa proteins. Combined, our results suggest that although Opa-CEACAM1 does not appear to affect the immunogenicity of OMV-based vaccines, this interaction may either promote antigenic retention and/or leukocyte retention at the injection site, thereby hindering the resolution of inflammation induced as an adverse event following immunization.

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The role of gonococcal Neisserial surface protein A in serum resistance and comparison of its factor H binding properties with that of its meningococcal counterpart

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Introduction: *Neisseria gonorrhoeae* (Ng) and *N. meningitidis* (Nm) bind the complement regulator factor H (fH) to evade killing by the innate immune system. Gonococcal porin molecules bind fH and binding is greatly enhanced by sialylation of lipooligosaccharide (LOS). In contrast, fH binds to fH binding protein (fHbp) and Neisserial surface protein A (NspA) in Nm. In many Ng strains fHbp lacks a signal peptide, is not expressed on the surface and does not contribute to fH binding. The Ng NspA (Ng-NspA) shares 95% identity with the Nm NspA (Nm-NspA). We sought to investigate the role of Ng-NspA in complement evasion and serum resistance and to compare the fH binding properties of Ng and Nm NspA.

Methods: Binding of fH, fH like protein-1 (FHL-1) and constructs containing fH domains fused to murine Fc (fH/Fc) to bacteria was assessed by FACS. Site directed mutagenesis (SDM) was used to alter specific amino acids in NspA and the wildtype and mutated NspA proteins were expressed in *E. coli* as membrane vesicles (MVs). Binding of fH, FHL-1 and fH/Fc constructs to NspA containing *E. coli* MVs was assessed using ELISA. Bactericidal assays were used to compare serum resistance of wildtype Ng and NspA deletion mutants.

Results: Four Ng strains expressing the L8 LOS epitope (lactose on HepI) bound fH and FHL-1. Deleting NspA dramatically decreased fH binding and was associated with increased sensitivity to killing by normal human serum.

Nm-NspA binds to fH and FHL-1 via fH domains 6 and 7. Consistent with this, Ng-NspA in *E. coli* MVs bound to fH domains 5-8/Fc and to FHL-1. Mutation of H337 in conjunction with either H360 or H371 of fH domain 6 decreased binding of domains 5-8 to NspA. Binding of fH to Nm-NspA is human specific; likewise rhesus fH 5-8/Fc did not bind to Ng-NspA. Interesting, and in contrast to Nm-NspA, Ng-NspA expressed in *E. coli* MVs did not notably bind full-length fH, possibly indicating that the interaction of Ng-NspA with fH is dependent on the Ng background.

Nm-NspA expressed in *E. coli* MVs bound fH and FHL-1 better than Ng-NspA. SDM indicated that V112 and D113 in surface loop 3 of Nm-NspA (A113 and H114 in Ng-NspA) were important for high affinity binding of Nm-NspA to fH and FHL-1.

Conclusions: Ng-NspA is important for binding of fH and FHL-1 to Ng and deletion of NspA decreases serum resistance. Nm-NspA binds fH and FHL-1 better that Ng-NspA; V112 and H113, specific to Nm-NspA, are critical for high affinity binding.

P 240

A Native Outer Membrane Vesicle (NOMV, GMMA) vaccine from recombinant strains against meningococcal disease in Africa

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Objective: *Neisseria meningitidis* causes reoccurring epidemics in sub Saharan Africa, mainly caused by strains belonging to capsular group A, but W-135 and X are currently also contributing to the burden of disease. Our goal at the Novartis Vaccines Institute for Global Health is the development of a safe and affordable vaccine based on native Outer Membrane Vesicles (renamed Generalized expression Modules for Membrane Antigens, GMMA) from genetically-engineered strains that could provide broad coverage against strains from all serogroups causing meningococcal disease in sub-Saharan Africa.
Methods: Our vaccine strategy is based on GMMA from a serogroup W-135 isolate expressing PorA subtype P1.5,2, which is highly predominant among African W-135 isolates. In order to increase breadth of cross protection, the strain was engineered to express increased levels of factor H binding protein in the variant 1 group [1] which is expressed by the majority of African serogroup A and X strains [2]. In order to attenuate virulence and increase safety of the vaccine, we deleted capsule biosynthesis and genetically-detoxified the lipooligosaccharide by deleting *lpxL1*. In order to increase the release of GMMA *gna33* was deleted [3]. We evaluated the release of GMMA from the mutant strains and measured serum bactericidal antibodies responses of mice immunized with GMMA from the mutant against invasive African serogroup A, W-135 and X strains.
Results: Deletion of *gna33* resulted in an approximately 15- fold increase in release of GMMA compared with the wildtype strain. In mice, the GMMA from the mutant engineered to over-express fHbp variant 1 elicited high anti fHbp v.1 antibody responses as measured by ELISA. When measured with human complement, the GMMA elicited high serum bactericidal antibody (SBA) titers against a W-135 strain expressing the homologous PorA and fHbp v.2. The vaccine also elicited serum bactericidal antibodies against serogroup A and X strains expressing a heterologous PorA and fHbp in the variant 1 group. Against such strains these bactericidal antibodies appear to be directed against fHbp as indicated by loss of serum bactericidal activity in mice immunized with GMMA without over-expressed fHbp v.1.
Conclusion: We have generated a recombinant African W-135 strain with deleted capsule expression, *lpxL1* and *gna33* genes, and engineered to overexpress fHbp v1. A GMMA based vaccine from this strain has the potential to provide broad coverage against strains from all serogroups causing meningococcal disease in Africa.
References:
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A trivalent outer membrane vesicle (OMV) vaccine against serogroup A, W-135 and X meningococcal disease

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Objective: The recent decade has demonstrated that there is a need for a vaccine to cover meningococci of serogroups A, W-135 and X to prevent most epidemic meningitis in the African meningitis belt. The current study explores the serogroup X specific immunogenicity in mice induced by combining X-OMV or X-polysaccharide (X-PS) with a serogroup A+W-135 OMV vaccine planned for clinical testing in Cuba.
Methods: Groups of NMRI mice were immunised with two doses of either a combination of the A+W-135 OMV vaccine with X-PS or X-OMV, or each of the monovalent vaccine products. Dose levels were 2.5 μg protein or PS per component, respectively, and all were formulated with $Al(OH)_3$. All deoxycholate extracted OMVs and the purified X-PS vaccines were produced by Finlay Institute. The OMVs were derived from wild-type disease isolates of serogroups A (strain Mk499/03), W-135 (strain Mk222/02) and X (strain BuFa2/97); for which the sequence types (STs) were 7, 11 and 751, respectively. Serum bactericidal activity (SBA) was evaluated using the homologous vaccine strains with 25% rabbit complement. Antibodies against target antigens were evaluated by ELISA.
Results: Immunisation of mice with X-OMV, either alone or in combination with A+W-135 OMVs induced serum bactericidal antibodies against the serogroup X target strain BuFa2/97; even after one dose. A combination of X-PS with the A+W-135 OMV vaccine did also induce SBA titers against the group X-strain, whereas the X-PS alone was not immunogenic in mice. Comparable SBA titers against serogroup A (vaccine strain) were induced in mice immunized with A-OMV as a monovalent vaccine or combined with W-OMV and X-PS/X-OMV.
Strong OMV specific antibody responses were induced against the corresponding OMVs, and a relatively low level of cross-reaction between serogroups was observed. Mixing the individual OMVs with each other did not seem to reduce the serogroup specific immunogenicity.
Conclusion: Both serogroup X OMV, or a mixture of X-PS or X-OMV with A+W135 OMVs, were shown to induce putatively protective antibodies against serogroup X meningococci in pre-clinical experiments. Moreover, addition of X-PS or X-OMV to an A+W135 OMV vaccine did not appear to decrease the immunogenicity against serogroups A or W-135. Thus, a trivalent AXW-135 vaccine, either as a combination of OMVs or OMVs and X-PS, may be able to prevent the majority of meningococcal disease in the African meningitis belt.

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Native outer membrane vesicles (NOMV) combined with a group A conjugate vaccine for prevention of epidemic meningococcal disease in Africa

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Background: A serogroup A conjugate vaccine has been introduced in Sub-Saharan Africa.W-135 and X strains also cause epidemics in the region. NOMV vaccines from mutants with genetically attenuated endotoxin and over-expressed (OE) fHbp (NOMV-fHbp) show promise for prevention of disease irrespective of capsular group. We investigated the vaccine-potential of a combination NOMV-fHbp/Men A conjugate vaccine for prevention of meningococcal disease in Africa.
Methods: Mice were immunized with a NOMV-fHbp vaccine from an African W-135 strain with OE mutant R41S fHbp (for impaired fH-binding), attenuated endotoxin (ΔLpxL1), and deleted capsular genes. For OE fHbp we chose lD 9 (prevalent in African W-135 strains with fHbp variant group 1) (v.1). For the combination vaccine, lyophilized MenA conjugate (Novartis) was reconstituted with liquid NOMV-fHbp. Control mice received the NOMV-fHbp vaccine alone, or an A,C,Y, W-135 conjugate vaccine (MCV4-CRM, Novartis), or a NOMV-ΔfHbp vaccine. All vaccines were adsorbed with $Al(OH)_3$.
Results: In study 1, mice given the NOMV-fHbp vaccine alone developed serum bactericidal responses (1/GMT) of 80 to 200 against 4/5 group A strains tested (v.1 fHbp ID 5); 300 and 2000 against group X strains (v.1 fHbp ID 74), and 28,000 and 2000 against W-135 strains with v.1 fHbp ID 9 or v.2 fHbp ID 22, respectively. Titers elicited by the NOMV-ΔfHbp vaccine were <1:10 against group A and X strains, and >1:1500 against W-135 strains with PorA matched to the vaccine (P1.5,2). In study 2, against a representative group A strain (Z1275) and a known anti-fHbp resistant strain (E23/03), 1/GMTs were similar for mice given the combination NOMV-fHbp/MenA conjugate vaccine or MCV4-CRM (P=NS), and >100-fold higher for the combination vaccine than the NOMV-fHbp vaccine alone (Fig 1, P<0.001). Against a group W-135 strain, 1/GMTs elicited by either vaccine containing NOMV were 10-fold higher than to MCV4-CRM (Fig 2, P<0.02). Against a group X strain, 1/GMT was lower for the combination NOMV vaccine than the NOMV-fHbp vaccine alone (Fig 2, P=0.07).
Conclusions: The NOMV-fHbp/MenA conjugate vaccine elicited broad bactericidal responses against epidemic group A and W-135 isolates from Africa with titers greater or equal than a licensed MCV4-CRM conjugate vaccine. The anti-fHbp bactericidal responses to the combination vaccine against a capsular X strain may have been lower than to the NOMV-fHbp alone, which suggests need for additional formulation work.

figure 1

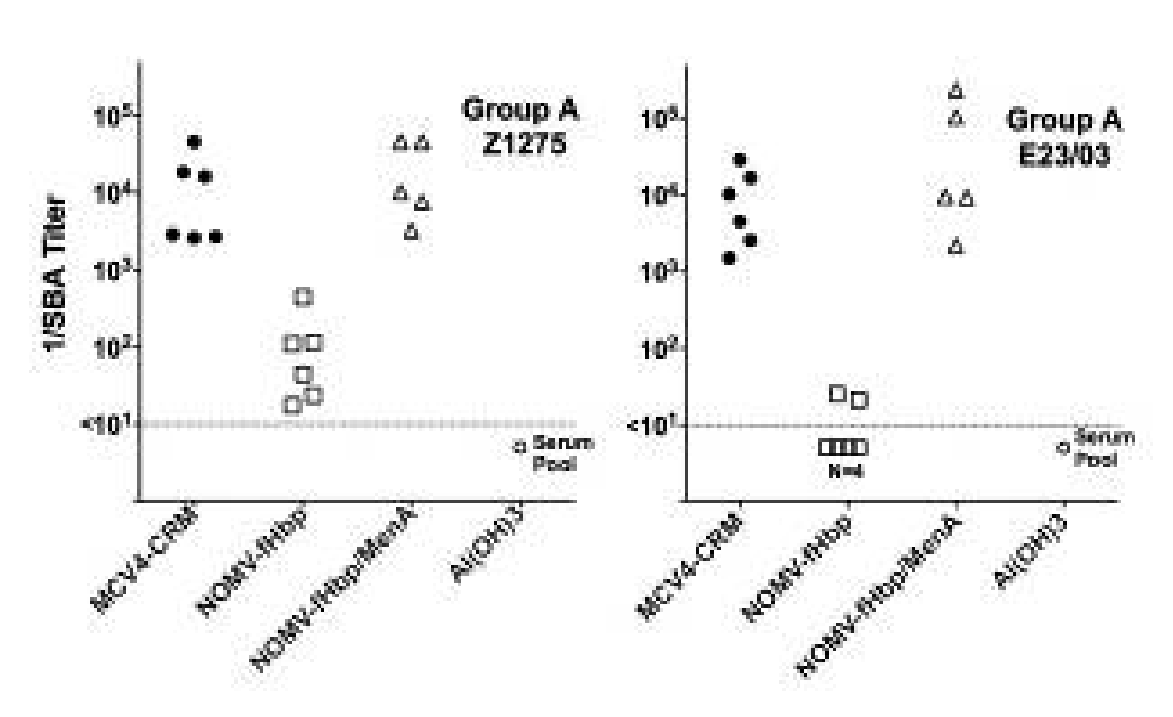
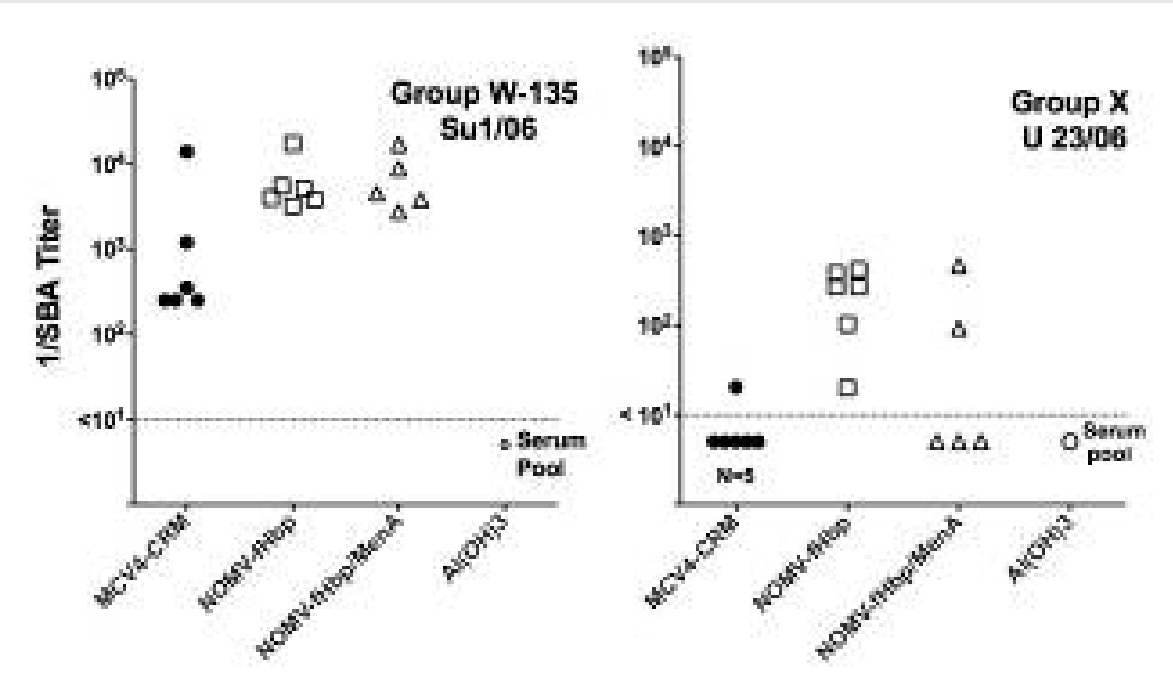


figure 2



P 243

Inconsistent correlation between antibody levels to outer membrane vesicles and serum bactericidal activity to group B meningococcus in adults

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Question: A protective response to vaccination for group B meningococcus in adults is presumed when there is a fourfold or greater increase in bactericidal activity using human complement (hSBA). The hSBA assay is resource intensive, as it requires human serum donors and screening against test strains to rule out intrinsic bactericidal activity. Standardization of immunogenicity testing could be improved if immune protection could be inferred without the use of human complement.
Previous seroepidemiologic and vaccine studies for group B meningococcus have suggested a correlation between the hSBA and antibody levels to outer membrane vesicles (anti-OMV) as measured by enzyme-linked immunosorbent assay (ELISA). We used sera from two previously conducted group B meningococcal vaccine trials to define the ability of anti-OMV to predict the hSBA response.
Methods: Sera were used from two previous phase I vaccine studies. One vaccine consisted of native OMV from an lpxL1 mutant of strain 8570 (HOPS-G, 36 vaccinees) and the other used native OMV from an lpxL2 mutant of strain 44/76 (MOS, 39 vaccineees). The hSBA and anti-OMV were measured using wild-type parent strains.
Results: Pre-vaccination antibody levels did not correlate with pre-vaccination hSBA for either vaccine. Post-vaccination, correlations were seen between antibody levels and hSBA for the HOPS-G vaccine (r^2=0.091, P=0.002), but fell short of significance for the MOS vaccine (r^2=0.060, P=0.078); correlations with a porA heterologous variant of the MOS vaccine strain were slightly better (r^2=0.091, P=0.030). For the HOPS-G vaccine, the fold-increase in antibody levels was higher in vaccine responders than non-responders (3.2 versus 1.9x, P<0.001) but these increases did not correlate generally with the fold-increases in hSBA. For this vaccine, the positive predictive value (PPV) of a twofold increase in anti-OMV for a fourfold increase in hSBA was 79%. For the MOS vaccine, the fold-increases in anti-OMV did not correlate with the fold-increases in hSBA, and the PPV of a twofold increase in anti-OMV was only 49%.
Conclusions: The correlation between anti-OMV and hSBA varies with the vaccine construct. Of the two vaccines considered, the more immunogenic gave a PPV of only 79%. These results suggests that antibody levels to OMV may be useful markers of immunogenicity in adults, but the degree of correlation needs to be defined for each vaccine construct.

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Optimization of critical process parameters for the production of Native OMV vaccine against Neisseria meningitidis

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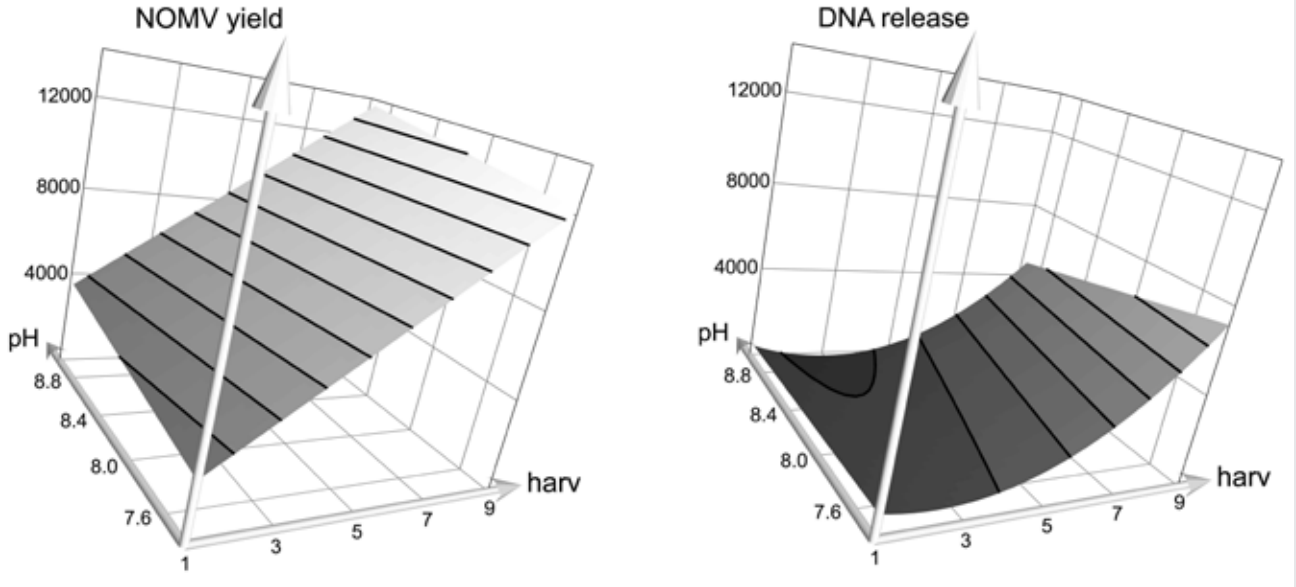
Introduction: Outer membrane vesicles (OMV) are used as a vaccine against Neisseria meningitidis serogroup B and are traditionally produced with detergent-extraction to remove endotoxin. Engineered strains with attenuated endotoxin allowed the use of Native OMV (NOMV) with improved stability and immunogenicity. The NOMV production process does not require detergents. Instead, vesicle release is stimulated with EDTA extraction (a chelating agent) to obtain a high yield. Many process parameters may affect the EDTA extraction efficiency, but it is unknown what the optimal ranges for these parameters are in terms of quality. The present study systematically optimized EDTA extraction and was representative for production at large-scale.
Results: Two critical process parameters were identified: harvest point of the cultivation (*harvest*) and pH of the extraction buffer (*pH*). They significantly changed yield (7-fold) and bacterial lysis (35-fold). Other important OMV quality attributes did not change. Optimization of *harvest* and *pH* revealed that the desired low bacterial lysis resulted in an intermediate but sufficient yield. High functional immunogenicity and low toxicity of the optimized vaccine were also confirmed.
Conclusion: The EDTA extraction is a robust process step which produces high quality OMV if harvest and pH are controlled within the optimized ranges.

figure 1



Effect of critical process parameters (*harvest* and *pH*) on OMV quality

P 245

Vaccine Potential of outer membrane vesicles from neisseria meningitides serogroup X

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Introduction: Meningococcal disease is caused mainly by serogroups A, B, C, Y, W_{135} of N. meningitidis. However, numerous cases of meningitis caused by N. meningitidis of serogroup X have recently been reported in several African countries. Currently, there are no licensed vaccines against this pathogen. Finlay Institute is experienced in production of an outer membrane vesicle (OMV) vaccine from N. meningitidis serogroup B, This vaccine have proven to be safe and effective in children, youth and adults. The aim of this work is to obtain, characterize and evaluate the vaccine potential of OMV derived from a serogroup X strain (OMVx).**Materials and Methods:** Three experimental lots of OMVx were obtained by detergent extraction method with deoxycholate from the N. meningitidis group X strain BuFa 2/97. Chemical and physical characterization was carried out to determine the size, morphology and the main antigens in vesicles. OMVx were adsorbed to aluminum hydroxide (OMVx/AL) and administered alone or in combination with capsular polysaccharide X (PsX) or polysaccharide A (PsA). Antigen specific IgG responses induced by these formulations to polysaccharides or OMVx were evaluated by ELISA, and by serum bactericidal assay (SBA).**Results:**The size of OMVx was between 90 and 120 nm determined by Dynamic Ligth Scattering and electron microscopy. The OpcA, RmpM and 70 kDa protein antigens were identified by SDS-PAGE and immunoblotting. Finally, BALB/c mice were immunized by subcutaneous route (0 and 21 days) with OMVx/AL and elicited high anti-OMVx antibodies responses with bactericidal activity. Co-administration of PsX or PsA with OMVx/AL enhanced the antibodies response to polysaccharides and bactericidal activity of sera against serogroup A and X strains. However, no immunogenicity was recorded from formulations of polysaccharide administered with Al adjuvant only.**Conclusion:** OMV obtained fromN meningitis serogroup X were immunogenic and potentiate the immune response to co administered PsX and PsA. These results suggest a switch from thymus (T) -independent to T-dependent response of polysaccharides. Novel formulations will be designed to protect against meningococcal disease in Africa.

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Immunogenicity in mice inoculated with different combinations of outer memberane proteins from a and W135 serogroups ans a Polysacharide

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Introduction: Serogroup A and W135 meningococcal disease causes a high burden of disease in many African countries. Plain polysaccharide vaccines are available, but they are poorly effective in young children, they do not induce memory immune response and polyvalent conjugate vaccines are considered to be too expensive for Africa. Outer membrane vesicle (OMV) vaccines from serogroup B have proven to be safe and immunogenic in various epidemic situations. Therefore a bivalent OMV vaccine against A and W135 serogroups could be an attractive alternative for "Meningitis Belt" in Africa. Materials and Methods: Different combinations of monovalent and bivalent OMV vaccines, with plain and conjugated A polysaccharide, were inoculated in Balb/c mice and compared with commercially available Men Afri Vac conjugated vaccine, in a two doses subcutaneous scheme. ELISA (anti OMVs and A polysaccharide) IgG titer and bactericidal titer assay (SBA) were used to measure immune response. Memory response after a 3rd dose was measured. Results: A high immune response was found in ELISA and SBA in groups inoculated with OMVs or conjugated polysaccharide, when the same serogroup was used as coating antigen or target strain. The group who received both OMVs plus plain polysaccharide gave better response than those that received plain A polysaccharide alone (no detectable response), measured against this antigen. The best titers, by ELISA, were achieved in the group inoculated with commercial vaccine, but the response was only against homologous serogroup. Memory response was observed after the third dose. Conclusions: A bivalent OMV vaccine could be more immunogenic than plain polysaccharide vaccine, and could be effective against a broader group of strains. This vaccine could be an interesting alternative to mono and multivalent conjugated vaccines for Africa.

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Neisseria meningitidis native outer membrane vesicles containing modified LPS have an adjuvant effect for meningococcal, non-meningococcal proteins, and polysaccharide antigens

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Question: Penta-acylatedNeisseria meningitidis LpxL1 lipopolysaccharide (LPS) could be used as an adjuvant for meningococcal vaccines as it has significantly reduced toxicity compared to wild-type LPS whilst maintaining a good adjuvant effect in mice. In addition, the disruption of *lgtB* has been shown to enhance the activation of human dendritic cellsin vitro. Therefore, we aimed to evaluate the adjuvant effects of native outer membrane vesicles (nOMVs) containing two different penta-acylated glycoforms of LPS (LpxL1 LPS and LgtB-LpxL1 LPS) on the immune responses elicited by various antigens.
Methods: Groups of mice were immunized with either recombinant serogroup B meningococcal protein PorA, tetanus toxoid (TT) or meningococcal serogroup C capsular polysaccharide (MenC PS) mixed with LPS-modified nOMVs or a licensed adjuvant, either monophosphoryl lipid A (MPL) or aluminium hydroxide ($Al(OH)_3$). Antigen-specific antibody responses were evaluated by ELISA and serum bactericidal antibody (SBA) assay using rabbit complement against a serogroup B or a serogroup C strain.
Results: Antigen-specific serum IgG concentrations against each of the three antigens were comparable, irrespective of the adjuvant used for immunization. However, lower IgG1/IgG2a ratios were elicited against PorA when the PorA protein was adjuvanted with either of the nOMVs containing modified LPS as compared to the ratio obtained with $Al(OH)_3$, along with higher SBA titres. Similarly, serogroup C-specific SBA titres induced following immunisation with MenC PS using nOMVs were higher than with $Al(OH)_3$.
Conclusions: Therefore, nOMVs with modified LPS (Lpxl1 and lgtB-Lpxl1) can act as adjuvants for protein and PS vaccine components, suggesting their possible use to adjuvant a variety of antigens.

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Neisseria lactamica antigens complexed with a novel cationic adjuvant

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Colonization of the nasopharynx by non-pathogenic Neisseria species, including *N. lactamica*, has been suggested to lead to the acquisition of natural immunity against *Neisseria meningitidis* in young children [1]. The aim of this study was to identify a model complex of antigens and adjuvant for immunological preparation against *N. meningitidis* B, based on cross reactivity with *N. lactamica* outer membrane vesicles (OMVs) antigens and the dioctadecyldimethylammonium bromide bilayer fragments (DDA-BF) adjuvant [2]. Complexes of 25 μg of OMVs in 0.1 mM of DDA-BF were colloidally stable, exhibiting a mean diameter and charge optimal for antigen presentation. Immunogenicity tests for these complexes were performed in mice. A single dose of OMV/DDA-BF was sufficient to induce a delayed type hypersensitivity (DTH) response, while the same result was achieved only after two doses of OMV/alum . In addition, to achieve total IgG levels that are similar to a single immunization with OMV/DDA-BF, it was necessary to give the mice a second dose of OMV/alum (Fig 1). Moreover, the antibodies induced from a single immunization with OMV/DDA-BF had an intermediate avidity, but antibodies with a similar avidity were only induced by OMV/alum after two immunizations. We evaluated the production of IgG1, IgG2a and IgG2b in immunized mice, 45 days after the first dose of the antigen (Fig 2) . The absorbance for each class of antibodies was subtracted from the mean absorbance of the non-immunized groupThe use of this novel cationic adjuvant for the first time with a *N. lactamica* OMV preparation revealed good potential for future vaccine design.

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figure 1

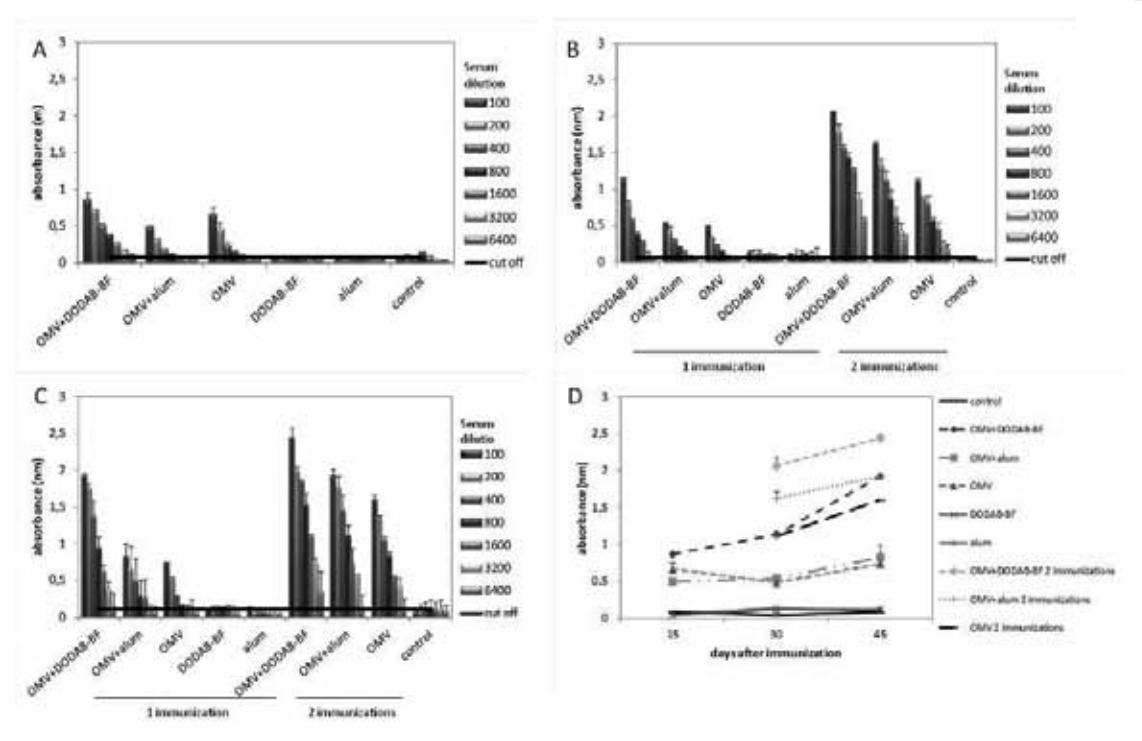
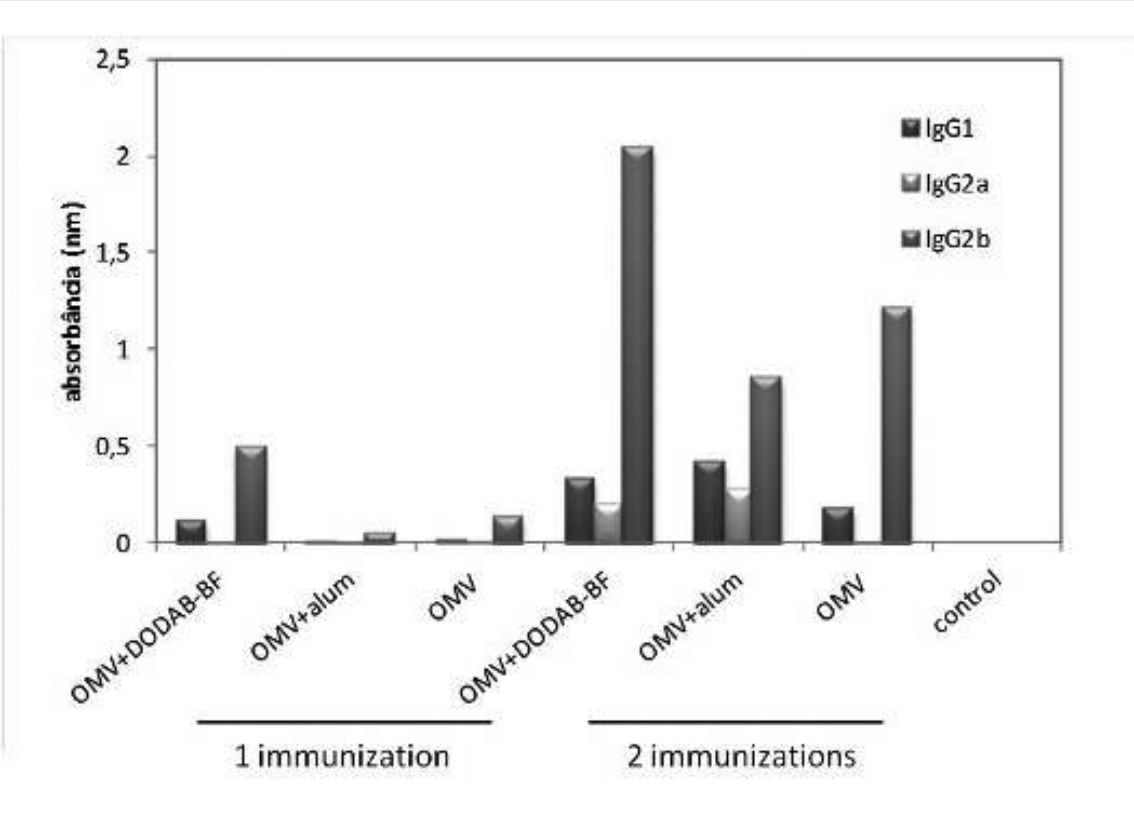


figure 2



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Study of the Antigenic cross-reactivity between *Neisseria meningitidis* and commensal Neisseria species using dioctadecyldimethylammonium bromide bilayer fragments (DDA-BF) as adjuvant

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OMVs from *N. lactamica* have emerged as an alternative to vaccination against meningococcal disease . Considering that this species has no capsule , OMVs vaccines based on *N. lactamica* are not serogroup or subtype specific, but they have been shown to protect mice against a lethal challenge in a model of meningitis, even in the absence of a bactericidal antibody response. Bilayer fragments of the cationic lipid dioctadecyldimethylammonium bromide (DDA-BF) can be obtained by dispersion of the white powder in an aqueous solution at low ionic strength after sonication at a temperature below 60°C. This low ionic strength allows the compound to remain stable due to the electrostatic repulsion of the molecules. DDA-BF can be used as an adjuvant, and its main advantage is that DDA-BF requires a lipid concentration lower than the concentrations traditionally used in liposomal formulations . Therefore, DDA-BF typically causes less toxicity. Aluminum compounds, which were identified as having immunostimulatory properties more than 70 years ago, remain the only adjuvant that is licensed worldwide . However, this adjuvant exhibits low colloid stability . Complexes of 25 μg of OMVs of *Neisseria lactamica* in 0.1 mM of DDA-BF were colloidally stable, exhibiting a mean diameter and charge optimal for antigen presentation. Immunogenicity tests for these complexes were performed in mice. Because vaccines based on *N. lactamica* OMVs aim to protect individuals against meningococcal infection, the cross-reactivity of the antibodies against *N. meningitidis* was tested by an ELISA, Dot-ELISA and immunoblot. In the present study, the immunogenicity of the OMVs of *N. lactamica* was tested in association with fragments of the lipid bilayer of dioctadecyldimethylammonium bromide (DDA-BF) used as adjuvant. In addition, DDA-BF was compared to alum, which had been implicated in the increased immunogenicity of OMVs vaccines to *N. meningitidis* B. The evaluation of the cross reactivity of the serum of the animals was performed 45 days after the first immunization. By Dot-ELISA they were tested against different meningococcal strains 39 strains of serogroup A, 42 strains of serogroup C (1972 to 1974), 131 strains of serogroup B (1990-2007) and 120 strains of serogroups B, C, W and Y (2011-2012) of Brazil. By Immunoblot we analysed the cross reactivity against OMVs of *N. meningitidis* A, B and C with different serotypes and subtypes and OMVs of Neisseria species. Results demonstrate by Dot ELISA for a total of 335 strains analyzed 93% of strains present reactivity with polyclonal serum from mice immunized with OMVs of *N. lactamica* and DDA-BF compared with 26% reactivity with aluminum . The cross reactivity of antibodies was also evaluated by immunoblot. Serum from immunized animals specifically recognized antigens of 20 to 130 kDa protein of *N.lactamica* and interestingly, this sera also recognized OMVs in *N. meningitidis* 10 to 85 kDa . In conclusion, OMVs of N. lactamica were effective in generating cross-reactive IgG antibodies to *N. meningitidis* . Interestingly, DDA-BF was superior to alum as an adjuvant for subcutaneous immunization with OMVs, both with measures of humoral and cellular immunity. Future work based on this study intends to examine any protective effects offered by the combination of OMV of *N. meningitidis* B-DDA-BF .

P 251

Immune response induced by liposome-associated porin/RmpM complexes alone or in combination with Alum and Freund´s adjuvants

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The refolding of neisserial porin complexes into liposomes allows the recovery of its native state and the induction of antibodies against conformational epitopes that can be crucial for a successful immune response. Previous studies showed that response against complexes including PorA, PorB and RmpM have interesting immunological properties.
As liposomes are appropriate protein carriers, with adjuvant properties and consistent and well defined composition, the aim of this work was to analyze the immunogenic characteristics of proteoliposomes carrying porin/RmpM complexes using Western-blotting and serum bactericidal activity.
Immunological analyses were performed using mouse sera against proteoliposomes that showed a high stability after preservation by lyophilization with glucose. The proteoliposomes obtained contained different combinations of recombinant PorA, PorB and RmpM, always minimizing the content of the hypervariable PorA. The need for higher adjuvant effects for proper responses was tested by using proteliposomes either alone or in combination with Alum or Freund´s adjuvant.
The results obtained showed that our proteoliposomes induced high bactericidal responses. Also, we found significant differences in protein recognition depending on the adjuvant used during immunization. While the sera against proteoliposomes alone or in combination with Alum displayed a good recognition of the three proteins incorporated in the liposomes, Freund's adjuvant did not allow an appropriate recognition of RmpM.
Therefore, we think that proteoliposomes containing porin/RmpM complexes should be further analysed as an alternative to current OMV vaccines against meningococcal serogroup B strains.

P 252

Modification of the AniA glycoprotein to enhance potential as a vaccine antigen for *Neisseria gonorrhoeae*

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Question: *O*-Glycosylation is emerging as a common post-translational modification of surface exposed proteins in bacterial mucosal pathogens. In recent studies we have identified an additional outer membrane glycoprotein in pathogenic *Neisseria*, the anaerobically induced nitrite reductase AniA, that is glycosylated in its C-terminal repeat region by the pilin glycosylation (*pgl*) pathway (1). AniA appears to be unique among bacterial nitrite reductases as it contains an N-terminal extension that includes a lipid modification site as well as this glycosylated C-terminal region. The glycosylation of AniA is not required for nitrite reductase function and its role is unknown. The aim of this study is to determine whether the glycosylation of AniA impacts on the host immune response to this glycoprotein.
Methods: Immuno-SEM and trypsin cleavage of surface proteins were used to determine if AniA was expressed on the cell surface. Animals were immunised with various glycoforms of the AniA protein to investigate the impact of the distinct glycoforms on the immune response to AniA. Recombinant AniA proteins with various truncations of the N- and C-termini expressed in an *E. coli* host were used as antigens to define the ideal antigen for generation of a cross reactive response.
Results: We have demonstrated that AniA is expressed on the cell surface in *N. gonorrhoeae* (Figure 1). Immunisation of animals with AniA glycosylated with the monosaccharide produced an antibody response directed predominantly towards the glycosyl moiety of the protein in a glycan-specific manner. In addition, when animals were immunised with a truncated form of AniA, completely lacking the glycosylated C-terminal region, the antibody response was directed against AniA regardless of the glycosylation state of the protein. These data show that when present the glycan of AniA appears to be immunodominant and only a weak immune response is elicited against the remainder of the protein. Furthermore, we found that a strong, non-native immune response against AniA can be generated by immunisation with truncated and/or non-glycosylated forms of this protein.
Figure 1. Examination of *N. gonorrhoeae* 1291 cells immunolabelled with pre-immune or anti-AniA polyclonal rabbit serum using SEM. 1291 cells grown aerobically immunolabelled with pre-immune rabbit serum (**A**) or anti-AniA polyclonal rabbit serum (produced in this study) (**B**). 1291 cells grown anaerobically immunolabelled with pre-immune rabbit serum (**C**) or anti-AniA polyclonal rabbit serum and (**D**). Scale bars indicate 500nm.
Conclusions: Our results suggest that the phase variable immunodominant *O*-linked glycan attached to AniA may confer an immune evasion mechanism in *N. gonorrhoeae* whereby the organism can switch between expression of different glycoforms of the AniA protein to escape detection by host antibodies specific to a particular glycan present on this protein. As AniA appears to be important to gonococcal host colonisation, we propose that recombinant modified AniA has potential as a vaccine antigen for *N. gonorrhoeae*.
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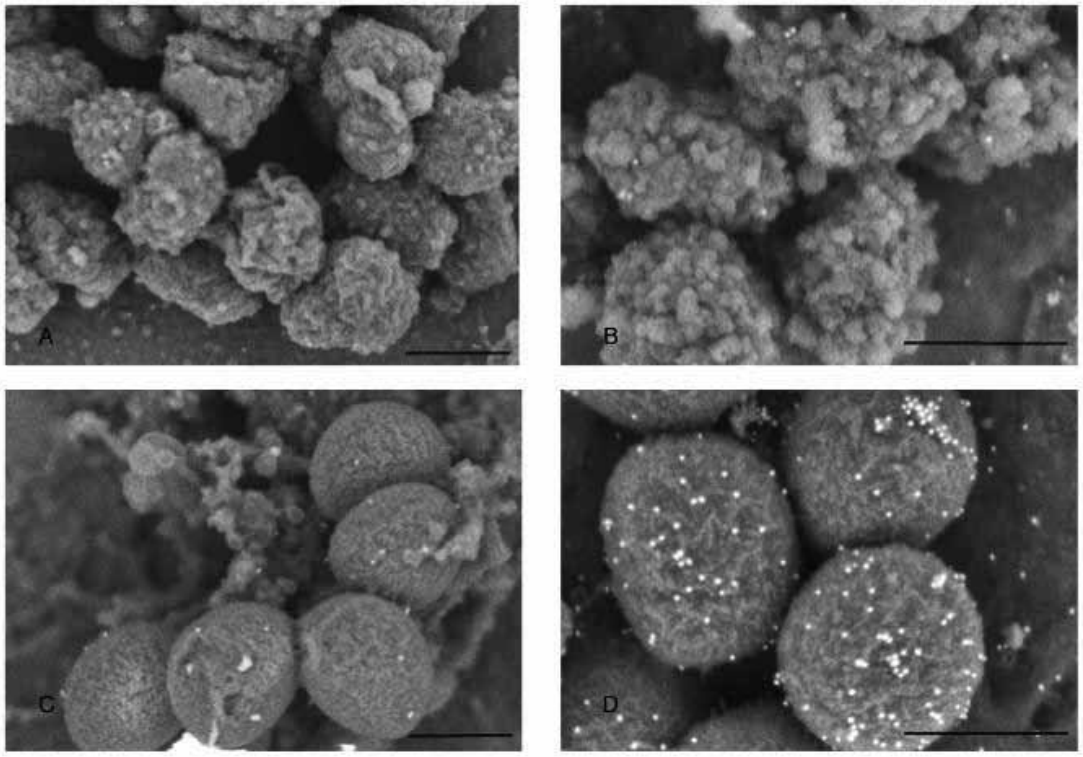
figure 1



P 253

Lipopolysaccharide as a vaccine component of a Tailor made Brazilian Meningococcal B vaccine

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Vaccines based on outer membrane vesicles (OMV) have been developed and successfully proven to be efficacious against serogroup B meningococcal disease in Cuba, Norway, New Zealand and France. However, these vaccines have induced better protection to homologous than heterologous strains. Many new vaccines against serogroup B have been focused in other antigens, including proteins that are important in epithelium attachment of the bacteria, others whose main role is in complement cascade and LPS. The inclusion of these antigens could increase the cross reactivity of the new products. Since 1990's Oswaldo Cruz Foundation, in Brazil, has been involved with the development of a Brazilian vaccine against serogroup B composed by detergent treated OMV and detoxified endotoxin (dLPS) from prevalent strains. The use of dLPS as a vaccine component was an approach to increase the cross-protection within heterologous strains. The dLPS was obtained from bacteria biomass, purified by hot phenol method and detoxified by treatment with NaOH. The kinetic of detoxification process was monitored by NMR, SDS-trycine gel, KDO quantification and pyrogen "in vivo" test. The purified and detoxified LPS profiles were evaluated by proton and phosphorus NMR. The analysis of signal intensity of the fatty acids regions in NMR ^{1}H spectra showed a signal decrease indicating the detoxification efficiency. Based on these results, the time of dLPS detoxification reaction was determined at 150 min. The changes observed in molecular weight and in the LPS quantification by each methodology studied demonstrated the antigen was detoxified appropriately and can be used as a vaccine component. This study is very relevant considering the Brazilian meningococcal B vaccine is the only vaccine based on OMV in Phase II trials that contain chemically detoxified LPS.

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Evaluation of Synthetic LPS-Core-Structure from Neisseria meningitidis as Potential Vaccine Candidate

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The human pathogen Neisseria meningitidis is an encapsulated, gram-negative bacterium transmitted by droplets and secretion. The serogroups A, B, C, W-135, X and Y are invasive and cause meningitis and/or sepsis worldwide. Currently available meningococcal vaccines are based on capsular polysaccharides. Such a vaccine cannot be developed for meningococci B which contains an autoantigenic [α2-8] polysialic acid capsule. So far no vaccine against meningococci B exists. Beside several capsular polysaccharides meningococci express 12 distinct lipopolysaccharides (LPS) in their outer membrane. The LPS-core structure α-GlcNAc-(1→2)-α-Hep-(1→3)-α-Hep-(1→5)-α-Kdo that is conserved in all meningococci has recently been synthesized by our group. Current work involves conjugation of this tetrasaccharide to carrier protein CRM_{197} and immunization experiments in BalB/C mice in presence of Freund's adjuvant. Significantly higher anti-LPS-core specific antibody titers were observed in mice immunized with glycoconjugate in comparison to unconjugated tetrasaccharide (end point titer for glycoconjugate is 10 fold higher than for unconjugated tetrasaccharide at 8^{th} week). Immunization with the glycoconjugate resulted in robust boosting responses. Specificity of sera and monoclonal antibodies against synthetic carbohydrate structures from different bacterial LPS was studied by microarray analysis revealing a specific response for the meningococcal tetrasaccharide. Flow cytometry and confocal fluorescence microscopy studies showed that antibodies are able to recognize meningococcal strains across all serogroups. The potential of these antibodies to promote opsonophagocytosis was evaluated by flow cytometry assay using fluorescence-labeled bacteria and serum-dependent enhancement in phagocytosis was demonstrated. Results of these studies show that the synthetic LPS-core structure is a potential vaccine candidate against meningococci B.

P 256

Targeting transferrin receptors for structure-based design of antigens against Neisseria meningitidis

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The ability of pathogenic *Neisseria* to reside in the human host relies on a surface receptor that mediates the acquisition of iron from human transferrin (Tf). The receptor is composed of transferrin-binding proteins A and B (TbpA and TbpB), an integral TonB-dependent outer membrane protein and a surface accessible lipoprotein, respectively. The critical role that these proteins play *in vivo* led to substantial efforts at developing a vaccine for prevention of meningococcal disease. Disappointing results in Phase I trials in humans with intact, lipidated TbpB reduced enthusiasm for it as a target for vaccine development. Using proof of concept experiments performed with receptor proteins from the porcine pathogen *Actinobacillus pleuropneumoniae* as a foundation, we have adopted a strategy of rational structure-based antigen design that integrates structural, functional and sequence information to optimize the ability of engineered antigens to induce a cross-protective antibody response. In this approach we use sequence information for *tbpB* and tbpA genes from a representative collection strains to map sequence variation onto TbpB (1) and TbpA (2) structures in order to identify potentially immunodominant variable regions as well as potential constraints on horizontal exchange. With the aid of this information, recombinant antigens are designed for enhanced immunological properties and used to immunize mice and rabbits. Analysis of sera obtained from mice and rabbits in a novel high-throughput solid-phase binding assay and a customized serum bactericidal assay is used to identify candidate antigens with enhanced immunological properties. The results of the immunological analyses are used for further iterations of antigen engineering.

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P 257

Vaccine potential of the Chaperonin60 (Chp60) protein of *Neisseria meningitidis*

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Question: Antibodies to the Chaperonin60 (Chp60, NMB1972) protein of *Neisseria meningitidis* were identified by immune-proteomics in sera from individuals following colonisation by meningococci (1). Based on this observation, we investigated the vaccine potential of Chp60 protein.
Methods: Recombinant (r)Chp60 was used to immunise mice and rabbits with a variety of different adjuvants and delivery vehicles suitable for human use. Anti-Chp60 sera were tested in ELISA against protein and outer membranes (OM) from the homologous strain MC58, in western blot and by immunofluorescence (IF) against MC58 and in bactericidal assays against a variety of different meningococcal strains. Variation in Chp60 was also examined in a collection of 13 strains isolated from colonised individuals and patients and in 200 strains in the BIGS database.
Results: Bio-informatic examination of meningococcal strains showed that the majority of strains expressed a Chp60 protein associated with alleles 1, 3 and 16. rChp60 (homologous allele 3 strain MC58) delivered in liposomes, detergent micelles and saline, with and without monophosphoryl lipid A adjuvant, or adsorbed onto $Al(OH)_3$, generated high titres of antibodies that reacted with the protein and with homologous OM. Antisera also recognised Chp60 in western blot and reacted with OM on the surface of meningococci as judged by IF. However, significant differences were observed in serum bactericidal activity (SBA): Chp60 induced significant SBA against the homologous strains MC58 and MC168 (SBA titres from 32 to 128), but reactivity was lower against a heterologous strain L2470 (allele 16; SBA titres 16-64) which differed by 2 amino acids. Moreover, no IF binding or SBA was detected against another heterologous strain MC161 (allele 1, SBA titre <4), which showed 4 amino acid changes compared to MC58.
Conclusions: The meningococcal Chp60 protein is capable of inducing bactericidal antibodies, but cross-protection is significantly reduced with increasing amino acid variability. Analysis of Chp60 variability with correlation to bactericidal activity suggests the position of the protective epitope(s). However, Chp60 shares significant sequence homology with human heat shock protein and is therefore likely to be precluded from inclusion in new meningococcal vaccines.
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P 258

Bactericidal antibody responses to Meningococcal recombinant outer membrane proteins

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Secretin PilQ is an antigenically conserved outer membrane protein which is present on most meningococci and PorA is a major protein that elicits bactericidal immune response in humans' serum following natural disease and immunization. In the present study, a 1095bp fragment of C-terminal coding sequence of pilQ(pilQ$_{406\text{-}770}$) and the full coding sequence of porA (1200bp) from serogroup B N. meningitidis were chosen and expressed into E. coli BL21 (DE3) and Origami B (DE3) as COOH terminal histidine fusion proteins, respectively. BALB/c mice were immunized subcutaneously with purified rPilQ$_{406\text{-}770}$ or PorA together with Freund's adjuvant. Serum antibody responses to serogroup A and B N. meningitidis whole cells or purified proteins and functional activity of antibodies were determined by ELISA and SBA, respectively. Serum IgG responses were significantly increased in immunized group with PilQ$_{406\text{-}770}$ or PorA together with Freund's adjuvant in comparison with control groups. IgG antibody response of mice immunized with PilQ$_{406\text{-}770}$ was higher than mice immunized with PorA ($P \leq 0.001$). The booster injections, especially the second booster, were effective to significantly increase the responses of anti-PilQ$_{406\text{-}770}$ or anti-PorA IgG ($P \leq 0.001$). Antisera produced against rPilQ$_{406\text{-}770}$ or rPorA demonstrated strong surface reactivity to serogroup B N. meningitides in comparison with control groups ($P \leq 0.001$). Antisera raised against PorA or PilQ$_{406\text{-}770}$ together with FA demonstrated SBA titers from 1/1024 to 1/2048 against serogroup B. The strongest bactericidal activity was detected in sera from mice immunized with PilQ$_{406\text{-}770}$ mixed with FA. These results suggest that rPilQ$_{406\text{-}770}$ is a potential vaccine candidate for serogroup B N. meningitidis.

Keywords: Bactericidal antibody, Neisseria meningitidis, PilQ$_{406\text{-}770}$, PorA

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The Macrophage Infectivity Potentiator (MIP) protein of *Neisseria meningitidis* induces cross-strain bactericidal activity and is a potential serogroup B vaccine antigen

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Question: Studies of the meningococcal outer membrane (OM) proteome identified the presence in relatively high abundance of a protein of Mr~29kDa, with homology to the macrophage infectivity potentiator (MIP) protein of *Legionella pneumophilia*, which we have termed the meningococcal MIP (1). We tested the ability of a recombinant (r)MIP to induce functional bactericidal antibodies, the generally accepted laboratory correlate of protection for serogroup B meningococci.
Methods: A gene encoding MIP protein from serogroup B strain MC58 was cloned and expressed in *Escherichia coli* and the purified soluble rMIP was used for immunisation studies in mice and rabbits. Animals were immunised with rMIP (1, 5, 10 and 20μg/animal) and a variety of different adjuvants and delivery vehicles suitable for human use. Anti-MIP sera were tested in ELISA against MIP and OM from MC58, in western blot and by immuno-fluorescence (IF) against MC58 and in bactericidal assays against a variety of different meningococcal strains. Variation in MIP was also examined in a collection of 13 strains isolated from colonised individuals and patients and in 200 strains in the BIGS database.
Results: Analysis of the predicted amino acid sequences of MIP from 13 well-characterised meningococcal strains differing in serogroup, serotype and subtype, showed that the protein was highly conserved (98-100%) and expressed at similar levels as judged by western blotting. Further interrogation of the BIGS database showed that there were four distinct sequence types found (designated I, II, III and IV) that covered ~95% of meningococcal strains. Immunisation of mice with type I MC58 rMIP in detergent micelles and liposomes containing monophosphoryl lipid A (MPLA) induced high levels of surface-reactive antibodies with serum bactericidal activity (SBA) titres of 1/1024 against the homologous strain. Bactericidal antibodies were also induced with the protein in saline alone and liposomes alone (titres of 1/128), but not following adsorption to $Al(OH)_3$. Doses of rMIP as low as 1-5μg/animal, delivered in saline or liposomes, were sufficient to induce significant bactericidal responses. Significantly, antisera raised against type I rMIP killed strains of heterologous sequence types II and III, with similar SBA titres (1/128-256). The biological response was specific, as anti-MIP antisera did not show reactivity against a MIP^- mutant strain.
Conclusions: Our studies show that the meningococcal MIP protein meets several important criteria for a potential meningococcal vaccine antigen - OM location, surface exposure, similar expression levels and conservation across meningococcal strains and the ability to induce high levels of cross-strain bactericidal activity. Taken together, these findings suggest that MIP is a serious candidate for inclusion in new vaccines against meningococcal infection.
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P 260

The *Neisseria meningitidis* macrophage infectivity potentiator protein (MIP) induces high serum bactericidal activity in the absence of adjuvants

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The poor antigenicity of the type B capsular polysaccharide leaded to focusing the development of effective vaccine for prevention of meningococcal disease caused by this serogroup on the study of alternative antigens, in particular outer membrane proteins. In previous works[1], we detected a highly immunogenic 33 KDa protein that belongs to a family of macrophage infectivity potentiators (MIPs). This family includes proteins with peptidyl-prolyl cis/trans isomerase activity and seems to be involved in the initiation of infection of macrophages. The gene encoding this protein was cloned and expressed in *Escherichia coli* and the purified recombinant MIP (rMIP) was used to immunize mice both alone and with $Al(OH)_3$ and Freund´s adjuvants. Western-blotting experiments showed that all sera obtained recognized the protein not only in the homologous strain, but also in all other type B strains analyzed. Bactericidal antibodies were induced in all sera, even when immunizing with rMIP without adjuvant, with serum bactericidal activity (SBA) titers higher than 1/256 against the homologous strain. Our results suggest that this protein could be a very good candidate for the development of new, effective and universal vaccines against serogroup B.

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P 261

The vaccine potential of a putative *Neisseria meningitidis* cell binding factor and NADP specific glutamate dehydrogenase enzyme identified by immuno-proteomics

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Question: Immuno-proteomic analyses revealed that colonisation with meningococci induced serum antibodies with bactericidal activity that reacted specifically with novel meningococcal OM antigens (1). Colonisation correlated with an increase in antibody levels to two proteins, NMB0345 (putative cell-binding factor, CBP) and NMB1710 (glutamate dehydrogenase, NADP specific, GDH), which were chosen for further study. The objectives were to investigate the immunogenicity of these antigens, in particular the ability of recombinant proteins to induce serum bactericidal antibodies.
Methods: the genes encoding NMB0345 and NMB1710 from strain MC58 were cloned into *E.coli* and the recombinant proteins expressed. Mice and rabbits were immunised with the proteins delivered with liposomes, detergent micelles, adsorbed to $Al(OH)_3$ or in saline only. Sera were tested for reactivity against recombinant proteins and outer membranes (OM) in ELISA and by western blotting and immuno-fluorescence (IF). Pooled sera were also tested for their ability to induce complement-mediated bactericidal activity against MC58.
Results: Both rCBP and rGDH induced high levels of antibodies that reacted with homologous recombinant protein in ELISA. Significant reactivity to MC58 OM was observed for rGDH delivered in all adjuvant formulations, whereas for rCBP, reactivity was observed with antisera raised using $Al(OH)_3$ and detergent micelles, but not in liposomes or saline. Significantly, antibody titres to OM were consistently higher with rGDH than with rCBP ($p<0.05$).The specificity of the humoral immune response elicited against rCBP and rGDH was investigated by Western immunoblotting with homologous MC58 OM. All antisera elicited against rCBP showed strong reactivity with a band of *Mr*31kDa (native CBP) and all antisera raised to rGDH reacted with a band of *Mr* 49 kDa (native GDH). The ability of the antisera to recognize and bind to the native antigens on meningococci was studied by IF: antisera raised to rGDH showed medium (++) IF signals, whereas antisera to rCBP did not react signficantly with the bacteria (+/-). Complement-mediated bactericidal activity towards the homologous strain MC58 was shown by antisera raised to rGDH delivered in saline or in detergent micelles (SBA titre of 1/16) and antisera to rCBP delivered in saline only (SBA titre of 1/32).
Conclusions: both recombinant proteins were antigenic in a variety of different adjuvant formulae, inducing high levels of antibodies that reacted with homologous recombinant protein and native protein in OM. However, bactericidal activity of antisera to rCBP and rGDH was weak using the current immunisation regimen; the incorporation of more effective adjuvants is likely to increase the bactericidal effect. Based on the current findings, both antigens merit further study for their vaccine potential and biological function.
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Transformation machinery components in vaccine development

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Background and objectives: In the course of our transformation studies in meningococci (Mc), we hypothesize that antigens identified by their affinity to negatively charged DNA are likely to possess regions with positive electrostatic charge, and these regions are often also those that are predicted to be, or turn out to be, antigenic. We therefore have devised strategies to identify all DNA binding components present in Mc cellular fractions. In this procedure, Mc cellular fractions are subjected to a screen for biophysical DNA binding through multiple types of DNA binding procedures. By using this strategy, we have also identified a number of conserved surface-exposed Mc components, including the Mc proteins PilQ, PilW and ComL.

With the exception of Mc serogroup B, conjugated polysaccharide vaccines against the major serogroups, A, C, Y and W135 have been shown to be immunogenic and safe in all age groups, even infants. However, serogroup B is still a major cause of meningitis and fatal sepsis worldwide. For that reason, the search for a safe and effective omnivalent vaccine against serogroup B is still a challenge.

We have previously shown that the PilQ complex has an immunoprotective potential in a mouse infection model. In this study, we are determining the immunoreactive / immunoprotective potential of the PilQ, PilW and ComL protein using a unique longitudinal set of human host derived samples.

Methods and results: Well defined sera from Mc meningitis patients enlisted in a longitudinal study were tested by enzyme-linked immunosorbent assay (ELISA). The acute phase and convalescence sera were tested, using recombinant as well as natively produced antigens, for antibodies active against the three outer membrane proteins.

All Mc patient sera tested so far showed over the analysis period significantly increased titers against PilQ and PilW but only few sera had increased titers against ComL. For the PilQ molecule, most of the patient sera exhibited higher titers against the N-terminal than the C-terminal part of the subunit.

Conclusions: Both Mc PilQ and PilW, induced a significant immunologic response in the human host and may be promising candidates for contributing to omnivalent Mc vaccine development.

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Structure and function of RmpM from Neisseria meningitidis

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Outer membrane vesicles (OMV) are currently the only effective vaccine against Serogroup B Neisseria meningitidis. To increase yield and remove endotoxic LOS, OMV are often prepared using the mild detergent sodium deoxycholate. However, this treatment also removes loosely anchored surface proteins, increases cellular contaminants and elicits a different immune profile to native OMV. Through genetic manipulation, it is hoped to increase OMV yield whilst maintaining outer membrane protein complexes and epitopes in their native conformation.

Deletion of rmpM increases native OMV formation. RmpM is proposed to bind to peptidoglycan through a C-terminal domain and from a link to the outer membrane (OM), via its N-terminus. The structure and precise function of the N-terminus of RmpM have not been resolved. Analysis of high molecular weight complexes in OMV using High Resolution Clear Native gel electrophoresis (hrCN-PAGE) demonstrated (Marzoa et al. 2010) the association of RmpM with PorA/PorB oligomeric complexes. It is hypothesised that RmpM is anchored to OM via the proteins PorA and PorB stabilising outer membrane and the porin complex and potently influencing the conformation of surface epitopes.

This aim of this study was to resolve the structure of N-terminus of RmpM (RMn) and its interaction with PorA, PorB and the outer membrane. Recombinant rRMn and rRmpM were expressed in E. coli and purified. Two dimensional 1H/15N NMR spectra with 15N labelled rRMn showed that the protein is unstructured in solution. Tryptophan fluorescence quenching and Size Exclusion Chromatography failed to identify any binding of rRMn or rRmpM to recombinant PorA and PorB. Further, rRmpM failed to interact with both recombinant PorA and PorB when the latter had been reconstituted into phospholipid vesicles. However, addition of rRmpM to PorA/PorB complexes from an rmpMΔ strain resulted in an increase in the size of the complex comparable to that of the WT strain, indicating rRmpN retains binding function. A mutant N. meningitidis strain expressing only RMn strain showed similar a growth rate to the wild type strain, but had a three-fold increase in OMV yield. We conclude that the interaction of RmpM with the OM appears to be highly dependent on protein and lipid environment, and may also require additional components which have yet to be identified.

Reference:

Marzoa J, Sánchez S, Ferreirós CM, Criado MT. Identification of Neisseria meningitidis outer membrane vesicle complexes using 2-D high resolution clearnative/SDS-PAGE. J Proteome Res 2010;9:611-9.

P 264

Modifying PorA to elicit a cross-reactive immune response

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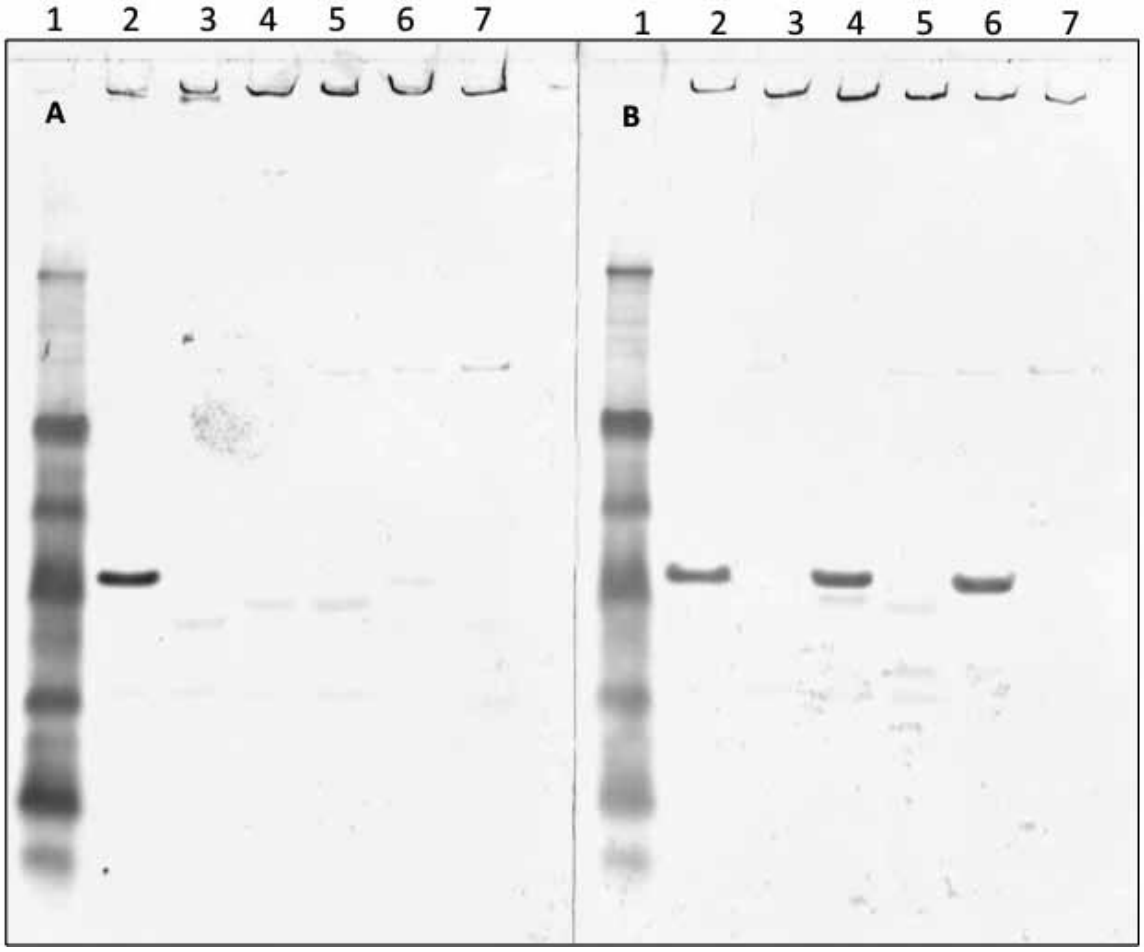
Background and Aim: *Neisseria meningitidis* is a bacterial pathogen that causes meningitis and septicemia. In a few per 100,000 of population, primarily infants and late teens, the organism causes invasive disease. Invasive disease is rapidly progressing, life threatening if untreated, and difficult to diagnose at early stages. The only effective public health response is vaccination. PorA is a major outer membrane protein in *N. meningitidis*. Currently registered vaccines, including the vaccine recently used by the New Zealand government (MeNZB) to eradicate a highly virulent clonal complex 41/44 strain of this pathogen, use PorA as a significant antigen generating the bactericidal immune response that protects against invasive disease. The regions of the PorA that generate this immune response vary from strain to strain, and define distinct serosubtypes. Vaccination with a particular serosubtype vaccine is thought to provide limited protection against strains with heterologous PorA. We have modified the PorA antigen, aiming to remove the serosubtype-specific immune response and enhance a cross-reactive immune response, opening the door to reconsideration of PorA as a cross protective vaccine antigen.
Question: Can modification of PorA be convert it from an antigen eliciting a strain-specific, to a cross-reactive response?
Methods: The gene encoding PorA was amplified, cloned, and modified by PCR and restriction digest methods to remove variable loops and replace with conserved loops. Animals were immunized with OMVs of strains expressing recombinant PorA and the antibody responses analysed against strains expressing different PorA serosubtypes.
Results: Antibodies raised against OMV with recombinant PorA recognized PorA from multiple strains, whereas wild-type (P1.7,16-2)-containing OMV elicited antibodies only recognizing autologous PorA.
Conclusion: PorA can be modified to elicit antibodies recognising heterologous PorA.

Figure legend:
Total cellular protein separated on 8-12% Bis-tris acrylamide gel prior to Western immunoblot and probing with pooled sera from 10 mice vaccinated with P1.7,16-2 containing OMV (Panel A) or with loop-replaced recombinant PorA (Panel B). Lanes: **1**) NEB protein marker: **2**) MC58 (P1.7,16-2): **3**) MC58ΔPorA **4**) BZ232 (P1.5-2,2-2): **5**) BZ232ΔPorA: **6**) 400 (P1.19,15): 7) 400ΔPorA.

figure 1



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The functional role of the meningococcal vaccine component GNA2091

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Backround: GNA2091 (Genome-derived Neisseria Antigen 2091) is an accessory protein present in the four component meningococcal serogroup B vaccine, 4CMenB. GNA2091 is fused to the antigen fHbp, and as such increases its immunogenicity. The function of GNA2091 has been poorly characterized to date due to its limited homology to known proteins.
Results: Deletion of the *nmb2091* gene resulted in a growth phenotype in Mueller Hinton (MH) media. Microarray and quantitative real-time PCR analysis of global gene expression in the wild type and deletion mutant (*Δnmb2091*) strains revealed an induction of the zinc regulon in the mutant strain, indicating a low internal zinc concentration. The decreased growth of the mutant strain in MH is restored by addition of glucose or zinc to the media. This suggests a direct or indirect role for GNA2091 in Zn-uptake in meningococcus. Furthermore the *Δnmb2091* mutant is sensitive to detergent stresses, suggesting the integrity of the envelope is compromised in the absence of the GNA2091 lipoprotein. GNA2091 is also important for meningococcal survival *in vivo*, with the *Δnmb2091* strain having decreased survival with respect to the wild type strain during a competitive index assay in the infant rat model of meningococcal infection.
Analysis of the *nmb2091* locus revealed that *nmb2091* is co-transcribed with *nmb2089* and *nmb2090* and that this operon is conserved in several Gram-negative bacteria, including the *yraNOP* operon in *Escherichia coli*. GNA2091 has 31% identity to the YraP lipoprotein, which in *E. coli* is thought to play a role in the periplasmic folding pathway of outer membrane protein (OMP) biogenesis.
Conclousions: Based on these data we propose that GNA2091 is involved in the homeostasis of the outer membrane, and that in the absence of GNA2091 the function of specific OM proteins may be compromised, such as ZnuD that is involved in high affinity Zn-uptake. As such, during growth under Zn-limitation in MH media, the inefficient uptake of Zn may result in reduced internal zinc concentrations, leading to the induction of the zinc regulon and the growth phenotype observed. We are currently investigating how the OM may be altered between the wild type and *Δnmb2091* strains. This insight into the function of GNA2091 is of interest due to its role in the physiology of *Neisseria meningitidis* and also due to its inclusion in the 4CMenB vaccine.

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Transcriptional Regulation of the *nadA* gene impacts on the prediction of coverage of the 4CMenB vaccine.

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Background: The NadA adhesin is one of the major components of the 4CMenB, a novel vaccine against meningococcus B. Its gene expression is finely regulated by both phase variation and the NadR repressor. Physiologically relevant small molecules such as 4-hydroxyphenylacetic acid (4HPA), secreted in human saliva, and 3-Cl-4-hydroxyphenylacetic acid (3Cl-4HPA), produced during inflammation, as well as human saliva itself, are able to induce *nadA* expression by alleviating the NadR-dependent repression. Recently, the regulon of NadR has been elucidated depicting it as a global regulator important for adaptation of meningococcus in response to host niche-specific signals.

Results: A new meningococcal antigen typing system (MATS), which correlates with killing in the serum bactericidal antibody assay (hSBA), is used to define the antigenicity and quantity of NadA on circulating strains and thus to predict the contribution of NadA to vaccine coverage. However, under the *in vitro* growth conditions used for MATS and hSBA, *nadA* is repressed by NadR. Interestingly, analysis of the infecting isolates from convalescent children whose sera were shown to recognize the NadA antigen, revealed strains with NadA MATS values too low to support bactericidal killing, suggesting that during infection NadA expression may be different from that observed *in vitro*.

In a strain panel covering a range of NadA levels, repression was relieved through deleting *nadR*. All *nadR*$^-$ strains expressed high levels of NadA and were efficiently killed by sera from subjects of different ages immunized with 4CMenB. A selected strain, namely NGP165, mismatched for other vaccine antigens, has a low NadA MATS value and is not killed by sera from immunized infants. However, in an *in vivo* passive protection model the same sera effectively protected infant rats from bacteraemia of NGP165. Furthermore, incubation of NGP165 with HPA molecules or saliva induces expression of NadA *in vitro* leading to high MATS values and positive titers in hSBA.

Conclusions: Our results suggest that *in vivo*, NadR repression is alleviated due to niche-specific signals, resulting in high levels of NadA expression from any *nadA*$^+$strain and therefore efficient killing by anti-NadA antibodies.

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Retrospective analysis of Meningococcal Antigen Typing System (MATS) on serogroup B meningococcal isolates in Greece

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Background and aims: In Greece, Neisseria meningitides serogroup B currently accounts for approximately 90% of IMD in Greece. An investigational multicomponent meningococcal serogroup B vaccine named 4CMenB contains three recombinant proteins, factor H binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA) and Neisserial adhesin A (NadA) formulated with outer membrane vesicles from the New Zealand epidemic strain NZ98/254. The aim of the study was to investigate the potential strain coverage of 4CMenB by the use of the meningococcal antigen typing system (MATS). **Material and methods:** A total of 148 serogroup B strains isolated for the time period 1999-2009 were analyzed by MultiLocus Sequence Typing (MLST) and characterised for 4CMenB antigen sequence diversity, and PorA genotypes. MATS assay was carried out for the three recombinant antigens. **Results:** Out of the 148 isolates tested by MATS, 68 (45,9%) belonged to ST-162 cc (which is the predominant lineage among the Greek serogroup B isolates), and 33 isolates (22,3%) belonged to clonal complex ST-269 . Also strains belonging to cc32 (n=16; 10.8%), ST-41/44 (n=16; 10.8%) were tested. The rest of strains (n=16) belonged to other clonal complexes. The global coverage, predicted by MATS, by at least one antigen (fHbp, NHBA, NadA or PorA) was 89,2% ((64,2% - 98,6%)CI0.95). The predicted coverage among the different clonal complexes was 86.7 % for the ST-162cc, 93,7% for the ST-41/44cc, 97% for the ST-269cc and 100% for the ST-32cc strains. The contribution of each antigen to coverage was variable among the different clonal complexes. NHBA showed the highest positive results (n=116; 78.4), followed by fHbp (n=78; 52,7), then PorA (n=12; 8.1%) and NadA (n=1; 0.7%). The percentage of fhbp contribution among the clonal complexes wide-ranged from 35.3% (ST-162cc) to 100% (ST-32cc). In contrast, the overall contribution of NHBA was higher, presenting coverage values ranging from 68.8% (ST-32cc) to 97% (ST-269cc). **Conclusions:** The estimate of coverage obtained in this study suggests that the 4CMenB vaccine is able to cover most of the circulating MenB strains in Greece belonging to different lineages. Estimate of coverage of MenB isolates from Greece showed similar coverage level as those initially tested from five European countries (England, France, Italy, Germany and Norway).

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Train Coverage of a Meningococcal multicomponent (4CMenB) Vaccine in Spain

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A multicomponent vaccine, 4CMenB, has been developed to prevent invasive disease caused by group B meningococci (MenB), being the four major components: factor H binding protein (fHbp), Neisseria Heparin Binding Antigen (NHBA), Neisserial Adhesin A (NadA), and outer membrane vesicles (OMV) derived from the strain NZ98/254. Recently the estimation of strain coverage of 4CMenB vaccine in five European countries (Euro-5) has proved that 78% of all MenB strains (95% CI 66% to 91%) will be potentially covered. In the current study, a panel of 300 invasive MenB strains isolated between December 2008 to May 2010 representing 50% of the confirmed group B cases inSpainduring that period. Meningococcal Antigen Typing System (MATS) was used to predict the potential for serum bactericidal antibody activity. Strains that meet a minimum threshold of reactivity to fHbp, NadA or NHBA in the MATS ELISA or that express the PorA P1.4 are expected to be covered by 4CMenB vaccine. The strain coverage estimated was 68.7% (95% CI 48.0% - 85.3%). Differences compared with the Euro-5 include distribution of clonal complexes in Spain, particularly with a lower prevalence of the cc41/44 complex (7% inSpainversus 37% in Euro-5) and a higher proportion of cc213 (17% versus 7%) (cc41/44 has been associated with high relative potencies (RPs) towards fHbp and NHBA, whereas cc213 strains are frequently showing RPs under the positive bactericidal threshold (PBT) for both antigens). In addition, a lower proportion of analyzed isolates with the PorA VR2 type 4 (8% inSpainversus 20% in Euro-5) was also observed. While there was a lower proportion of fHbp variant 1 (50% versus 69% in Euro-5 panel), the proportion of fHbp genotype 1.1 (subvariant included in the vaccine) was much higher for thespian panel (40%) compared with 20% in the Euro-5 panel. Such homology could result in protection in early age groups, especially infants under one year of age. Overall, analysis of recent Spanish isolates associated with clinical cases of invasive meningococcal disease predict high coverage of 4CMenB of close to 70%. The meaning of the differences observed is being investigated, including coverage by age groups and Spanish regions. The observed data suggest that country-by country investigations of strain coverage and of clonal complexes distribution are important, particularly in countries with high number of isolates.

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Use of the Meningococcal Antigen Typing System (MATS) to Assess the Australian Meningococcal Strain Coverage with a Multicomponent Serogroup B Vaccine

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Objective: A multicomponent vaccine (4CMenB) under development by Novartis is proposed for the prevention of invasive serogroup B (MenB) meningococcal disease. The vaccine is composed of three protein antigens (fHbp, NHBA and NadA) and Outer Membrane Vesicles (OMVs) of a New Zealand MenB strain.
Methods: The Meningococcal Antigen Typing System (MATS) was used to evaluate strain coverage by 4CMenB. MATS predicts the potential for bactericidal activity of sera from immunized 13-month-olds, based on the quantity of and cross-reactivity with, vaccine-induced immune responses to the four vaccine components. Men B Clinical Isolates collected over five years were included in the study to assess local strain diversity and public health impact. Preliminary data for MenB isolates collected from January 2007 to June 2011 by five National Neisseria Network reference laboratories were initially analyzed by MATS. Men B clinical isolates subjected to MATS also have been analysed by multi-locus sequence typing (MLST) to further assess the Australian meningococcal epidemiology. MATS testing of Australian non-serogroup B meningococcal isolates have also commenced and will be complete by June 2012.
Results: A total of 373 Australian meningococcal isolates were tested by MATS. Based on these results, the overall estimate of meningococcal strain coverage is 76% (95%CI: [63, 87]). As MATS does not account for the activity of bactericidal antibodies generated from non-PorA components of OMVs or the synergistic effects of the multiple components of 4CMenB, this is considered to be a conservative estimate of strain coverage. To date, 238 isolates have also been analysed by MLST. A total of 83 different MLST types were observed with five MLST types accounting for more than half of the isolates; MLST types 32 (n=49 isolates), 154 (n=36), 740 (n=13), 42 (n=12), 213 (n=9) and 479 (n= 8).
Conclusions: The results of this large series of Australian Meningococcal B isolates show that the 4CMenB has the potential to protect against a majority of the MenB strains that have recently caused invasive disease in the country over the last five years. It is anticipated that with completion of the MATS testing of Australian non-serogroup B meningococcal isolates that a more accurate picture of the true impact of the 4CMenB vaccine will be obtained. A more detailed knowledge of the epidemiology of invasive Meningococcal B disease in Australia is now also possible.

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Meningococcal antigen typing system (MATS) based coverage prediction for the 4CMenB vaccine in United States

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Background: A candidateNeisseria meningitidis serogroup B (MenB) protein vaccine (4CMenB, Novartis) contains 4 major components: an outer membrane vesicle (OMV) and 3 proteins selected by genome screening; factor H binding protein (fhbp v1.1), Neisserial adhesin A (NadA) and Neisserial heparin binding antigen (NHBA). MATS analysis is used to predict vaccine coverage.
Methods: MATS, an ELISA based meningococcal antigen typing system, is used to quantify surface-exposed 4CMenB proteins (phenotype) of MenB isolates. A panel of 442 MenB US clinical isolates (2000-2008) was selected from the Active Bacterial Core surveillance (ABCs, CDC, Atlanta). The sample set was down weighted with respect to outbreak strains isolated from Oregon. The amount of each protein expressed on standard strains, as measured by MATS, was used to determine the relative potency (RP) for test isolates. Positive bactericidal threshold (PBT), the RP cut-off that predicts the serum bactericidal activity for an antigen, was used to estimate vaccine coverage.
Results: Based on the serum bactericidal activity (SBA) and RP correlation, RP over 2.1%, 29.4% and 0.9% was set as the PBT for fhbp, NHBA and NadA, respectively. MATS predicted 91.2% (72.2% - 95.9%)coverage for 4CMenB in the panel of 442 MenB isolates. While 194 isolates (43.9%) had PBT values for one antigen, 183 isolates (41.4%) had PBT for 2 antigens. Only 5.9% (26/442) of isolates expressed all 3 vaccine antigens with PBT values above cut-off. MATS analysis suggests that NHBA is likely to confer 82.6% coverage (365/442) singly or in combination with other antigens followed by fhbp v1.1 (234; 52.9%).
Conclusion: MATS predicted high coverage of US MenB isolates for 4CMenB vaccine based on immune reactivity to vaccine components. MATS testing of a panel of non-MenB neisserial US isolates is in progress to understand the expanded coverage potential of 4CMenB vaccine.

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Characterisation of the 4CMenB vaccine components on strains isolated during a carrier survey in Italy, by MATS and Molecular Epidemiology analysis

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Introduction: 4CMenB is a multicomponent protein vaccine proposed for prevention of meningococcal disease. It is based on three main protein antigens (fHbp, NHBA and NadA) and the Outer Membrane Vesicles (OMVs) of the New Zealand MenB epidemic strain. We used Meningococcal Antigen Typing System (MATS) and MolEpi characterisation to evaluate presence, diversity and expression levels of the 4CMenB components in N. meningitidis isolates collected during the carriage survey described by Panatto et.al (poster SIE-12-130). This is the first MATS study performed on a carrier strain panel.
Methods - The relative level of expression and antigenic reactivity of fHbp, NadA and NHBA where measured by MATS. PorA characterisation was performed by sequencing, which is part of the MATS assay [1]. Sequence diversity of vaccine components was assessed by automated sequencing [2].
Results: All tested isolates showed detectable expression levels of NHBA. As for fHbp, all strains but one had fHbp-2 subvariants. The only strain harbouring a fHbp-1 subvariant yielded an undetectable MATS result. NadA coding gene has been detected in one isolate and its expression was confirmed by MATS. All results corresponding to isolates from multiple swabs from the same individual were used for tracing each strain carriage persistency. MolEpi and MATS data confirmed that in all cases of multiple positive swabs, the colonisation was due to the same respective strains.
Discussion and Conclusions: MATS and MolEpi profiles were assessed in all strains isolated during a carrier survey in Italy in order to verify both presence and expression of 4CMenB components. All data from the two approaches (phenotypic and genotypic) were consistent to each other. The presence of an identical profile was used for tracing all strains spreading in this present cohort, demonstrating this approach could be useful for other similar future studies.
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MATS based coverage prediction for the 4CMenB Vaccine on *Neisseria meningitidis* B (MenB) Brazilian invasive strains

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A serogroup B multicomponent vaccine candidate, 4CMenB, has four immunogenic components: factor H binding protein (fHbp), Neisserial Adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA) and PorA serosubtype P1.4 as part of outer membrane vesicles derived from the strain NZ98/254.
The Meningococcal Antigen Typing System (MATS), was used to evaluate strain coverage by 4CMenB. MATS predicts the potential for bactericidal activity of sera from immunized 13-month-olds based on quantity and cross-reactivity with vaccine-induced immune responses to the three protein antigens and the genotype of PorA.
We have performed MATS in a collection of 99 MenB invasive strains isolated in 2010 in Brazil which accounts for approximately 53% of the MenB cases. On the basis of MATS, 80.8% (80/99) were positive for one or more of the four major antigens: 21.2% (21/99) were NHBA positive, 7.0% (7/99) fHbp positive, 1.0% (1/99) porA positive, 42.4% (42/99) were positive for both fHbp and NHBA, 3.0% (3/99) fHbp and NadA positive, 1.0% (1/99) porA and NadA, and 5.1% (5/99) were positive for fHbp, NHBA and NadA. A percentage of 19.2% (19/99) did not match the vaccine antigens.
In this study the strain coverage predicted by MATS was 80.8% [CL95%:70.7% - 94.9%]. In conclusion, 4CMenB has the potential to protect against a significant proportion of the invasive MenB strains recently isolated in Brazil.

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Potential coverage of the 4CMenB vaccine in non-B meningococci

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The multi-component vaccine against group B meningococci (4CMenB) comprises three major recombinant antigens including Neisserial adhesin A (NadA), factor H binding protein (fHbp), and Neisserial Heparin Binding Antigen (NHBA) as well as outer-membrane vesicles of a New Zealand MenB strain inducing antibodies against PorA P1.4. For 1052 invasive MenB strains isolated in England and Wales, France, Germany, Norway, and Italy 2007/2008 the strain coverage of 4CMenB which predicts the proportion of strains that would be killed by post-vaccination sera was estimated by the application of the recently published Meningococcal Antigen Typing System (MATS, Donnellyet al. 2010) to be 78% of all MenB strains (95% confidence interval [CI] 66-91%) (Manuscript in preparation).

The purpose of this study was to estimate the effect of 4CMenB on meningococci expressing capsular groups other than B (non-B). Two hundred twenty-seven representative non-B isolates from three countries (England and Wales (n=78; 34.4%), France (n=56; 24.7%) and Germany (n=93; 41.0%)), mostly belonging to groups C (n=130; 57.3%), W-135 (n=36; 15.9%) and Y (n=50; 22.0%), were analysed for antigen expression by MATS and antigen conservation by DNA sequencing.

The correlation analysis of MATS and hSBA (Serum Bactericidal Assay using human complement) was conducted using MenB strains and has not yet been performed for non-B strains. For this reason, hSBA data for non-B strains will be needed to confirm whether the positive bactericidal thresholds (PBTs) derived for MenB strains will apply also to non-B strains. Nonetheless, the overall predicted strain coverage of 4CMenB among non-B meningococcal isolates was 68% (95% CI 44-79%) obtained applying PBTs established for MenB. The capsular group-specific strain coverage was 80% (95% CI 58-88%) for group C, 83% (95% CI 44-94%) for group W-135, and 22% (95% CI 12-38%) for group Y. The contribution of each antigen to the coverage was 20.3% for fHbp, 43.2% for NHBA, 26.4% for NadA and 0.4% for PorA. MATS data for fHbp correlated with sequence data, i.e. fHbp variant 1 is present in 28% of the non-B isolates. The NHBA peptide contained within 4CMenB was not found in any of the non-B isolates which indicates that the most prevalent NHBA peptides identified in non-B, i.e. peptides 20 and 29, cross-react with the anti-NHBA MATS antibody.

The study is limited by the fact that PBTs need to be formally established for non-B strains. Nevertheless, the results are a first indication that 4CMenB might provide added value with regards to the prevention of MenC and MenW135 disease. The effects are of interest for countries without MenC vaccine recommendation or those without an effective adolescent vaccination programme. Moreover, cost-effectiveness models for 4CMenB should include the impact of the vaccine on all meningococci rather than only on MenB.

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Strain coverage of a multicomponent meningococcal vaccine (4cMenB) in Western Europe

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A novel multicomponent vaccine against invasive disease caused by meningococcal group B (MenB), 4CMenB, contains four major components: factor H binding protein (fHbp),Neisseriaheparin binding antigen (NHBA), Neisserial adhesin A (NadA), and outer membrane vesicles derived from the strain NZ98/254 (PorA P1.4). The potential coverage of 4CMenB is unknown given the diversity of MenB strains.

The strain coverage in a defined target population of MenB invasive disease isolates collected in five European countries was estimated by sequencing genes encoding fHbp, NHBA and NadA by using the Meningococcal Antigen Typing System (MATS, Donnelly Jet al.2010), which provides an estimate of killing by bactericidal antibody activity by immune sera. MATS was performed at each national meningococcal reference laboratory following an interlaboratory standardisation study. MATS also takes into account the presence of the PorA antigenic variant P1.4, the immunogenic component of outer membrane vesicles.

We analysed 1052 MenB strains representing recent isolates (2007-2008) obtained from England and Wales, France, Germany, Italy and Norway which accounts for approximately two-thirds of the MenB cases reported in Europe in 2007/8 to assess predicted coverage of 4CMenB. Although some differences were observed between countries, overall results provided substantial evidence that 4CMenB would cover the majority of circulating strains in Europe and each individual country, based MATS data. All 1052 MenB strains contained a gene encoding at least one major 4CMenB antigen. MATS predicted that 78% (73% to 87% in individual country panels) of all MenB strains (95% CI 66% to 91%); would be killed by postvaccination sera. Half of all strains (and 64% of covered strains) could be targeted by bactericidal antibodies against two or more 4CMenB antigens. MATS data were also linked to antigen sequence data and Multilocus Sequence Typing (MLST). There was some association of MATS results with fHbp genotypes but genetic typing alone was not sufficient to predict MATS results, suggesting that monitoring of antigen expression rather than solely sequence typing is needed in the future.

In conclusion, 4CMenB has the potential to protect against a significant proportion of the invasive MenB strains recently isolated in Western Europe.

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Strain coverage of a multi-component meningococcus B vaccine in the Czech Republic and in Germany

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The multi-component vaccine 4CMenB has been developed to target the antigenically variable population of serogroup B meningococci. The immune-dominant components of the vaccine are: FHbp (factor H binding protein), NHBA (Neisserial heparin binding antigen), NadA (Neisserial adhesion A), and outer membrane vesicles. Aim of the study was to assess the strain coverage of 4CMenB in serogroup B meningococci of the Czech Republic and to compare the data with those from German presented at the EMGM meeting 2011.

Relative potencies of FHbp, NHBA, and NadA were assessed by the MATS ELISA (Donnellyet al. PNAS 2010) as well as antigen sequence typing of the variable region 2 of porin A (PorA). Multi-locus sequence typing was performed on 222 German serogroup B strains isolated 2007/2008 and 108 Czech serogroup B strains isolated 2007-2010.

The strain populations differed with regard to the ST-41/44 complex, which was more prevalent in Germany, and the ST-18 and ST-35 complexes which were more prevalent in the Czech Republic. Relative potencies above the positive bactericidal threshold were observed for FHbp in 69% of the German and 67% of the Czech isolates, for NHBA in 63% of the German and 42% of the Czech isolates, and for NadA in 3% of the German and 4% of the Czech isolates. Antigen sequence typing revealed the presence of PorA P1.4 in 21% of the German and 1% of the Czech isolates. In total, the strain coverage of 4CMenB was estimated to be 81% (95% confidence interval 71-93%) for the German and 74% (95% CI 59-87%) for the Czech isolates.

In conclusion, three quarters of the Czech MenB isolates were shown to be potentially covered by 4CMenB. There was no significant difference of strain coverage between both countries. The slightly lower coverage in the Czech Republic may be related to the low prevalence of the ST-41/44 complex.

P 276

Meningococcal Antigen Typing System (MATS) was a conservative estimate of killing when confirmed in a serum bactericidal assay using human complement (hSBA)

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Background: The meningococcal antigen typing system (MATS) predicts the strain coverage of a multicomponent meningococcal serogroup B vaccine (4CMenB) based on quantity of and crossreactivity with vaccine-induced immune responses of factor H binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA), and Neisserial Adhesin A (NadA), and on the genotype of PorA. MATS estimated that 4CMenB would cover 78% (95%CI: 66-91%) of 1052 isolates collected in five European countries (73-87% by country) during a specified time period. We compared MATS predictions with actual hSBA results for an unbiased representative set of isolates selected from the previously presented panel.
Methods: All 528 serogroup B strains isolated between July 2007-June 2008 by the Health Protection Agency had been evaluated by MLST and MATS, and genotyped for fHbp, NadA, and NHBA. A panel of 40 isolates was selected using stratified proportional sampling to account for coverage distribution and epidemiologically relevant strains by controlling for MATS scores and relevant genetic characteristics: MLST type and genotype of vaccine antigens. The 40 strains were then tested in hSBA using pooled postvaccination sera from infants or adolescents. Results were compared with MATS data.
Results/Conclusion: The 40-strain panel selected for hSBA testing provided an unbiased sample that was proportionally representative of 98% of MATS phenotypes and >80% of MLST and vaccine antigen genotypes.
Based on MATS data, 73% (95%CI: 59-88%) of 528 strains of UK panel were predicted to be killed in the hSBA, while 70% (95%CI: 55-85%) of 40 selected strains were predicted to be killed in the hSBA. Experimental validation revealed that 88% (95%CI: 78-98%) and 88% (95%CI: 78-98%) of the selected strains were killed by pooled infant and adolescent sera respectively confirming that MATS is a conservative measure of killing in the hSBA.

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In vitro Surface Expression of Factor H Binding Proteins: Correlation of the Meningococcal Antigen Surface Expression (MeASURE) Assay with Western Immunoblot Analysis

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Background and Goals: Factor H binding protein (fHBP) or LP2086 is the primary target of two vaccines currently in development for the prevention of meningococcal meningitis caused by serogroup BNeisseria menigitidis (MnB). Polyclonal antisera recognizes fHBP on the surface of N. meningitidis and activates the classical complement pathway resulting in bacteriolysis. This process can be measured by anin vitroassay with human complement (hSBA). Measurable responses in hSBA are the accepted surrogate of protection against MnB . Flow cytometry is an accurate and reliable method for determiningin vitrosurface expression of bacterial antigens and to study cell surface receptors in humans and other species. While polyclonal antisera are useful reagents to identify surface expression of antigens of interest, monoclonal antibodies can be more consistent and reproducible reagents to identify individual antigens. The goal of our study was to investigate the relationship between total bacterial cell expression of fHBP and the amount of fHBP localized on the bacterial surface.
Methods: MnB strains expressing fHBP from both subfamilies were examined by flow cytometry and quantitative western immunoblot analysis using the monoclonal antibody MN86-994-11 and rabbit polyclonal antisera, respectively. Purified rLP2086 variants identical to those expressed by the bacteria were used to prepare standard curves to determine total cell expression of fHBP.
Results: Western immunoblots of total fHBP expression will be shown for several strains from both LP2086 subfamilies. The correlation of surface expression of fHBP determined by the MEASURE assay with total cell expression of fHBP will also be demonstrated and discussed.
Conclusion: Surface expression of fHBP measured by flow cytometry using the monoclonal antibody MN86-994-11 correlates with total fHBP expression determined by quantitative western immunoblot analysis. Therefore, bacterial surface expression of fHBP measured by flow cytometry accurately reflects fHBP expression by MnB strains expressing either subfamily of LP2086.

P 278

Clonal features of Czech serogroup B meningococci in prospect of a vaccination

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Background and aims: Serogroup B strains represent a major continuous cause of meningococcal disease in the Czech Republic in the current period. Detailed characterization of serogroup B isolates from invasive disease identifies clonal shape of strains in the host population supposed to be protected by a vaccine formulation being under pharmaceutical development.
Material and methods: All serogroup B isolates (n=360) referred to the NRL by clinical laboratories in 2001-2012 period were typed by MLST and *porA*, *fetA*, *penA* sequencing. In a hundred of recent isolates from 2009-2011 period, structure of FHbp (factor H binding protein), the component common to vaccine formulations being developed, was assessed.
Results and conclusions: Among sixteen clonal complexes representing 87% of the collection, cc41/44 was the most frequent (25% in the whole, 9% related to ST-41 subancestor only), followed by cc32 (22%), cc18 (15%), cc269 (13%), cc213 and cc35 (4% each). The *penA* features correlates with good penicillin G susceptibility of Czech isolates. P1.7-2,4 specificity related to OMV-PorA vaccine component was found in 2% of serogroup B isolates - in some of ST-41 subancestor-attributed strains of cc41/44. FHbp antigenic variant 1 (also reported as subfamily B), common to formulations of vaccines under development, harbored 64% of isolates screened, whether FHbp variants 2 or 3 (corresponding together to subfamily A) were represented by 31% or 5%, respectively. Overal assessment of NHBA and NadA experimental vaccine components shall complete the onsight on feasibility of vaccine formulation for a pespective eradication of spread of serogroup B strains in Central European region.
The work was supported in part by grant NT11424-4/10 of the Internal Agency of the Ministry of Health of the Czech Republic.

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The Relationship Between IgG Subclass Responses, Antibody Repertoire, and Serum Bactericidal Activity in Mice Immunized with Recombinant Factor H Binding Protein (fHbp) or Native Outer Membrane Vesicle (NOMV) Vaccines

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Background: Anti-fHbp antisera elicitedbyNOMV vaccines with over-expressed fHbp (NOMV-fHbp) have broader complement-mediated serum bactericidal activity than antibodies elicited by recombinant fHbp vaccines. The basis for this difference is not understood.
Methods: Mice were immunized with a recombinant fHbp ID 9 vaccine (rfHbp) in variant group 1, or a NOMV-fHbp ID 9 vaccine, or a control NOMV-ΔfHbp vaccine. We measured serum bactericidal responses (human complement) against a heterologous serogroup A strain (fHbp ID 5, variant group 1), IgG sub-class-specific anti-fHbp titers (ELISA), and the ability of the antisera to inhibit binding of factor H to fHbp, and binding of different anti-fHbp monoclonal antibodies (mAbs).
Results: The rfHbp vaccine elicited 2-fold higher serum total IgG anti-fHbp titers than the NOMV-fHbp vaccine ($P<0.04$). While anti-fHbp antibodies elicited by either vaccine inhibited binding of fH to fHbp, only the antibodies elicited by the NOMV-fHbp vaccine had bactericidal activity against a heterologous strain (1/GMT, 156 vs. <10 in the rfHbp group). Antibodies elicited by the control NOMV-ΔfHbp vaccine lacked bactericidal activity. In the rfHbp vaccine group, the IgG1 subclass predominated the IgG anti-fHbp antibody responses, and inhibition of binding of anti-fHbp mAb502 to fHbp was greater than in the NOMV-fHbp group ($P<0.05$). In the NOMV-fHbp vaccine group, IgG2a and IgG2b subclasses predominated, and inhibition of binding of anti-fHbp mAb JAR 5 was greater than in the rfHbp group ($P<0.05$). In mice immunized with the NOMV-fHbp vaccine, total IgG and IgG2a anti-fHbp titers, the ability of the antibodies to inhibit binding of mAb JAR 5 (Fig 1), and inhibit fH binding (Fig 2) all correlated with serum bactericidal titers ($r>0.6$; $P<0.05$). A multivariable model incorporating all four variables was highly predictive of individual bactericidal titers in the NOMV-fHbp group ($r^2=0.8$, $P<0.01$).
Conclusions: Anti-fHbp antibodies elicited by the rfHbp vaccine lacked cross-bactericidal activity, while sera from mice immunized with the NOMV-fHbp vaccine were bactericidal. The anti-fHbp antibodies induced by the NOMV-fHbp vaccine had different functional profiles than the rfHbp vaccine including better inhibition of mAb JAR 5 binding, less inhibition of mAb502, higher IgG2a and lower IgG1 sub-classes. Profiling anti-NOMV-fHbp antibody responses merits further investigation for studying human immune response to fHbp-based meningococcal vaccines.

figure 1

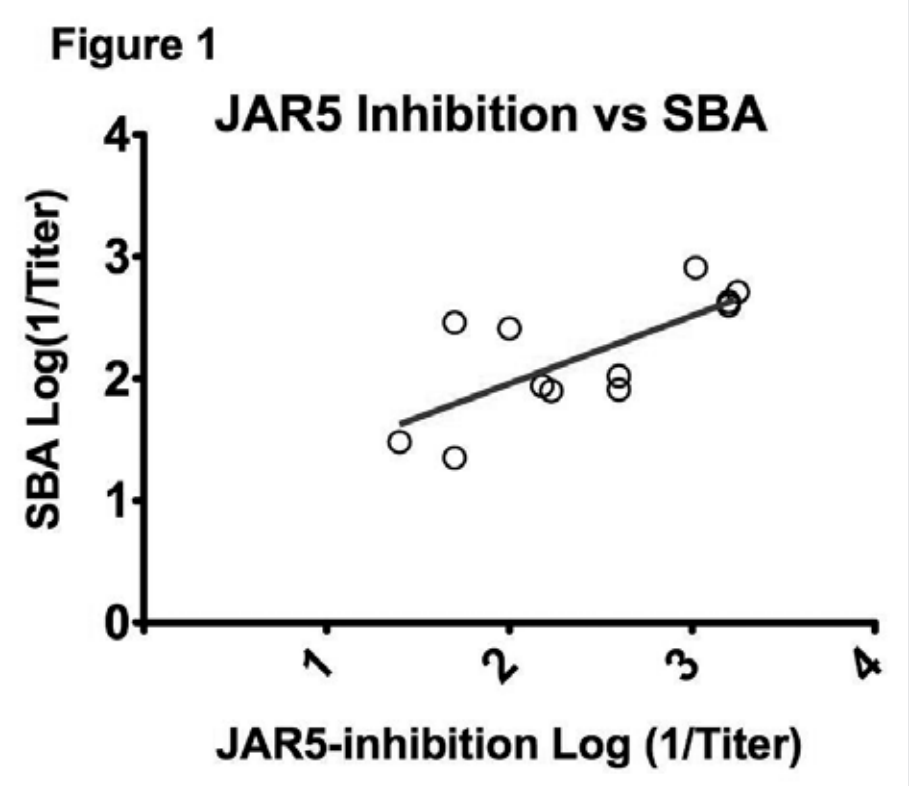

figure 2

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Mapping of epitopes recognized by monoclonal antibodies against Factor H binding protein (fHbp)

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Background: Factor H binding protein (fHbp) is a 28 kDa surface lipoprotein expressed by the vast majority of *Neisseria meningitidis* strains. fHbp is a good target for bactericidal antibodies and one of the most important candidates for the development of protein-based vaccines against serogroup B N. meningitidis. Recently, it was discovered that this protein is able to bind human fH, a negative regulator of the complement cascade, thus allowing the survival and proliferation of bacteria in the bloodstream. The three-dimensional structure of the fHbp:fH complex has been solved showing that both lobes of fHbp are involved in the interaction. Primary sequence of fHbp is variable among strains and three different variants were identified, sharing very limited cross-protection, but all able to bind fH to very similar extent.

Methods & Results: In order to map regions of the fHbp that are important for the generation of variant-specific or broadly protective antibody response, we used different variants of fHbp to immunize mice and generate monoclonal antibodies (mAbs). A series of mAbs belonging to different isotypes and with different fHbp variant specificities were generated, including some that were broadly reactive on all variants. The mAbs were tested for their ability to inhibit binding of fH to fHbp as well as in the serum bactericidal assay (SBA), either alone or in combination. Finally, mapping of the fHbp regions targeted by each of the mAbs was achieved using an array of different experimental methodologies, including mutagenesis, the standard PepScan analysis, the ELISA-based GyroLab assay, the Phage Display technique and the more sophisticated Hydrogen-Deuterium Exchange method.

Conclusions: The results indicate that the protective epitopes are generally conformational and are located both within the N-and C-terminal region of fHbp. Furthermore, we confirm that a single experimental approach is not sufficient to reliably map the position of epitopes on a folded molecule.

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Mapping of epitopes recognized by synergic pairs of monoclonal antibodies directed against the factor H binding protein of Neisseria meningitidis

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The factor H binding protein (fHbp) is a lipoprotein of Neisseria meningitidis that plays a major role for the bacterium survival in human plasma, preventing the complement-mediated bacteriolysis. Antibodies against fHbp are known to induce a robust protective immunity, and for this reason this protein has been included into the Bexero vaccine against meningococcus B. In this study, we map on the three-dimensional structure of fHbp the epitopes recognized by Mab502, JAR4 and JAR5, three monoclonal antibodies that cooperate in inducing an effective complement-mediated bacterium killing. For example, the concentrations of human IgG1 chimeric JAR5 or mAb502 required for 50% killing of group B strain H44/76 with human complement were 9 and >50 μg/ml. A 1:1 mixture of the two mAbs required <0.2 μg/ml. Our three-dimensional structural analysis of fHbp the epitopes shows that the epitopes recognized by cooperative antibody pairs are localized in well defined and distinct regions of the fHbp surface. The mapping at atomic level of such synergically bactericidal epitopes provides the structural basis to understand how binding to non-overlapping epitopes can confer to pairs of monoclonal antibodies the ability to engage C1q and trigger the classical complement pathway bactericidal response.

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Diversity of noncapsular antigens among *Neisseria meningitis* isolates of serogroups C, W_{135} and Y in Brazil – implications for vaccine prevention

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Background: FHbp, NadA and NhbA are novel meningococcal vaccine candidates. No data regarding noncapsular antigens in non-serogroup B *N. meningitidis* isolates from Brazil have been published. The aim of this study was to determine the diversity of the vaccine antigens in invasive *N. meningitidis* isolates of serogroups C, W_{135} and Y.

Methods: Invasive meningococcal isolates collected from 1988 to 2009 from patients in Rio de Janeiro State underwent serogrouping. Molecular characterization was performed by multilocus sequence typing (MLST). The noncapsular antigen genes *fHbp,nadA and nhbA* were amplified and sequenced.

Results: A total of 160 (118 C, 32 W135 and 10 Y) isolates were studied. All 160 isolates harbored *fHbp (*A/V2-3 or B/V1*)*, 99% (138/139) harbored *nhbA*, while 39% (54/139) harbored *nadA* (major variants 1, 2 or 3). Of the C isolates, 58% (67/118) were *fHbp* A/V2-3 and 35% (35/99) were *nadA* positive. Of W_{135} isolates, 88% (28/32) were *fHbp* A/V2-3 and 56% (18/32) were *nadA* positive. Of Y isolates, 70% (7/10) were *fHbp* A/V2-3 and 13% (1/8) were *nadA* positive. Interestingly, a disrupted *nhbA* gene (IS*1301* insertion) was found in a serogroup C (cc103) isolate, which was *fHbp* A/V2 and nadA negative. Disrupted *nadA* genes were commonly found in serogroup C (cc11 and cc32) isolates.

Conclusions: *FHbp* was universally detected, *nhbA* was frequently detected in and *nadA* was frequently absent from serogroups C, W_{135} and Y Brazilian meningococcal disease isolates. Although most isolates potentially contain at least two candidate antigens, the findings of disrupted genes are of concern and should be monitored.

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Design and evaluation of meningococcal vaccines through structure-based modification of host and pathogen molecules

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Factor H binding protein (fHbp) is a key antigen that elicits protective immunity against the meningococcus, and is currently being assessed in several vaccine trials. However, fHbp recruits the host complement repressor factor H (fH), and this may affect immune responses. Our aim was to generate non-functional fHbps as vaccine candidate and define the key interactions between fHbp and fH by alanine substitution of amino acids in the interface of fHbp from three variant groups, V1, V2, and V3. Our results showed that the distribution of important amino-acids for the interaction with fH differ significantly between the three fHbp variant groups, although some amino acids were required for high affinity interactions with fH in proteins from all groups. Furthermore, several mutants with increased binding to fH were identified as well.

To provide insights into the differences in the interaction between fHbp and fH, we are undertaking structure:function studies of fHbp V2 and V3 in complex with fH, and comparing the results with the V1:fH structure.

Non-functional fHbps were further characterized and their immunogenicity assessed in transgenic mice, expressing a chimeric fH that preserves the precise complement regulatory function of this molecule while binding fHbp. These findings provide the basis for the rational design of next generation meningococcal vaccines containing non-functional fHbps.

P 284

Biophysical Characterization of LP2086 Subfamily A Factor H Binding Protein and Its Interactions with Human Factor H

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Background and Objectives: The factor H binding protein (fHBP or LP2086) is a 28 kDa outer membrane lipoprotein of N. meningitidis, which has been identified as a vaccine candidate for the prevention of meningococcal disease. The fHBP protein family can be divided into two subfamilies, A and B. Since LP2086 elicits a largely subfamily specific bactericidal response, a bivalent vaccine will offer broad protection against serogroup B disease. LP2086 is utilized by meningococci to bind human Factor H (hFH), a key regulator of the alternative complement pathway that prevents autologous complement attack. Recruitment of hFH to the bacterium surface is important for the survival of the bacteria as it encounters the innate immune system of the human host. In our previous work on the LP2086 B subfamily, the solution structure was determined by NMR[1]. This protein structure was characterized as a novel fold, composed of a "taco shaped" N-domain and a "cannoli shaped" C-domain. Although the LP2086 A subfamily shares 60% sequence homology with the B subfamily, the subfamily A proteins appear somewhat less highly ordered. Also, it is interesting that the majority of the residues involved in contact points between hFH and the B subfamily member with which it was crystalized are not conserved in subfamily A proteins. The main goal of our study was to determine the structural features of the subfamily A variant A05, using NMR spectroscopy and to understand how LP2086 from this subfamily interacts with hFH relative to the counterpart from subfamily B.

Methods: The structural features of an rP2086 subfamily A member were determined using NMR. The interaction of rP2086 with hFH were studied using both full length hFH and the rhFH6-7 protein fragment. NMR, SPR, titration calorimetry, and analytical ultracentrifugation were utilized to study the interactions of these proteins.

Results and Conclusions: Structural elements of LP2086 subfamily A will be presented and compared to the B subfamily structure. Additionally, the effects of hFH binding on the structure of fHBP subfamily A and the relative affinities of hFH will be discussed.

[1] A. Mascioni, et. al., JBC, 284, 13, 8738 (2009)

P 285

Meningococcal Strains Engineered to Express Divergent Factor H Binding Protein Variants with Similar Expression Levels

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Background: Factor H binding protein (fHbp) is a part of two vaccines in late stage clinical development for prevention of meningococcal group B disease. Based on amino acid sequence diversity, three variant groups (1, 2 and 3) have been described. While susceptibility of a strain to anti-fHbp bactericidal activity is variant-group specific, the extent of cross-reactive bactericidal activity among isolates within a variant group is unknown. The sequence of fHbp and the level of expression in the selected test strains are predicted to affect the extent of cross-protection. The aim of the present study was to generate an isogenic panel of strains with different fHbp sequences, but similar levels of fHbp expression, to test bactericidal cross-reactivity in a defined system.

Methods: We chose two fHbp sequence variants (ID 1 and 55) that are in clinical development and five other fHbps in variant group 1 that are prevalent in invasive isolates (ID 4, 9, 13, 14 and 15). The genes encoding these fHbp variants were amplified from the genomic DNA of the appropriate strains by PCR and were cloned into a pBluescript plasmid containing an erythromycin resistance cassette, a stabilized PorA promotor, and 5' and 3' sequences flanking fHbp for integration into the meningococcal chromosome. The resulting plasmids were confirmed by DNA sequencing.

Results: To generate an isogenic panel of strains expressing different fHbp variants, we transformed strain NZ98/254 with the seven plasmids and selected for erythromycin-resistant colonies. The recombinant fHbp encoded in the genomic DNA was confirmed by DNA sequencing. Expression of the recombinant fHbps in meningococci was tested by Western blotting with anti-fHbp mAbs that were specific for subsets of the fHbp sequences. Broadly cross-reactive mAbs confirmed that the levels of surface expression of the recombinant fHbps were comparable by flow cytometry. The strains tested thus far are susceptible to serum bactericidal activity elicited in mice immunized with the matched fHbp variant, or a closely related sequence.

Conclusions: The isogenic strain collection will be useful for assessing the effects of fHbp sequence variation on vaccine coverage against strains. In ongoing studies, we are determining the extent of cross protective antibodies elicited in mice by seven fHbp variants: ID 1, 4, 9, 13, 14, 15 and 55. The strains also will be valuable for testing the breadth of protective anti-fHbp antibodies elicited in humans.

P 286

Complement evasion by disease-causing meningococcal strains with absent factor H binding protein (fHbp) genes or frameshift mutations – Implications for vaccines that target fHbp

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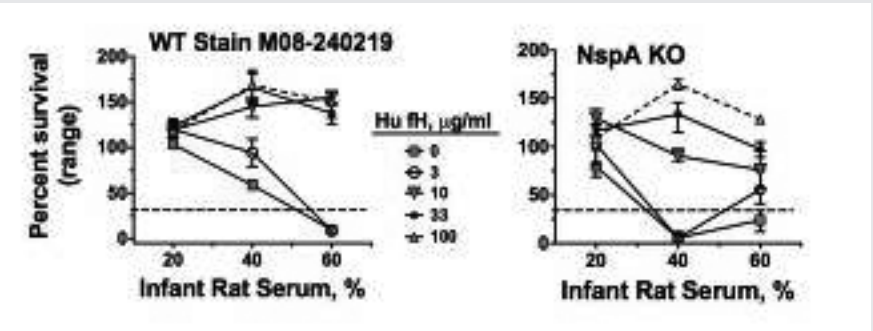
Background: Meningococci bind fH to down-regulate complement activation and evade the host. Two known fH ligands are fHbp and NspA. Binding to each is specific for human fH. Recently Lucidarme et al reported 18 meningococcal isolates with absent fHbp genes or frameshift mutations, which were from blood or CSF (CVI 2011). No fH binding was detected by far Western blot; a role for fH enabling these isolates to cause invasive disease was not identified. **Aim:** We investigated 7 serogroup B isolates from the Lucidarme study for fH binding and evasion of complement. **Results:** By flow cytometry, 4 isolates (3 with no detectable fHbp genes and 1 with a frameshift mutation) bound human fH; fH binding was decreased but detectable in all 4 isogenic NspA knockout (KO) mutants. Each wild-type isolate that bound human fH and their NspA KO mutant had >100% survival (compared to time 0) after incubation in up to 60% IgG-depleted human serum, or in infant rat serum (the highest concentrations tested). The addition of human fH to infant rat serum increased growth of 2 of the 4 isolates. Of the remaining 3 isolates with no detectable fH binding (all frameshift mutants), 1 was killed by 20% IgG-depleted human serum. A 2nd WT isolate and its NspA KO mutant had >100% survival in human serum. Both were killed by infant rat serum but rescued by human fH (Fig). The 3rd WT isolate and its NspA KO mutant had >100% survival in human serum or infant rat serum without human fH; but growth of the WT isolate in rat serum was enhanced by human fH. In a human fH transgenic infant rat model, IP challenge with ~1000 CFU of either of the two WT isolates or NspA KO mutants that survived in human serum caused similar levels of bacteremia. In contrast, in human fH-negative littermates the WT isolate killed by infant rat serum did not give bacteremia, and the other WT isolate had 10-fold lower bacteremia than in transgenic rats. **Conclusions:** Some meningococcal strains can survive in human serum and cause bacteremia in human fH transgenic rats in the absence of both fHbp and NspA (implies neither is essential for pathogenesis). Human fH increases survival of some strains in infant rat serum in the absence of both fHbp and NspA (implies an additional human fH ligand). Since fHbp is not an essential virulence factor, immune pressure from fHbp vaccination may select for escape mutants with low or absent fHbp expression that do not require this ligand for fH binding and evasion of host defenses.

figure 1



P 287

Heterogeneity of binding of Rhesus macaque factor H to meningococcal factor H binding protein

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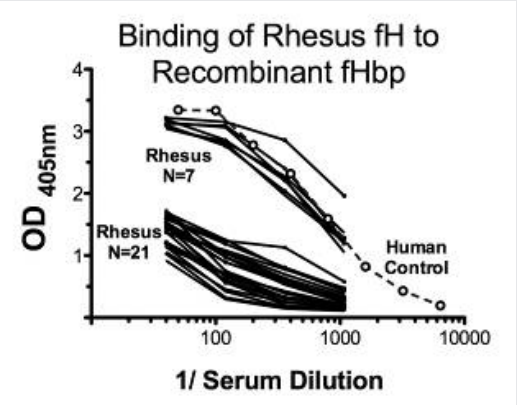
Question: The meningococcal vaccine candidate, fHbp, is an important ligand for factor H (fH). Binding of fH to fHbp has been reported to be specific for human fH. Further, mutant fHbp vaccines that did not bind human fH elicited higher serum bactericidal antibody responses in human fH transgenic mice than control fHbp vaccines that bound human fH. For predicting human antibody responses to fHbp vaccines, a non-human primate immunogenicity model might be better than human fH transgenic mouse models. In previous studies, however, rhesus macaque or baboon fH was reported not to bind to fHbp, and chimpanzee fH bound only weakly. In the present study we tested sera from 28 rhesus macaques, aged 3 months, for binding of rhesus fH to fHbp and down-regulation of complement. **Results:** By ELISA using immobilized fHbp, concentration-dependent binding of fH in 7 of the rhesus sera (25%) was indistinguishable from fH binding in the control human serum (Figure). The remaining 21 rhesus sera showed >50-fold lower fH binding to fHbp. By an anti-fH mAb capture ELISA, the concentrations of rhesus fH were similar in rhesus sera containing fH that bound or did not bind to fHbp. Binding of rhesus fH to fHbp was eliminated by single (R41S) or double (E218A/E239A) amino acid substitutions in fHbp, which are known to decrease binding of human fH. A recombinant protein comprising human fH domains 6-7 fused to murine Fc bound fHbp and showed similar inhibition of binding of rhesus or human fH to *N. meningitidis* serogroup B strain H44/76. Binding of the recombinant fH domain 6-7 inhibitor also blocked rhesus fH or human fH down-regulation of alternative pathway-mediated C3b deposition on the bacterial surface. The addition of human or rhesus fH also down-regulated bactericidal activity of a human IgG1 chimeric anti-fHbp mAb mediated by fH-depleted human complement. **Conclusions:** ~25% of rhesus macaque sera contain fH that can bind to fHbp via fH domains 6-7; fH binding in these rhesus sera was indistinguishable from fH binding in human sera, and binding of rhesus fH to the bacterial surface down-regulated complement activation and increased resistance of meningococci to complement-mediated killing. Studies are in progress to define the polymorphism(s) in rhesus fH responsible for binding to fHbp, and to determine whether rhesus macaques whose serum fH binds to fHbp have lower antibody responses to fHbp vaccines than rhesus macaques whose serum fH binds minimally to fHbp.

figure 1



P 288

Influence of factor H-binding protein (fHbp) sequence divergence on cross-protective antibody responses to meningococcal fHbp vaccines

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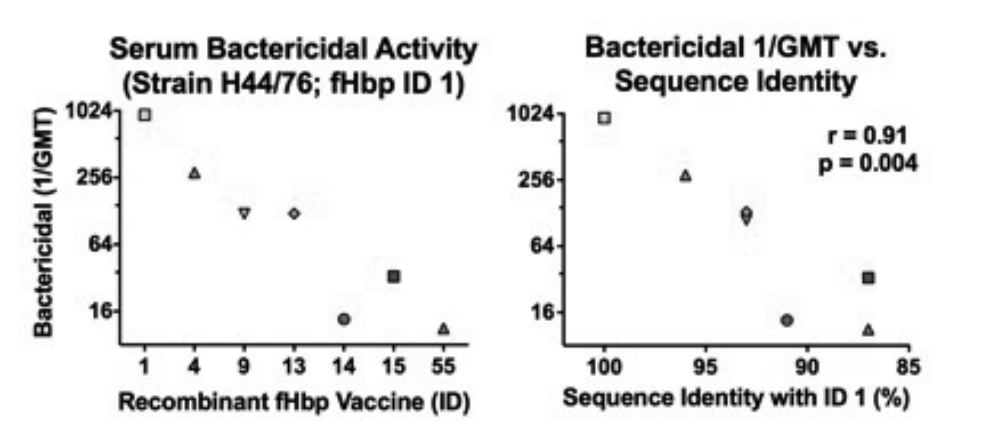
Question: fHbp sequence variants can be divided into two sub-families, A and B. Antibodies to sub-family A do not protect against strains with sub-family B, and vice versa; the extent of cross-protection within a sub-family is controversial. Two sub-family B vaccine antigens are in development, ID 1 (Novartis) and ID 55 (Pfizer). Based on phylogenic network analyses of 325 sub-family B sequences, these antigens are among the most divergent (87% identity). More central fHbp variants such as ID 9 may elicit broader protection. **Methods:** CD-1 mice were immunized with recombinant fHbp vaccines ID 1 and 55, and five other prevalent sub-family B variants: ID 4, 9, 13, 14 and 15. Control mice were immunized with native outer membrane vesicle (NOMV) vaccines from W-135 mutants with over-expressed fHbp ID 9 or fHbp KO. We measured serum IgG anti-fHbp titers by ELISA and human complement-mediated serum bactericidal activity. Bactericidal data are available against three strains with similar respective expression of different fHbp sequence variants matched to vaccine variants. **Results:** By ELISA, the IgG GMT to each of the seven recombinant fHbp vaccines decreased with increasing sequence divergence of the three target antigens tested, ID 1, 9 and 55 ($r \geq 0.92$, $p \leq 0.003$). For bactericidal activity, the recombinant fHbp ID 1 vaccine elicited GMTs of 938, 36 and 5 against strains with ID 1, 9 or 55, respectively. For the fHbp ID 55 vaccine, the respective GMTs were 11, 14 and 51; and for the ID 9 vaccine, were 121, 387 and 7. Overall, the bactericidal GMT for each of the seven recombinant fHbp vaccines correlated with the percent sequence identity of fHbp in the test strain (for ID 1 [Figure] and ID 9 strains, $r \geq 0.80$, $p \leq 0.03$; for ID 55 strain, $r = 0.68$, $p = 0.10$). Serum bactericidal GMTs of mice immunized with the NOMV vaccine from the mutant with over-expressed fHbp ID 9 were uniformly high (640, 1559 and 450, against strains with ID 1, 9 or 55, respectively). The respective GMTs elicited by the NOMV vaccine from the fHbp KO mutant were <10. **Conclusions:** Serum antibody responses to recombinant fHbp vaccines are sensitive to small differences in sequence identity between immunogen and target antigens. A phylogenically more central fHbp variant such as ID 9 may elicit broader reactivity than more divergent sub-family B sequence variants currently in development. Compared with recombinant fHbp vaccines, an NOMV vaccine with over-expressed fHbp ID 9 elicited the broadest protection.

figure 1



P 289

Persistance of bactericidal antibodies following early infant immunisation with investigational serogroup B meningococcal vaccines and immunogenicity of pre-school booster doses

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Question: At least 93% of infants given 4 doses of an investigational serogroup B meningococcal vaccine containing recombinant-proteins and an outer-membrane vesicle (4CMenB) had serum bactericidal antibody (SBA) titres ≥1:4 against 3 reference strains (44/76-SL, NZ98/254, 5/99) by 13 months of age. Participants immunized with a vaccine containing recombinant proteins alone (rMenB) generated bactericidal antibodies only 2 of these strains. We evaluated persistence of these antibodies to 40 months of age and the increase in antibodies following a booster dose of vaccine.

Methods: Participants given 4 infant doses of 4CMenB or rMenB received a fifth dose of the respective vaccines at 40 months (groups 4CMenB246-12 and rMenB246-12). Toddlers given one dose of these vaccines at 12 months were immunized at 40 & 42 months (groups 4CMenB-12 and rMenB-12). MenB vaccine-naïve participants received 4CMenB at 40 and 42 months (control group). Sera obtained at 40 months and 1 month after each immunisation was assessed against 4 reference strains: 44/76 -SL (assessing vaccine antigen fHbp), 5/99 (NadA), NZ98/254 (OMV/PorA) and M10713 (NHBA). Bactericidal activity against four additional strains (UKP1.7-2.4, GB101, GB355 and GB364) was also assessed.

Results: At 40 months the proportions of participants in the 4CMenB246-12 group with SBA titres ≥ 1:4 were 65% (95% CI 38-86%) for strain 44/76, 76% (50-93%) for 5/99, 41% (18-67%) for NZ98/254 & 67% (38-88%) for M10713 (N=15-17). For rMenB246-12 recipients these proportions were 43-68% except for NZ98/254 (3%, 95% CI 0.09-18%) (N=28-29). Pre-booster, 0-38% 4CMenB-12 recipients had SBA titres ≥ 1:4 for these reference strains (N=8), compared with 7-57% rMenB -12 recipients (N=13-14). For controls (N=40) proportions were < 3% for strains 5/99 and NZ98/254, 63% (46-77%) for strain 44/76-SL and 68% (51-81%) for strain M10713. For the additional four strains the percentages of 4CMenB246-12 with SBA titres ≥ 1:4 were at least 53% except for one strain not expressing vaccine antigens (GB355), for which this value was 6% (0-29%). A booster dose in the 4CMenB246-12 group increased the proportions of participants with SBA titres ≥ 1:4 to 89-100% for all strains except GB355 (50% (26-74%)).

Conclusion: SBA titres wane following infant immunization with rMenB or 4CMenB but there is an anamnestic response to a booster dose. Booster doses of 4CMenB may be required to maintain immune protection through childhood and adolescence.

P 290

Benefit of vaccination against meningococcal capsular group B (MenB), in addition to chemoprophylaxis, for prevention of secondary cases among close contacts of cases and more widely during clusters

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Introduction: It is current UK practice to provide antibiotic prophylaxis to close contacts of single cases of invasive meningococcal disease (IMD) and in the management of IMD clusters in institutional settings. Vaccination of contacts is also recommended where a vaccine preventable strain is identified. Most IMD cases in the UK are due to capsular group B (MenB) and several MenB vaccines are nearing licensure. It is not yet known how these vaccines will be used as part of immunisation programmes or in case and cluster management.

Methods: We investigated whether current UK recommendations will be applicable to MenB disease. A review of the international literature was performed to explore the incidence and characteristics of secondary cases and clusters. Published data on MenB vaccine immunogenicity and potential strain coverage were used to estimate numbers of contacts needed to vaccinate (NNV) to prevent one case of IMD.

Results: In settings where antibiotic chemoprophylaxis is used routinely for close contacts of IMD cases, the residual estimated secondary attack rate (> one month post exposure to the index case) is 20-90 per 100,000 household contacts. A 4-component MenB vaccine is predicted to protect against 73% of UK MenB strains. We found no immunogenicity data for one MenB vaccine dose in infants or toddlers. After two doses, 64% of infants achieved protective antibody titres. In adolescents, 93-96% achieved protective antibody titres following a single dose. Using these data, the estimated NNV in infants ranges between 2,400-10,700 and, in adolescents, between 1,600-7,200. In a cluster setting, almost all tertiary cases occur within 7 days of the second case, suggesting that MenB vaccination is less likely to offer any additional protection. On the other hand, MenB vaccines may have a role in prolonged outbreaks, where the circulating strain is demonstrated to be vaccine preventable, although vaccine effectiveness will depend on whether the vaccines also protect against carriage, which has yet to be demonstrated.

Conclusions: There is currently insufficient evidence of benefit to recommend routine MenB vaccine use in contacts of IMD cases in the household or institutional settings. This recommendation should, however, be revised when further immunogenicity, carriage and vaccine coverage data are available. MenB vaccines may have a role in the management of prolonged outbreaks in an institution setting or the wider community, where the circulating strain is potentially vaccine preventable.

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Reactogenicity of Investigational Serogroup B Meningococcal Vaccines given at 40 months of age to primed and vaccine naïve children

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Question: An investigational serogroup B meningococcal vaccine (4CMenB), consisting of recombinant proteins with detoxified outer membrane vesicles, has recently been submitted for licensure. Infant studies have shown that 51-61% of infants developed fever when 4CMenB was given with routine vaccines compared with 26-41% when 4CMenB was given alone. We evaluated the reactogenicity of this vaccine, and another investigational vaccine consisting of recombinant proteins only (rMenB) when given at 40 months of age.

Methods: In 2 previous studies children received 4CMenB or rMenB in early infant (2, 4, 6, 12 months), late infant (6, 8 12 months) or toddler (12 months) schedules. In 2 follow up studies we evaluated the reactogenicity of additional doses of vaccine at 40 months of age (1 dose of 4CMenB or rMenB for early and late infant schedules; 2 doses for toddler schedules). In each study a MenB vaccine naïve control group received 2 doses of 4CMenB 2 months apart. Parents were provided with a diary card to record solicited and unsolicited reactions.

Results: Following immunisation with a dose of 4CMenB at 40 months of age, fever was reported by 5% (1/19) of participants previously immunised with 4CMenB in early infancy, 7% (1/14) for late infant recipients, 0% (0/8) for toddler recipients, 10% (4/42) and 15% (6/41) of the two cohorts of MenB vaccine naive (control) participants. For rMenB recipients these proportions were 3% (early infancy, 1/29), 25% (late infant, 4/16) and 7% (toddler, 1/14). Irritability and sleepiness were the most commonly reported systemic reactions for both vaccines. For recipients of two doses of 4CMenB fever rates were similar at both doses. Across the two studies severe arthralgia (transient) was experienced by ten 4CMenB and two rMenB recipients. Pain was reported by 74-100% of 4CMenB follow-on participants, 11-29% of these reactions were considered severe (defined as inability to perform daily activity). In control group participants these proportions were 83-93 % (any pain) and 7-21% (severe pain). In rMenB recipients these were 21-63% (any pain) and 0-3% (severe pain). For 4CMenB follow-on recipients other local reactions rates were erythema 100%, induration 36-63%, swelling 25-43%; for 4CMenB control participants erythema 93-98%, induration 33-41%, swelling 27-48%; and rMenB follow-on recipients erythema 81-97%, induration 21-69%, swelling 21-56%. In neither study did systemic reaction rates appear to increase with increasing numbers of prior doses.

Conclusion: These data suggest if there were a need for 4CMenB immunisation at age 3-4 years to boost waning immunity or as part of a 'catch up' campaign, few participants would develop fever, however local reactions of limited duration might be relatively common.

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Production and control of a Tailor made Brazilian Meningococcal B vaccine to Phase II trial in children

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In Brazil the meningococcal disease has been a public health problem since groups A and C epidemics occurred in the 1970's. Since 1995 Oswaldo Cruz Foundation, in Brazil, has been working to develop a group B meningococcal vaccine composed by detergent treated outer membrane vesicles (OMV) and detoxified endotoxin (dLPS) from Neisseria meningitidis grou B prevalent strains. The vaccines were developed and tested in Phase I studies in adults with promising results. Currently, the vaccine is prepared to Phase II trial in children. The physico-chemical and biological controls were established based on what has been described, in literature, to similar vaccines. Three experimental vaccines were used in a sequential study with increasing antigens dose as was performed in the Phase I in adults. The both vaccine OMVs contain the main class 1, 2, 3 and 5 proteins, some minor iron regulated proteins and 5-10% of residual lipopolysaccharide related to total protein amount. The detoxified LPS was added in each vaccine in a half amount of protein dose. The pyrogenicity of final products, based on the residual LPS in OMV, varies from 1.25 to 5.0 ng LPS/kg rabbit. The three experimental vaccines were highly immunogenic in mice. The results suggested better performance for higher antigen concentrations in bactericidal activity using as the target vaccine strains. Using adsorption assay the main induced antibodies in mice were against OMV antigens but antibody against lipopolysaccharide was also important in detected vaccine potency. The produced lots of experimental vaccines were approved by Brazilian regulatory agency (ANVISA) and the Phase II study has been conducted since last January. It will be finished in November of 2012.

P 293

Functional impacts of the diversity of the meningococcal factor H binding protein

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Question: *Neisseria meningitidisis* a human pathogenic bacterium responsible for life threatening and rapidly evolving invasive infections where several bacterial virulence factors play primordial role during host-bacteria interactions. The meningococcal factor H binding protein, fHbp interacts with the complement negative regulator, factor H (fH), to enhance meningococcal survival. fHbp is also a major component in the recombinant vaccines under development against serogroup B isolates. In 2010, we detected variations in this gene during an outbreak due to serogroup C isolates belonging to the same clonal complex, ST-11. We therefore explored invasive meningococcal isolates inFranceduring 2009-2010 (n=680 representing 88% of all invasive isolates).
Methods: DNA sequencing offHbpgene, the level of expression of fHbp at the bacterial surface by ELISA using anti-fHbp antibodies and the interaction of fHbp with human fH were determined as well as the deposit of C3b complement component.
Results: We observed a high level of sequence diversity of fHbp in particular within regions known to interact with fH that was observed. The distribution offHbpalleles differed among meningococcal serogroups and clonal complexes. This diversity affected directly binding of fH to fHbp and seems to influence the deposit of the complement C3b component on the bacterial surface. However, bacterial killing by anti-fHbp antibodies was still achieved and required a minimum level of expression at the bacterial surface regardless the binding to fH or sequence diversity.
Conclusions: These data have impacts on our understanding of meningococcal pathogenesis and surveillance of meningococcal variants of fHbp that is targeted by recombinant vaccines under clinical trials. They also provide view on the diversity offHbpprior to the vaccination era.

P 294

Dried blood spots for the serologic evaluation of meningococcal disease and vaccine failures in Africa

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Backround: Dried blood spot (DBS) samples have been used in a variety of diagnostic and immunologic assays, including newborn screening, HIV and measles. DBS require smaller volumes of blood, fewer collection materials, and a simplified storage and transport protocol compared to serum collection and thus provides substantial advantage in rural and resource limited settings. In 2010, a new serogroup A meningococcal conjugate vaccine, MenAfriVac, was introduced to Burkina Faso, Mali, and Niger. Measurement of serum bactericidal activity (SBA) and IgG ELISA antibody concentration using DBS may offer a less expensive, more feasible approach to evaluate antibody persistence and immune responses to carriage and disease following the implementation of MenAfriVac.
Objectives: To develop and validate a process for antibody elution from DBS for use in previously validated SBA and IgG ELISA assays.
Methods: Blood was collected by both finger-stick and venipuncture from (meningococcal vaccinated or negative control) adults. DBS were created from venipuncture blood; allowed to dry; transported, and stored at -20°C. DBS were eluted (O/N, 4°C, with rotation) and SBA titer against Men A and anti-Men A IgG ELISA antibody measurements were determined with paired serum, on multiple days. DBS were evaluated (n=30) against paired serum relative to recovery, correlation, and equivalency using SBA assays and ELISAs.
RESULTS: DBS eluted serum and venipuncture serum samples were highly correlated (r_{SBA}=0.907, P_{SBA}<0.05; r_{ELISA}=0.996, P_{ELISA}<0.05) independently for SBA and ELISA. Recovery for the DBS eluted sera varied less than one dilution for SBA and less than 15% for ELISA as compared to serum. Meningococcal A polysaccharide specificity was similar for both types of serum samples. No statistically significant difference was found between the DBS and serum samples by either SBA or ELISA (P_{SBA}=0.07; and P_{ELISA}=0.442).
Conclusions: Serum-based assays used for the measurement and evaluation of immune responses to meningococcal vaccination or natural infection can be converted to work with DBS samples with excellent comparability.

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In vitro Relative Potency Assay (IVRPA) with functional monoclonals to *Neisseria meningitidis* as a surrogate for immunogenicity evaluation of vaccines

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The development of *in vivo* potency assays for clinical batch release requires repeated immunogenicity studies with the use of a large number of laboratory animals, which instead should be reduced whenever possible. Moreover immunogenicity in laboratory animals is difficult to correlate with antibody response in humans.
We sought to replace immunogenicity evaluation of vaccines in mouse models with an *in vitro* assay based on the specific binding of functional monoclonal antibodies to the vaccine components.
We developed an antibody binding assay based on the specific interaction of bactericidal monoclonal antibodies and capsular polysaccharides of serogroups A, C, W-135 and Y meningocci.
The assay is a competitive ELISA in which the binding of a monoclonal antibody to the native polysaccharide is specifically inhibited by the quadrivalent ACWY conjugate vaccine (Menveo®).
The read-out of the assay is a relative potency determination, calculated by comparing the inhibition curve of a reference vaccine of proven clinical efficacy with the curve of an unknown vaccine sample.
The method was successfully validated and is now routinely applied for batch release.
An *in vitro* relative potency assay is now under development to adapt the method to a recombinant protein-based vaccine against *N. meningitidis* serogroup B. We are planning to screen a panel of monoclonal antibodies for each antigen to select mAbs that are specific for each vaccine component and recognize immunological relevant epitopes. Results are promising and highlight the usefulness and applicability of the method to various vaccines.

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Improved high-throughput assay for evaluating the antibody response against surface antigens from Neisseria meningitidis

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One of the primary challenges in developing protein-based vaccines against pathogens likeNeisseria meningitidisis to generate an immune response capable of providing protection against most or all disease isolates. Currently there is no simple and convenient screening system for comparing the ability of various recombinant antigens to generate a cross-reactive response that would aid in selection and optimization for vaccine development. Two strategies that are commonly employed are screening against a panel of strains in whole cell ELISA assays or screening against a panel of purified target antigens isolated from representative strains. The former has the disadvantage of not being able to conclusively attribute the immune response to the target antigen, and the latter has the disadvantage of being very labor intensive. An additional disadvantage of the latter approach that we discovered while working with transferrin binding protein B (TbpB) is that the binding of purified protein to the ELISA plate surface is non-random, such that epitopes on one surface of the protein are not detected. To overcome this limitation we developed a strategy involving expression of the target antigens in customized expression vectors that include an N-terminal tag capable of binding to streptavidin and using streptavidin-coated ELISA plates to capture the recombinant antigen. We have demonstrated that crude extracts expressing recombinant antigens can be used directly to coat streptavidin-coated plates with results that are identical to using previously purified antigen. This observation has enabled us to develop the screening assay in a high-throughput format capable of rapidly screening reactivity against a diverse panel of strains and variants.

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Large-scale generation of a universal human complement source for functional bacterial immunoassays

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The interaction of antibody and complement has long been known to be important for protection against meningococcal disease and the serum bactericidal assay (SBA) using human complement has been established as a correlate of protection for meningococcal disease in a number of studies using both capsular polysaccharide and outer membrane vesicle vaccines. Acquiring a source of human complement for use in the SBA and other immunoassays assessing vaccine-induced immunity to meningococcal disease can be difficult, due to high levels of antibodies which have resulted from nasopharyngeal carriage of *Neisseria meningitidis* or commensal *Neisseria* species. Most adults have cross-reactive antibodies can evoke meningococcal bacteriolysis against a number of different *N. meningitidis* strains in the absence of test antiserum, requiring a large number of individuals to be screened to identify a suitable source of complement for a particular test strain.

We have developed a reproducible method for antibody depletion of pooled 300ml batches of human plasma using protein G Sepharose. Plasma anti-coagulated with either heparin or lepirudin was compared to serum in a number of immunoassays to establish the optimum initial source of complement, with the greatest complement activity retained using lepirudin. Anti-coagulated plasma was IgG-depleted and then assessed for classical and alternative pathway complement activity using radial immunodiffusion assays, which demonstrated good retention of complement activity. IgM and IgG subclass levels were assessed following protein G Sepharose chromotography by ELISA, and IgG levels were reduced to undetectable levels. IgG-depleted plasma retained very high levels of haemolytic complement activity and has been successfully used in *N. meningitidis* SBA, opsonophagocytosis and antibody-mediated complement C3 and C5b-9 binding assays.We have used this IgG-depleted plasma as a complement source to assess functional immunity to a range of meningococcal serogroups and strains, as well as for a variety of isolates of *Bordetella pertussis*, *Haemophilus influenzae* and Group B Streptococcus.

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Development of an intrinsic killing assay for identifying susceptible individuals to Neisseria meningitidis serogroup B strains

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Neisseria meningitidis is a major cause of bacterial meningitis, septicemia, and remains an important public health problem. Polysaccharide conjugate vaccines are available against infections caused by *N. meningitidis* serogroups A, C, Y and W135; however, no vaccines are licensed in the U.S. to prevent disease caused by serogroup B (MenB) strains. To test MenB vaccines, serum bactericidal assays will be utilized to investigate sero-responses based on the original Goldschneideret. al studies, where bactericidal antibodies in sera were measured using either exogenous or endogenous human complement. Only individuals whose sera lacked bactericidal killing were at risk for developing disease. Screening healthy individuals for intrinsic bactericidal activity can serve two purposes: first, to identify sources of exogenous complement that will not interfere with the results of the bactericidal antibody assay; and second, to assess the population for broadly protective bactericidal activity. We developed and explored the feasibility of a micro titer plate-based intrinsic killing assay to screen healthy adult blood donor sera for bactericidal activity against a collection of MenB isolates. In a pilot study of 24 sera and up to 5 diverse MenB strains, we tested intrinsic killing at dilutions of 1:4 and 1:8 in triplicate and observed that the results amongst the dilutions were consistent: the triplicate wells were all concordant for the 50% killing threshold for 92 of 94 serum-strain pairs at the 1:4 dilution, and 84 of 94 were concordant at the 1:8 dilution. When comparing bacterial cell counts using data from the 1:8 and 1:4 dilutions, the 1:8 dilution had adequate complement activity for complete killing of MenB. Seventeen sera (71%) showed intrinsic bactericidal activity against all strains at both dilutions. On the contrary, 3 sera were unable to kill one or more strains at the 1:4 dilution and 4 additional sera had <50% killing at 1:8. Thus this population, even though broadly protected, has individuals, whose sera lack substantive intrinsic activity, including some that might serve as complement sources for broad spectrum vaccine testing. Our approach is currently being applied to screen a subset of over 400 active sera from healthy adult blood donors (average age 40 years) against diverse and epidemiologically relevant MenB strains. This micro-titer intrinsic bactericidal assay will be useful for identifying complement donors, for testing the breadth of strains affected by bactericidal antibody to specific antigens and for determining the susceptibility of individuals to MenB strains. Additionally, the ability to determine changes in individual susceptibility to a large number of strains in a simple assay using intrinsic complement will help evaluate the impact group B vaccines may have on the targeted populations.

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Predicting serum bactericidal responses using a high-throughput flow-cytometric complement deposition assay – Sensitivity and Specificity

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Serum bactericidal activity (SBA) has long been established as a correlate of protection for capsular polysaccharide and outer membrane vesicle-based meningococcal vaccines, with SBA titres of ≥1:4 measured using human complement established as providing protection. Thus potential vaccine efficacy is widely assessed by the measurement of serum bactericidal antibodies and this method is currently required for vaccine licensure. However, the serum bactericidal assay requires large volumes of sera and can be laborious to perform. For new serogroup B vaccines it is vitally important to determine whether protection will extend to all strains that can cause disease. The limited volume of serum available, particularly from paediatric clinical trials, limits the number of strains that can be assessed. Thus the development of high-throughput assays, which require very low volumes of serum, is important to determine the potential effectiveness of a vaccine in a particular setting.

We have developed a flow cytometric assay measuring antibody-mediated complement deposition. The assay uses fixed meningococci, IgG-depleted human plasma as the complement source and fluorescent-conjugated antibodies to measure deposition of C3b/iC3b and C5b-9 (membrane attack complex) on the surface of *Neisseria meningitidis*. The assay uses a single dilution containing 5µl serum per assay, which is considerably less than that required for a standard SBA. We have analysed antibody-mediated complement deposition and SBA with a panel of 40 human sera and 5 diverse serogroup B meningococcal strains. We have established a preliminary cut-off value for C5b-9 deposition that will predict SBA. Using this value we have demonstrated that this flow cytometric assay can predict SBA activity with 65-89% accuracy, depending on strain. To determine the utility of this approach for new vaccines, further analysis with larger panels of sera and strains will be required.

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Factors Affecting Neisseria Meningitidis Serogroup B Bactericidal Killing

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With the introduction of meningococcal conjugate vaccines and the low burden of disease, conventional assessment of efficacy of a novel vaccine is unrealistic. To overcome this, serological correlates of protection are used to demonstrate immunogenicity and efficacy for licensure. Serum bactericidal assays (SBA) measure the ability of functional antibodies to kill meningococci in the presence of complement. To combat the lack of a vaccine against serogroup B meningococcal disease, the majority of vaccines use PorA as a component. Although expression is variable, PorA is one of the most predominant proteins of the outer membrane and induces bactericidal antibodies. There have been many reports in the literature on the synergy between minor antigens resulting in bactericidal killing. The aim of this study was to investigate various factors which could influence the SBA titer using monoclonal antibodies to PorA with known quantity, affinity and binding target.

Seven H44/76 strains were constructed, each containing a modified *porA* promoter. The spacer sequence of the wild type promoter between the -10 and -35 sites containing the poly C tract was replaced to eliminate variation through slip strand misparing. Strains were engineered with spacer sequences varying in length from 14 to 20bp to provide a range of PorA expression. PorA protein from these strains was quantified by SDS-page electrophoresis and slot blot.

In a standard SBA, no titre was achieved in a *porA* Δ and the strains expressing low levels of the PorA protein. For the remaining strains, regardless of expression level, %killing increased with rising antibody concentration. However, while high porA expressing strains achieved 100% bactericidal killing, strains expressing an intermediate level, killing reached a plateau below 100%. Addition of a second monoclonal antibody, boosted the %killing above the level generated by the individual antibodies alone, indicating a synergistic effect. The ability to achieve complete killing, with saturating concentrations of antibody was also dependent on incubation time. In high-expressing strains killing was rapid and complete within 20 minutes. Strains expressing intermediate levels took longer to reach 100% killing, up to 3 hours, indicating time required for complete killing was dependent on PorA expression level. Additional factors such as monoclonal antibody affinity also influenced induction of complement-mediated bacterial killing.

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Comparison of flow-cytometry (FACS) and meningococcal antigen typing system (MATS) as predictors of serum bactericidal activity using human complement (hSBA) on pathogenic isolates collected during an endemic period in Norway (2005 – 06)

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Question: Vaccines against meningococcal serogroup B (MenB) disease may not cover all circulating strains because of genotypic and phenotypic variability of surface protein antigens. The meningococcal antigen typing system (MATS) measures a combination of antibody cross-reactivity and quantity of antigens expressed by an isolate, which are included in a multicomponent MenB vaccine (4CMenB). MATS correlates with killing of strains by serum bactericidal activity using human complement (hSBA): strains with MATS values above a positive bactericidal threshold have a probability of ≥89% for being killed by 4CMenB induced antibodies. Exceptions may be explained by the use of lysed bacteria in MATS, which may correlate imperfectly with the amount of antigen accessible on the surface of live bacteria.
Methods: We compared MATS with flow-cytometry (FACS) measurements of 4CMenB antigens expressed by 64 pathogenic MenB isolates from Norway in 2005-06. These were highly heterogeneous when assessed by MLST, FetA, PorA and sequence variability of 4CMenB antigens. We used MATS and FACS, with the same respective rabbit polyclonal antibodies, to assess fHbp variant group 1, NadA, and NHBA. Variant group 2 and 3 fHbp, GNA2091 and GNA1030 were assessed by FACS only. A subset of 26 strains was also tested in the hSBA using pooled sera from 4CMenB vaccinees.
Results: Studying all the five recombinant antigens of 4CMenB by FACS showed fHbp to be the most diversified antigen; a number of strains were high expressers, while quite a few gave a FACS signal below the cut-off level, (nearly all those harbouring variant group 2 or 3). For the other antigens the surface expression levels were generally lower. This was in particular the case for GNA2091 and GNA1030, where 75% of the strains were below the cut-off level. A positive correlation ($p<0.05$) was seen in FACS for some antigen combinations which suggested an effect of strain-specific characteristics at the cell surface that modulated accessibility of antigens for antibodies in FACS. This effect was not detected in MATS. For fHbp variant group 1 and NHBA, MATS predicted protective hSBA titres in pooled infant sera.
Conclusions: Correlations between FACS and MATS results were weak, but statistically significant for fHbp and NadA; no correlation was seen for NHBA. Both FACS and MATS measure relative antigen expression. However, FACS seems to be less sensitive to sequence variation than MATS, which may explain why MATS correlated better with hSBA than FACS.

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Evaluation of the immunological properties of the Neisserial Heparin Binding Antigen (NHBA)

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Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein ofNeisseria meningitidis which binds heparin-like molecules.NHBA is an antigen of the multicomponent 4CMenB vaccine able to induce bactericidal antibodies in laboratory animals and humans. The aim of this study is to investigate the immunological properties and potential cross protection of NHBA-induced bactericidal antibodies against a panel of N. meningitidis strains.

We used various approaches to investigate the level of cross protection mediated by human anti-NHBA antibodies. In order to characterize only the immunological properties of NHBA we selected N. meningitidis strains mismatched for the other vaccine antigens (fHbp, NadA and PorA1.4). These strains have been tested in a Serum Bactericidal Assay using human complement (hSBA) and human sera from different age groups vaccinated with the 4CMenB vaccine. To further prove that the immune response was directed against NHBA, we performed a competitive hSBA using the NHBA recombinant antigen and also generated NHBA deletion mutants in different genetic backgrounds.

The hSBA analysis showed that human sera raised against the 4CMenB vaccine are able to kill natural N. meningitidsstrains harboring different NHBA amino acidic sequences. We also demonstrated that the addition of recombinant NHBA antigen or the deletion ofnhbagene abolished or significantly decreases bactericidal titers.

To evaluate the contribution of amino acid sequence variability to vaccine coverage, we constructed a strain that is susceptible to bactericidal killing only by anti-NHBA antibodies and engineered it to express equal levels of different NHBA peptides under an inducible promoter. This ongoing approach will be useful to further evaluate the level of cross-protection of NHBA and assess the relation between level of expression and bactericidal killing mediated by NHBA. Moreover, to evaluate the most immunogenic regions of NHBA protein, ELISA experiments are ongoing using both sera from animals and from humans vaccinated with the 4CMenB vaccine.

The results obtained so far demonstrate that NHBA is an important vaccine antigen able to induce cross-protective bactericidal antibodies against genetically different strains in different age groups vaccinated with the 4CMenB vaccine.

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Safety and immunogenicity of a four component meningococcal group B vaccine (4CMenB) and a quadrivalent meningococcal group A, C, W135 and Y conjugate vaccine (Menveo) in UK laboratory workers with potential occupational exposure to meningococci.

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Laboratory safety to prevent exposure of laboratory staff to meningococci should primarily rely on procedures preventing exposure to aerosols containing viable meningococci. Despite this, vaccination is also a key component of protection against this and other infectious diseases in the occupational setting. There is currently no licensed vaccine for meningococcal capsular group B (MenB) and at the time of trial commencement, no licensed conjugate vaccine available in the UK for meningococcal capsular groups A, C, W135 and Y. We therefore undertook a Phase II trial in UK laboratory workers, to investigate the safety and immunogenicity of a novel four component group B vaccine (4CMenB) and a quadrivalent group A, C, W135 and Y conjugate vaccine (Menveo).

Subjects enrolled were adults aged 18-65 years at the Public Health Laboratory, Manchester who may have a potential occupational exposure risk to meningococci. 4CMenB was administered at 0-2-6 months in the non-dominant arm and Menveo concomitantly at 0 months in the dominant arm. Blood samples were taken pre- and post- each vaccination and analysed in the serum bactericidal antibody (SBA) assay against a panel of seven diverse MenB strains and A, C, W135 and Y strains.

In total 38 staff were enrolled and received the first vaccinations with 31 completing the trial per protocol. Both vaccines were proven safe, with local reactogenicity being more commonly reported following 4CMenB than Menveo. High proportions of subjects had putative protective SBA titres pre-vaccination, with 61-84% and 61-87% protected against A, C, W135 and Y strains and seven diverse MenB strains, respectively. Post-vaccination, SBA titres increased with 95-100% and 90-100% of subjects with protective SBA titres against A, C, W135 and Y strains and seven diverse MenB strains, respectively.

These data suggest that 4CMenB and Menveo are safe and have the potential to provide protection in laboratory workers against the five pathogenic meningococcal groups.

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Guillain-Barre Syndrome Among Adolescents Receiving Meningococcal Conjugate Vaccines – United States, January 2005 – May 2012

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Background: Guillain-Barre Syndrome (GBS) has been reported temporally following meningococcal conjugate vaccination, which is categorically included in package inserts for Menactra (Sanofi-Pasteur Inc.) [MCV4-D] and Menveo (Novartis Vaccines and Diagnostics) [MCV4-CRM_{197}]. The vaccines were licensed in theUS in 2005 and 2010, respectively. In December 2011, safety data from a large post-marketing study were reviewed as part of a labeling update. In addition, surveillance data are monitored through the Vaccine Adverse Event Reporting System (VAERS).
Methods: The risk of GBS following receipt of MCV4-D was evaluated in a retrospective cohort study, conducted from 2005 to 2008. The primary study population consisted of 9,578,688 individuals 11-18 years of age, of whom 1,431,906 (15%) received MCV4-D. Potential cases of GBS were confirmed by medical chart review. In VAERS, passive surveillance methods were used to identify reports of GBS in MCV4 (MCV4-D and MCV4-CRM_{197}) recipients.
Results: Of 72 medical chart-confirmed GBS cases, none had received Menactra vaccine within 6 weeks prior to symptom onset. Of 18 potential cases, 9 cases were confirmed to be non GBS cases and 9 other cases could not be confirmed or excluded due to absent or insufficient medical chart information. The binomial exact method was used to estimate the upper one-sided 95% confidence limit of the attributable risk estimate. Imputation of 1 or 2 missing cases would result in 2.8 and 3.9 excess cases per million doses, respectively. Imputation of the maximum number of cases (n=9) would result in an attributable risk of 10.6 excess cases per million doses. However, since the observed case confirmation rate was consistent with experiences with similar healthcare databases, an excess of 4.9 cases of GBS per million doses was likely a more reasonable estimate.
From February 2010 to May 2012, no cases of GBS were reported to VAERS among MCV4 recipients within 60 days of vaccination.
Conclusions: The possibility of GBS following receipt of MCV4-D could not be ruled out due to missing data. The attributable risk of GBS ranged from 0 to 5 additional cases of GBS per 1,000,000 vaccinees within the 6-week period after vaccination. No new concerns have been identified through VAERS. Considerations about MCV4 vaccination can take into account the benefits of preventing meningococcal disease and possible risks of GBS.

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Meningococcal Vaccine-loaded nanoparticles induce robust autophagy

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Background: *Neisseria meningitidisis* a leading cause of bacterial meningitis and sepsis associated with a high mortality rate. Capsular polysaccharides(CPS)are a major virulence factor and form the basis for serogroup designation and protective vaccines. Meningococcal vaccines are available but are very expensive and require chemical conjugation. Therefore, the search for novel vaccine formulations that overcome the limitations of the current conjugate vaccines is important. We designed a novel meningococcal vaccine formulation consisting of meningococcal CPS polymers encapsulated in albumin-based biodegradable nanoparticles that slowly release antigen and induce robust innate immune responses. Vaccines that elicit innate immunity are reported to have enhanced and protective adaptive immune responses.
Methods: Autophagy, an ancient homeostasis mechanism for macromolecule degradation, recently has been recognized to play a role in host defense and antigen presentation. Since autophagy induction has been reported to enhance adaptive immune responses by enhancing memory cells, we investigated autophagy induction in macrophages exposed to meningococcal vaccine-loaded nanoparticles using RAW264 cells stably transfected with a construct encoding the autophagy marker LC3 protein.
Results: We report that meningococcal CPS-loaded nanoparticles, but not the empty nanoparticles, induced the release of IL-8, TNF-α and IL-1β, and enhanced phagocytic capacity in macrophages. Further, the CPS-loaded nanoparticles, but not the empty nanoparticles, strongly induced autophagic vacuoles in a dose-dependent manner. Autophagic vacuoles were also induced by CPS polymers that have not been encapsulated in the albumin-based matrix. However, when compared to CPS polymers alone, CPS-loaded nanoparticles induced much larger cytosolic autophagic vacuoles. The large autophagic vacuoles in the cytosolic compartment of macrophages indicate the uptake, slow release and the immunostimulatory activity of the vaccine-loaded nanoparticles that lead to functional antigen presentation.
Conclusion: Our data show that the novel meningococcal vaccine nanoparticles are taken up by macrophages and induce robust autophagy formation, thereby enhancing antigen presentation which is a pre-requisite for inducing adaptive immunity.

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Inter-laboratory comparison of the meningococcal capsular group B serum bactericidal antibody assay between Novartis Vaccines Marburg and Health Protection Agency Manchester

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Background: There is a need to bridge serum bactericidal antibody (SBA) assays in order to evaluate investigational meningococcal group B vaccines from different trials. In order to compare data between laboratories and with different strains, a collaborative study was conducted to compare SBA data from clinical trials of 4CMenB between Manchester, Health Protection Agency (MC, HPA) and in Marburg, Novartis (MB, Novartis).

Methods: A total 110 pre - and post-vaccination residual serum samples from previous clinical trials were selected for this study, consisting of 25 adults who received MeNZB®(n=15), rMenB (n=5) or 4CMenB (n=5) and 30 infants who received rMenB (n=11) or 4CMenB (n=19), respectively. Based on the sera availability samples were assayed in a standardised SBA assay against three MenB strains 44/76-SL, NZ 98/254 and 5/99 in both laboratories.

Results: Strong correlations were observed between MC and MB laboratories. Correlation coefficients (r) were 0.718 to 1.00 for strains NZ98/254 and 5/99 across all vaccine groups in both adults and infants prior to and after the vaccination. For strain 44/76-SL sera from subjects who received 4CMenB had good correlations in both adult and infant groups (r, 0.700 to 1.000). However, a poorer correlation for pre-vaccination samples from infants who had received rMenB (r=-0.316) and moderate correlation for adults received MeNZB® (r=0.558) were observed

No significant differences were found between laboratories when comparing the proportion of subjects with a four-fold or greater rise in SBA titres against strains 44/76-SL, NZ98/254 and 5/99 which was determined for 80%, 71% and 73% of subjects at MC, and 82%, 65% and 75% of subjects at MB respectively. The proportions of subjects with SBA titres ≥4 were comparable between MC and MB laboratories. However SBA GMTs varied between MC and MB for pre-and post- vaccination samples, in which MC's GMTs were significantly higher than the MB's GMTs for strains 44/76-SL and NZ98/254 in the adult MeNZB® group for post-vaccination sera. For strain 5/99 no significant differences were found for pre- and post-vaccination sera between the two laboratories.

Conclusion: The SBA data assay demonstrated a good agreement between the two laboratories.

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A novel factor H-Fc chimeric immunotherapeutic molecule against *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted infection gonorrhea, has become resistant to almost every conventional antibiotic, thus attaining 'superbug' status. There is an urgent need to develop novel therapies against this pathogen. The gonococcus binds human factor H (fH), an inhibitor of the alternative complement pathway, through fH domains 18-20.
We have shown previously that a chimeric molecule comprising fH domains 18-20 fused to murine IgG2a Fc (fH18-20/Fc) bound to gonococci and mediated complement-dependent killing. However, the use of native (unmodified) fH domains 18-20 in such a molecule could result in binding to host cells, complement activation and subsequent tissue damage. Mutations in fH domains 19 and 20 that alter binding of fH to polyanions and/or C3b occur in persons with atypical hemolytic uremic syndrome (aHUS).
We created four fH18-20/Fc mutant proteins that incorporated individual aHUS mutations and evaluated them for binding to and complement-dependent killing of gonococci. Prior work had shown that rH 19-20 molecules that displayed one of these mutations, D1119G, was totally incapable of inhibiting native fH-mediated protection of anti-CD59-treated human RBCs from complement-mediated lysis (Ferreira et al, J Immunol, 2009, 182(11):7009). The D1119G mutant (fH18-10 (D1119G)/Fc) showed binding and killing of gonococci comparable to the wild-type fH18-20/Fc. In contrast to the wild-type fH18-20/Fc, fH18-20(D1119G)/Fc did not cause hemolysis of anti-CD59-treated human RBCs and showed less C3 deposition on retinal pigment epithelial cells compared to fH18-20/Fc wt.
These data have identified fH18-20 (D1119G)/Fc as a promising anti-gonococcal immunotherapeutic. Ongoing in vitro studies show less activation of complement on host cells than fH18-20/Fc wt, indicating a favorable safety profile. Several pathogens bind the C-terminus of fH and the utility of fH 18-20(D1119G)/Fc against these microbes merits investigation.

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The factor H binding protein of Neisseria meningitidis interacts with xenosiderophores in vitro

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Neisseria meningitidisis an obligate human pathogen that can cause severe and sometimes fatal septicaemia and meningitis. Meningococcal serogroup B has long been identified as an important cause of disease in many parts of the world; however, vaccine development has been hindered by the biochemistry of its capsular polysaccharide and the difficulties inherent in identifying appropriate surface protein antigens. The use of reverse vaccinology to select antigens suitable for vaccine development led to identification of a number of novel proteins. As a result a multicomponent vaccine, Bexsero, has been proposed, which includes among the others the factor H binding protein (fHbp), a surface anchored lipoprotein previously referred genome-derived neisserial antigen 1870 (GNA1870) or LP20286.

The fHbp is prone to sequence variability, so that three distinct variants of the protein can be recognised in the meningococcal population. Each variant is however able to bind human factor H, a negative regulator of the alternative complement pathway. Such interaction enables the meningococcal cells to evade the innate immunity of the host.

The three dimensional structure of fHbp free or in complex with human factor H showed that this molecule consists of two domains connected by a flexible linker. While the N-terminal domain adopts an elongated 'barrel-like' structure characterized by the presence of long flexible loops and by extensive flexibility, the C-terminal portion of the molecule is arranged in a well defined beta barrel stabilized by a regular network of hydrogen bonds. The structure of the C-terminal domain shows a remarkable similarity to lipocalins. Lipocalins form a wide family of proteins expressed by plants, fungi, bacteria, vertebrates and invertebrates that participate in many biological functions including binding to siderophores, organic chelators with strong affinity for ferric iron known for their capacity to feed microorganisms with this metal.

The structural similarity to lipocalins prompted us to investigate on the possible role of fHbp as siderophore binding protein. In this work we analyzed the affinity of recombinant meningococcal fHbp to different siderophores. Our results show that the protein is able to bind enterobactin in vitro, a feature shared with its horthologue in Neisseria gonorrhoeae. These observations suggest that fHbp could be involved in iron uptake, and for this reason play an important role for bacterial survival in the human plasma.

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