IPNC 2006 Australia

Program and Abstract Book

15th International Pathogenic Neisseria Conference

10 -15 September 2006

Cairns Convention Centre
Queensland Australia

IPNC06

15th international pathogenic neisseria conference

Published for the 15th International Pathogenic Neisseria Conference (IPNC 2006 Australia)

Conference Co-Convenors
Professor John Davies
Professor Michael Jennings

Editor
Janette Sofronidis, Conference Manager

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Welcome to IPNC 2006 Australia!

A warm welcome to the **15th International Pathogenic Neisseria Conference (IPNC 2006 Australia)!**

For the first time, this conference is being held outside of Europe or North America and, for many delegates, it will be their first visit to Australia. We hope that you take the opportunity to explore a little of our beautiful country, as well as enjoy excellent presentations here at the conference.

We would like to thank all those individuals who willingly served on the scientific panels for their advice and help on the program. This has included several people who were unable to attend, yet still gladly offered us their time and services. We are also deeply appreciative of the generous support provided by our Meeting Partners. As a direct result of their support, we have been able to subsidise the travel of many younger researchers, who otherwise would have found it difficult, if not impossible, to attend and participate in the program. Finally, we would also like to thank our Conference Manager, Janette Sofronidis and her team within the Conference Management Division of the Australian Society for Microbiology – their professional assistance along the way has made our involvement with this conference a pleasure.

We truly look forward to an exciting meeting and exploring the latest findings regarding these two human-specific bacterial pathogens.

Let the tradition continue . . .

John Davies
Michael Jennings
Co-Convenors, IPNC2006 Australia

15th International Pathogenic Neisseria Conference (IPNC 2006, Australia)

Cairns Convention Centre, Queensland Australia 10 - 15 September 2006

CONFERENCE TIMETABLE (printed 30 August 2006)

All workshops & oral presentations will be held in the Plenary Hall, Cairns Convention Centre

Sunday 10 Sept

MEETING OPEN - 11:00am

Open: Registration Desk, Speaker Preparation Room & Internet Café
(11:00am - 6:00pm)
* *Collect name badge & delegate satchel*
* *Submit oral presentations to technicians*

3:30 - 6:00 Set-Up Posters

3:30 - 5:30 WORKSHOP:
Meningococcal Vaccines for Africa and Beyond
Chair: David Stephens
Co-Chair: Robert Booy

Workshop sponsored by an Educational Grant from GlaxoSmithKline Biologicals

Monday 11 Sept

8:00 - 5:30 Open
* *Registration Desk*
* *Speaker Prep Room*
* *Internet Café*

8:00 ARRIVAL TEA / COFFEE

8:00 - 10:10 Continue Poster Set-Up

8:30 - 10:10 **S01: Bacterial Genetics, Physiology & Metabolism: Part 1**
Chair: Jeff Cole
* Elizabeth Stohl
* Hong Wan
* Tonje Davidsen
* Susan Grogan
* Jeff Cole

10:10 - 10:40 *MORNING TEA BREAK*
POSTER VIEWING OPPORTUNITY

10:40 - 12:00 **S02: Bacterial Genetics, Physiology & Metabolism: Part 2**
Chair: Jeff Cole
* Stuart Hill
* Joseph Dillard
* Alastair McEwan
* Virginia Clark

12:00 - 2:00 *LUNCH BREAK*
POSTER VIEWING OPPORTUNITY

2:00 - 3:40 **S03: Surface Structures**
Chair: Charlene Kahler
* Simon Kroll
* Martine Bos
* Seetha Balasingham
* Vladimir Pelicic
* Robert Nicholas

3:40 - 4:10 *AFTERNOON TEA BREAK*
POSTER VIEWING OPPORTUNITY

4:10 - 5:30 **S04: Antibiotic Resistance**
Chair: William Shafer
* Jason Folster
* Marilyn Roberts
* Marcin Kadlubowski
* John Tapsall

Tuesday 12 Sept

8:00 - 5:30 Open
* *Registration Desk*
* *Speaker Preparation Room*
* *Internet Café*

8:00 ARRIVAL TEA / COFFEE

8:30 - 10:10 **S05: Genomics & Gene Expression: Part 1**
Chair: Caroline Genco
* Xavier Nassif
* JR Mellin
* Yih-Ling Tzeng
* Eun Hee Lee
* Deborah Tobiason

10:40 - 12:00 **S06: Genomics & Gene Expression: Part 2**
Chair: Caroline Genco
* Cindy Arvidson
* Michael Jennings

S07: Cellular Microbiology: Part 1
Chair: Xavier Nassif
* Darryl Hill
* Thomas Rudel

2:00 - 3:40 **S08: Cellular Microbiology: Part 2**
Chair: Xavier Nassif
* Michael Apicella
* Sarika Agarwal
* Jennifer Edwards
* Jo-Anne Dillon
* Sarah Follows

4:10 - 5:30 **S09: Epidemiology**
Chair: Dominique Caugant
* Ana Belen Ibraz Pavon
* Robert Booy
* Peter Beernink
* Carina Brehony

Wed 13 Sept

Free Day
Own Activities
-
Conference venue closed

Thursday 14 Sept

8:00 - 5:30 Open
* *Registration Desk*
* *Speaker Preparation Room*
* *Internet Café*

ARRIVAL TEA / COFFEE

S10: Vaccinology: Part 1
Chair: David Stephens
* Jane O'Hallahan
* Rino Rappuoli
* Dominic Kelly
* Ray Borrow
* Matthew Snape

MORNING TEA BREAK

S11: Vaccinology: Part 2
Chair: David Stephens
* Jamie Findlow
* Gregory Moe
* Jan Poolman
* Joyce Plested

LUNCH BREAK

S12: Vaccinology: Part 3
Chair: David Stephens
* Victoria Davenport
* Andrew Cox
* Henry Shinefield
* Duzhang Zhu
* Germie van den Dobbelsteen

AFTERNOON TEA BREAK

S13: Vaccinology: Part 4
Chair: David Stephens
* Steven Black
* Diana Martin
* Gunnstein Norheim
* Tom Mendum

LATE START / Friday 15 Sept

8:30 - 10:50 Open
* *Reg Desk* * *Speaker Preparation Rm*
8:30 - 12:15 Open
* *Internet Café*

8:30 ARRIVAL TEA / COFFEE

9:00 - 10:20 **S14: Host Response: Part 1**
Chair: Lee Wetzler
* Jo Anne Welsch
* Heather MacLeod
* Tie Chen
* Laura Plant

10:20 - 10:50 *MORNING TEA BREAK*

10:50 - 11:50 **S15: Host Response: Part 2**
Chair: Lee Wetzler
* Daniel Stein
* Jutamas Ngampasutadol
* Alison Criss

11:50 - 12:00 Closing Comments

MEETING CLOSE - 12:00pm

Evening Functions

Welcome Reception
6:00pm - 8:00pm
Outdoor Plaza
Cairns Convention Centre

Welcome Reception is sponsored by Novartis Vaccines

International Collaboration on Gonococci
Chair: John Tapsall
5:30pm - 7:30pm
Plenary Hall
Cairns Convention Centre

Poster Discussion Session
7:30pm - 10:30pm
Poster Display Hall
Cairns Convention Centre

10:30pm - All posters to be removed (uncollected posters will be discarded)

Conference Dinner & Dance
7:00pm - 11:30pm
Cairns Convention Centre

CONTENTS

Meeting Partners

IPNC 2006 Australia and its organisers gratefully acknowledge the generous support provided by the following companies

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(including Internet Café Sponsor, Welcome Reception Sponsor, Local Signage Sponsor)

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Searching for a vaccine

History of the International Pathogenic Neisseria Conferences

In the 1970's a series of conferences were held dealing with issues of meningococcal epidemiology and vaccination.

Some of these conferences were held in Milano, St. Paul de Vence and Marseille. However the first *official* Neisseria conference was held in San Francisco, California, 1978.

1st International Pathogenic Neisseria Conference
1978, San Francisco, California, USA
Chair: GF Brooks

2nd International Pathogenic Neisseria Conference
1980, Hemavan, Sweden
Chairs: S Normark and D Danielsson

3rd International Pathogenic Neisseria Conference
1982, Montreal, Canada
Chair: I WDeVoe

4th International Pathogenic Neisseria Conference
1984, Asilomar; California, USA
Chair: GK Schoolnik

5th International Pathogenic Neisseria Conference
1986, Noordwijkerhout, The Netherlands
Chair: JT Poolman

6th International Pathogenic Neisseria Conference
1988, Pine Mountaine, Georgia, USA
Chair: SA Morse

7th International Pathogenic Neisseria Conference
1990, Berlin, Germany
Chair: M Achtman

8th International Pathogenic Neisseria Conference
1992, Cuernavaca, Mexico
Chair: CI Conde-Glez

9th International Pathogenic Neisseria Conference
1994, Winchester, England
Chair: MCJ Maiden and I Feavers

10th International Pathogenic Neisseria Conference
1996, Baltimore, Maryland, USA
Chair: CE Frasch

11th International Pathogenic Neisseria Conference
1998, Nice, France
Chair: X Nassif

12th International Pathogenic Neisseria Conference
2000, Galveston, Texas, USA
Chair: F Sparling and P Rice

13th International Pathogenic Conference
2002, Oslo, Norway
Chair: E Wedege

14th International Pathogenic Conference
2004, Milwaukee, Wisconsin, USA
Co-Convenors: MA Apicella and H Seifert

15th International Pathogenic Neisseria Conference

10 - 15 September 2006

Cairns Convention Centre

Cairns, North Queensland, Australia

Co-Convenors: John K Davies and Michael P Jennings

Conference Co-Convenors

Professor John K Davies
Department of Microbiology
Monash University, Clayton, Victoria, Australia
Email: John.Davies@med.monash.edu.au

Professor Michael P Jennings
School of Molecular & Microbial Sciences
University of Queensland, St Lucia, Queensland, Australia
Email: jennings@uq.edu.au

Abstract Review Committees

Travel Award Review Committee
- **William Shafer *(Chair)*** Emory University, USA
- **Charlene Kahler** University of Western Australia, WA Australia
- **Ian Peak** Griffith University, QLD Australia
- **John Davies** Monash University, VIC Australia (IPNC 2006 Co-Convenor)

International Collaboration on Gonococci
- **John Tapsall *(Session Chair)*** University of New South Wales, NSW Australia

Epidemiology
- **Dominique Caugant *(Session Chair)*** National Institute of Public Health, Norway
- **John Tapsall** University of New South Wales, NSW Australia
- **Cathy Ison** Health Protection Agency, UK
- **Robert Booy** National Centre for Immunisation Research & Surveillance, Australia

Antibiotic Resistance
- **William Shafer *(Session Chair)*** Emory University, USA
- **Rob Nicholas** University of North Carolina, USA
- **Jo-Anne Dillon** University of Saskatchewan, Canada

Vaccinology
- **David Stephens *(Session Chair)*** Emory University, USA
- **Dan Granoff** Children's Hospital Oakland Research Institute, USA
- **Rino Rappuoli** Novartis Vaccines, Italy
- **Martine Bos** Utrecht University, The Netherlands
- **Andrew Pollard** University of Oxford, UK
- **Jan Poolman** GlaxoSmithKline Biologicals, Belgium
- **Milan Blake** Food and Drug Administration, USA
- **Remy Teyssou** Sanofi Pasteur, France

Genomics and Gene Expression
- **Caroline Genco *(Session Chair)*** Boston University, USA
- **Nigel Saunders** University of Oxford, UK
- **Cindy Arvidson** Michigan State University, USA

Cellular Microbiology
- **Mumtaz Virji *(Session Chair)*** University of Bristol, UK
- **Jos van Putten** Utrecht University, The Netherlands
- **Maggie So** Oregon Health Sciences University, USA
- **Tie Chen** University of Illinois at Chicago, USA
- **Michael Apicella** University of Iowa, USA
- **Xavier Nassif** Hôpital Necker-Enfants Malades, France
- **Ann-Beth Jonsson** University of Uppsala, Sweden

Bacterial Genetics, Physiology and Metabolism
- **Jeff Cole *(Session Chair)*** University of Birmingham, UK
- **Cynthia Cornelissen** Virginia Commonwealth University, USA
- **Joe Dillard** University of Wisconsin, USA
- **Stuart Hill** University of Northern Illinois, USA
- **Hank Seifert** Northwestern University, USA
- **Tone Tonjum** University of Oslo, Norway

Host Response
- **Lee Wetzler *(Session Chair)*** Boston University, USA
- **Ann Jerse** Uniformed Services University of the Health Sciences, USA
- **Thomas Rudel** Max-Planck Institut für Infektionsbiologie, Germany
- **Dlawer Ala'Aldeen** University Hospital, Nottingham UK

Surface Structures
- **Charlene Kahler *(Session Chair)*** University of Western Australia, WA Australia
- **Tony Schryvers** University of Calgary, Canada
- **Simon Kroll** Imperial College, UK
- **Katie Forest** University of Wisconsin, USA
- **Peter van der Ley** Netherlands Vaccine Institute, The Netherlands

Conference Management

The Australian Society for Microbiology
bringing Microbiologists together

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Melbourne Victoria 3004 Australia

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Conference Manager - Janette Sofronidis
Email: janette@theasm.com.au
Mobile: 0402 428 842

Registration Services - Meg Lukies
Email: meg@theasm.com.au
Mobile: 0423 007 144

Conference Venue

The Cairns Convention Centre is a world class meetings venue in the tropical paradise of Cairns, fringed by the Great Barrier Reef and World Heritage Rainforest. Cairns is in the very heart of the Asia Pacific region and its International Airport makes it the most convenient of convention locations.

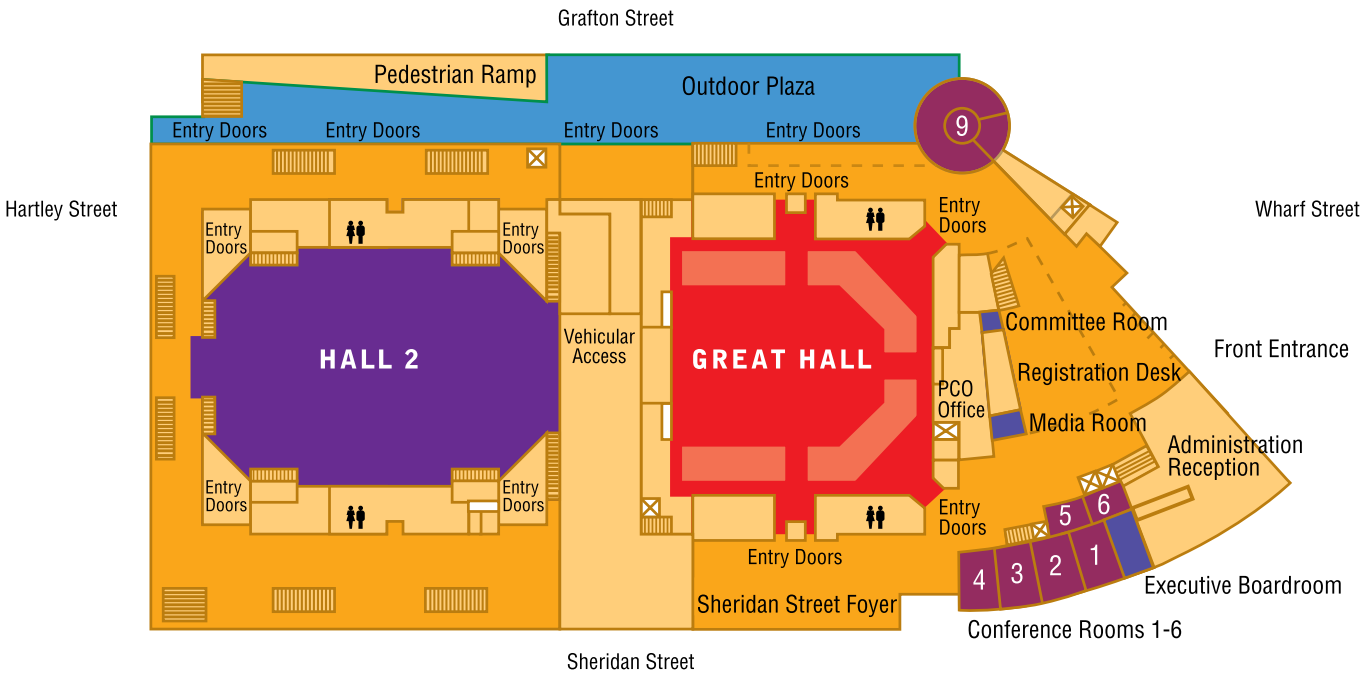
The Centre's expert team regularly receives acclaim from highly satisfied clients and all of the best industry surveys.

Cairns Convention Centre
Corner Wharf & Sheridan Streets
Cairns, Queensland, Australia 4870
Tel: +61 7 4042 4200
Fax: +61 7 4052 1152
Email: sales@cairnsconvention.com.au

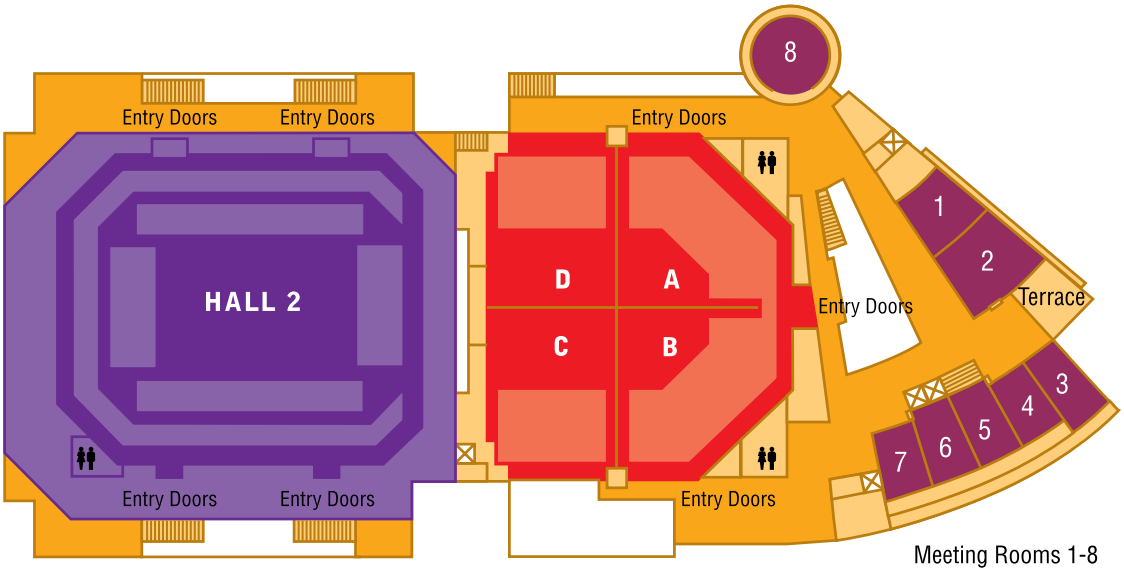
Image courtesy of Queensland Tourism.

Conference Venue Floor Plan

Exhibition Level



Mezzanine Level



Program Topic Outline

Refer to the **Detailed Scientific Program** that is printed in this book for information on presenting authors, their presentation times and titles; the **Conference Timetable** (distributed separately) for further information on the oral and poster programs; and the **Addendum** for any last minute program and speaker changes.

Sunday 10 September 2006

11.00am	Meeting Opens
3.30pm	Workshop: Meningococcal Vaccines for Africa and Beyond (sponsored by an Educational Grant from GlaxoSmithKline Biologicals)
6.00pm	Welcome Reception

Monday 11 September 2006

8.30am	Bacterial Genetics, Physiology & Metabolism
2.00pm	Surface Structures
4.10pm	Antibiotic Resistance
5.30pm	International Collaboration on Gonococci

Tuesday 12 September 2006

8.30am	Genomics & Gene Expression
11.20am	Cellular Microbiology
4.10pm	Epidemiology
7.30pm	Poster Discussion Session

Wednesday 13 September 2006

Free day, own activities – conference venue closed

Thursday 14 September 2006

All Day	Vaccinology
7.00pm	Conference Dinner & Dance

Friday 15 September 2006

9.00am	Host Response
11.50am	Closing Comments
12.00pm	Meeting Close

IMPORTANT INFORMATION FOR ORAL PRESENTERS

Speaker Preparation Area

On arrival at the conference you are required to visit the Speaker Preparation Area so that technicians can check your presentation and load it onto the server. Preferably you should do this as soon as you've collected your name badge from the Registration Desk. However if that's not possible or there's a queue, you can try again later - ***but not leaving it any later than either the day before or at least 4 hours before you are scheduled to speak.***

The Speaker Preparation Area will be located to the side of the main foyer area of the Cairns Convention Centre and will be open during the following hours:

Sunday 10 September	11.00am to 6.00pm
Monday 11 September	8.00am to 5.30pm
Tuesday 12 September	8.00am to 5.30pm
Wednesday 13 September	*Closed*
Thursday 14 September	8.00am to 5.30pm
Friday 15 September	8.30am to 10.50am

You should refer to the scheduling information emailed to you by the conference organisers for your exact scheduling and presentation time details.

Audio Visual Equipment

The meeting room will be set up for data projection using **Windows for PC based software** and will be supported by a professional a/v technician. You will be able to use your laptop in the Speaker Preparation Area but not in the meeting room. Please also note that slide projectors, overhead projectors and VCRs are not available. (Please let the conference organisers know as soon as possible if you will require any other form of a/v equipment.)

Please avoid having to make changes or modifications to your presentation when onsite.

If your presentation is in a platform other than Windows for PC based, please ensure that you either save it for Windows or convert it **prior** to your arrival at the conference. Also, if you have movie files within your presentation, please remember to bring these additional files, plus your presentation, with you and to let the conference organisers know if you require sound.

The International Collaboration on Gonococci – ICG

Monday 11 September 2006
5.30pm – 7.30pm
Cairns Convention Centre

This is an informal group with a special interest in optimising laboratory contributions to the control of gonorrhoea.

It had an early focus on surveillance of antimicrobial resistance in *Neisseria gonorrhoeae,* and this subsequently expanded to include interests in laboratory methods for confirmation of gonococcal infection and systems for typing gonococci.

The IPNC provides an opportunity for less-formal discussions amongst ICG members on these and other related topics.

At this conference the ICG group will revisit the practical applications of gonococcal typing with input from **Jo-Anne Dillon, Margaret Bash** and **Magnus Unemo.** Additionally **David Whiley** will offer thoughts on the status of nucleic acid amplification assays for the diagnosis of gonorrhoea and a review of some recent data on antimicrobial resistance **(Sunil Sethi)** and its implications will be provided **(John Tapsall). Ming Min Liao** will provide a demonstration of the latest version of the ICG website and **Lai King Ng** will expand on the use of the ICG web based discussion tool.

The meeting is open, and any attending conference delegates with an interest in the above matters are most welcome to attend – pre-registration is not required.

Sunday Workshop Meningococcal Vaccines in Africa and Beyond

Sunday 10 September 2006
3.30pm – 5.30pm
Cairns Convention Centre

There is no additional fee to attend this workshop – all delegates with a genuine interest in the subject topic are welcome to attend.

Workshop is sponsored by an Educational Grant from GlaxoSmithKline Biologicals

Poster Discussion Session

Tuesday 12 September 2006
7.30pm – 10.30pm

All scientific posters will be displayed in the Poster Display Hall of the Cairns Convention Centre.

Delegates will have the opportunity to view posters at their leisure on Monday & Tuesday during the morning & afternoon tea and lunch catering breaks. Delegates should use these times to identify posters of interest in preparation for the informal discussions with presenting authors that will take place during Tuesday evening's Poster Discussion Session.

Note: All posters must be removed by their presenting authors at the end of Tuesday evening's Poster Discussion Session.

During the Poster Discussion Session, there will be 3 main discussion periods – this has been provided so that presenting authors can also roam the poster displays and discuss the work that is being presented by other presenting authors who are in the same category.

Times for the 3 main discussion periods are:
From 8.00pm to 8.45pm
From 8.45pm to 9.30pm
From 9.30pm to 10.15pm

Presenting authors should refer to the information provided to them by the conference organisers for the times when they need to be standing next to their poster for informal discussions.

Important Information for Poster Presenters
All posters must be original work and the presenting author will be required to attend the Poster Discussion Session on Tuesday evening (12 Sept) to discuss their poster during informal discussions.

During their allocated discussion period, presenting authors are required to stand next to their poster to be available for informal discussions.

Set Up
It is a requirement for poster participation in the conference, that posters be on continuous display in the Poster Display Hall from the Morning Tea break on Monday (11 Sept) until the end of the Poster Discussion Session on Tuesday (12 Sept) evening.

Accordingly, posters should be set-up either on Sunday (10 Sept) between 3.30pm - 6.00pm or on Monday morning (11 Sept) between 8.00am – 10.10am.

Presenters should supply their own:

- Velcro dots/strips (Blu Tac or similar won't be appropriate), thumb tacs or push pins to attach their poster to their allocated poster board
- Approx 60 handouts placed inside a clear plastic sleeve that is attached (using velcro dots, etc) to the foot of their poster board

Removal
All posters must be removed **at the end** of the Poster Discussion Session on Tuesday evening (12 Sept). It will be the presenter's responsibility to remove their poster and there is no guarantee that uncollected posters will be available post-conference.

Social Program

Welcome Reception
Sunday 10 September 2006
6.00pm – 8.00pm
Cairns Convention Centre

Enjoy an evening on the outdoor plaza of the Cairns Convention Centre at sunset to mix with old acquaintances and make new ones! An excellent networking opportunity.

Entertainment and refreshments provided. Admission includes all food being served and standard beverages (beer, wine & soft drinks).

Admission
- Included with all Full and Student Registrations (name badges required)
- Additional Tickets:
  - Adult AU$70
  - Child (4 - 14 yrs) AU$25
    (Additional tickets must be pre-purchased)

Poster Discussion Session
Tuesday 12 September 2006
7.30pm - 10.30pm
Cairns Convention Centre, Poster Display Hall

An evening dedicated to poster review and informal discussions with poster presenting authors. Opportunity is available for all posters to be previewed (without presenting authors) at leisure during the catering breaks on Monday 11 Sept and Tuesday 12 Sept.

Entertainment and refreshments provided. Admission includes all food being served and standard beverages (beer, wine & soft drinks).

Admission
- Included with all Full and Student Registrations (name badges required)
- Additional Tickets:
  - Adult AU$70
  - Child N/A (function not suitable for children)
    (Additional tickets must be pre-purchased)

Conference Dinner & Dance
Thursday 14 September 2006
7.00pm - 11.30pm
Cairns Convention Centre, Dinner Hall

After a fine gourmet dinner and fine Australian wine, kick up your heels and dance the night away!! Always a fun night!

Admission includes all food being served and standard beverages (beer, wine & soft drinks).

Admission
- Included with all Full and Student Registrations (name badges required)
- Additional Tickets:
  - Adult AU$125
  - Child (4 - 14 yrs) AU$55
    (Additional tickets must be pre-purchased)

Conference Hotels

Country Comfort Sunlodge Cairns
Cnr Florence & Abbott Street
Cairns QLD 4870
Tel: +61 7 4051 5733
Fax: +61 7 4031 2298

Il Centro Apartments Suites
26 Sheridan Street
Cairns QLD 4870
Tel: +61 7 4031 6699
Fax: +61 7 4031 6777

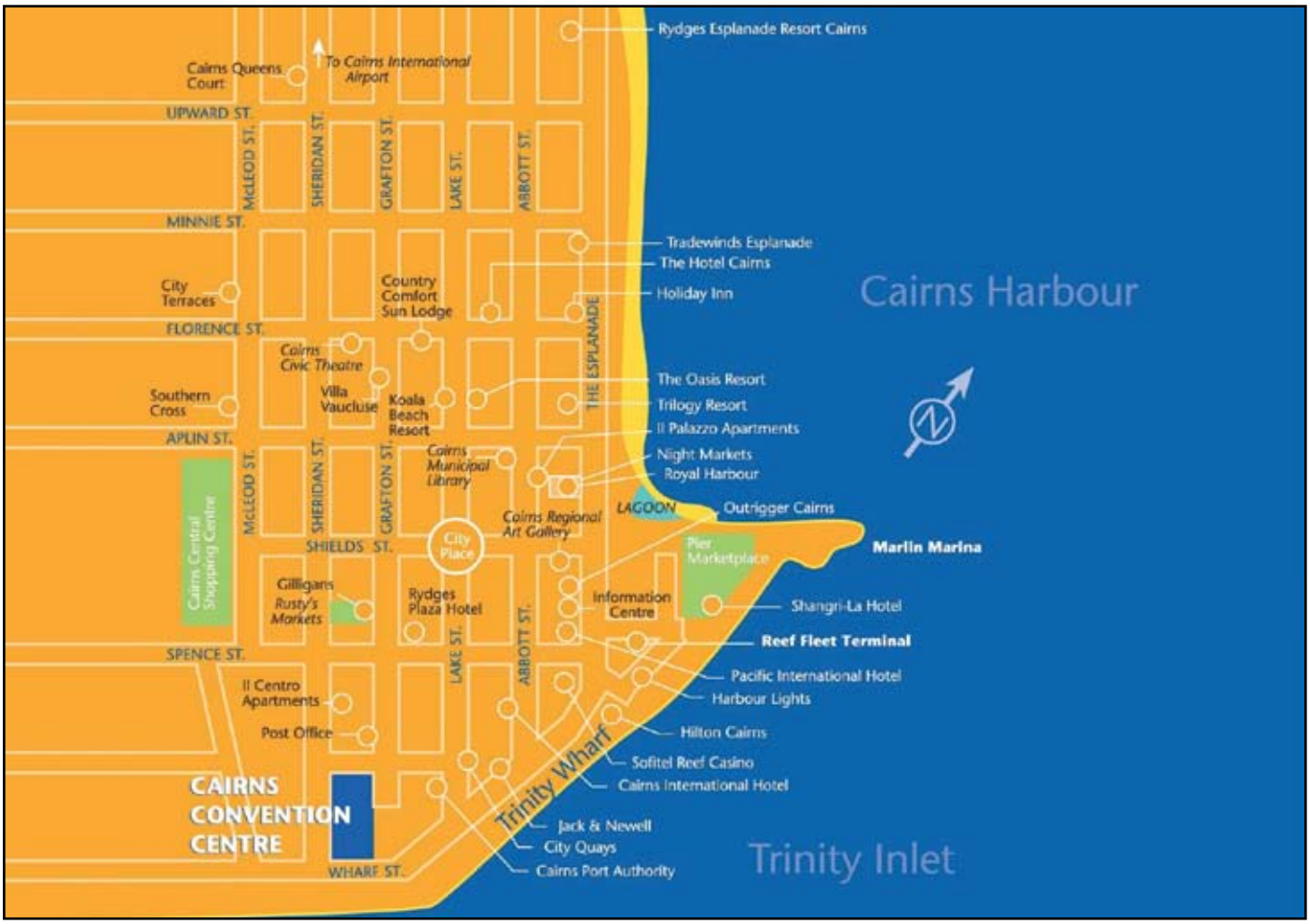
Oaks City Quays
6 Lake Street
Cairns QLD 4870
Tel: 1300 655 319
Fax: +61 7 4031 7227

Oaks Piermonde Hotel
3 – 4 Lake Street
Cairns QLD 4870
Tel: +61 7 4042 6500
Fax: +61 7 4041 6026

Shangri La Hotel, The Marina Cairns
Pier Point Road
Cairns QLD 4870
Tel: +61 7 4031 1411
Fax: +61 7 4031 3226

Rydges Tradewinds Cairns
137 The Esplanade
Cairns QLD 4870
Tel: +61 7 4053 0300
Fax: +61 7 4051 8649

Rydges Plaza Hotel Cairns
Cnr Grafton & Spence Street
Cairns QLD 4870
Tel: +61 7 4041 1022
Fax: +61 7 4041 1033



General Information

Admittance & Name Badges

Registrants are required to collect their name badge from the Registration & Information Desk located in the main foyer of the Cairns Convention Centre immediately upon arrival at the venue. Name badges must be worn at all times to gain entry to scientific & social sessions. For security purposes, the conference organisers reserve the right to ask venue security to escort from the venue any persons not wearing an official conference name badge.

Business Centre / Novartis Vaccines Internet Cafe

Neither the Cairns Convention Centre or the conference will have a Business Centre for delegate use. However the **Novartis Vaccines IPNC 2006 Internet Cafe** will provide delegates with free internet access and is located near the main foyer of the venue.

Messages

Registration Desk Tel: +61 7 4042 4300

Messages for delegates attending the conference can be left with staff at the conference Registration & Information Desk. Messages will be posted to an adjoining Message Board and it will be the responsibility of delegates attending the conference to check for messages on a regular basis. There is no guarantee that messages will be delivered personally.

People with Special Needs

Every effort will be made to ensure that people who have special needs are adequately catered for.

Smoking Policy

The Cairns Convention Centre and other venues/areas of the conference are non-smoking areas.

What to Wear

Smart casual attire will be suitable for all scientific sessions and social events.

Catering

All morning/afternoon teas and lunches will be served in the main foyer area of the conference venue. Refer to the Social Program for other catering information.

General Terms & Conditions / Disclaimer

The conference organisers & the Local Organising Committee will not be responsible for registrations and/or abstract submissions not received whether via the conference website due to Internet Service outages, hardware or software delays, power outages or natural disasters or via another format. It will be the registrant's own responsibility to ensure that an official confirmation of registration/abstract submission is received from the conference organisers.

As a condition of registration and attendance, the conference organisers & the Local Organising Committee will not be held responsible for damages of any nature sustained by participants or their guests, visitors or accompanying persons for personal injury or loss or damage to their personal property as a result of activities associated with the running of the conference or its related events. Participants and attendees should make their own arrangements with regard to appropriate personal and property insurance.
Attendees to the conference should arrange their own medical, travel and personal insurance as the conference organisers & the Local Organising Committee will not be responsible for claims by attendees. Insurance should be purchased in your state/country of origin. It is strongly recommended that at the time of submitting your registration and making your travel arrangements that you also take out an appropriate insurance policy/s.

Image courtesy of Queensland Tourism.

Detailed Scientific Program

ORAL PRESENTATION PROGRAM

15th International Pathogenic Neisseria Conference 2006 (IPNC 2006 Australia)

As at 31 July 2006; Refer to the Addendum for any program updates

S01

0830 - 1010 Monday 11 September

Bacterial Genetics, Physiology & Metabolism - Part 1

Room: Plenary Hall (Halls A+B)

Chair: Jeff Cole, University Of Birmingham, BIRMINGHAM, UNITED KINGDOM

S01.1 0830 0850 **Neisseria gonorrhoeae RecA: Protein Biochemistry and Antioxidant Function**
Dr. Elizabeth Stohl
Northwestern University Feinberg School Of Medicine, CHICAGO IL USA

S01.2 0850 0910 **New Perspectives of Meningococcal Disease Revealed by in vivo Bioluminescence Imaging**
Dr Sjölinder Hong
Uppsala University, UPPSALA SWEDEN

S01.3 0910 0930 **Role of DNA Repair in Meningococcal Genome Dynamics**
Ms Tonje Davidsen
University Of Oslo, OSLO NORWAY

S01.4 0930 0950 **Characterization of a Neisseria gonorrhoeae strain F62 fur mutant and novel genes under Fur control**
Dr Susan Grogan
Boston University Medical School, BOSTON MA USA

S01.5 0950 1010 **Indirect Sensing of Nitrite by the Nitric Oxide-Sensitive Repressor, NsrR, Mediates Co-regulation of Genes in the FNR and NarQ-NarP Regulons Essential for Adaptation of Neisseria gonorrhoeae to Oxygen-Limited Growth**
Professor Jeff Cole
University Of Birmingham, BIRMINGHAM UNITED KINGDOM

S02

1040 - 1200 Monday 11 September

Bacterial Genetics, Physiology & Metabolism - Part 2

Room: Plenary Hall (Halls A+B)

Chair: Jeff Cole, University Of Birmingham, BIRMINGHAM, UNITED KINGDOM

S02.1 1040 1100 **pilE mRNA Processing Reveals a Potential Micro RNA Regulatory Mechanism for Transcript Stability**
Dr Stuart Hill
Northern Illinois University, DEKALB ILLINOIS USA

S02.2 1100 1120 **Characterizing DNA Donation in Neisseria gonorrhoeae: Autolysis, Allolysis, and Type IV Secretion**
Dr Joseph Dillard
University Of Wisconsin, MADISON WI USA

S02.3 1120 1140 **Manganese-Dependent Defense Against Oxidative Stress in Neisseria gonorrhoeae**
Prof Alastair McEwan
University Of Queensland, BRISBANE QLD AUSTRALIA

S02.4 1140 1200 **Regulation of gonococcal nitric oxide reductase (norB/norZ) by NsrR, an Rrf2 family transcriptional repressor**
Dr. Virginia Clark
University Of Rochester, ROCHESTER NEW YORK USA

S03

1400 - 1540 Monday 11 September

Surface Structures

Room: Plenary Hall (Halls A+B)

Chair: Charlene Kahler, University Of Western Australia, NEDLANDS, AUSTRALIA

S03.1 1400 1420 **Exploring the Role of a TPR-encoding Meningococcal Gene in Bacterial Adherence to Human Epithelial Cells**
Professor Simon Kroll
Imperial College London, LONDON UNITED KINGDOM

S03.2 1420 1440 **Identification of Lipopolysaccharide Transport Components in Neisseria meningitidis**
Dr Martine Bos
Utrecht University, UTRECHT NETHERLANDS

S03.3 1440 1500 **Meningococcal Lipoprotein PilP: its structure and role in pilus biogenesis and transformation**
Dr Seetha Balasingham
University Of Oslo, OSLO OSLO NORWAY

S03.4 1500 1520 **Definition, by a Systematic Analysis in Neisseria meningitidis, of the Pil Proteins that are Required for the Assembly, Functionality, Stabilization and Export of Type IV Pili**
Mr Vladimir Pelicic
Filiale de L'institut National de la Sante et la Rechercvhe Meducale, PARIS PARIS FRANCE

S03.5 1520 1540 **Characterization of Intragenic and Intergenic Mutations that Disrupt Antibiotic Influx Through the PilQ Secretin in Neisseria gonorrhoeae**
Dr. Robert Nicholas
University Of North Carolina At Chapel Hill, CHAPEL HILL NORTH CAROLINA USA

ORAL PRESENTATION PROGRAM

15th International Pathogenic Neisseria Conference 2006 (IPNC 2006 Australia)

As at 31 July 2006; Refer to the Addendum for any program updates

S04

1610 - 1730 Monday 11 September

Antibiotic Resistance

Room: Plenary Hall (Halls A+B)

Chair: William Shafer, Emory University School Of Medicine, ATLANTA, USA

S04.1 1610 1630 **Differential Regulation of ponA and pilM Expression by the Gonococcal MtrR Transcriptional Regulatory Protein**
Dr Jason Folster
Emory University, DECATUR GA USA

S04.2 1630 1650 **Characterization of High-level Erythromycin-Azithromycin Multidrug Resistant Neisseria gonorrhoeae and Therapy Failure**
Prof Marilyn Roberts
University Of Washington, SEATTLE WA USA

S04.3 1650 1710 **Correlation between the penA Gene Sequence and Penicillin Susceptibility of Polish Isolates of Neisseria meningitidis**
Mr Marcin Kadlubowski
National Reference Centre For Bacterial Meningitis, WARSAW POLAND

S04.4 1710 1730 **Antimicrobial Resistance in N. gonorrhoeae in Australia: Results of 25 years of Continuous, Standardised National Surveillance**
Prof John Tapsall
SEALS, RANDWICK NSW AUSTRALIA

S05

0830 - 1010 Tuesday 12 September

Genomics & Gene Expression - Part 1

Room: Plenary Hall (Halls A+B)

Chair: Caroline Genco, Boston University

S05.1 0830 0850 **A chromosomally integrated bacteriophage in invasive meningococci**
Prof Xavier Nassif
Inserm U570, PARIS FRANCE

S05.2 0850 0910 **Identification of a Novel, Fur-Regulated Small RNA in Neisseria meningitidis Serogroup B**
Mr. J.R. Mellin
Boston University, BOSTON MA USA

S05.3 0910 0930 **The MisRS Two-Component Regulon in Neisseria meningitidis**
Dr. Yih-Ling Tzeng
Emory University School Of Medicine, ATLANTA GA USA

S05.4 0930 0950 **The Role of Integration Host Factor in the Regulation of Genes Involved in Transport System Used by Neisseria gonorrhoeae**
Dr. Eun Hee Lee
Emory University, ATLANTA GA USA

S05.5 0950 1010 **Neisseria gonorrhoeae is Polyploid**
Dr. Deborah Tobiason
Northwestern University, CHICAGO IL USA

S06

1040 - 1120 Tuesday 12 September

Genomics & Gene Expression - Part 2

Room: Plenary Hall (Halls A+B)

Chair: Caroline Genco, Boston University

S06.1 1040 1100 **Sublethal Concentrations of Ciprofloxacin Induces Genes Implicated in Horizontal Gene Transfer in Neisseria gonorrhoeae**
Dr Cindy Arvidson
Michigan State University, EAST LANSING MI USA

S06.2 1100 1120 **Defining the phase variable regulons 'phasevarions" of pathogenic Neisseria.**
Prof Michael Jennings
University Of Queensland, BRISBANE QLD AUSTRALIA

S07

1120 - 1200 Tuesday 12 September

Cellular Microbiology - Part 1

Room: Plenary Hall (Halls A+B)

Chair: Mumtaz Virji, University Of Bristol, BRISTOL, UNITED KINGDOM

S07.1 1120 1140 **Structural Analysis of a CEACAM-Binding Recombinant Polypeptide that Inhibits the Interactions of Neisseria meningitidis and Neisseria gonorrhoeae with Human Target Cells**
Dr Darryl Hill
University Of Bristol, BRISTOL BRISTOL UNITED KINGDOM

S07.2 1140 1200 **Identification of a new receptor for PorBIA of neisseria gonorrhoeae**
Mr Dr Thomas Rudel
Max Planck Institute For Infection Biology, BERLIN GERMANY

S08

1400 - 1540 Tuesday 12 September

Cellular Microbiology - Part 2

Room: Plenary Hall (Halls A+B)

Chair: Mumtaz Virji, University Of Bristol, BRISTOL, UNITED KINGDOM

S08.1 1400 1420 **Biofilm Formation by Neisseria gonorrhoeae during Cervical Infection**
Dr. Michael Apicella
University Of Iowa, IOWA CITY IA USA

S08.2 1420 1440 **Role of Factor H and Complement Receptor 3 in Mediating Attachment of Neisseria gonorrhoeae to Epithelial Cells**
Dr. Sarika Agarwal
University Of Massachusetts, WORCESTER MA USA

S08.3 1440 1500 **Hormonal Modulation of CR3-mediated Neisseria gonorrhoeae Infection of Primary, Human, Cervical Epithelial Cells**
Dr. Jennifer Edwards
Columbus Children's Research Institute, COLUMBUS OHIO USA

S08.4 1500 1520 **To Divide or Not to Divide: Cell Division Proteins in Neisseria gonorrhoeae and their Roles in Dynamic Localization and the Formation of a Bacterial Cytoskeleton**
Dr Jo-Anne Dillon
University Of Saskatchewan, SASKATOON SASKATCHEWAN CANADA

S08.5 1520 1540 **Human Cervical Epithelial Cells are Resistant to Apoptosis upon Infection with Live Neisseria gonorrhoeae.**
Ms. Sarah Follows
Boston University, BOSTON MA USA

S09

1610 - 1730 Tuesday 12 September

Epidemiology

Room: Plenary Hall (Halls A+B)

Chair: Dominique Caugant, Norwegian Institute of Public Health, OSLO, NORWAY

S09.1 1610 1630 **Geographical and Temporal Distribution of Meningococcal Genotypes Isolated from 53,234 Healthy Teenage Carriers in the United Kingdom Collected Between 1999 and 2001**
Miss Ana Belen Ibarz Pavon
University Of Oxford, OXFORD UNITED KINGDOM

S09.2 1630 1650 **Outcome and medical follow up in adolescent survivors of meningococcal disease: a prospective matched cohort study**
Prof Robert Booy
National Centre For Immunisation Research, WESTMEAD NSW AUSTRALIA

S09.3 1650 1710 **Prevalence of NadA and Factor H-Binding Protein (GNA1870) Variants in Neisseria meningitidis Group B Isolates from California and Maryland, USA: Implications for Design of a Multicomponent Group B Vaccine**
Dr. Peter Beernink
Children's Hospital Oakland Research Institute, OAKLAND CA USA

S09.4 1710 1730 **Distribution of Hyper-invasive lineages of Neisseria meningitidis in Europe over 3 years**
Miss Carina Brehony
University Of Oxford, OXFORD OXFORDSHIRE UNITED KINGDOM

S10

0830 - 1010 Thursday 14 September

Vaccinology - Part 1

Room: Plenary Hall (Halls A+B)

Chair: David Stephens, Emory University School Of Medicine, ATLANTA, USA

S10.1 0830 0850 **An epidemic of Group B Meningococcal disease controlled by a vaccine: The final chapter**
Dr Jane O'Hallahan
Ministry Of Health, WELLINGTON NEW ZEALAND

S10.2 0850 0910 **A Universal Vaccine for Serogroup B Meningococcus**
Dr Rino Rappuoli
Novartis Vaccines, SIENA ITALY

S10.3 0910 0930 **Serogroup C Meningococcal Polysaccharide-Specific Plasma and Memory B-cells after Immunisation of Infants with a Glycoconjugate Vaccine**
Dr Dominic Kelly
University Of Oxford, OXFORD OXFORDSHIRE UNITED KINGDOM

S10.4 0930 0950 **An Update on Meningococcal Group C Conjugate Vaccination Programme in England and Wales: Vaccine Efficacy, Antibody Persistence and Serological Correlates**
Dr Ray Borrow
Health Protection Agency, MANCHESTER UNITED KINGDOM

S10.5 0950 1010 **Immunogenicity and Safety in infancy of a Novel Tetravalent Meningococcal Glyco-conjugate Vaccine**
Dr Matthew Snape
Oxford Vaccine Group, OXFORD OXFORDSHIRE UNITED KINGDOM

S11

1040 - 1200 Thursday 14 September

Vaccinology - Part 2

Room: Plenary Hall (Halls A+B)

Chair: David Stephens, Emory University School Of Medicine, ATLANTA, USA

S11.1 1040 1100 **Comparison and correlation of Neisseria meningitidis serogroup B immunological assays used in a study of the 'Norwegian' meningococcal outer membrane vesicle vaccine, MenBvac**
Dr Jamie Findlow
Health Protection Agency, MANCHESTER UNITED KINGDOM

S11.2 1100 1120 **Non-Autoreactive Anti-N-Propionyl N. meningtidis group B Polysaccharide (N-Pr NmB PS) mAbs Bind to Membrane Proteins of NmB and E. coli K1 Bacteria**
Dr. Gregory Moe
Children's Hospital Oakland Research Institute, OAKLAND CA USA

S11.3 1120 1140 **Serogroup B Neisseria Meningitidis Vaccine Development**
Dr Jan Poolman
GlaxoSmithKline Biologicals, RIXENSART BELGIUM

S11.4 1140 1200 **Opsonophagocytosis in the Absence of Serum Bactericidal Activity Confers Protection against Meningococcal Disease**
Dr Joyce Plested
Children's Hospital Oakland Research Institute, SAN FRANCISCO CA USA

S12

1400 - 1540 Thursday 14 September

Vaccinology - Part 3

Room: Plenary Hall (Halls A+B)

Chair: David Stephens, Emory University School Of Medicine, ATLANTA, USA

S12.1 1400 1420 **Vaccination Re-Programmes Naturally Acquired Mucosal Immunity Against Neisseria Meningitidis**
Dr Victoria Davenport
University Of West Of England, BRISTOL UNITED KINGDOM

S12.2 1420 1440 **Investigating the potential of LPS-based vaccines to protect against meningococcal disease**
Dr Andrew Cox
Inst For Biological Sciences National Research Council, OTTAWA ON CANADA

S12.3 1440 1500 **Safety and Immunogenicity of a Tetravalent Meningococcal ACWY Glycoconjugate vaccine in Toddlers 12-23 Months of Age**
Dr Henry Shinefield
University Of California San Francisco, SAN FRANCISCO CALIFORNIA USA

S12.4 1500 1520 **Effective Immunization Strategy Against Group B Neisseria meningitidis Using Purified Recombinant Lipidated P2086 Protein**
Dr. Duzhang Zhu
Wyeth Vaccines Research, PEARL RIVER NY USA

S12.5 1520 1540 **Preclinical Immunogenicity of a 9 valent PorA OMV/13 valent Pneumococcal Conjugate Combination Vaccine**
Dr Germie van den Dobbelsteen
Netherlands Vaccine Institute, BILTHOVEN NETHERLANDS

S13

1610 - 1730 Thursday 14 September

Vaccinology - Part 4

Room: Plenary Hall (Halls A+B)

Chair: David Stephens, Emory University School Of Medicine, ATLANTA, USA

S13.1 1610 1630 **Safety and Immunogenicity of a Tetravalent Meningococcal ACWY Glycoconjugate Vaccine in Healthy Children 2-10 Years of Age**
Dr. Steven Black
Kaiser Permanente Vaccine Study Center, OAKLAND CALIFORNIA USA

S13.2 1630 1650 **How do we Determine a Biologically Meaningful Protective Antibody Level**
Dr Diana Martin
Institute Of Environmental Science And Research, PORIRUA NEW ZEALAND

S13.3 1650 1710 **Serological Response to Epidemic Serogroup A ST-7 Meningococcal Meningitis in Ethiopians**
Mr Gunnstein Norheim
Norwegian Institute Of Public Health, OSLO NORWAY

S13.4 1710 1730 **Investigating the 2D Immunoproteome of Neisseria meningitidis**
Dr Tom Mendum
University Of Surrey, GUILDFORD SURREY UNITED KINGDOM

S14

0900 - 1020 Friday 15 September

Host Response - Part 1

Room: Plenary Hall (Halls A+B)

Chair: Lee Wetzler, Boston University School Of Medicine, BOSTON, USA

S14.1 0900 0920 **A Novel Mechanism for Complement-mediated Killing of Encapsulated Neisseria meningitidis Elicited by Monoclonal Antibodies to Factor H-binding Protein (Genome-derived Neisserial antigen 1870)**
Dr Jo Anne Welsch
Children's Hospital Oakland Research Institute, OAKLAND CA USA

S14.2 0920 0940 **Differential Use of MAPKS by N. Meningitidis PorB and Los in Costimulatory Molecule Expression and Cytokine Production**
Ms. Heather MacLeod
Boston University, BOSTON MA USA

S14.3 0940 1000 **DC-SIGN (CD209) Recognition of Neisseria gonorrhoeae is Circumvented by Lipooligosaccharide Variation**
Dr. Tie Chen
University Of Illinois At Chicago (UIC), ROCKFORD ILLINOIS USA

S14.4 1000 1020 **Non-Lipoligosaccharide Mediated Signalling through TLR4 causes Meningococcal Sepsis via the MyD88 Dependent Pathway**
Dr Laura Plant
Karolinska Institutet, STOCKHOLM SWEDEN

S15

1050 - 1150 Friday 15 September

Host Response - Part 2

Room: Plenary Hall (Halls A+B)

Chair: Lee Wetzler, Boston University School Of Medicine, BOSTON, USA

S15.1 1050 1110 **Role of H8 and LOS in Gonococcal Macrophage Interactions.**
Dr Daniel Stein
University Of Maryland, COLLEGE PARK MARYLAND USA

S15.2 1110 1130 **Serum Resistance of Neisseria gonorrhoeae is Restricted to Humans; A Possible Explanation for the Species Specificity of Gonococcal Infections**
Dr. Jutamas Ngampasutadol
University Of Massachusetts Medical School, WORCESTER MA USA

S15.3 1130 1150 **Killing of Neisseria gonorrhoeae by Human Polymorphonuclear Leukocytes Occurs Non-Oxidatively**
Dr Alison Criss
Northwestern University, CHICAGO IL USA

Image courtesy of Queensland Tourism.

POSTER DISCUSSION SESSION

15th International Pathogenic Neisseria Conference 2006 (IPNC 2006 Australia)

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P01

Antibiotic Resistance

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P1.1.01 **Quinolone-Resistant Neisseria gonorrhoeae (QRNG) Surveillance: Integrating Molecular Resistance Testing into Clinical Care**
Margaret Bash
Center For Biologics Evaluation And Research, ROCKVILLE MD USA

P1.1.02 **The MtrE Outer Membrane Protein and Not the TolC-like Protein of Neisseria meningitidis Is Used by the Mtr Efflux System**
Nazia Kamal
Emory University, DECATUR GA USA

P1.1.03 **Antimicrobial Susceptibility of Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae Isolates Causing Meningitis at the Far East of Russia in 2003-2004**
Alina Martynova
Epidemiology Department, VLADIVOSTOK RUSSIA

P1.1.04 **Vancomycin Reaches its Site of Action in the Periplasm by Diffusing Through the Gonococcal PilQ Pore**
Robert Nicholas
University Of North Carolina At Chapel Hill, CHAPEL HILL NORTH CAROLINA USA

The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P1.2.02 **Cost-effective Sampling of Pharyngeal Meningococci to Predict Antimicrobial Resistance in Invasive N. meningitidis: a Ten-Year Study**
John Tapsall
SEALS, RANDWICK NSW AUSTRALIA

P1.2.03 **Total Variation in the penA Gene of Neisseria meningitidis. Correlation Between Susceptibility to b-lactam Antibiotics and penA Gene Heterogeneity.**
Magnus Unemo
Örebro University Hospital, ÖREBRO SWEDEN

P1.2.04 **Rapid and Sensitive Identification of Reduced Susceptibility to Penicillins in Neisseria meningitidis by Real-time PCR and Pyrosequencing technology**
Magnus Unemo
Örebro University Hospital, ÖREBRO SWEDEN

The Discussion Session for the following posters is from:
2130 - 2215 Tuesday 12 September

P1.3.01 **Real-Time Multiplex PCR Assay Detecting penA and ponA Genotypes In Neisseria gonorrhoeae**
Frédérique Vernel-Pauillac
Institut Pasteur, NOUMÉA CEDEX NEW CALEDONIA

P1.3.02 **Is the Mosaic penA Sequence Solely Responsible for Decreased Ceftriaxone Susceptibility in Neisseria gonorrhoeae?**
David Whiley
Queensland Paediatric Infectious Diseases Laboratory, HERSTON QLD AUSTRALIA

P1.3.03 **Antimicrobial Susceptibility and Molecular Determinants of Quinolone Resistance in Neisseria gonorrhoeae Isolates from Shanghai**
Yang Yang
Shanghai Skin Disease And STD Hospital, SHANGHAI CHINA

P02

Bacterial Genetics, Physiology & Metabolism

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P2.1.01 **Gonococcal AHU Strains Establish a Nitric Oxide Steady State Level Within an Anti-inflammatory Range**
Kenneth Barth
University Of Rochester, ROCHESTER NEW YORK USA

P2.1.02 **The Gonococcal Genetic Island Provides a Bypass Mechanism to TonB-Dependent Intracellular Survival of Neisseria gonorrhoeae within Cervical Epithelial Cells**
Cynthia Cornelissen
Virginia Commonwealth University Medical Center, RICHMOND VIRGINIA USA

P2.1.03 **Identification and Characterization of Three Neisseria gonorrhoeae-Specific Gene Clusters that are of Putative Bacteriophage Origin**
Mary Fantacone
Michigan State University, EAST LANSING MICHIGAN USA

P2.1.04 **A TlpA Homologue from Neisseria gonorrhoeae Plays a Role in Defense against Oxidative Stress**
Amanda Hamilton
University Of Queensland, ST LUCIA QLD AUSTRALIA

P2.1.05 **Detection of Two Genetic Elements Associated with Increased Incidence of Serogroup C Neisseria meningitidis Infection and Antigenic Shift in the United States during the 1990s by Representational Difference Analysis (RDA)**
Lee Harrison
University Of Pittsburgh, PITTSBURGH PA USA

P2.1.06 **PilQ Missense Mutations Distinguish Multimer Stability, DNA Transformation, and Pilus Expression from Host-Cell Adherence and Colony Morphology in N. gonorrhoeae**
Allen Helm
Northwestern University, CHICAGO IL USA

P2.1.07 **Natural transformation in Neisseria lactamica**
Paul Langford
Imperial College, LONDON UNITED KINGDOM

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The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P2.2.01 **Construction and characterisation of a nitrite reductase-deficient mutant of Neisseria meningitidis**
Jay Laver
University Of Sheffield, SHEFFIELD SOUTH YORKSHIRE UNITED KINGDOM

P2.2.02 **Nucleotide Excision Repair in Neisseria Gonorrhoeae**
Brian LeCuyer
Northwestern University Feinberg School Of Medicine, CHICAGO IL USA

P2.2.03 **The MntABC Transport System of Neisseria gonorrhoeae**
Karen Lim
University Of Queensland, ST LUCIA QLD AUSTRALIA

P2.2.04 **Gonococcal Biofilms and Membrane Blebbing**
Brock Neil
University Of Iowa, IOWA CITY IOWA USA

P2.2.05 **Identification of an Autotoxin Gene in Neisseria Meningitidis**
Jane Newcombe
University Of Surrey, GUILDFORD SURREY UNITED KINGDOM

P2.2.06 **Auxotype Classifications of Neisseria gonorrhoeae Represent the Overall Genetic Content of Individual Strains**
Lai King Ng
Canadian Science Centre For Human And Animal Health, WINNIPEG MB CANADA

P2.2.07 **Molecular Typing of N. Gonorrhoeae Using Clinical Specimens for PCR Diagnosis**
Lai King Ng
Canadian Science Centre For Human And Animal Health, WINNIPEG MB CANADA

The Discussion Session for the following posters is from:
2130 - 2215 Tuesday 12 September

P2.3.01 **The NmlR Defence Systems Against Oxidative Stress in Neisseria gonorrhoeae and Neisseria meningitidis**
Adam Potter
University Of Queensland, ST LUCIA QLD AUSTRALIA

P2.3.02 **Characterisation of Periplasmic Oxidoreductase DsbA3 of Neisseria meningitidis**
Jessica Scoullar
University Of Western Australia, NEDLANDS WA AUSTRALIA

P2.3.03 **Regulation of Denitrification in Neisseria meningitidis by Nitric Oxide and Repressor NsrR**
Melanie Thomson
University Of York, HESLINGTON YORKSHIRE UNITED KINGDOM

P2.3.04 **Regulation of c-type Cytochromes of Neisseria gonorrhoeae**
Nicholas Tovell
Birmingham University, BIRMINGHAM ENGLAND UNITED KINGDOM

P2.3.05 **The Use of Microarray in Assessing the Role of IHF in Neisseria meningitidis Gene Regulation**
Sally Turner
Monash University, MONASH UNIVERSITY VIC AUSTRALIA

P2.3.06 **The aerobic respiratory chain of Neisseria gonorrhoeae**
Rachel van den Hoven
University Of Queensland, ST LUCIA QLD AUSTRALIA

P2.3.07 **Characterization of N. meningitidis (Serogroup B) Wild-Type and Isogenic Knock Out Mutants by Proteomic Analysis**
Daniele Veggi
Novartis Vaccines & Diagnostics, SIENA ITALY

P03

Cellular Microbiology

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P3.1.01 **Neisseria gonorrhoeae Escape from Cervical Epithelial Cells to Promote Bacterial Persistence**
Samuel Bish
Unversity Of Maryland, SILVER SPRING MD USA

P3.1.02 **The genetic structure and diversity of human CEACAM genes: implications for Opa function and susceptibility to invasive meningococcal disease.**
Martin Callaghan
University Of Oxford, OXFORD OXON UNITED KINGDOM

The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P3.2.01 **Uncommon CEACAM Targets of Respiratory Pathogens**
Darryl Hill
University Of Bristol, BRISTOL BRISTOL UNITED KINGDOM

P3.2.02 **The Roles of Meningococcal OPA, OPC and PILI in Modulating T Cell Proliferation and Function and the Effect of Recombinant CEACAM-Binding Bacterial Adhesion Blocking Peptides**
Darryl Hill
University Of Bristol, BRISTOL BRISTOL UNITED KINGDOM

The Discussion Session for the following posters is from:
2130 - 2215 Tuesday 12 September

P3.3.01 **Opa Negative Gonococci Possess Enhanced Virulence**
Daniel Stein
University Of Maryland, COLLEGE PARK MARYLAND USA

P3.3.02 **Search for meningococcal DNA binding proteins involved in transformation**
Afsaneh Vahdani-Benam
University Of Oslo, OSLO NORWAY

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P3.3.03 **Using CD46 Cyt1 and Cyt2 Monoclonal Antibodies to Study CD46 During Infection of Epithelial Cells with Neisseria gonorrhoea**
Nathan Weyand
Oregon Health And Science University, PORTLAND OREGON USA

P3.3.04 **T-cell Stimulating Protein A of Neisseria meningitidis is required for Optimal Adhesion to Human Cells**
Karl Wooldridge
University Of Nottingham, NOTTIGHAM UNITED KINGDOM

P04

Epidemiology

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P4.1.01 **Epidemiological Study of Neisseria gonorrhoeae Isolates by Auxotyping and Serotyping**
Kuhulika Bhalla
Jamia Milia Islamia University, NEW DELHI DELHI INDIA

P4.1.02 **Research up in a puff of smoke- are we asking the right questions?**
Robert Booy
National Centre For Immunisation Research, WESTMEAD NSW AUSTRALIA

P4.1.03 **Meningococcal Disease Epidemiology Update – United States, 2005**
Thomas Clark
Centers For Disease Control And Prevention, ATLANTA GEORGIA USA

P4.1.04 **Surveillance of Meningococcal Disease in New Zealand**
Heather Davies
Institute Of Environmental Science And Research, PORIRUA NEW ZEALAND

P4.1.05 **Antigenic expression of LPS from Brazilian meningococcal strains, production of monoclonal antibodies as subsidy to epidemiological studies**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P4.1.06 **Genotypic Characterisation of Non-invasive Neisseria meningitidis in Scotland 1997 - 2004**
Mathew Diggle
Greater Glasgow Health Board, GLASGOW STRATHCLYDE UNITED KINGDOM

P4.1.07 **Global Incidence and Case Fatality Rates of Meningococcal Disease due to Different Neisseria meningitidis Serogroups**
Martha Doemland
Sanofi Pasteur, SWIFTWATER PA USA

P4.1.08 **Investigation of the Basis for Persistent Neisseria gonorrhoeae Porin VR Types in Community Infections**
Lotisha Garvin
Uniformed Services University Of The Health Sciences, BETHESDA MD USA

The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P4.2.01 **Risk Factors for Meningococcal Disease in U.S. High School Students**
Lee Harrison
University Of Pittsburgh, PITTSBURGH PA USA

P4.2.02 **Integrated Bacteriophage and Invasive and Carriage Isolates of Neisseria meningitidis**
Rhonda Hobb
Emory University, DECATUR GEORGIA USA

P4.2.03 **The Epidemiology and Surveillance of Meningococcal Disease in England and Wales since 1995**
Edward Kaczmarski
Health Protection Agency, MANCHESTER UNITED KINGDOM

P4.2.04 **Surveillance of Serogroup Y Meningococcal Infection in England and Wales: Characterising a Recent Increase in Cases**
Edward Kaczmarski
Health Protection Agency, MANCHESTER UNITED KINGDOM

P4.2.05 **Clonal Analysis of Neisseria meningitidis Serogroup C Responsible for Invasive Disease in Poland**
Marcin Kadlubowski
National Reference Centre For Bacterial Meningitis, WARSAW POLAND

P4.2.06 **Epidemiological study of gonococcal isolates based on diversity of the opa gene**
Pejvak Khaki
Maulana Azad Medical College - Delhi University, NEW DELHI DELHI INDIA

P4.2.08 **Genetic Diversity of porB for 174 Neisseria gonorrhoeae Isolates from Shanghai: Impact on Molecular Epidemiology and Antimicrobial Resistance**
Mingmin Liao
Vaccine & Infectious Disease Organization, SASKATOON SASKATCHEWAN CANADA

P4.2.09 **Epidemiology of Neisserria meningitis Infection at the Far East of Russia**
Alina Martynova
Epidemiology Department, VLADIVOSTOK RUSSIA

P4.2.10 **Invasive Meningococcal Disease in Italian Children and Adolescents**
Paola Mastrantonio
Istituto Superiore Di Sanità, ROME LAZIO ITALY

The Discussion Session for the following posters is from:
2130 - 2215 Tuesday 12 September

P4.3.01 **Distribution of multi-locus sequence types of N. meningitidis serogroup B in the US: 2000-2005**
Leonard Mayer
Centers For Disease Control & Prevention, ATLANTA GEORGIA USA

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P4.3.02 **Neisseria meningitidis PorA Subtype Distribution in the South of Brasil**
Leonard Mayer
Centers For Disease Control & Prevention, ATLANTA GEORGIA USA

P4.3.03 **Carriage of serogroup A meningococci on the peak of an NmA epidemic in Burkina Faso**
Berthe-Marie Njanpop-Lafourcade
Agence De Médecine Préventive, PARIS CEDEX 15 FRANCE

P4.3.04 **Variability in Meningococcal Serogroups Causing Invasive Infection in Children in Canada,2002-2005**
David Scheifele
BC Children's Hospital/ University Of British Columbia, VANCOUVER BRITISH COLUMBIA CANADA

P4.3.05 **Changes in Serogroup Distribution of Agents of Invasive Meningococcal Disease in Australia Following a Selective Publicly-Funded Programme of Vaccination with Serogroup C Conjugate Vaccine**
Helen Smith
Queensland Health Scientific Services, COOPERS PLAINS QLD AUSTRALIA

P4.3.06 **Neisseria meningitidis: Carriage in Africa**
Montse Soriano-Gabarro
GSK Biologicals, RIXENSART BELGIUM

P4.3.07 **Survival of Meningococci Outside of the Host: Implications for Acquisition**
Claire Swain
Environmental Science & Research, PORIRUA NEW ZEALAND

P4.3.08 **Intact-cell MALDI-TOF Mass-spectrometry for Identification and Subtyping of Pathogenic Neisseria**
Vladimir Vereshchagin
Institute Of Phisico-Chemical Medicine, MOSCOW RUSSIA

P4.3.09 **W135 meningococccal disease: an emerging problem in Singapore related to the Hajj pilgrimage**
Annelies Wilder-Smith
Travellers' Health & Vaccination Centre, SINGAPORE

P4.3.10 **Meningococcal Meningitis in China: Status in 2005**
Zun Dong Yin
Chinese Center For Diseases Control & Prevention, BEIJING CHINA

P05

Genomics & Gene Expression

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P5.1.01 **The Neisserial DNA Uptake Sequence Revisited: Locational Bias and Quantitation of Effect on Transformation**
Ole Herman Ambur
University Of Oslo, OSLO NORWAY

P5.1.02 **Overexpression of GNA1870, a conserved surface-exposed protein, in group B Neisseria meningitidis and in E. coli.**
Mikhail Donets
Walter Reed Army Institute Of Research (WRAIR), SILVER SPRING MD USA

P5.1.03 **Transcriptional Profiling of Neisseria gonorrhoeae Biofilm**
Megan Falsetta
University Of Iowa, IOWA CITY IOWA USA

P5.1.04 **A mutation in the gonococcal pilT has a global influence on the Transcriptome of Neisseria gonorrhoeae**
Alexandra Friedrich
Max Planck Institute For Infection Biology, BERLIN GERMANY

P5.1.05 **Transcriptome of Neisseria meningitidis serogroup B grown in Human Plasma - an Ex Vivo Model of Infection**
Asa Hedman
Imperial College London, LONDON UNITED KINGDOM

The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P5.2.01 **MtrA Regulation of Gene Expression in Neisseria gonorrhoeae**
Paul Johnson
Emory University, DECATUR GA USA

P5.2.02 **The Attenuation of Meningococcal NMB1966 Mutant and its Transcriptome analysis**
Ming-Shi Li
Imperial College London, LONDON UNITED KINGDOM

P5.2.03 **Regulatory network involved in the human nasopharyngeal colonization by Neisseria meningitidis**
Patricia Martin
Inserm, PARIS FRANCE

P5.2.04 **The Gonococcal Ferric Uptake Regulator (Fur) and Fur-dependent Genes are Induced During Experimental Gonococcal Genital Tract Infection of Female Mice**
Mathanraj Packiam
Uniformed Services University, ROCKVILLE MARYLAND USA

P5.2.05 **A Leading/Lagging Strand Bias in Homopolymeric Tracts and the Development of a GFP Reporter Construct for the Analysis of Phase Variation**
Peter Power
University Of Oxford, OXFORD OXON UNITED KINGDOM

The Discussion Session for the following posters is from:
2130 - 2215 Tuesday 12 September

P5.3.01 **Transcriptional Regulation of the pilE Gene of Neisseria gonorrhoeae**
Catherine Ryan
Monash University, VERMONT SOUTH VIC AUSTRALIA

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P5.3.02 **Comparative Genomics of Pathogenic and Apathogenic Meningococci**
Christoph Schoen
Insitut For Hygiene And Microbiology, WÜRZBURG GERMANY

P5.3.03 **Defining the Phasevarion of Pathogenic Neisseria**
Yogitha Srikhanta
University Of Queensland, ST LUCIA QLD AUSTRALIA

P5.3.04 **A Type I Protein Secretion System Is Controlled by the MisR/MisS Two-Component Regulatory System in Neisseria meningitidis**
Yih-Ling Tzeng
Emory University School Of Medicine, ATLANTA GA USA

P5.3.05 **Mutation of MtrR and MtrA, Transcriptional Regulators of the MtrCDE Efflux Pump System, Alters the Fitness of Neisseria gonorrhoeae for Experimental Genital Tract Infection**
Douglas Warner
Uniformed Services University Of The Health Sciences, BETHESDA MARYLAND USA

P5.3.06 **Proteomic Analysis of Manganese Regulation of Neisseria gonorrhoeae**
Hsing-Ju (Hilda) Wu
Core Facilities For Proteomics Research, TAIPEI TAIWAN

P06

Host Response

The Discussion Session for the following posters is from:
2000 - 2045 Tuesday 12 September

P6.1.01 **Macrophage responses to C-reactive protein-opsonised Neisseria meningitidis**
Rosalyn Casey
University Of Surrey, GUILDFORD SURREY UNITED KINGDOM

P6.1.02 **Glial and Neuronal Responses to Meningococcal Meningitis**
Cesilie Castellanos
Rikshospitalet-Radiumshospitalet Medical Center, OSLO NORWAY

P6.1.03 **The inflammatory response of human meningeal cells to Neisseriae and the role of neuropeptides**
Myron Christodoulides
University Of Southampton, SOUTHAMPTON HAMPSHIRE UNITED KINGDOM

P6.1.04 **Role of nitric oxide in cellular damage to human Fallopian tubes following infection with Neisseria gonorrhoeae.**
Myron Christodoulides
University Of Southampton, SOUTHAMPTON HAMPSHIRE UNITED KINGDOM

P6.1.05 **Gonococcal Lipooligosaccharide Phosphoethanolamine Substitutions Modulate Serum Resistance and C4bp Binding**
Lisa Lewis
University Of Massachusetts Medical School, WORCESTER MA USA

P6.1.06 **Neisserial Lipooligosaccharide and Complement Activation: Contrasting Effects of Heptose II Substitutions in Gonococci and Meningococci**
Lisa Lewis
University Of Massachusetts Medical School, WORCESTER MA USA

P6.1.07 **Induction of an Inflammatory Response but not a Humoral Response During Experimental Gonococcal Genital Tract Infection of Estradiol-treated Mice**
Ann Jerse
Uniformed Services University, BETHESDA MD USA

P6.1.08 **Neisseria gonorrhoeae Induces Cyclin Down Regulation and an early G1 Arrest in Human Epithelial Cells**
Allison Jones
Uppsala University, UPPSALA SWEDEN

The Discussion Session for the following posters is from:
2045 - 2130 Tuesday 12 September

P6.2.01 **Differential Responses of Human Dendritic Cells to Live and Killed Neisseria meningitidis**
Hannah Jones
Institute Of Child Health, LONDON UNITED KINGDOM

P6.2.02 **Use of monoclonal Antibodies on the lipopolysaccharide (LPS) antigen selection from epidemic strain of Neisseria meningitidis B: nasal immunization in rabbits**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P6.2.03 **Use of monoclonal antibodies in the selection of LPS from the epidemic strain B:4:P1.15 of Neisseria meningitidis B: Nasal immunization in mice**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P6.2.04 **The Anti-apoptotic Effect of Meningococcal PorB Does Not Require TLR2-mediated NF-êB Activation**
Paola Massari
Boston University School Of Medicine, BOSTON MA USA

P6.2.05 **A Novel Interaction between Factor H SCR 6 and the Meningococcal Vaccine Candidate GNA1870: Implications for Meningococcal Pathogenesis and Vaccine Development**
Sanjay Ram
University Of Massachusetts, WORCESTER MA USA

P6.2.06 **Differential Gene Expression After Infection of Neisseria Meningitidis in Host Cerebrovascular Endothelial and Respiratory Epithelial Cell Lines**
Andrew Rogers
University Of Nottingham, NOTTINGHAM UNITED KINGDOM

P6.2.07 **Different Forms of Penta-acylated Meningococcal LPS have Distinct Biological Activity**
Peter van der Ley
Netherlands Vaccine Institute, BILTHOVEN NETHERLANDS

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P6.2.08 **Unravelling the Human CD4+ T cell Response to Neisserial PorA Antigens: Selective Processing and Presentation of Epitopes caused by Serosubtype Variation**
Cecile van Els
Netherlands Vaccine Institute, BILTHOVEN NETHERLANDS

P6.2.09 **Different Regulation of B Cell Responses against Strongly and Weakly Immunogenic PorA antigens**
Cecile van Els
Netherlands Vaccine Institute, BILTHOVEN NETHERLANDS

The Discussion Session for the following posters is from:
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P6.3.01 **Mucosal Immunity to Neisseria lactamica and the Development of Cross-reactive T-cell Memory to Neisseria meningitidis**
Andrew Vaughan
University Of Bristol, BRISTOL AVON UNITED KINGDOM

P6.3.02 **Effect of Meningococcal Carriage on Human Humoral Immune Response**
Jeannette Williams
University Of Southampton, SOUTHAMPTON HAMPSHIRE UNITED KINGDOM

P6.3.03 **Dynamics of Human Humoral Immune Response to Serogroup B Neisseria meningitidis Infection: Analysis of Pre-infection, Acute and Convalescent Sera from the same individual**
Jeannette Williams
University Of Southampton, SOUTHAMPTON HAMPSHIRE UNITED KINGDOM

P6.3.04 **Effect of Mannose-binding lectin on epithelial cell responses to Neisseria gonorrhoeae**
James Wing
University Of Sheffield, SHEFFIELD UNITED KINGDOM

P6.3.05 **Contribution of Catalase, Cytochrome C Peroxidase, Methionine Sulfoxide Reductase, and Manganese Transport in Survival of Neisseria gonorrhoeae during Lower Genital Tract Infection of Female Mice**
Hong Wu
Uniformed Services University, BETHESDA MARYLAND USA

P07

Surface Structures

The Discussion Session for the following posters is from:
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P7.1.01 **Effect of Pilin Glycosylation Alterations on Adhesion Invasion and Transmigration of N. gonorrhoeae**
Asesh Banerjee
New York Medical College, VALHALLA NEW YORK USA

P7.1.02 **Neisseria meningitidis NhhA is a multifunctional trimeric autotransporter adhesin**
Beatrice Arico
Novartis Vaccines & Diagnostics, SIENA ITALY ITALY

P7.1.03 **Human lactoferrin cleaves and inactivates Neisseria meningitidis surface antigens**
Beatrice Arico
Novartis Vaccines & Diagnostics, SIENA ITALY ITALY

P7.1.04 **A Novel Serogroup W135 Strain Causing Disease in New Zealand**
Amanda Beddek
Victoria Univeristy/ESR Ltd, PORIRUA NEW ZEALAND

P7.1.05 **Primary Human Nasopharyngeal Epithelial Cell Receptor Expression and Interactions with Phenotypic Variants of Neisseria meningitidis**
Claudia Cunha
University Of Bristol, BRISTOL UNITED KINGDOM

P7.1.06 **Molecular Interactions of the Meningococcal Opc Further Explored - Identification of á-actinin as an Opc-Binding Protein**
Claudia Cunha
University Of Bristol, BRISTOL UNITED KINGDOM

P7.1.07 **Investigation of the Role of Iron Acquisition Genes in Meningococcal Colonisation of Nasopharyngeal Tissue**
Rachel Exley
Imperial College London, LONDON UNITED KINGDOM

The Discussion Session for the following posters is from:
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P7.2.01 **Meningococcal secretin PilQ: Actions and Interactions**
Stephan Frye
Rikshospitalet-Radiumhospitalet HF, OSLO NORWAY

P7.2.02 **Analysis of Murine Immune Responses to Conserved- and Hypervariable-regions of Neisseria gonorrhoeae Pilin**
Johanna Hansen
University Of Wisconsin, MADISON WI USA

P7.2.03 **Meningococcal Autotransporter Proteins (App and MspA) mediate Adherence to Human Brain Microvascular Endothelial Cells**
David Turner
University Of Nottingham, NOTTINGHAM NOTTINGHAMSHIRE UNITED KINGDOM

P7.2.04 **O-acetylation of the terminal N-acetylglucosamine of the lipooligosaccharide inner core in Neisseria meningitidis: influence on inner core structure and assembly**
Charlene Kahler
University Of Western Australia, NEDLANDS WA AUSTRALIA

P7.2.05 **Hemagglutinin related genes encode novel adhesins of Neisseria meningitidis**
Oliver Kurzai
Institute Of Hygiene And Microbiology, WUERZBURG GERMANY

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P7.2.06 **Simultaneous Mutations in Two Meningococcal dsbA Genes Compromise Transformation**
Paul Langford
Imperial College, LONDON UNITED KINGDOM

P7.2.07 **Liquid Chromatography MS/MS Analysis of the Protein Content of Outer Membranes and Vesicles Derived from MC58 and a Lipopolysaccharide-Deficient Mutant**
Jeannette Williams
University Of Southampton, SOUTHAMPTON HAMPSHIRE UNITED KINGDOM

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P7.3.01 **Crystal Structure of the N-Terminal Domain of Human CEACAM1: Binding Target of the Opacity Proteins During Invasion of Neisseria meningitidis and N. gonorrhoeae**
Robert Nicholas
University Of North Carolina At Chapel Hill, CHAPEL HILL NORTH CAROLINA USA

P7.3.02 **The Neisseria meningitidis Outer Membrane Lipoprotein FrpD Binds the RTX Protein FrpC**
Katerina Prochazkova
Academy Of Sciences Of The Czech Republic, PRAGUE CZECH REPUBLIC

P7.3.03 **Cross Reactivity of Lacto-N-neotretraose Epitopes on N. meningitidis LPS and Human Blood Cells**
Deborah Schmiel
Walter Reed Army Inst. of Res. (WRAIR)/CRM, Inc., SILVER SPRING MD USA

P7.3.04 **Characterization of the Interaction Between Transferrin and Transferrin Binding Protein B**
Stephen Shouldice
University Of Calgary, CALGARY ALBERTA CANADA

P7.3.05 **The Role of Gonococcal PilC in Pathogenesis: Preparation for Experimental Human Challenge**
Christopher Thomas
University Of North Carolina At Chapel Hill, CHAPEL HILL NC USA

P7.3.06 **Involvement of NMB0065 in Translocation of Serogroup B Capsule Polysaccharide in Neisseria meningitidis**
Rhonda Hobb
Emory University, DECATUR GEORGIA USA

P7.3.07 **Characterization of pIII protein of Neisseria gonorrhoeae**
Laura Ciucchi
Novartis Vaccines & Diagnostics, SIENA ITALY ITALY

P7.3.08 **Variation in the Repertoire and Sequence of Lipopolysaccharide Genes of Neisseria meningitidis and the Implications for Vaccine Development**
Judith Claire Wright
University Of Oxford, OXFORD UNITED KINGDOM

P08

Vaccinology

The Discussion Session for the following posters is from:
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P8.1.01 **Secreted Proteins of Neisseria meningitidis Protect Mice against Infection**
Dlawer Ala'Aldeen
University Of Nottingham, NOTTINGHAM UNITED KINGDOM

P8.1.02 **Characterization of LP2086 Expression in Neisseria meningitidis**
Karita Ambrose
Wyeth, PEARL RIVER NY USA

P8.1.03 **Postmarketing Safety of MCV-4**
Roger Baxter
Kaiser Permanente Vaccine Study Center, OAKLAND CA USA

P8.1.04 **NMR-Based Assay for Tracking the Industrial Process and the Structural Characterization of Meningococcal A, C, W135 and Y Conjugate Vaccines**
Francesco Berti
Novartis Vaccines, SIENA ITALY

P8.1.05 **The B Cell Response to a Booster Dose of MenC Vaccine at 1 Year of Age After Three Dose Priming in Infancy**
Geraldine Blanchard
Oxford University, OXFORD OXON UNITED KINGDOM

P8.1.06 **A Phase I, Double Blind, Randomized Study to Evaluate a New Meningococcal Group A Conjugate Vaccine in Healthy Indian Adults**
Ray Borrow
Health Protection Agency, MANCHESTER UNITED KINGDOM

P8.1.07 **Immunogenicity and Reactogenicity of a Combined Haemophilus influenzae type b and Neisseria meningitidis Serogroup C and Y-Tetanus Toxoid Conjugate (Hib-MenCY-TT) Vaccine Administered at 2, 3 and 4 Months and as a Booster dose at Second Year of Life**
Dominique Boutriau
GlaxoSmihtKline Biologicals, RIXENSART BELGIUM

P8.1.08 **A novel DTPw-HBV/Hib-MenAC Conjugate Combination Vaccine Administered to Infants in Northern Ghana is Safe and Induces Immune Memory**
Abraham Hodgson
Navrongo Health Research Centre, NAVRONGO GHANA

P8.1.09 **Preparation and characterization of murine monoclonal antibodies against three antigens of Group B Neisseria meningitidis**
Brunella Brunelli
Novartis Vaccines & Diagnostics, SIENA ITALY

P8.1.10 **Development of a Multiplex Assay using X-MAP Technology for the Evaluation of Protein-Based Meningococcal Vaccines**
Hannah Chan
National Institute For Biological Standards And Control, POTTERS BAR HERTS UNITED KINGDOM

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P8.1.11 **Protection by Immunization with Alphavirus Replicon Particles against Gonorrhea in a female Mouse Model**
Ching-ju Chen
University of North Carolina at Chapel Hill, CHAPEL HILL NORTH CAROLINA USA

P8.1.12 **Investigation of the Protective Potential of Antibodies Directed against Surface-Exposed Opacity Protein Loops of Neisseria gonorrhoeae**
Jessica Cole
USUHS, BETHESDA MD USA

P8.1.13 **Mucosal immunization against meningococci: induction of bactericidal antibodies and cellular immunity in C3H/HeJ and C3H/He Pasteur mice following intranasal immunization with native outer membrane vesicles**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P8.1.14 **Immunogenicity of intranasally administered class 5C protein of Neisseria meningitidis in mice with different adjuvants**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P8.1.15 **Identification of a surface protein in Neisseria commensal strains and evaluation of its immunogenic and protective capacity**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P8.1.16 **Improved purification of native meningococcal porin (Por B) and studies using intranasal route in neonates mouse**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P8.1.17 **Antibody response in rabbits intranasally administered with Neisseria commensal strains**
Elizabeth de Gaspari
Adolfo Lutz Institute, SAO PAULO BRAZIL

P8.1.18 **MenA Natural Immunity and SBA Tests with A:L10 and A:L11 Target Strains**
Isabel De Vleeschauwer
GlaxoSmithKline Biologicals, RIXENSART BELGIUM

P8.1.19 **Natural Immunity to Neisseria meningitidis Serogroup C during the First Year of Life**
Richarda De Voer
RIVM, BILTHOVEN NETHERLANDS

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P8.2.01 **Comparing the Avidity of the IgG response to Neisseria meningitidis Group C Polysaccharide Conjugate Vaccine by Inhibition ELISA or Chaotropic ELISA**
Philip Fernsten
Wyeth Vaccines Research, PEARL RIVER NEW YORK USA

P8.2.02 **Outer Membrane Vesicles Expressing L3,7 or lgtB LOS but not galE LOS Induce Cross-Protection in Mice**
Christiane Feron
GlaxoSmithKline Biologicals, RIXENSART BELGIUM

P8.2.03 **Three cases of invasive meningococcal disease in Burkina Faso caused by a capsule null locus strain circulating among healthy carriers**
Helen Findlow
Health Protection Agency, MANCHESTER UNITED KINGDOM

P8.2.04 **Characterisation of the Antigenic Components and Immune Responses to a Vaccine Based on Neisseria lactamica Outer Membrane Vesicles**
Michelle Finney
Health Protection Agency, SALISBURY WILTS UNITED KINGDOM

P8.2.05 **The Role of PorA and PorB in the Antibody Response to Outer Membrane Vesicle Vaccines Determined by Antibody Binding, Complement Deposition and Opsonophagocytosis assays**
Andrew Gorringe
Health Protection Agency, SALISBURY WILTSHIRE UNITED KINGDOM

P8.2.06 **Human Monoclonal Antibodies with Specificities against Several Epitopes on the Surface of Neisseria Meningitides can be Isolated from Vaccinees using a Novel Recombinant Approach**
Øistein Ihle
Norwegian Institute Of Public Health, OSLO NORWAY

P8.2.07 **Sialylation Plays a Detectable Role in Protecting Neisseria gonorrhoeae from Bactericidal Antibodies Induced by Immunization in vitro but not in vivo**
Ann Jerse
Uniformed Services University, BETHESDA MD USA

P8.2.08 **Serum IgG Response Induced by a Bivalent Recombinant LP2086 Provides Broad Protection against Serogroup B Neisseria meningitidis**
Han-Qing Jiang
Wyeth Research, PEARL RIVER NEW YORK USA

P8.2.09 **Development of a Luminex-based Meningococcal rLP2086-specific Human IgG Assay**
Thomas Jones
Wyeth, PEARL RIVER NY USA

P8.2.10 **Expression of the meningococcal vaccine antigen NadA and the Mycobacterium tuberculosis Acr2 antigen in commensal Neisseria**
Paul Langford
Imperial College, LONDON UNITED KINGDOM

P8.2.11 **Preclinical evaluation of a recombinant protein MenB vaccine with various adjuvants**

Giuliani Marzia Monica
Novartis Vaccines & Diagnostics, SIENA ITALY

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P8.2.12 **Meningococcal vaccine components' diversification in a worldwide strain panel**
Maurizio Comanducci
Novartis Vaccines & Diagnostics, MARBURG GERMANY

P8.2.13 **Systemic and Mucosal Immunogenicity in Healthy Humans Immunized with Meningococcal Serogroup C Conjugate Vaccine MenC/P64k using P64k Recombinant Protein Carrier**
Tamara Menendez
Center For Genetic Engineering And Biotechnology, HAVANA CUBA

P8.2.14 **Molecular Mimetics of Meningococcal Serogroup A and C Capsular Polysaccharides Selected with Human Sera are Able to Elicit Bactericidal Activity against Neisseria meningitidis.**
Tamara Menendez
Center For Genetic Engineering And Biotechnology, HAVANA CUBA

P8.2.15 **Immunization with Molecular Mimetics of Meningococcal Serogroup B Capsular Polysaccharide induces Bactericidal Activity against Neisseria meningitidis**
Tamara Menendez
Center For Genetic Engineering And Biotechnology, HAVANA CUBA

P8.2.16 **Transmission of Maternal Antibodies To Offspring In Response To Immunisation With Glyconjugate Vaccines**
Elena Mori
Novartis Vaccines & Diagnostics, SIENA ITALY

P8.2.17 **Immunogenicity of Fractional Dose Tetravalent A/C/Y/W135 Meningococcal Polysaccharide Vaccine: Results from a Non-inferiority Trial in Uganda**
Lisbeth Meyer Næss
Norwegian Institute Of Public Health, OSLO NORWAY

P8.2.18 **Identification and Characterisation of a T-Cell and B-Cell-Stimulating protein B (TspB) of N. meningitidis**
Karl Wooldridge
University Of Nottingham, NOTTIGHAM UNITED KINGDOM

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P8.3.01 **A New Attractive Method for Refolding and Increasing the Immunogenicity of Recombinant antigens**
Olivia Niebla
Vaccine Division, CIGB, HAVANA CITY HAVANA CITY CUBA

P8.3.02 **The Antibody Response to Recombinant Protein NMB1126 From Neisseria meningitidis with Different Adjuvants**
Olivia Niebla
Vaccine Division, CIGB, HAVANA CITY HAVANA CITY CUBA

P8.3.03 **Plasmid DNA as Adjuvant for Meningococcal Outer Membrane Vesicles**
Olivia Niebla
Vaccine Division, CIGB, HAVANA CITY HAVANA CITY CUBA

P8.3.04 **Immunogenicity of Meningococcal Outer Membrane Vesicles in Neonatal Mice is Influenced by the Route and Schedule of Immunization**
Rolando Pajón
CIGB, CUBANACÁN, PLAYA HAVANA CITY CUBA

P8.3.05 **Detection Of Beta-Barrel Outer Membrane Proteins In Gram-Negative Bacterial Proteomes**
Rolando Pajón
CIGB, CUBANACÁN, PLAYA HAVANA CITY CUBA

P8.3.06 **From current OMV-based vaccines to broadly cross-reactive Recombinant Protein-based Vaccines: challenges and realities on the Cuban strategy**
Rolando Pajón
CIGB, CUBANACÁN, PLAYA HAVANA CITY CUBA

P8.3.07 **The transferrin-supplemented mouse model of meningococcal infection revisited**
Rolando Pajón
CIGB, CUBANACÁN, PLAYA HAVANA CITY CUBA

P8.3.08 **Recombinant Antigen NMB0088 as Vaccine Candidate Against Neisseria meningitidis**
Rolando Pajón
CIGB, CUBANACÁN, PLAYA HAVANA CITY CUBA

P8.3.09 **The Application of Physicochemical Methods of Analysis to the Development of Conjugate Vaccines Against Meningococcal Group A Bacteria**
Neil Ravenscroft
University Of Cape Town, RONDEBOSCH SOUTH AFRICA

P8.3.10 **OxyR and Fur Regulate the Oxidative Stress Response in Neisseria meningitidis**
Kate Seib
Novartis Vaccines & Diagnostics, SIENA ITALY

P8.3.11 **A Sustained Increase in Meningococcal Serogroup C Specific IgG Following Glycoconjugate or Plain Polysaccharide Meningococcal Vaccines in Adolescents Previously Immunised With Serogroup C Meningococcal Glycoconjugate Vaccine.**
Matthew Snape
Oxford Vaccine Group, OXFORD OXFORDSHIRE UNITED KINGDOM

P8.3.12 **The Diversity of Meningococcal Vaccine Components in a Worldwide Strain Panel**
Stefania Bambini
Novartis Vaccines & Diagnostics, SIENA ITALY

P8.3.13 **Construction of a Neisseria gonorrhoeae Vaccine by Fusion of a Peptide Mimic of a Lipooligosaccharide Derived Carbohydrate Epitope with Complement C3d**
Xiaohong Su
University Of Massachusetts Medical School, WORCESTER MA USA

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Presenting Authors will be available for informal discussions in the Poster Display Hall on Tuesday 12 September 2006, as follows.
There will be 3 main Discussion Sessions: From 2000hrs - 2045hrs From 2045hrs - 2130hrs From 2130hrs - 2215hrs

P8.3.14 **Adjuvant Activity of Different TLR Agonists in Combination with LPS-deficient Meningococcal Outer Membrane Complexes**
Peter van der Ley
Netherlands Vaccine Institute, BILTHOVEN NETHERLANDS

P8.3.15 **N. Meningitidis PorB Induces an Antigen-Specific Eosinophil Recall Response: Potential Adjuvant for Helminth Vaccines?**
Lee Wetzler
Boston University School Of Medicine, BOSTON MA USA

P8.3.16 **Combination of Minor Outer Membrane Proteins Inducing Bactericidal Antibodies in Mice**
Vincent Weynants
GlaxoSmithKline Biologicals, RIXENSART BELGIUM

P8.3.17 **Complement Regulatory Proteins Attenuate the Functional Effect of Human Antibody Elicited by an Outer Membrane Derived Vaccine Prototype that Contains 2C7 Epitope Expressing Lipooligosaccharide**
Jutamas Ngampasutadol
University Of Massachusetts Medical School, WORCESTER MA USA

P8.3.18 **Immunisation with live Neisseria lactamica elicits protective immunity against meningococcal challenge**
Qian Zhang
Imperial College London, LONDON UNITED KINGDOM

P8.3.19 **Specificity of Cross-Reactive Bactericidal Antiobodies in Normal and Convalescent Human Sera**
Wendell Zollinger
Walter Reed Army Institute Of Research, SILVER SPRING MARYLAND USA

Image courtesy of Queensland Tourism.

Abstracts Section

S01

Bacterial Genetics, Physiology & Metabolism: Part 1

Monday 11 September 8.30am

Plenary Hall (Halls A+B)

S01.1

Neisseria gonorrhoeae RecA: Protein Biochemistry and Antioxidant Function

Elizabeth A. Stohl[1], Shelley L. Lusetti[2], Michael M. Cox[2], H. Steven Seifert[1]

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RecA is ubiquitous in all organisms and is absolutely required for the cellular processes of DNA transformation, pilus antigenic variation, and DNA repair in *Neisseria gonorrhoeae* (Gc), all of which are critical for the success of Gc as a human pathogen. Gc is naturally competent, and the ability to take up exogenous DNA from the environment and incorporate it into its genome likely contributes to the rapid emergence of antibiotic-resistant strains of Gc. Antigenic variation of the pilus subverts the human acquired immune system, allowing a person to become infected repeatedly and never develop immunity against reinfection. Although Gc *recA* has been known to be important for repair of DNA damaged by UV light, naladixic acid, and γ-irradiation, *recA* was previously reported to not be involved in resistance to oxidative damage, the preponderant type of damage Gc is likely to encounter during human infection. We show that RecA, genes of the RecBCD and RecF-like pathways, and genes encoding Holliday junction processing enzymes confer resistance to hydrogen peroxide, likely by repairing oxidatively damaged DNA through the process of recombinational DNA repair. To explore the mechanisms by which Gc RecA participates in these cellular processes, we purified the Gc RecA and SSB (single-stranded binding) proteins to homogeneity to begin a study of the biochemistry of recombination by Gc. We show that Gc RecA is able to catalyze DNA strand exchange, the *in vitro* measurement of RecA to promote homologous recombination, more efficiently than the *E. coli* RecA protein. The enhanced recombinase activity of Gc RecA reflects the pivotal importance of recombination in Gc genetics and pathogenesis.

S01.2

New Perspectives of Meningococcal Disease Revealed by in vivo Bioluminescence Imaging

Hong Wan and Ann-Beth Jonsson

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Neisseria meningitidis causes severe sepsis and/or meningitis with high mortality. The disease scenario is rapid and much remains unknown about the disease process and host-pathogen interaction *in vivo*. CD46 is a human cell surface complement regulator that interacts with *Neisseria* and several other microbes. Mice expressing human CD46 are susceptible to meningococcal disease and offer a model system to study pathogenesis *in vivo*.

A bioluminescence labeled serogroup C *N. meningitidis* strain FAM20 was constructed in order to monitor meningococcal disease in CD46 transgenic mice. *In vivo* bioluminescence imaging demonstrated that *N. meningitidis* accumulated in the thyroid gland, and succeeded to reach the mucosal surface of the upper respiratory tract after blood invasion of humanized mice. Impaired thyroid function was associated with meningococcal infection. The meningococcal adhesin PilC1 was essential for bacterial translocation from blood to the respiratory mucosal surface, but was dispensable for meningococcal growth in blood and for crossing of the blood-brain barrier. Further, *in vivo* real-time imaging revealed that bacterial signals sometimes decreased to undetectable levels, and then reappeared either as high-level whole body bacteremia or distinct meningitis in mice. Reappearance of bacteria was associated with turn on of the phase-variable Opa proteins, and argues that bacteria are capable of rapid growth in CD46 transgenic mice, in contrast to nontransgenic mice.

Taken together, this real-time *in vivo* study reveals novel perspectives of meningococcal disease that might lead to new strategies to improve the clinical outcome in human patients, and presents a potent tool for further investigations of meningococcal pathogenesis and vaccines *in vivo*.

S01.3

Role of DNA Repair in Meningococcal Genome Dynamics

Tonje Davidsen[1,2], Hanne Tuven[1], Einar Rødland[1], Magnar Bjørås[1] and Tone Tønjum[1,2].

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[2]Centre for Molecular Biology and Neuroscience and Institute of Microbiology, Rikshospitalet-Radiumhospitalet Medical Centre, N-0027 Oslo, Norway.

Neisseria meningitidis (the meningococcus, Mc) is an important commensal, pathogen and model organism that faces up to the environment in its exclusive human host with a small but hyperdynamic genome. DNA repair pathways are most important for maintaining Mc genome stability, however, such mechanisms are poorly characterized in this bacterium. We constructed a panel of single and double Mc mutants disrupting the genes encoding the DNA glycosylases MutY and Fpg, as well as the genes encoding MutS, UvrA, DinB and RecA representing the base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), translesion synthesis (TLS) and recombinational repair (REC) pathways, respectively. As opposed to what is found in *Escherichia coli*, the *mutS* mutant exhibited a less pronounced mutator phenotype than that of the Mc *mutY* mutant. Indeed, the highest spontaneous mutation rate among the Mc single mutants was found in the MutY deficient strain. A synergistic interplay between the two DNA glycosylases MutY and Fpg demonstrated that the GO system, which is made up of MutY, Fpg and MutT, is the major pathway for correcting Mc spontaneous lesions. Interestingly, an interaction of MutY-initiated BER with MMR, TLS and recombinational repair was not identified. The phenotypes of the Mc DNA repair mutants reveal differences from what is found in *E. coli*, which is the most thoroughly characterised system to date, implying that the *E. coli* paradigm might not be representative for all bacterial species. More importantly, the frequent genomic alterations and polymorphisms resulting from the balance between Mc genome fluidity and stability have profound consequences for the interaction of this microorganism with its host, impacting structural and antigenic changes in critical surface components relevant for adherence and invasion as well as antibiotic resistance and vaccine development.

S01.4

Characterization of a *Neisseria gonorrhoeae* strain F62 *fur* mutant and novel genes under Fur control

Susan Grogan[1], Sulip Goswami[1], Yazdani B. Shaik[1] and Caroline Attardo Genco[2]

Department of Medicine, Section of Infectious Diseases[1], and Department of Microbiology[2], Boston University School of Medicine, Boston, MA 02118 USA.

Transcriptional regulation in response to iron in Gram-negative bacteria is mediated primarily by the ferric uptake regulatory protein Fur. In its most basic form Fur together with iron binds to a specific sequence in the promoter regions of genes under its control, acting as a repressor of gene transcription. The *Neisseria gonorrhoeae fur* gene was thought to be an essential gene and the lack of availability of a *fur* mutant strain has hampered the ability to define the gonococcal *fur* regulon. In this study we have succeeded in constructing a *N. gonorrhoeae* strain F62 *fur* gene knockout strain and a corresponding strain in which the *fur* gene has been complemented in trans. We did not observe any deleterious growth defects of the *fur* mutant strain and its ability to proliferate in either iron replete or deplete growth conditions is similar to the parental wild type strain during aerobic growth. In addition to confirming the role of Fur in controlling gene transcription of previously identified Fur repressed genes, we have determined that Fur positively controls expression of the gonococcal *aniA* and *norB* and genes in response to iron. Likewise we have demonstrated that Fur controls expression of the *opaB* gene and that this is independent of iron. Analysis of the *N. gonorrhoeae fur* mutant using antisera specific for OpaB confirmed the lack of OpaB protein expression. Furthermore, OpaB expression was restored in the corresponding strain in which the *fur* gene was complemented in trans. Taken together these studies have begun to address the role of the gonococcal Fur protein as a global regulatory protein and have identified a novel role for this protein as both an activator and repressor of gene transcription. Furthermore our studies indicate that Fur can function to control gene transcription without iron as a co-regulator.

S01.5

Indirect Sensing of Nitrite by the Nitric Oxide-Sensitive Repressor, NsrR, Mediates Co-regulation of Genes in the FNR and NarQ-NarP Regulons Essential for Adaptation of Neisseria gonorrhoeae to Oxygen-Limited Growth

Tim Overton[1], Rebekah Whitehead[1], Ying Li[1], Nigel Saunders[2], Harry Smith[1], Jeff Cole[1]

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[2]University of Oxford UK

Neisseria gonorrhoeae can grow anaerobically by reducing nitrite using a copper-containing nitrite reductase, AniA. Microarray analysis revealed that the gonococcal FNR controls a small regulon by activating transcription of *aniA* and six other genes, and repressing transcription of five genes that include *dnrN* (NG0653) encoding a putative NO-response regulator, and *norB* (NG1275), the structural gene for the nitric oxide reductase. Possible FNR-binding sites were identified upstream of seven of these genes, and binding of FNR to most of these promoters was confirmed by chromatin immunoprecipitation using a FLAG-tagged FNR protein. Although 43 transcripts were more abundant and 7 transcripts were less abundant in a *narP*$^+$ strain than in a *narP* mutant, many of the genes more highly expressed in the *narP*$^+$ strain encoded ribosomal and other growth-rate-dependent proteins. Potential NarP-binding sites were identified in the promoter regions of only five genes differentially expressed in the *narP*$^+$ and *narP* strains, including the regulatory region between *aniA* and *norB*.

Although the *aniA* promoter is activated by NarP and by nitrite, NarP does not activate *aniA* expression in response to nitrite. A multi-genome bioinformatic study identified a putative Rrf2 family member similar in sequence to the NsrR proteins of *E. coli* and *Nitrosomonas europea* in *N. gonorrhoeae*. Expression of p*aniA* was unaffected by nitrite in an *nsrR* mutant, suggesting that NsrR is the only nitrite-sensing transcription factor acting at this promoter, and that in the absence of NsrR, NarP constitutively activates p*aniA*. Both *norB* and *dnrN* were also expressed constitutively at high level even in the absence of nitrite in the *nsrR* strain. As NO rather than nitrite is the signal to which the gonococcal NsrR responds, NsrR plays a key role in enabling gonococci to evade NO generated as a host defence mechanism.

S02

Bacterial Genetics, Physiology & Metabolism: Part 2

Monday 11 September 10.40am
Plenary Hall (Halls A+B)

S02.1

***pilE* mRNA Processing Reveals a Potential Micro RNA Regulatory Mechanism for Transcript Stability**

Stuart Hill , Theresa Dinse and Sandra Hoambrecker

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Piliation is an important virulence determinant for *Neisseria gonorrhoeae.* The promoter structure of *pilE* is complicated, yet, to date, no regulatory element has been demonstrated despite an apparent potential for genetic regulation due to Integration Host Factor (IHF) binding at the *pilE* promoter. In this study, we demonstrate that as gonococci proceed through their growth cycle *pilE* mRNA is absent when cells are harvested in the stationary-growth phase indicating either transcriptional regulation or some form of processing.

When the *pilE* mRNA was further examined, a novel small RNA species was identified that was derived from the 3' end of the *pilE* transcript. The 5' endpoint of the novel RNA species was determined and was found to be located within the hypervariable segment of the transcript and that the excised RNA is predicted to form a thermodynamically-stable structure that appears to be independent of *pilE* sequence variability.

If the small RNA species was involved in regulation, it was reasoned that a *pilE* constant region sequence within the excised RNA would have to be any potential regulatory element. RNA hybridization-modeling analysis was then performed using the *pilE* constant sequence contained within the novel RNA species against the rest of the *pilE* transcript. This sequence was predicted to bind to the very 5' end of the *pilE* transcript (ΔG = - 12.7 kcal/mol).

As IHF binding induces DNA bending, hybridization-modeling analysis was again employed to see whether any DNA sequence within the *pilE* promoter is also predicted to bind to the 5' end of the transcript. A DNA sequence was identified on the non-coding strand of the DNA duplex immediately upstream of the IHF binding site that is predicted to bind to the 5' end of the transcript (ΔG = - 13.61 kcal/mol). Consequently, the novel RNA species has potential to be a novel micro RNA regulatory element.

S02.2

Characterizing DNA Donation in *Neisseria gonorrhoeae* – Autolysis, Allolysis, and Type IV Secretion

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Horizontal transfer of DNA occurs by natural transformation in Neisseria. These bacteria are prone to autolysis, suggesting that lysis provides DNA. Most *N. gonorrhoeae* strains and a few *N. meningitidis* strains contain a genetic island that encodes a type IV secretion system (T4SS). Mutations in T4SS genes reduce DNA donation and the appearance of free DNA in cultures. To characterize DNA donation by T4S, we made mutations in the putative relaxase gene *traI*. Both an insertion and point mutations in *traI* reduced DNA secretion. Two tyrosine residues in TraI were required for DNA secretion, suggesting that the relaxase uses one for initial cleaving of DNA, and the second for a second cleavage and rejoining reaction.

To characterize autolysis, we deleted *amiC*, the gene for the predicted major autolysin. *amiC* mutants were deficient in lysis under non-growth conditions, but still lysed in culture. Characterization of peptidoglycan released during autolysis showed amidase-specific and transglycosylase-specific fragments. These results indicate that AmiC is not the only autolysin and that mutation of *amiC* does not prevent DNA donation.

Two peptidoglycan transglycosylases (AtlA and LtgX) are encoded in the gonococcal genetic island. Mutation of *atlA* eliminated DNA secretion, but mutation of *ltgX* had no effect. Biochemical characterization showed that AtlA degrades peptidoglycan to monomers, consistent with a role as a lytic transglycosylase. As predicted for other T4SSs, this lytic transglycosylase might make a space in the cell wall for T4SS assembly. Another possibility is that AtlA lyses the donor cell (autolysis) or lyses other cells in the culture (allolysis). By measuring release of total nucleic acid, we found that wild-type strains release more nucleic acid into the supernatant than *atlA* mutants. Mixing experiments are being used to determine if the nucleic acid comes only from wild type cells or can also be harvested from other cells.

S02.3

Manganese-Dependent Defense Against Oxidative Stress in *Neisseria gonorrhoeae*

Alastair McEwan[1], Huei Lim[1], Rachel vandenHoven[1], Kate Sieb[1], Hsing-Ju Wu[2], Stephen Kidd[1], Michael Jennings[1]

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Manganese(II) ions are recognized as being antioxidants as a consequence of their ability to quench reactive oxygen species such as superoxide and hydrogen peroxide. In *Neisseria gonorrhoeae* higher levels of intracellular Mn have been correlated with increased ability to survive oxidative stress. An ABC-cassette transporter MntABC, composed of a membrane transporter MntAB and a periplasmic binding protein MntC, is central to the transport of manganese into the cell. Mutation of *mntC* did not affect growth of cells except at low manganese concentrations. *mntC* mutants could be rescued by addition of excess manganese, consistent with the view that MntC is required for high affinity Mn uptake. Mutation of *mntAB* resulted in cells that were unable to grow in liquid medium and could not be rescued by addition of manganese. However, these cells could grow in liquid culture following the addition of quenchers of reactive oxygen species such as mannitol. These results show that the MntABC transporter is the major Mn transport system in *N. gonorrhoeae*. *N. gonorrhoeae* only possesses a Fe-Sod (SodB) and so it is assumed that the antioxidant activity of Mn is due to non-enzymic quenching of reactive oxygen species. Although the nature of the Mn complex which operates in the bacterium is yet to be confirmed using physical methods, it was observed that mutation of the *ppa* gene, which encodes inorganic pyrophophatase, resulted in a phenotype that was highly resistant to oxidative killing by the superoxide generator paraquat. These data are consistent with a role for Mn-pyrophosphate complexes in protection against reactive oxygen species. Proteomic analysis showed that Mn affected the level of a number of proteins involved in the oxidative stress response, intermediary carbon metabolism and at the cell surface.

S02.4

Regulation of gonococcal nitric oxide reductase (*norB/norZ)* by NsrR, an Rrf2 family transcriptional repressor

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Neisseria gonorrhoeae grows anaerobically by anaerobic respiration using nitrite reductase (AniA) to convert nitrite to nitric oxide (NO) and NO reductase (NorB) to convert NO to nitrous oxide. Neither of the transcriptional regulators that regulate *aniA*, FNR and NarP, are involved in the regulation of *norB*, which is induced by NO. To identify the *norB* regulator, we analyzed the the functional regions of the *norB* promoter. We found that the promoter contains an extended –10 motif (TGNTACAAT) that is required for high level expression but not for anaerobic regulation. Deletion and substitution analysis revealed that the sequences upstream of the extended –10 are not involved in regulation, but the replacement of the sequences between the extended –10 and the ATG start site gave high level aerobic expression of a *norB:lacZ* fusion and eliminated anaerobic regulation. Since these results suggested that *norB* is regulated by a repressor, we identified an inverted repeat that could be a site for regulation. Computational analysis identified a gonococcal gene, NGO1519, that had homology to the half-site of the inverted repeat between its –10 region and its ATG start site. NGO1519 is a putative Rrf2 transcriptional repressor with homology to *nsrR* of *Nitrosomonas europaea*, which responds to nitrite, and *nsrR (yjeB*) of *E. coli*, which responds to NO. A cloned NGO1519 gene repressed *norB* in *E. coli* and insertional inactivation of NGO1519 in *N. gonorrhoeae* resulted in high level expression of *norB* aerobically and loss of anaerobic regulation. We conclude that NGO1519 encodes a transcriptional repressor that regulates *norB* expression and propose that this gene be named *nsrR*.

S03

Surface Structures

Monday 11 September 2.00pm

Plenary Hall (Halls A+B)

S03.1

Exploring the Role of a TPR-encoding Meningococcal Gene in Bacterial Adherence to Human Epithelial Cells

Simon Kroll, Ming-Shi Li, Asa Hedman, Paul Langford

Imperial College London

NMB0419 of *Neisseria meningitidis* strain MC58, encoding a protein containing 4 imperfect repeats of a 36 a.a. domain, has been identified as a member of the family of SEL1-like tetratricopeptide repeat (TPR) proteins, involved in protein/protein interactions, including chaperoning. In *Legionella pneumophila* such a protein is involved in adherence/invasion, and we have explored a similar possible function for NMB0419. Adherence of meningococci to human epithelial cells is substantially reduced on mutation of NMB0419. To explore the mechanism, we have transferred our attention to *E. coli*.

Expression of NMB0419 in *E. coli* resulted in increased adherence to epithelial cells in a striking "stacked brick" pattern, while EM revealed rigid pili on the bacterial surface. Adherence was abolished by D-mannose, suggesting that these were type 1 pili, their formation facilitated by NMB0419.

Transcriptional analysis revealed that expression of NMB0419 in *E. coli* was accompanied by upregulation of the *fim* operon, encoding the structural proteins and accessory factors needed for formation of type 1 pili. NMB0419 appears to play a transcriptional role, beyond merely chaperoning subunits into the multi-molecular fimbrial structure.

All strains in a large collection of *Neisseria meningitidis* and *N. lactamica* contained an NMB0419 homologue, but the number of SEL-1-like repeats varied, from one (commonly) to >11. It has previously been suggested that ≥3 domains are necessary for typical TPR function. To investigate this in the present context, we repeated the previous *E. coli* adherence experiments with strains bearing respectively a cloned "single-repeat" gene and one with an in-frame deletion removing the only SEL-1-like repeat. The single domain was sufficient to confer the same level of enhanced adherence seen with NMB0419, while the in-frame deleted gene did not enhance adherence at all.

We suggest that NMB0419 and its homologues may play an important part in meningococcal-host interactive biology, for example in adhesion/invasion; that the functional domain includes the SEL-1-like repeat; and that contrary to our initial expectations, its role may be transcriptional rather than simply as a molecular chaperone of fimbrial components.

S03.2

Identification of Lipopolysaccharide Transport Components in *Neisseria meningitidis*

Martine P. Bos and Jan Tommassen

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The biosynthesis of lipopolysaccharide (LPS) has been elucidated mostly through work in *E. coli* and *Salmonella*. However, the transport of its site of synthesis, i.e. the inner leaflet of the inner membrane, to its final destination, the outer leaflet of the outer membrane (OM), is still poorly understood. This is partly due to the fact that *E. coli* cannot survive without LPS, and therefore deletion of LPS biogenesis genes is often lethal. In contrast, *Neisseria meningitidis* can survive without LPS and tolerates inactivation of genes encoding LPS-transport components. This property of *N. meningitidis* allowed us previously to identify an OM component, called Imp, required for transport of LPS to the neisserial cell surface. Now we report the identification of novel components involved in the periplasmic LPS transport. Inactivation of the ORFs designated NMB0339 and NMB0355 in *N. meningitidis* strain H44/76 resulted in viable bacteria that produced only very little LPS, but demonstrated normal porin assembly. Immunoblot analyses showed that the LPS produced was modified by enzymes at the periplasmic side of the inner membrane, showing that transport across this membrane was not hampered. These features were also found for the *imp* mutant, indicating that both NMB0339 and NMB0355, like Imp, are involved in LPS transport. NMB0339 was previously designated ght by Rasmussen *et al*., for gene for hydrophobic agent tolerance because a *ght* mutant showed enhanced sensitivity to hydrophobic agents. This phenomenon is likely caused by the absence of LPS in the OM, consistent with our proposed role for ght in LPS transport. The NMB0355 sequence indicates it is a soluble periplasmic protein; thus it might act as a chaperone for LPS transport through the periplasm. Thus, *N. meningitidis* is an excellent model organism to identity LPS transport components, which might be valuable targets for development of antimicrobial agents.

S03.3

Meningococcal Lipoprotein PilP: its structure and role in pilus biogenesis and transformation

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Neisseria meningitidis is constitutively competent for transformation throughout its life-cycle. Transformation in neisserial species is particularly important for genetic exchange and is coupled to the expression of type IV pili (Tfp). Tfp are present on the cell surface as bundled filamentous appendages, are also required for autoagglutination and adherence, and are assembled, extruded and retracted by pilus biogenesis components. The secretin PilQ is a macromolecular outer membrane complex which mediates the passage of Tfp through the outer membrane. PilQ requires auxiliary

proteins for its assembly, membrane association and stabilization; one of these is the lipoprotein PilP.

We have characterized the interaction between meningococcal PilP and PilQ using molecular biological tools and structural analysis. PilP knock-out mutants were non-piliated, non-competent and exhibited reduced amounts of oligomerized PilQ. Native PilP co-purified with inner membrane proteins and had a tendency to form dimers and oligomers. The solution structure of a recombinant fragment of PilP was determined by NMR and revealed a compact, core domain of about 80 residues with mainly a beta fold. Transcription analysis of the genes encoding PilP and PilQ suggested that they are co-transcribed. Direct interaction was detected between N-terminal PilP and the central domain of PilQ by far-Western analysis. The 3D structure of the PilP-PilQ interacting complex was determined using single particle averaging, applied to TEM images collected in cryonegative stain. PilP bound to the 'cap' region of PilQ, suggesting that the PilQ oligomer spans the entire periplasm. Furthermore, bandshift analyses showed that PilP binds DNA with a preference for single-stranded DNA (ssDNA). Based on these findings, we suggest that PilP has a role in binding to and stabilizing the conformation of the PilQ oligomer within the periplasmic space and that it binds ssDNA during transformation.

S03.4

Definition, by a Systematic Analysis in *Neisseria meningitidis*, of the Pil Proteins that are Required for the Assembly, Functionality, Stabilization and Export of Type IV Pili

Etienne Carbonnelle, Sophie Helaine, Xavier Nassif, Vladimir Pelicic

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Although type IV pili (Tfp) are among the commonest virulence factors in bacteria, how they are assembled by complex machineries of 12-15 proteins and thereby how they function remains poorly understood. Some of these proteins, however, have been shown to antagonize the continuous retraction of the fibers powered by PilT, since piliation could be restored in their absence by a mutation in the *pilT* gene. We therefore defined the contribution in Tfp biogenesis of each of the 15 *N. meningitidis* dedicated Pil proteins by systematically introducing a *pilT* mutation into the corresponding non-piliated mutants and characterizing them for the rescue of Tfp and Tfp-mediated virulence phenotypes. Unexpectedly, we found that in addition to the pilin, the main constituent of Tfp, six proteins only are required for the assembly of the fibers, since apparently normal fibers could be restored in the remaining mutants. Restored fibers were surface-exposed, except in the *pilQ/T* mutant where they were trapped in the periplasm, suggesting that the PilQ secretin is the sole Pil component necessary for their emergence on the surface. Importantly, although in most of the mutants, including those previously characterized, the restored Tfp were not functional, the *pilG/T* and *pilH/T* mutants could form bacterial aggregates and adhere to human cells, indicating that Tfp stabilization and functional maturation are two discrete steps. These findings have numerous implications for understanding Tfp biogenesis/function and provide a useful groundwork for the characterization of the precise function of each Pil protein in this process.

S03.5

Characterization of Intragenic and Intergenic Mutations that Disrupt Antibiotic Influx Through the PilQ Secretin in *Neisseria gonorrhoeae*

Sobhan Nandi, Shuqing Zhao, and Robert A. Nicholas

Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC USA

We have shown previously that a mutation in PilQ (*pilQ2*; E666K) or a PilQ deletion increases penicillin resistance in gonococcal strains that also contain the *penA, mtrR,* and *penB* resistance determinants, suggesting that PilQ forms a pore in the outer membrane through which antibiotics diffuse into the periplasm. Using this system, we isolated spontaneously arising penicillin-resistant mutants and, as expected, a vast majority of these contained deletions or mutations in PilQ. Western blots of PilQ in some of these mutants revealed a phenotype similar to *pilQ2*, whereby the mutations disrupted formation of the SDS-resistant PilQ dodecamer. All of these mutations were located in the C-terminal domain of PilQ, consistent with this region being involved in multimer formation. We also investigated the effects of a deletion in PilW, a PilQ pilotin that is required for formation of the SDS-resistant PilQ multimer. Even though SDS resistance of the PilQ dodecamer was completely abrogated in a *pilW* deletion strain, antibiotic resistance did not increase, suggesting that SDS resistance in itself is not requisite for formation of the PilQ pore. We also isolated penicillin-resistant mutant strains that expressed wild-type levels of SDS-resistant PilQ dodecamers. Gene mapping experiments in two of these mutant strains revealed frame-shift mutations in the *pilM* gene, which is upstream of the *pilQ* gene in the *pilMNOPQ* operon. Strains containing directed frameshift mutations in *pilM*, *pilN*, or *pilO* were created, and although these strains expressed wild-type levels of SDS-resistant PilQ dodecamers, they had levels of penicillin resistance similar to those of strains with a *pilQ2* mutation or *pilQ* deletion. These data suggest that PilM, PilN and PilO affect antibiotic influx by regulating the ability of PilQ to exist in an "open" conformation.

S04

Antibiotic Resistance

Monday 11 September 4.10pm

Plenary Hall (Halls A+B)

S04.1

Differential Regulation of *ponA* and *pilM* Expression by the Gonococcal MtrR Transcriptional Regulatory Protein

Jason Folster, Robert Nicholas, and William Shafer

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Resistance of *N. gonorrhoeae* to hydrophobic agents (HAs), including antibiotics, is mediated by the *mtrCDE* locus, which encodes an energy-dependent efflux pump that exports host-derived antimicrobials. The *mtr* system is important for the pathogenesis of *N. gonorrhoeae*, as it provides for resistance to HAs present at the sites of infection. MtrR is the major transcriptional repressor of the *mtr* locus. Besides repressing *mtr* expression, MtrR has been shown to repress transcription of *mtrF*, a putative inner membrane transporter necessary for high level HA

resistance and *farR*, which encodes a repressor of the *farAB*-encoded efflux system that enhances gonococcal resistance to antibacterial long chain fatty acids. Based on our observations that MtrR can regulate genes other than *mtrCDE,* we hypothesize that MtrR is a global regulator of gonococcal gene expression. In support of this concept, we now report that MtrR activates expression of the *ponA* gene encoding penicillin-binding protein 1 (PBP 1). Western blot analysis confirmed that PBP 1 levels increased in the presence of MtrR. In contrast to the increase in *ponA* expression, MtrR was found to repress expression of the divergently transcribed *pilM* gene, the first gene in the *pilMNOPQ* operon. Specific binding of purified MtrR protein to the *ponA-pilM* intergenic region was confirmed by electrophoretic mobility shift assays (EMSAs). EMSAs were performed on a series of overlapping probes within the intergenic region, resulting in the identification of a 25 basepair region required for MtrR binding. Comparison of this region with the known MtrR binding site within the *mtrC* promoter region revealed a potential MtrR binding site. Mutation of the potential binding site disrupted MtrR binding, confirming the identity of the MtrR binding site. These studies have identified additional targets of MtrR regulation and have shown that MtrR may act as a repressor or activator of multiple gonococcal genes.

S04.2

Characterization of High-level Erythromycin-Azithromycin Multidrug Resistant *Neisseria gonorrhoeae* and therapy failure

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Four recent gonococcal isolates over a two month period were recovered from the pharynx of a man attending the Seattle STD Clinic. The patient failed therapy with cefpodoxime, 400 mg and azithromycin, 1 gram. Isolates were resistant to ciprofloxacin (MIC, 16 μg/ml), erythromycin (>32 μg/ml), azithromycin (Az >16 μg/ml) with somewhat higher values to ceftriaxone (0.06 μg/ml). These isolates and three other (Kansas City, San Francisco, Seattle) macrolide-resistant isolates (Az MIC 2->16 μg/ml) were characterized. Amino acid (aa) changes to their *mtrR*, *penA*, *penB* and *ponA* genes, the presence of the adenine (A) deletion in the 13 bp *mtrR* promoter, the 153 bp insertion sequence between the *mtrR*/*mtrC* promoter and *mtrC* gene were determined. The presence of acquired macrolide rRNA methylases (*erm* genes) and macrolide efflux gene *mef*(A) was evaluated. By pulsed-field gel electrophoresis using 3 enzymes, the isolates from the man who failed therapy were indistinguishable from each other and unrelated to the other isolates. These isolates and the San Francisco isolate had the A deletion, and the same aa changes including a single unique aa change in the PonA protein, 20 aa changes (3 not previously identified) in the PenB protein, a single aa change in the MtrR protein and multiple changes in PenA protein. All strains carried an acquired *erm*(B) gene which was associated with a conjugative element that conferred macrolide resistance to transconjugants. Neither the San Francisco nor Seattle isolates carried the 153 bp insertion. These findings suggest that multiple mechanisms may result in macrolide resistance 8-fold higher than has been previously reported in North America. Although the recent patient with the pharyngeal infection was successfully treated with ceftriaxone, 125 mg IM, this multiple drug resistant strain challenges the limited choice of gonococcal therapies.

S04.3

Question marks about the Correlation between the *penA* Gene Sequence and Penicillin Susceptibility of Polish Isolates of *Neisseria meningitidis*

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Introduction. The most common mechanism of reduced susceptibility to penicillin (penicillin-intermediate phenotype, Pen^I) in *Neisseria meningitidis* are alterations in the *penA* gene sequence, encoding the PBP2. The aim of this study was to characterize the mechanism of Pen^I phenotype in Polish isolates of meningococci.

Methods. The study included 46 isolates of *N. meningitidis* collected in the National Reference Centre for Bacterial Meningitis in Poland in the period 1997-2004. Antibiotic susceptibility was determined by Etest, bacterial serogroups – by PCR, serotypes and serosubtypes – by the Whole Cell ELISA. The relatedness of isolates was determined by RFLP-PFGE and MLST. For all isolates the *penA* and *mtrR* genes were sequenced, as well as the *penA* upstream region, corresponding to the promoter of this gene. Expression of the PorA class1 protein was analyzed by sequencing the encoding gene and its promoter.

Results. Twenty-nine penicillin susceptible isolates (MIC <= 0,064 mg/L) possessed wild-type *penA* genes. In eight of Pen^I isolates (MIC 0,094-0,5 mg/L) specific, already described changes in the *penA* gene sequence were found. Nevertheless, 9 penicillin-intermediate isolates (MIC 0,094-0,19 mg/L) didn't show changes in this gene. No correlation between susceptibility to penicillin and other antibiotics was observed. There was also no relationship between serogroup, serotype or serosubtype and penicillin-susceptibility phenotype. The sequence analysis of the *porA* gene and its promoter excluded its decreased expression. Analysis of *mtrR* gene and promoter region of *penA* also didn't explain the Pen^I phenotype of these nine isolates.

Conclusion. Although decreased susceptibility to penicillin isn't very common in Polish meningococci, its mechanism seems to be unusual in more than a half of Polish isolates showing Pen^I phenotype. Well-known mechanisms, such as *penA* mutations, decreased level of expression of PorA porine, *mtrCDE* efflux pump and changes in upstream region of *penA* were excluded, therefore further analysis of this phenomenon is required.

S04.4

Antimicrobial Resistance in *N. gonorrhoeae* in Australia – Results of 25 years of Continuous, Standardised National Surveillance

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Background: Gonorrhoea in urban Australia has a similar distribution to that reported in other Western industrialised countries, namely, moderate, but rising disease rates, mainly in

homosexually active men, with increasing rates of antimicrobial resistance (AMR). In contrast rural settings have high disease rates, but low AMR levels.

Methods. The Australian Gonococcal Surveillance Programme (AGSP) network of reference laboratories undertakes AMR surveillance in each Australian jurisdiction. In 1981, the AGSP introduced a standardized methodology, a programme-specific EQAS and analysed data quarterly and annually.

Results. More than 100,000 gonococci (GC) were examined by the AGSP over 25 years. In urban populations resistance to the penicillins increased as both PPNG and CMRNG proliferated and by 2005 up to 50% of gonococci were resistant. In rural settings, despite constant and higher generalised use of beta-lactams, less than 5% of isolates were resistant in 2005. Quinolone resistance (QRNG) was first detected in 1984 and the number of QRNG, centres with QRNG and MIC levels of QRNG all increased substantially so that nationally in 2005, 30% of GC were high-level QRNG. This proportion was 50% in some urban centres but about 2% in rural settings. Quinolones were rarely used in Australia, even for gonorrhoea. An increasing but low proportion of multi-resistant isolates also displayed decreased susceptibility to ceftriaxone. Spectinomycin resistance was rare.

Conclusions: Increasing problems with AMR exist in urban settings in Australia despite rational antibiotic use for gonorrhoea. Low AMR rates continue in remote locations, including to penicillins which retain their clinical effectiveness. Antibiotic use/misuse seems to have played little part in generation of resistant strains in Australia and AMR in GC resulted from repeated importation of subtypes of resistant isolates, some of which expanded in particular urban patient sub-populations. Continuing surveillance and analysis of disaggregated data is required to optimise treatment options for gonorrhoea.

S05

Genomics & Gene Expression: Part 1

Tuesday 12 September 8.30am

Plenary Hall (Halls A+B)

S05.1

A chromosomally integrated bacteriophage in invasive meningococci

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These authors contributed equally to this work

Neisseria meningitidis has the capacity to invade the host tissue and cause life-threatening disease. The reasons for this breaking down of the normal commensal relationship are not well understood, but are certainly dependant on both host and bacterial factors.

To search for the genetic determinants of the elevated pathogenic potential of certain strains of meningococcus, we used DNA arrays to compare strains belonging to recognised invasive clonal complexes with strains belonging to complexes having no association with disease. A single group of genes of 8kb was significantly associated with invasive isolates. This gene cluster, the "Meningococcal Disease-Associated island" (MDA), was present in 100% of the invasive isolates and absent from 90% of the non invasive isolates.

This association with increased invasiveness was confirmed using a collection of 293 well-characterised meningococci obtained from patients and asymptomatic carriers in the Czech Republic during 1993. Considering the presence or absence of MDA in each strain (as determined by PCR) in relation to its clonal group and its isolation from a case or carrier, the results confirmed the association with invasive lineages and furthermore demonstrated a highly significant association of the island with disease. In addition, we studied a population of 707 bacteria isolated in the United Kingdom from healthy carriers and patients. In this group also the MDA was highly represented among hyperinvasive clonal complexes, and more generally its presence among disease isolates was significantly higher than in isolates obtained from carriers.

Further sequence analysis of the ten open reading frames showed similarities to bacteriophages of the type M13 and to CTX-Phi of *Vibrio cholerae*. Biological investigations showed that the MDA island indeed has the characteristics of a filamentous bacteriophage. Bacteria harbouring the MDA secrete a circular form of the element in a nuclease-resistant state via the type IV pilin secretin.

S05.2

Identification of a Novel, Fur-Regulated Small RNA in *Neisseria meningitidis* Serogroup B

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Iron is an essential nutrient for a number of bacteria, yet at high concentrations it can catalyze the formation of reactive oxygen species which are toxic to a bacterium. Thus iron homeostasis is a tightly regulated process. In the pathogenic *Neisseria*, iron regulation has largely been thought to be mediated by an iron-responsive transcriptional repressor termed the Ferric Uptake Regulator (Fur). Whole genome microarray studies in our laboratory identified a number of iron-activated genes in *N. meningitidis* MC58, which raised the possibility that Fur could also function as a direct activator of transcription. Studies in other pathogenic bacteria recently identified small RNA's (sRNA) which are regulated by Fur and involved in iron-dependent gene activation in these organisms. To determine if sRNA's also function in this regard in *N. meningitidis,* we utilized *in silico* and *in vitro* approaches to search for sRNA's in the *N. meningitidis* MC58 genome, which also had the potential to be regulated by Fur. Using a pattern search algorithm, the genome was parsed for consensus Fur binding sites (Fur boxes) within 250 base-pairs of predicted rho-independent terminator sequences, a common feature of majority of bacterial sRNA's. This approach identified 22 candidate regions. The *in vivo* presence of these sRNA's in *N. meningitidis* MC58

cultures, grown under both high and low-iron conditions, was investigated by S1 nuclease protection and Northern blot analyses. We observed the presence of a novel sRNA in *N. meningitidis,* which is transcribed in a low-iron environment and is repressed by Fur in a high-iron environment. Regulation of this sRNA by Fur was confirmed using a *N. meningitidis fur*⁻ knockout strain and the corresponding strain in which the *fur* gene was complemented in trans. Taken together, our results provide the first example of a sRNA in *N. meningitidis* which is iron-repressed via a Fur-mediated mechanism.

S05.3

The MisRS Two-Component Regulon in *Neisseria meningitidis*

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Two-component regulatory systems modulate processes important for bacterial pathogenesis. The *misR*/*misS* (*phoP*/*phoQ*) system is one of only four two-component regulatory systems encoded in the *Neisseria meningitidis* genome. Inactivation of this system in meningococcus results in the loss of lipooligosaccharide inner core phosphorylation and attenuates meningococcal infection in a mouse model. The transcriptional profiles of the *misR::erm* mutant and its parental wild type meningococcal strain NMB were compared using microarrays derived from serogroup A and B genomes (Eurogentec). Genes were identified as regulatory targets (95% confidence) of MisR upon exhibiting more than two-fold transcriptional change in at least two of three independent biological replicates. Approximately 40 genes were identified by microarray analysis and represent the minimal *misRS* regulon. The functions of the MisR regulatory targets include protein folding, transcriptional regulation, metabolism, and transport. Quantitative real time PCRs were performed to confirm the changes observed by microarray analysis. In addition, MisR regulation of several target genes *in vivo* was demonstrated by reporter assays comparing the β-galactosidase activities in the wild type and the misRS mutant backgrounds. To distinguish between direct and indirect targets, electrophoretic mobility shift assays (EMSA) were carried out using purified MisR-6xHis protein either with or without phosphorylation by acetyl phosphate. Of the 22 genes examined by EMSA, MisR binds directly to promoter regions of twelve genes. Several of these promoters were further examined by DNase I protection assays and a MisR-binding consensus sequence has been proposed. Thus, the direct regulatory targets of MisR were identified and a minimal regulon of the meningococcal MisR/MisS two-component signal transduction system has been characterized. These data suggest that the MisRS system controls a wide range of biological functions in *N. meningitidis* either directly or via intermediate regulatory proteins.

S05.4

The Role of Integration Host Factor in the Regulation of Genes Involved in Transport System Used by *Neisseria gonorrhoeae*

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Integration host factor (IHF) is a histone-like DNA-binding and -bending protein. It plays an important role in local DNA structural organization and transcriptional regulation in gram negative bacteria. In *Neisseria*, IHF modulates expression of genes that encode proteins (e.g., pilin and efflux pumps) likely required for virulence. We have determined that IHF can bind DNA sequences upstream of genes that encode distinct efflux pumps and certain ABC transporters. Among these IHF-target genes is an operon encoding the *farAB* efflux pump, which exports antimicrobial long chain fatty acids. We used it as a model system to better understand how IHF can regulate gene expression in gonococci. *In vitro* transcription and DNA bending assays indicated that IHF-binding to the *farAB* promoter region could inhibit its transcription and that such binding induced a bending of the target DNA, which we propose to be important in regulating expression of this operon. The *farAB* operon is also negatively regulated by the FarR repressor and results from experiments using a *farAB-lacZ* reporter fusion system and those obtained from DNase I protection/competition assays, suggested that in the presence of FarR, a bend of DNA accentuated by IHF-binding could stabilize binding of FarR to its target sites. This process served to enhance repression of *farAB* expression mediated by the FarR repressor. Thus, while repression of the *farAB* operon can be mediated by IHF or FarR alone, maximal repression involves the synergistic activity of both repressors. Thus, our findings support the concept that transport systems in *Neisseria* can be controlled at the level of transcription by the dual action of distinct DNA-binding proteins.

S05.5

***Neisseria gonorrhoeae* is Polyploid**

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Neisseria gonorrhoeae (gonococcus, Gc) is a diplococcal bacterium, though the reason for a diplococcal state has not been explained. We set out to determine chromosome number and distribution in the monococcal and diplococcal forms of Gc. Using flow cytometry, fluorescent microscopy and cell sorting, we determined that exponentially growing gonococci have three to six Gc genome equivalents per monococcal cell. To ascertain whether the DNA content observed is due to multiple replication forks on a single chromosome or multiple, complete chromosomes per coccal unit, gene dosage of markers near the origin and terminus were measured by microarray analysis and quantitative PCR, which showed that a single replication initiation event per chromosome occurs per round of cell division. Quantitative PCR of sites near the terminus also revealed that exponentially growing Gc contain on average three chromosomes per coccal unit, hence six chromosomes per diplococcus. Based upon these findings, we propose a model for gonococcal DNA replication and cell division in which a minimum of two chromosomes exist per coccal unit, and

the chromosomes replicate in unison to produce four chromosomes during cell division. Thin section electron microscopy indicates that both halves of the diplococcus contain nucleoids. We do not know how multiple chromosomes are segregated during cell division particularly in light of the diplococcal form, and to begin to address this issue, we are attempting to transform two markers into a single chromosomal location in order to monitor segregation. We propose that the polyploid nature of the gonococcus may be necessary for pilin antigenic variation; however, the exact role of polyploidy in gonococcal pathogenesis remains to be determined.

S06

Genomics & Gene Expression: Part 2

Tuesday 12 September 10.40am

Plenary Hall (Halls A+B)

S06.1

Sublethal Concentrations of Ciprofloxacin Induces Genes Implicated in Horizontal Gene Transfer in *Neisseria gonorrhoeae*

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Overuse and inappropriate use of antibiotics has led to widespread antibiotic resistance in many pathogens, including *Neisseria gonorrhoeae* (GC). Increasing levels of resistance as well as acquisition of multiple resistances is a continuing problem. We hypothesized that antibiotics present during disease therapies might be in concentrations low enough to avoid complete killing of *N. gonorrhoeae*, yet still influence the ability of the bacterium to establish a productive infection, mediate asymptomatic carriage, cause disease, and/or develop resistance to multiple antibiotics.

The effects of sub-lethal concentrations of the fluoroquinolone antibiotic, Ciprofloxacin (Cipro) on gene expression in *N. gonorrhoeae* strain MS11 were determined using a gonococcal DNA microarray. A total of 140 genes were found to be significantly differentially expressed in Cipro-treated gonococci, with 64 genes reduced and 76 genes increased in expression. Many of those repressed (41/64) are predicted to encode proteins involved in transcription or translation, consistent with the slower growth rate of Cipro-treated bacteria. Of those up-regulated following exposure to Cipro, two groups of genes were notable in that they encoded genes related to horizontal gene transfer. The first set includes multiple genes known or suspected to play a role in gonococcal transformation, including recombination, type IV pilus biosynthesis, and competence genes. The second includes genes that are apparently of bacteriophage origin.

A testable hypothesis resulting from these observations was that Cipro treatment of GC could lead to an increase in transformation efficiency. Transformation assays of GC treated and not treated with Cipro showed that Cipro increased transformation efficiency 3-4 fold, irrespective of transforming DNA. Thus, Cipro is a DNA damaging antibiotic that kills gonococci in high concentrations, yet increases transformation efficiency at low concentrations, leading to the real possibility that DNA damage can lead to the acquisition of resistance to multiple antibiotics, and potentially other characteristics as well.

S06.2

Defining the phase variable regulons – "phasevarions" – of pathogenic Neisseria

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We have previously reported that the phase variable type III Restriction-Modification (R-M) system of *Haemophilus influenza* strain Rd mediates the coordinated random switching of a "regulon" of genes (phasevarion) via differential methylation of the genome (PNAS 2005 102:5547-51). Similar, phase variable type III R-M methyltransferases are also present in pathogenic Neisseria. To investigate whether these gene were also involved in mediating phase variable expression of distal genes, we made mutant strains lacking the methyltransferase (*mod*) associated with a phase variable type III R-M system of *Neisseria meningitidis* MC58 and *N. gonorrhoeae* FA1090 and analyzed their phenotypes. Microarray analysis identified a number of genes that were either up- or down-regulated in the *mod* mutant strain in each organism, some of which were known virulence factors. This suggests that the phasevarion may be a commonly used system mediating coordinated phase variation of multiple genes in bacterial pathogens.

S07

Cellular Microbiology: Part 1

Tuesday 12 September 11.20am

Plenary Hall (Halls A+B)

S07.1

Structural Analysis of a CEACAM-Binding Recombinant Polypeptide that Inhibits the Interactions of *Neisseria meningitidis* and *Neisseria gonorrhoeae* with Human Target Cells

Darryl J. Hill[1], Rebecca Conners[2], Anthony R. Clarke[2], Richard B. Sessions[2], John Rea[2], R. Leo Brady[2] & Mumtaz Virji[1].

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The pathogenic Neisseria spp. *Neisseria meningitidis* and *N. gonorrhoeae*, as well as two other mucosal pathogens, *H. influenzae* and *M. catarrhalis*, share the property of targeting the carbohydrate deficient face of the N-domain of human Carcinoembryonic antigen (CEA)-related cell adhesion molecules (CEACAMs). CEACAMs are signalling receptors implicated in cell adhesion and regulation of several physiological functions and are expressed on a variety of tissues including apical surfaces of respiratory epithelia, a site of residence of *N. meningitidis*. CEACAM targeting by pathogens can lead to tissue invasion. Although the CEACAM-binding ligands of the Neisseria spp., *H. influenzae* and *M. catarrhalis* are structurally and antigenically diverse, they target an overlapping site on the receptor centered around Ile 91, whose mutation abrogates the binding of these pathogens. We have generated a recombinant polypeptide (rD-7) based on the CEACAM-binding *M. catarrhalis* ligand UspA1 that blocks the interactions of multiple mucosal pathogens with human epithelial cells. Recent studies have shown that rD-7 can also prevent cellular invasion of piliated-Opa-expressing *N. meningitidis*

in which pili act synergistically with opa proteins to augment cellular invasion. Due to its efficiency as a blocking agent for multiple CEACAM binding pathogens in a number of model systems employed *in vitro*, we undertook to analyse the structural features and requirements for binding of rD-7 to the receptor. A complete understanding of such interactions could ultimately lead to the development of novel intervention strategies useful in the prevention of neisserial and other infections. We have crystallised the polypeptide and obtained X-ray crystallographic information. By molecular modelling and mutational analysis of the receptor as well as rD-7, the structural features that enable the polypeptide to engage with the receptor at the common site of bacterial adhesion are being defined.

S07.2

Identification of a new receptor for $PorB_{IA}$ of *neisseria gonorrhoeae*

Cindy Rechner[1], Christiane Kühlewein[1], Hans-Jörg Schild[2], Thomas Rudel[1]

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Neisseria gonorrhoeae express numerous variant surface proteins including type IV pili and the opacity associated outer membrane (Opa) proteins mediating adherence to and invasion into target cells. The invariant major outer membrane porin of serotype A ($PorB_{IA}$) triggers invasion into various host cells also in the absence of pili and Opa proteins. A special feature of the $PorB_{IA}$-mediated interaction is that it requires an environment of low phosphate. Since gonococci expressing $PorB_{IA}$ are frequently isolated from patients with severe disseminating infections the interaction initiated by the porin may be of major relevance for the development of this serious disease.

Using genetically defined *Neisseria* strains, potential host cell receptors interacting with $PorB_{IA}$ but not with $PorB_{IB}$ were purified and identified by mass spectrometry. One of these proteins was subsequently identified as $PorB_{IA}$ specific receptor. $PorB_{IA}$, but not $PorB_{IB}$ expressing gonococci bound to purified native or recombinant $PorB_{IA}$-receptor. Unexpectedly, depletion of the $PorB_{IA}$-receptor from HeLa cells prevented adherence but significantly triggered invasion of gonococci. Invasion via $PorB_{IA}$ in the absence of $PorB_{IA}$-receptor was insensitive to phosphate suggesting the involvement of a second receptor involved in the uptake of gonococci. The addition of purified native PorB receptor protein to epithelial cells depleted of endogenous $PorB_{IA}$-receptor restored adherence and interfered with the uptake of $PorB_{IA}$-expressing gonococci. Interestingly, depletion of the PorB receptor from human monocytic cells increased the phagocytosis of non-piliated, Opa-negative bacteria which were otherwise resistant to their uptake by these phagocytes.

Our data suggest a two-step interaction of $PorB_{IA}$ expressing gonococci with epithelial cells involving the phosphate-sensitive adherence via the $PorB_{IA}$-receptor and the phosphate-insensitive interaction with a thus far unknown receptor triggering the uptake by the epithelial cell. We will discuss the possible relevance of the $PorB_{IA}$-receptor interaction in the different infection scenarios.

S08

Cellular Microbiology: Part 2

Tuesday 12 September 2.00pm

Plenary Hall (Halls A+B)

S08.1

Biofilm Formation by *Neisseria gonorrhoeae* during Cervical Infection

Michael Apicella, Megan Falsetta, Brock Neil

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Neisseria gonorrhoeae has been shown to form biofilms in continuous flow chambers. In order to determine if biofilm formation was a component of natural cervical infection, cervical biopsies from 10 infected women were studied using transmission and immunoelectron microscopy. In addition, studies were instituted to determine if *N. gonorrhoeae* could produce biofilms over human cervical epithelial cells in a continuous flow system. These studies were performed using confocal, light and scanning electron microscopy (SEM). Our studies demonstrated that evidence of biofilm formation could be detected in 3 of the 10 patients. Biofilms were manifest by clusters of organisms oriented above cervical epithelial cells. There organisms were embedded in a membranous matrix similar to that seen when gonococcal biofilms were studied in continuous flow chambers over glass surfaces. Immunoelectron microscopy using monoclonal antibody 2C3 which binds to the H.8 protein of pathogenic *Neisseria* demonstrated that organisms within the biofilm structures were *N. gonorrhoeae*. In order to simulate gonococcal growth as in natural infection, a continuous flow chamber was developed which allowed perfusion of tissue culture fluid over *N. gonorrhoeae* strain 1291 infected human cervical epithelial cells. Confocal microscopy demonstrated progression of bacterial growth on the epithelial cell surface from microcolonies to biofilm-like structures over 48 hours. SEM studies showed extensive blebbing of the organisms within the biofilms with fusion of these blebs into extensive membranous structures that ultimately covered much of the biofilm surface. Similar membranous structures could be seen within the biofilm itself. Studies of biofilm formation by strain 1291 *msbB, lgtB* and *laz* mutants demonstrated formation of defective biofilm by each of these mutants over human cervical epithelia. These studies demonstrate that biofilms are a part of the biology of gonococcal pathogenesis during cervical infection. Understanding of the role of biofilm in persistence of infection and the evolution of antimicrobial resistance is ongoing.

S08.2

Role of Factor H and Complement Receptor 3 in Mediating Attachment of *Neisseria gonorrhoeae* to Eukaryotic Cells

Sarika Agarwal, Sanjay Ram, Sunita Gulati and Peter A. Rice

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Human factor H, an alternative pathway regulatory protein, is instrumental in converting complement component C3b to the inactivate form, iC3b, enabling gonococci to evade killing by human complement. In addition to iC3b, Factor H, itself, binds to specific receptors on different cell types and in association with complement receptor 3 (CR3, also known as CD11b/CD18) plays a role in adherence to PMNs. Recently it has been reported that CR3 is also present on primary cervical epithelial cells. We hypothesized a potentially cooperative role between factor H and CR3 in

gonococcal adherence to epithelial cells that may facilitate their entry into cells. In a model system, we examined association of a factor H binding strain of *Neisseria*. *gonorrhoeae*, called 252, and the non-factor H binding strain, F62, to Chinese hamster ovary (CHO) cells, transfected with and without the CR3 receptor. In addition to unsialylated gonococcal strains used in the study, cultures were also sialylated with the addition of 5'-cytidinemonophospho-*N*-acetyl neuraminic acid (CMP-NANA). A fraction of sialylated and unsialylated cultures were each pre-incubated with factor H, then added to CR3 transfected and non-transfected CHO cells. Using either FACS or fluorometer analysis, we measured a significant increase in adherence to CHO cells by GFP expressing gonococci with bound factor H in CR3 transfected CHO cells compared to diminished adherence to non-transfected CHO cells using gonococci with or without bound factor H (ratios range between 2.8 and 5 in gonococci that bind factor H compared to a ratio of ~1 for gonococci that do not bind factor H). Specificity of the factor H/CR3 interaction in CHO cells was corroborated by increased binding of free factor H and resultant decreased exposure of CD18 on CR3 transfected cells. These results indicate that binding to CR3 bearing cells is enhanced in *N. gonorrhoeae* with affixed factor H, thereby providing an additional mechanism of adherence of gonococci to host cells.

S08.3

Hormonal Modulation of CR3-mediated *Neisseria gonorrhoeae* Infection of Primary, Human, Cervical Epithelial Cells

Jennifer L. Edwards

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Complement Receptor 3 (CR3)-mediated endocytosis is a primary mechanism by which *Neisseria gonorrhoeae* elicit membrane ruffling and invade primary cervical epithelial (pex) cells. It is known that the susceptibility of women to develop ascending gonococcal infection varies with the menses cycle. However, how steroid hormones function to modulate progressive gonococcal disease is poorly understood. Thus, we initiated studies to address this question. We show that low levels of estradiol promote CR3 surface expression on pex cells. A parallel increase in the ability of gonococci to associate with pex cells was also observed. Our previous studies demonstrate that gonococcal phospholipase D (NgPLD) modulates cervical infection, in part, through its ability to directly interact with host cell Akt kinase and, consequently, subvert cervical cell signaling. Consistent with these data, we also found that increasing concentrations of progesterone augmented the ability of wildtype, but not PLD-deficient, gonococci to survive intracellularly. In addition to the ability of progesterone to promote gonococcal survival in a NgPLD-dependent manner, we found that NgPLD and progesterone had an additive effect on Akt activation. One downstream effector of Akt is nitric oxide synthase (NOS). Our data suggest that NOS2 and NOS3 may play distinct roles in gonococcal cervicitis, which are, in part, dependent upon the specific signaling pathways triggered with CR3 engagement as well as the presence of varying concentrations of steroid hormones. Collectively, these studies provide new insights into the role of steroid hormones and innate immune effectors in gonococcal cervicitis.

S08.4

To Divide or Not to Divide: Cell Division Proteins in *Neisseria gonorrhoeae* and their Roles in Dynamic Localization and the Formation of a Bacterial Cytoskeleton

Jo-Anne R. Dillon

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The cell division protein, FtsZ, is the first cytoskeleton-forming protein identified in bacteria. Gonococcal FtsZ localizes to the midcell in both *E. coli* and *N. gonorrhoeae,* defines the cell division plane in the gonococcus, and forms helical structures. The placement of FtsZ at the midcell (Z-ring formation) is, in part, specified by the Min (minicell) proteins MinC, MinD and MinE. The Min proteins are essential for normal gonococcal cell division. The gonococcal Min proteins oscillate from one end of the cell to the other when expressed in rod-shaped *E. coli*; and the oscillation plane changes in round bacterial cells which divide in alternating perpendicular planes such as round *E. coli* and *N. gonorrhoeae* cells. MinC binds to MinD, forming a complex which reversibly associates with the membrane. Membrane association is regulated by MinE which binds to and stimulates the ATPase activity of MinD, causing the release of MinC from MinD and subsequently the dissociation of Min C/D complex from the membrane. Since MinC can deploymerize FtsZ, the presence of MinC and MinD at specific membrane locations prevents Z-ring formation at those sites. The time averaged concentration of oscillating MinD and MinC has been shown to be lowest at midcell, thereby allowing FtsZ to form a cytokinetic ring and to initiate cell division there. Min proteins form a helical array in both round and rod-shaped *E. coli* cells, implicated in the dynamic bacterial cytoskeleton. Specific mutations in both MinD and MinE alter array formation. Thus, certain gonococcal cell division proteins have an important role in dynamic localization and the formation of subcellular structures.

S08.5

Human Cervical Epithelial Cells are Resistant to Apoptosis upon Infection with Live *Neisseria gonorrhoeae*

S. A. Follows[1], P. Massari[2], L.M. Wetzler[1,2], and C.A. Genco[1,2]

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[2]Department of Medicine, Section of Infectious Diseases, Boston University School of Medicine

Neisseria gonorrhoeae infects the mucosal epithelium of both men and women, commonly causing asymptomatic disease in women. The interactions of *N. gonorrhoeae* with host cells has been studied extensively *in vitro*, most often in cancer cell lines such as HeLa cells, a cervical adenocarcinoma cell line which may not accurately reflect what is occurring *in vivo*. To study *N. gonorrhoeae* effects on target epithelial cells, a newly derived cell line was utilized in an attempt to more accurately model female gonococcal infection. Previously, it has been demonstrated that *N. meningitidis* and *N. gonorrhoeae* have the ability to modulate apoptosis in HeLa cells and male urethral cells, respectively. We hypothesized that the lack of symptomatic disease in females could be due to an immune evasion strategy in which this organism inhibits the host-cell apoptotic response. In this study, cell lines derived from the ectocervix and endocervix of the lower female genital tract were infected with *N. gonorrhoeae* strains FA1090B, a derivative of FA1090 which expresses only OpaB, and F62-pEG2, a GFP-expressing derivative of F62. After infection for 24h, epithelial cells were cultured under normal or apoptotic conditions. Our results

indicate that following gonococcal infection, cervical epithelial cells are resistant to the apoptotic processes of caspase-3 activation, mitochondrial depolarization, and DNA degradation. Additionally, the anti-apoptotic response is dependent on live bacteria, as heat-killed and gentamicin-killed *N. gonorrhoeae* did not display the same results. Infections utilizing GFP-expressing bacteria also demonstrated that this response is specific to individually infected cells. These results indicate that *N. gonorrhoeae* may establish infection in women by inhibiting the apoptotic response to infection, thereby resisting killing from both the host cell and the innate immune response. Furthermore, prolonged survival of the host cell potentially allows the bacteria to successfully invade cervical tissue, eventually transcending to the upper genital tract.

S09

Epidemiology

Tuesday 12 September 4.10pm

Plenary Hall (Halls A+B)

S09.1

Geographical and Temporal Distribution of Meningococcal Genotypes Isolated from 53,234 Healthy Teenage Carriers in the United Kingdom Between 1999 and 2001

Ana Belén Ibarz Pavón[1], Martin C.J. Maiden[1], The UK Meningococcal Carriage Study Group.

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As part of the UK meningococcal carriage study, which monitored the effect of MCC vaccine introduction on the meningococcal population, a total of 8,592 isolates were collected over three years (1999-2001) and characterized by MLST. For each isolate, geographic information (town, school, and postcode) were collected, along with risk factors for carriage for each participant. These data represent a valuable resource for the investigation of meningococcal population biology.

A number of analyses (F-statistics (F_{ST}) and AMOVA) were applied to the MLST data (both STs and nucleotide sequences) to assess the gene flow among the meningococci obtained: (i) over the three years of the study; (ii) in different locations; (iii) in different schools; and (iv) in different postcode districts.

The results indicated that 0.083% of the population structuring observed was an effect of the collection year (*p*-value<0.0001). Pairwise comparison revealed 0.093% variation between isolates obtained in 1999 and 2000 (*p*-value<0.0001), and 0.146% variation between those from 1999 and 2001 (*p*-value<0.0001), in contrast to a 0.025% variability between 2000 and 2001 (*p*-value 0.01802). Further analysis revealed that 0.12% (*p*-value<0.0001) of the population structure observed could be an effect of the introduction of the vaccine, the first measure of such an effect by population genetic methods.

A 0.24% variation was observed among different locations, compared to 1.07% seen among schools and 0.94% among postcodes. When schools were grouped into three categories according to the number of pupils, the variation seen was 1.12%, suggesting that is the closeness rather than the size of a community what defines the boundaries of a meningococcal subpopulation. The geographic results were consistent with those obtained in a study conducted in Bavaria, Germany. The results further demonstrate that public health interventions can generate a detectable effect on the bacterial population structure.

S09.2

Outcome and medical follow up in adolescent survivors of meningococcal disease: a prospective matched cohort study

Jennie Borg,[1] Deborah Christie,[1] Pietro G Coen,[1] Robert Booy,[2] Russell M Viner[1]

[1] Department of Paediatrics, Royal Free & University College Medical School, University College London, UK.
[2]NCIRS, The Children's Hospital Westmead, NSW, Australia

Despite the introduction in some countries of the serogroup C conjugate vaccine, meningococcal disease (MD) remains a major source of mortality and morbidity in adolescence. We examined the physical, cognitive, educational, social and psychological outcomes of meningococcal disease (MD) in adolescence and demographic and disease factors associated with outcome.

Population-based matched cohort study: 101 sex and age matched case-control pairs (aged 15-19 years at disease) from 6 regions of England were followed up 18-36 months after MD (46% males). Educational, social and vocational function, mental health, social support, self-efficacy, and quality of life data were collected using standardised questionnaires and neuropsychological tests.

57% (N=58) of cases had major physical sequelae. Survivors had greater fatigue (p=0.04), lower social support (p=0.01), poorer mental health function (p=0.07) and reduction in quality of life (p=0.01), and lower educational attainment (p=0.02) compared with controls. Global cognitive function (Cognitive summary score (CSS)) was 0.5 SD less than controls (p<0.0001). Those with serogroup C disease had greater physical sequelae than those with B disease. Greater cognitive deficit was associated with female gender (p = 0.055) and a younger age at diagnosis (p < 0.01). Only 53/101 cases had any medical follow-up after MD.

Survivors of MD in adolescence have a disturbing series of deficits including poorer physical and mental health, quality of life, cognitive function and educational achievement. Serogroup C is associated with poorer outcome. Of concern, medical care is poor after discharged from hospital. Routine follow up of adolescent survivors is essential for improving physical and psychological outcomes of MD.

S09.3

Prevalence of NadA and Factor H-Binding Protein (GNA1870) Variants in *Neisseria meningitidis* Group B Isolates from California and Maryland, USA: Implications for Design of a Multicomponent Group B Vaccine

Peter T. Beernink[1], Jo Anne Welsch[1], Lee H. Harrison[2], Arunas Leipus[1] and Dan M. Granoff[1]

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[2]University of Pittsburgh School of Medicine, Department of Medicine, Pittsburgh, PA USA.

Two important antigens in a promising meningococcal group B recombinant protein vaccine are GNA1870 (renamed Factor-H binding protein [FHBP] to reflect its critical function as a ligand for a key complement-regulatory protein), and NadA, an adhesin. *N. meningitidis* strains can be divided into three FHBP variant groups based on divergent protein sequences and antibody cross-reactivity. To develop a NadA- and FHBP-based group B vaccine, the prevalence of these antigens is important since some strains

lack *nadA* and antibodies against FHBP are bactericidal primarily against strains within a variant group. We used quantitative PCR to identify *nadA* and to assign FHBP genes into variant groups. Among 48 group B isolates from patients from 22 counties in California (2003-04), 83%, 13% or 4%, respectively, had variant (v.) 1, 2 or 3 FHBP, and 77% had *nadA*. The respective percentages among 50 group B isolates from the State of Maryland (1995-2005) were 52%, 44% or 4% for FHBP ($P<0.003$), and 32% for *nadA* ($P<0.001$). v.1 FHBP and the presence of *nadA* were prevalent in ST32/ET5 strains while v.2 FHBP and absence of *nadA* were prevalent among ST44 complex/Lineage 3 strains. Mab JAR1 reacts with a subset of v.1 FHBP proteins; in both collections, *nadA* segregated with JAR1-positive isolates (48/54 [89%] vs. 1/12 JAR1-negative v.1 isolates [8%], and 5/32 v.2 and v.3 isolates [16%], $P<0.0001$). In mice, a v.1 FHBP recombinant protein vaccine elicits serum bactericidal activity primarily against JAR1-positive but few JAR1-negative strains. A vaccine containing JAR1-positive v.1 FHBP could potentially prevent 73% and 38% of disease, respectively, in California and Maryland. The addition of NadA could prevent 83% and 42%, whereas the addition of v.2 FHBP could potentially prevent 90% and 86%. Regional antigenic differences among strains underscore the need to include multiple antigens for a broadly protective group B vaccine.

S09.4

Distribution of Hyper-invasive lineages of *Neisseria meningitidis* in Europe over 3 years

Carina Brehony[1], Martin Maiden[1], Keith Jolley[1], and the EUMenNet Consortium

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The aim of the study is to characterise meningococcal disease isolates from the 18 participating countries of the EUMenNet project, a pan-European infrastructure for the research and surveillance of European meningococcal disease. The European Meningococcal Multi-Locus Sequence Typing (MLST) Centre (EMMC) was set up as part of the EUMenNet and provides the infrastructure for a centralised high-throughput low-cost facility for MLST and PorA-typing of meningococcal isolates. It allows for the characterisation of representative isolates which will give an overview of the lineages of *Neisseria meningitidis* responsible for meningococcal disease throughout Europe. A representative sample of 3000 disease isolates was chosen from the 18 EU countries from the years 2000, 2001 and 2002 and have been typed by MLST and PorA.

A total of 982 different sequence types (STs) were identified; despite this diversity, 5 STs (ST-11, ST-32, ST-8, ST-269, ST-41) accounted for 40% of the isolates. Further, the isolates were resolved into 31 distinct clonal complexes, the main ones being ST-41/44 complex (Lineage 3), ST-11 (ET-37) complex, ST-32 (ET-5) complex, ST-8 complex (Cluster A4) and ST-269 complex. The distributions of clonal complexes found were broadly similar, although prevalence of each was slightly different among countries. Over the three years there were no major changes in the prevalence of clonal complexes across Europe. Some changes were an increase in the prevalence of the ST-11 clonal complex (19% to 21%) and a decrease in ST-8 complex (9% to 5%) over the three years. A total of 188 PorA types were found with 6 accounting for over half of isolates. Calculations of F_{ST}s for allelic profiles and concatenated locus sequences have shown a significant presence of structure.

The data have shown the distribution of a relatively small number of STs, clonal complexes and PorA types that account for a large proportion of disease-associated isolates in Europe. Continuing analysis and linkage to epidemiological data will provide a comprehensive look at those lineages associated with meningococcal disease in Europe. This will assist in the development of future vaccine strategies.

S10

Vaccinology: Part 1

Thursday 14 September 8.30am

Plenary Hall (Halls A+B)

S10.1

An Epidemic of Group B Meningococcal Disease Controlled by a Vaccine – The Final Chapter

O'hallahan J, Martin D, Oster P

Ministry of Health, Institute of Environmental Science and Research Ltd (ESR) Wellington, Chiron Vaccines Siena

Background and aims

In response to a Meningococcal B epidemic, a case was prepared for new health funding, and a new OMV strain-specific vaccine (MeNZB™) developed. Following clinical trials undertaken to show MeNZB™ is immunogenic, a national implementation strategy was prepared and ultimately MeNZB ™ was delivered to more than one million young people in New Zealand.

Methods

MeNZB™ was developed by Chiron in collaboration with the Norwegian Institute of Public Health and trialled in New Zealand in the target groups ie from 6 weeks to 20 years. On the basis of safety and immunogenicity, a provisional consent was granted. Additional safety and effectiveness data have been collected in the post-marketing phase. A national immunisation register has been implemented at the same time. This enables us to accurately report on vaccine coverage and estimate vaccine efficacy by calculating attack rates in vaccinated populations and unvaccinated populations.

Results

Over a two year period 80% of all those under 20 in New Zealand have completed the course of MeNZB™ and high coverage has been achieved in Pacific children who suffer disproportionately from the disease. The comprehensive safety surveillance has not revealed any significant safety concerns. Most rewardingly we are seeing the early, promising reduction in cases of disease and vaccine effectiveness calculations confirm an effective vaccine.

Conclusions

The mass vaccination programme to control a 15 year epidemic of meningococcal has been a success. Central to this success has been a public private partnership that shared the risks of the vaccine development, clinical trials and roll out. We have faced the same challenges that are common to other developed countries introducing new vaccines - distrust of authority and a vocal anti-immunisation group spreading misinformation.

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S10.2

A Universal Vaccine for Serogroup B Meningococcus

Marzia M. Giuliani, Jeannette Adu-Bobie, Maurizio Comanducci, Beatrice Aricò, Silvana Savino, Mariagrazia Pizza and Rino Rappuoli

Novartis Vaccines, Via Fiorentina 1, 53100 Siena, Italy

Meningitis and sepsis caused by serogroup B meningococcus are two severe diseases that still cause significant mortality. To date there is no a universal vaccine which prevents this diseases. In this work, five antigens discovered by reverse vaccinology were expressed in a form suitable for large-scale manufacturing and formulated with adjuvants suitable for human use. The vaccine adjuvanted by aluminium hydroxide induced bactericidal antibodies in mice against 78% of a panel of 85 meningococcal strains representative of the global population diversity. The strain coverage could be increased to 90% and above by the addition of CpG oligonucleotides or by using MF59 as adjuvant. The vaccine has the potential to conquer one of the most devastating diseases of childhood.

S10.3

Serogroup C Meningococcal Polysaccharide-Specific Plasma and Memory B-cells after Immunisation of Infants with a Glycoconjugate Vaccine

Dominic Kelly[1] , Mathew Snape[1], Kirsten Perrett[1], Elizabeth Clutterbuck[1], Ly-mee Yu[2], Linda Diggle[1], Viola Schultze[3], Astrid Borkowski[3], Richard Moxon[1], Andrew Pollard[1].

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[2]Centre for Statistics in Medicine, Oxford University, Oxford UK
[3]Novartis Vaccines, Marburg, Germany

The rate of infection due to serogroup C *Neisseria menigitidis* (MenC) has fallen since the introduction of MenC vaccines in the UK in 1999. However, serum antibody levels wane rapidly after infant immunisation with MenC vaccines and may be associated with a decrease in vaccine efficacy despite the presence of immunological memory. The phenotype, kinetics and quantity of B-cells that determine the antibody response to immunisation with glycoconjugate vaccines in infants are unknown.
We examined the kinetics of MenC-specific plasma and memory B-cell frequency in peripheral blood using a B-cell ELISPOT following 3-dose infant immunisation with a MenC glycoconjugate vaccine at 2, 3 and 4 months of age. Blood sampling was undertaken 4, 6, 8, 10, 12, 16 and 30 days following the 2 month and 4 month doses of the vaccine in separate cohorts of at least 5 infants for each time point.

After the 2 month vaccine administration, low numbers of MenC-specific plasma cells were detected in a minority of individuals between days 8 and 14. By contrast, following the 4 month dose, plasma cells were detected as early as day 4 after immunisation and appeared to decline thereafter with few detected beyond day 12. MenC-specific memory B-cells were detected in low numbers on days 16 and 30 after the 2 month dose but were readily detected in the majority of individuals at all time points after the 4 month dose of vaccine, peaking at the end of the first week.

This study demonstrates the feasibility of detecting B-cell responses to protein-polysaccharide conjugate vaccines in young infants and describes the differences in the nature of the B-cell response after 1 and 3 doses in infancy. Further studies are underway to test the hypothesis that there is a relationship between the early B-cell response and serological evidence of sustained protection from meningococcal disease.

S10.4

An Update on Meningococcal Group C Conjugate Vaccination Programme in England and Wales: Vaccine Efficacy, Antibody Persistence and Serological Correlates.

Ray Borrow[1,3], Mary Ramsay[2], Ed Kaczmarski[3], Nick Andrews[2], Helen Campbell[2], Liz Miller[2].

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[2] Immunisation Division, Centre for Infections, Health Protection Agency, Colindale, London, UK.
[3] Meningococcal Reference Unit, Health Protection Agency North West, Manchester Laboratory, Manchester Royal Infirmary, Manchester, U.K.

Meningococcal group C conjugate (MCC) vaccine was introduced into the UK immunisation schedule in 1999 with a catch-up as a single dose to all children aged 1-18 years. The number of group C (MenC) cases rapidly fell in the targeted age groups, and early analyses showed high vaccine effectiveness in all ages. Evidence of herd immunity was determined by comparing incidence of MenC disease in unvaccinated individuals before and after the MCC campaign. Although early analyses showed high vaccine effectiveness in all age groups, when effectiveness was measured again more than one year after vaccination there was a significant decline in the combined 1-18 year age groups, but this was still 89% (81 to 94) up to the end of June 2005. The most marked decline was in infants vaccinated in the routine infant programme, for whom there was no demonstrable efficacy after only 1 year. Vaccine effectiveness in toddlers fell to 71% (-40 to 93) by 30 June 2005 from 98% (64 to 97) in the first year, this was not significant . However, good disease control was maintained in the U.K. Of 464 cases of confirmed group C disease identified in those eligible for vaccination, information on vaccination history was obtained for 99.4%; of these, 53 were vaccine failures. MenC serum bactericidal antibody (SBA) titres in convalescent sera were significantly higher in vaccine failures than in unvaccinated cases. The antibody response in the vaccine failures was consistent with an anamnestic response to MenC disease suggesting that disease occurred despite MCC vaccine priming for immune memory. The assumption that immune memory was predictive of long term protection in the absence of a booster was shown to be incorrect, at least after vaccination in infancy and persistence of antibody paramount together with herd immunity. The short term correlate of protection has been previously demonstrated to be a SBA titre of 1:8 (using baby rabbit complement). Following a large seroepidemiology study involving the assay of over 2,800 sera long term correlates have been established.

S10.5

Immunogenicity and Safety in Infancy of a Novel Tetravalent Meningococcal Glyco-conjugate Vaccine

Snape, MD[1], Perrett, KP[1], Ford, KJ[1], Waterhouse, TM[1], Pace, D[1], Langley, JM[3], McNeil, S[3], Ceddia, F[2], Anemona, A[2], Halperin, S[3], Dobson, S[4], Pollard, AJ[1]

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[2] Novartis Vaccines, Siena, Italy
[3] Clinical Trials Research Center, Department of Pediatrics, Dalhousie University, Halifax, Canada
[4] Vaccine Evaluation Center, Department of Pediatrics, British Columbia's Children's Hospital, Vancouver, Canada

Introduction
Serogroup C meningococcal glycoconjugate (MenC) vaccines have been highly effective in controlling serogroup C meningococcal disease. However, disease caused by other serogroups, including serogroups A, W-135 and Y, remains a threat to global public health. We evaluated the response of healthy UK and Canadian infants to a novel tetravalent glycoconjugate adjuvanted vaccine utilising CRM_{197} as a carrier protein (MenACWY).

Methods
UK participants received MenACWY (2, 3, 4 months), MenACWY (2, 4 months) or a MenC vaccine (Menjugate®; 2, 4 months) concomitantly with DTaP-Hib-IPV. Canadian participants received MenACWY (2, 4 months) or MenACWY (2, 4, 6 months) concomitantly with DTaP-Hib-IPV, HBV and 7-PnC. Serum was obtained at baseline and 1 month following the immunisation course. The serological marker of protection was a serum bactericidal activity titre of ≥ 1:4 using human complement (hSBA).

Results
225 UK and 194 Canadian infants were enrolled. Serogroup A, C, W-135 and Y specific hSBA titres of ≥ 1:4 were achieved by (respectively) 93%, 96%, 97% and 94% of MenACWY (2, 3, 4 months) and 83%, 98%, 99% and 98% of MenACWY (2, 4, 6 months) recipients. At least 83% of UK and Canadian MenACWY (2, 4 months) recipients achieved an hSBA titre of ≥ 1:4 for each of serogroups C, W-135 and Y, as did 61% (UK) and 65% (Canada) for serogroup A. No significant difference in the percentage of participants achieving serogroup C specific hSBA titres of ≥ 1:4 was observed following 2 doses of Menjugate® or MenACWY. hSBA geometric mean titres were higher following Menjugate® than MenACWY (325 (95% C.I. 198 – 532) and 52 (95% C.I. 36 – 74) respectively). MenACWY and Menjugate® reactogenicity was comparable.

Discussion
MenACWY is well tolerated and immunogenic in the first year of life and has the potential to protect against serogroup A, C, W-135 and Y meningococcal disease from early infancy.

S11

Vaccinology: Part 2

Thursday 14 September 10.40am
Plenary Hall (Halls A+B)

S11.1

Comparison and correlation of *Neisseria meningitidis* serogroup B immunological assays used in a study of the 'Norwegian' meningococcal outer membrane vesicle vaccine, MenBvac.

Jamie Findlow[1], Stephen Taylor[2], Audun Aase[3], Rachel Horton[4], Robert Heyderman[4], Jo Southern[5], Nick Andrews[5], Rita Barchha[1], Ewan Harrison[1], Ann Lowe[1], Emma Boxer[2], Charlotte Heaton[2], Paul Balmer[1], Ed Kaczmarski[6], Philipp Oster[7], Andrew Gorringe[2], Ray Borrow[1], Elizabeth Miller[5].

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[5] Health Protection Agency, Centre for Infections, U.K.
[6] Meningococcal Reference Unit, Health Protection Agency North West, Manchester Laboratory, U.K.
[7] Chiron Vaccines, Italy

The prediction of efficacy of *Neisseria meningitidis* serogroup B (MenB) vaccines is currently hindered due to the lack of an appropriate correlate of protection. For outer membrane vesicle (OMV) vaccines, immunogenicity has primarily been determined by the serum bactericidal antibody (SBA) assay and OMV enzyme-linked immunosorbent assay (ELISA). However, the opsonophagocytic assay (OPA), surface labelling assay, whole blood assay (WBA) and salivary antibody ELISA have been developed although correlation with protection is presently undetermined. Therefore, the aim of the study was to investigate further the usefulness of, and relationships between, MenB immunological assays with heterologous MenB target strains.

A phase II trial of the candidate OMV vaccine, "MenBvac", with proven efficacy was initiated to compare immunological assays incorporating the vaccine strain and six heterologous strains. Correlations were achieved between the SBA assay, OMV ELISA and OPA when using human polymorphonuclear leukocytes and human complement but not between an OPA using HL60 phagocytic cells and baby rabbit complement. Correlations between the surface labelling assay and SBA assay and OMV ELISA were promising although target strain dependent. Modest changes in salivary antibody were detected in the salivary antibody ELISA and therefore correlations with other assays were poor. Correlations to the WBA were prevented as many samples had results greater than the range of the assay.

These results emphasise the complexity of the assessment of protein based vaccines and the need for standardised methodologies, including a standard meningococcal strain panel which would allow a more robust comparison of assays between laboratories and promote their further evaluation as correlates of protection against MenB disease.

S11.2

Non-Autoreactive Anti-N-Propionyl *N. meningtidis* group B Polysaccharide (N-Pr NmB PS) mAbs Bind to Membrane Proteins of NmB and *E. coli* K1 Bacteria

George S. Mittendorf, Andrew M. Fergus, Hardeep Kaur, Gregory R. Moe

Children's Hospital Oakland Research Institute, Oakland, CA, USA

Murine IgG monoclonal antibodies (mAbs) produced by immunization with an N-Pr NmB PS-tetanus toxoid conjugate vaccine mediate bactericidal activity against NmB in the presence of exogenous human complement and are protective in an infant rat model of NmB bacteremia. However, the mAbs have no or minimal cross-reactivity with purified NmB PS or chemically identical polysialic acid antigens expressed in human tissues. To determine the identity of antigens recognized by each of four anti-N-Pr NmB PS mAbs that are representative of different fine antigenic specificity groups, we prepared and purified cell fractions of NmB and *E. coli* K1. By inhibition ELISA and immuno dot blots, the antigens recognized by the mAbs were located in the membrane fraction but not capsular polysaccharide, periplasmic, or intracellular fractions. The membranes were solubilized in SDS, partially purified by SEC and separated by SDS-PAGE. On Western blots, one mAb bound to one set of proteins while the other three recognized a separate distinct set. Evidently, some of the proteins are modified with an as yet unknown alpha (2→8) linked sialic acid derivatives since one subset of mAbs recognize native or denatured proteins boiled in SDS, but do not bind to the same proteins similarly prepared from an NmC strain or an isogenic NmB strain that can not make sialic acid. None of the antigens were cross-reactive with an anti-group B capsular mAb. The proteins and modifying sialic acids are currently in the process of being characterized. The results suggest a previously unrecognized role for sialic acid derivatives in the biology and pathogenesis of NmB and a novel approach for developing sialic acid-based vaccines for preventing disease caused by all NmB strains without risk of also eliciting autoantibodies.

S11.3

Serogroup B *Neisseria meningitidis* Vaccine Development

Jan Poolman, Philippe Denoel, Christiane Feron, Karine Goraj, Vincent Verlant, Vincent Weynants

GlaxoSmithKline Biologicals, Rixensart, Belgium

Due to the dominant immune response mediated by PorA, outer membrane vesicles (OMVs) derived from serogroup B meningococcal wild-type strains of *Neisseria meningitidis* (MenB) confer only limited cross-protection against circulating PorA-heterologous wild-type strains. The partial cross-protection induced by wild-type OMV appears to be due to some minor and well conserved outer membrane proteins (OMPs) and lipooligosaccharide (LOS)

Strain H44/76 was genetically modified to down-regulate the expression of the major variable proteins PorA and FrpB and to up-regulate the expression of minor conserved OMPs. Different preparations of OMVs were produced from genetically modified H44/76 strains expressing either L3,7 LOS or truncated versions such as *lgtB* or *galE* LOS.

The positive impact of the up-regulation of minor conserved OMPs was demonstrated by ELISA using sera of mice immunized with (in a *galE* LOS background) OMP-up-regulated OMVs. By mixing sera from animals separately immunized with OMVs over-expressing different OMPs we have observed that anti-OMP antibodies synergize to mediate bactericidal activity

Separate murine immunization experiments have demonstrated that OMVs with high content of L3,7 or *lgtB* LOS were capable to induce bactericidal antibodies. OMVs with high content of *galE* LOS did induce comparable ELISA antibody levels, but such antibodies were found to be devoid of bactericidal activity.

Finally, we demonstrated that anti-minor OMP and anti-LOS antibodies show an additive effect with respect to cross-reactive bactericidal activity.

S11.4

Opsonophagocytosis in the Absence of Serum Bactericidal Activity Confers Protection against Meningococcal Disease

Joyce S. Plested [1], Jo Anne Welsch [1] and Dan M. Granoff [1].

[1]Children's Hospital Oakland Research Institute, Oakland, CA 94609

Background: Complement-mediated serum bactericidal activity (SBA) confers protection against meningococcal disease. The protective role of opsonic activity (OPA) is controversial.

Methods: We measured age-acquisition of human serum passive protective activity (PPA) against meningococcal group B bacteremia in the infant rat model. Group B and C PPA of adult sera were correlated with serum OPA measured by killing with human complement and human neutrophils.

Results: Multiple lines of evidence supported a protective role for opsonic activity. 1. Although the incidence of group B disease in adults is low, only 10% to 20% of adult sera were bactericidal against different group B strains. 2. In contrast, the age–acquisition of PPA against bacteremia in infant rats by group B strain NZ98/254 correlated inversely with the age-incidence of disease (PPA in 9% (34 sera) of 6-12 month olds; 22% (27 sera) of 2-year olds; 75% (20 sera) of 5-year olds; 80% (15 sera) of 10-year olds; and 92% (25 sera) of adults; P<0.001 for trend). 3. The presence of OPA in adult sera correlated with PPA (7 of 7 SBA-negative, OPA-positive adult sera tested conferred PPA against group B bacteremia compared with 1 of 7 sera negative for both SBA and OPA (P<0.001). 4. The corresponding results for group C were 10/10 and 2/9 (P<0.001) 5. Anti-capsular and anti-porin Abs conferred protection against group C bacteremia in C6-deficient infant rats. Since the serum complement of these animals supports opsonization (via Fc and C3b receptors), but not bacteriolysis (which requires C6 to form the membrane attack complex), the mechanism of PPA is opsonophagocytosis. Naturally-acquired group B and C OPA in adult serum is directed at the capsule since it was eliminated by absorption with homologous but not heterologous polysaccharide.

Conclusions: Serum OPA confers protection against meningococcal disease in the absence of bactericidal activity.

S12

Vaccinology: Part 3

Thursday 14 September 2.00pm

Plenary Hall (Halls A+B)

S12.1

Vaccination Re-Programmes Naturally Acquired Mucosal Immunity Against *Neisseria Meningitidis*

Victoria Davenport[1], Eleanor Groves[2], Rachel Horton[2], Terry Guthrie[2], Chris Hobbs[2], Jamie Findlow[3], Ray Borrow[3], Lisbeth Meyer-Naess[4], Phillip Oster[5], Neil Williams[2], Rob Heyderman[2],

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Carriage of *N. meningitidis* serogroup B (MenB) is thought to induce a naturally acquired immune response which is protective in most adults. We have previously shown that in the nasopharynx, the mucosal cellular arm of this response is largely CD4 T cell mediated, compartmentalised and Th1-biased. Whether systemic introduction of meningococcal antigen boosts pre-existing naturally-acquired mucosal immunity, changes the phenotype of immunity, or induces immunological tolerance is unknown.

In order to address this question, adults aged 18-34 years were vaccinated i.m. with two doses of MenB OMV vaccine (NZMenB™ based on the New Zealand epidemic strain ZN98/254) prior to routine tonsillectomy. The effects on pre-existing immunity against a range of MenB strains in both mucosal and systemic compartments was investigated by examining T cell proliferative responses, CD4+ T helper cell bias, and humoral responses (salivary IgG and IgA; serum IgG; and serum bactericidal activity) compared with unvaccinated controls.

Systemic immunisation with NZMenB™ resulted in an increase in peripheral T cell proliferation but little change to the pre-existing mucosal response. However, the phenotype of the CD4+CD45RO+ T cells responding to MenB in the mucosa and peripheral blood was found to have switched following vaccination. Peripheral blood responders showed more prominent IL-18R expression and interferon gamma secretion, suggestive of a Th1 bias. Tonsillar responders had a more balanced phenotype with higher proportions of cells expressing CRTH2 and higher levels of IL-5/ IL-10 secretion. Pre-existing salivary IgA and IgG levels were not influenced by i.m. vaccination. No clear relationship was found between pre-existing mucosal immunity and vaccine induced cellular or humoral responses in the periphery.

Overall, systemic vaccination against MenB was found to re-programme naturally acquired T cell mediated immunity in both the mucosal and systemic compartments. The implication of this switch requires further clarification in order to fully inform future vaccine development.

S12.2

Investigating the potential of LPS-based vaccines to protect against meningococcal disease

Andrew Cox[1], Frank St. Michael[1], Margaret Anne Gidney[1], Suzanne Lacelle[1], Dhamodharan Neelamegan[1], Wei Zou[1], Claire Wright[2], Joyce Plested[2], Katherine Makepeace[2], Phillip Coull[2], Richard Moxon[2] and James Richards[1]

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Despite the success of glycoconjugate vaccines based on the capsular polysaccharides of several serogroups of *Neisseria meningitidis*, the quest for an effective vaccine to combat meningococcal Group B (*Nm*B) disease continues. Similarity between the α-2,8 polysialic acid capsule of *Nm*B and the post-translational sialylated proteins of certain cell-surface expressed molecules of human cells has raised serious safety issues. Alternative immunogens have therefore been sought. Of these, outer membrane proteins of *Nm*B are leading candidates. However, fulfilling the several requirements of a protective antigen, including conservation, consistent expression, immunogenicity and induction of bactericidal antibodies has been difficult. In particular, the extensive antigenic variability of candidate cell surface expressed *Nm*B proteins has proved problematical. We are considering approaches based on the candidacy of lipopolysaccharide (LPS). The outer core of LPS contains several host-like structures and this potential cross-reactivity with human cell surface antigens coupled with the variability of the outer core structures precludes their consideration as vaccine candidates. In contrast to the outer core structures, the inner core is relatively conserved. We have demonstrated that a monoclonal antibody (designated mAb B5), directed to an inner core epitope on an immunotype L3 *galE* mutant, reacted with 76% of *Nm*B strains and could bind to the surface of intact *Nm*B bacteria. Additionally, using bactericidal assays and passive protection studies, this mAb was shown to kill *Nm*B *in vitro* and to reduce or eliminate bacteremia *in vivo* in an infant rat model. To investigate if active immunisation with a glycoconjugate displaying this conserved inner core structure could induce specific antibodies with protective activity, we have explored several conjugation strategies to optimise appropriate presentation of LPS inner core epitopes. We have found that conjugation strategies involving coupling through the detoxified lipid A region could elicit a functional immune response.

S12.3

Safety and Immunogenicity of a Tetravalent Meningococcal ACWY Glycoconjugate vaccine in Toddlers 12-23 Months of Age

Henry Shinefield[1], Steven Black[2], Kathy Ensor[2], Jitendra Ganju[3], Lisa Danzig[3], Peter Dull[3]

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[2]Kaiser Permanente Vaccine Study Center, Oakland CA
[3]Novartis Vaccines, Emeryville CA

Background: The meningococcus causes life threatening illness in young children, adolescents and adults. Currently a conjugate vaccine is licensed and recommended for adolescents in the US; no such vaccine is available for younger children despite significant disease risk in this age group. The objective of this study was to

evaluate the safety and immunogenicity of an investigational meningococcal ACWY polysaccharide CRM_{197} conjugate vaccine (MenACWY) in toddlers.

Methods: Healthy toddlers aged 12 – 23 months received a single dose of MenACWY. As no meningococcal vaccine is licensed in the US for children under 2 years of age, an immunogenicity comparison group of children 3-5 years of age receiving meningococcal ACWY plain polysaccharide vaccine (Menomune®) was enrolled. The immune response at day 29 was assessed by a serum bactericidal assay using human complement (hSBA). The data presented are interim immunogenicity data from 91 toddlers and 35 children.

Results: In the MenACWY group, erythema, tenderness and induration in the vaccinated arm were reported in 35%, 24% and 22% with <3% graded as severe. For immunogenicity in the MenACWY (todlers0 vs Menomune (3-5 yo) respectively, the percent with SBA titer ≥1:4 was: 82% vs 54%, p=0.004 for group A, 90% vs 43%, p=<0.001 for group C, 86% vs 80%, p<0.47 for group W, and 66% vs 60%, p=0.54 for group Y. Similarly for GMT response (ug/ml), GMT = 18 vs 8.3, pp=0.004 for group A, 22 vs 7, p<0.001 for group C, 21 vs 13 p=0.086 for group W and 13 vs 11, p=0.72 for group Y.

Conclusion: As measured by the percentage of subjects with an SBA titer ≥1:4 and by GMT, MenACWY was at least as immunogenic in toddlers as Menomune was in children 3-5 years of age. Novartis MenACWY conjugate vaccine offers promise for the prevention of meningococcal disease in toddlers 12 – 23 months of age.

S12.4

Effective Immunization Strategy Against Group B *Neisseria meningitidis* Using Purified Recombinant Lipidated P2086 Protein

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Neisseria meningitidis is a major causative agent of bacterial meningitis in human beings, especially among young children (≤ 2 years) and adolescents. Prevention of group B meningococcal disase represents a particularly difficult challenge in vaccine development, due to the poor immune response against the capsular polysaccharide of these strains and the possibility of developing an immune response to developing brain tissue. Since *N. meningitidis* is a respiratory pathogen, a mucosal immune response may play an important role in the defense against meningococcal infections. Therefore, mucosal immunization may be a useful route of immunization. In this study, mice were immunized intranasally with purified recombinant lipidated P2086 protein (rLP2086) adjuvanted with either CT-E29H, a genetically modified cholera toxin that is significantly reduced in enzymatic activity and toxicity or RC529-AF, a synthetic immunostimulant molecule in aqueous formulation. We demonstrated that intranasal immunization with rLP2086 adjuvanted with either CT-E29H or RC529-AF significantly reduced nasal colonization in mice challenged with either of two different strains of group B *N. meningitidis* that express different allelic forms of LP2086 proteins within the same subfamily. To identify an immunological correlate for the reduction of bacterial colonization in nasal tissues we compared the levels of serum and mucosal IgG and IgA antibodies specific to rLP2086 and serum IgG antibodies to various strains of group B *N. meningitidis* whole cells, as well as the functionality of the immune sera. We show that mice immunized with rLP2086 produced strong systemic and mucosal rLP2086-specific IgG and IgA antibody responses. The serum antibodies elicited by rLP2086 cross-reacted to the whole cells of multiple strains of group B *N. meningitidis* and had functional serum bactericidal activity against several strains of group B *N.* meningitidis. These results demonstrate that an intranasal immunization with rLP2086 protein formulated with a detoxified cholera toxin or RC529-AF could prevent the initial colonization of group B meningococcus and become an effective immunization strategy against group B *N. meningitidis*.

S12.5

Preclinical Immunogenicity of a combination vaccine containing 9-valent PorA Outer Membrane Vesicle Vaccine and 13-valent Pneumococcal Conjugate Vaccine

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Neisseria meningitidis (Men) and *Streptococcus pneumoniae* (Pn) are both human pathogens causing severe diseases like meningitis and septicaemia in young children. For endemic MenB disease a 6-valent PorA Outer Membrane Vesicle (OMV) vaccine (HexaMen), initially evaluated in UK children, has been extended to a 9-valent PorA OMV vaccine (NonaMen) to increase serosubtype coverage of MenB strains. A 13-valent pneumococcal conjugate vaccine formulation (13vPnC) as follow-up for the successful 7-valent PnC vaccine is currently being tested in clinical studies. To reduce the number of injections in infants, the development of combination vaccines should receive strong emphasis.

The aim of this study was to investigate the pre-clinical immunogenicity of a combination vaccine containing NonaMen (serosubtypes P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1 and P1.18-1,3,6) and 13vPnC vaccine (Serotypes 1, 3, 4, 5, 6A ,6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM197) and to evaluate any potential immunological interference between MenB and pneumococcal components of the vaccine.

NIH mice were immunized twice subcutaneously with the vaccines combined in one syringe, or given individually. Combining 13vPnC vaccine with NonaMen had no negative effect on the induced antibody response against any pneumococcal serotypes or MenB serosubtypes. On the contrary, we showed that the anti-pneumococcal antibody responses were enhanced when both vaccines were combined in one syringe compared to individual vaccination. Furthermore, New Zealand white rabbits were immunized with the combination of NonaMen/13vPnC vaccine with or without DTaP/IPV-Hib vaccine given concurrently at a different injection site. Comparable anti-PorA and anti-pneumococcal polysaccharide antibody titres were found in the two groups.

Conclusion: Preclinical studies in mice and rabbits indicate that NonaMen can be combined with 13vPnC vaccine without causing immunological interference. These studies support the development of a combination vaccine for MenB and pneumococci, thereby potentially reducing the number of injections in the early childhood immunization schedule.

S13

Vaccinology: Part 4

Thursday 14 September 4.10pm

Plenary Hall (Halls A+B)

S13.1

Safety and Immunogenicity of a Tetravalent Meningococcal ACWY Glycoconjugate Vaccine in Healthy Children

Steve Black[1], Henry Shinefield[2], Kathy Ensor[1], Jitendra Ganju[3], Lisa Danzig[3], Peter Dull[3]

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Background: The meningococcus causes life threatening illness in young children, adolescents and adults. Currently a conjugate vaccine is licensed and recommended for persons age 11 - 55, but no such vaccine is available for younger children despite significant disease risk in this age group. The objective of this study was to compare the safety and immunogenicity of an investigational meningococcal ACWY polysaccharide CRM_{197} conjugate vaccine (MenACWY) with the safety and immunogenicity of a meningococcal ACWY plain polysaccharide vaccine (Menomune®).

Methods: In this randomized, single-blind, controlled study, healthy 2 – 10 year olds participants were randomized 1:1 to received either MenACWY or Menomune. The immune response at day 29 was assessed by a serum bactericidal assay using human complement (hSBA). The data presented include interim immunogenicity analyses from 311 enrolled subjects.

Results: Pain, erythema, and induration in the vaccinated arm were reported by 34%, 17%, and 14% of the MenACWY group and by 26%, 8%, and 4% of the Menomune group, respectively. Severity was mild (≤3% graded as severe) and self-limited. For immunogenicity in the MenACWY vs Menomune respectively, the percent with SBA titer ≥1:4 was: 80% vs 45%, p<0.001 for group A, 77% vs 59%, p=0.005 for group C, 94% vs 71%, p<0.001 for group W, and 92% vs 66%, p<0.001 for group Y. Similarly for GMT response (ug/ml), GMT = 36 vs 6.2, p<0.001 for group A, 28 vs 14, p=0.025 for group C, 74 vs 13 p<0.001 for group W and 74 vs 16, p<0.001 for group Y.

Conclusion: Both vaccines were well tolerated. The immune response to MenACWY, as measured by the percentage of subjects with an SBA titer ≥1:4 and by GMT, was superior to Menomune for all four vaccine serogroups. Novartis MenACWY conjugate vaccine offers promise for the prevention of meningococcal disease in young children.

S13.2

How do we determine a biologically meaningful protective antibody level?

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The progression of meningococcal disease from the earliest non-specific symptom to death can be very rapid. Vaccination induces immune memory with antibodies protecting against infection. Vaccines developed against group B meningococci incorporate surface-exposed outer membrane proteins from the vaccine strain. Such a vaccine, MeNZB™, has recently been used for the control of an epidemic of meningococcal disease in New Zealand. The PorA protein VR2 epitope has been shown to be the immunodominant antigen targeted by the serum bactericidal antibody (SBAb) responses to the vaccine. However, levels of SBAb post-vaccination are not maintained for long periods and the question arose as to how fast a measurable increase in SBAb occurred following antigen stimulation. The opportunity arose to measure this in a small group of adults 15 months after receiving their primary vaccination. Six adults participated in this study. Each was given one dose of MeNZB™ containing 25μg of OMV NZ98/254 antigen. A baseline serum sample was taken prior to vaccination and then at 12h, 24h, 48h, 4days, 7d, 14d, 21d, and 42d later. IgG antibody unit levels were measured by ELISA assay using NZ98/254 vesicles for antibody capture and SBAb levels were determined using a validated serum bactericidal assay. By 4 days none of the subjects had shown an increase over pre-vaccination levels in either ELISA IgG units or SBAb. After 7 days an increase had become apparent in all five subjects sampled. Anti-NZ98/254 SBAb GMTs increased from a baseline of 14 (95%CI:1.87-101) at day 1 to 37(2.3- fold from baseline) on day 8, to 66(4.8-fold from baseline) at 21 days and 42 days later. Similar results were obtained by the analysis of ELISA IgG Units. The results of this study raise interesting questions around the sensitivity of the serum bactericidal antibody assay and what constitutes a biologically meaningful protective antibody level.

S13.3

Serological Response to Epidemic Serogroup A ST-7 Meningococcal Meningitis in Ethiopians

Gunnstein Norheim[1], Abraham Aseffa[2], Mohammed A. Yassin[3], Getahun Mengistu[4], Afework Kassu[4], Dereje Fikremariam[5], Wogene Tamire[5], Elisabeth Fritzsønn[1], Yared Merid[3], E. Arne Høiby[1], Morten Harboe[2,6], Degu Abebe[2], Tsegaye Alebel[7], Tangen T[1], Caugant DA[1] and Einar Rosenqvist[1]

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[7]North Gondar Zonal Health Bureau, Gondar, Ethiopia

Few studies have characterized the antibody response following disease caused by the serogroup A meningococci. In order to elucidate the importance of critical components of protective serological responses induced by the natural course of disease, we performed a study in Ethiopian meningococcal meningitis patients during outbreaks in 2002-03. Sera were obtained from 71 patients confirmed positive with serogroup A ST-7 meningococci (A:4/21:P1.20,9), at up to three time points after onset of disease, as well as from 113 Ethiopian controls. The sera were analyzed in ELISA for IgG levels against serogroup A polysaccharide (APS), outer membrane vesicles (OMVs), lipooligosaccharide (LOS) L11 and NadA protein and for serum bactericidal activity (SBA). Results showed that despite relatively high pre-existing SBA titers and levels of IgG against APS, OMVs, LOS and NadA in acute phase patient sera, significant increases were seen during early convalescent phase. Except for IgG against LOS, levels returned to the acute phase levels in late convalescent phase at median 8.5 months afterwards. On immunoblotting the main antigens targeted were the PorA, PorB, RmpM, OpcA and LOS. Some degree of correlation was observed between SBA with rabbit complement using the ST-5 strain F8238 (rSBA) and SBA with human complement using an ST-7 strain (hSBA) (r_s = 0.4 – 0.5). In early convalescent sera, rSBA correlated best with anti-APS IgG (r_s = 0.6) while the hSBA correlated best with anti-OMV IgG (r_s = 0.8). Considering SBA as a correlate of protection, undergoing disease apparently did not confer long-term protection beyond levels observed in controls. Proportions of patient sera with both rSBA titer ≥1:128 and hSBA titer ≥1:4 were 29 – 74%, whereas proportions with hSBA titers ≥1:4 and rSBA <1:128 were up to 15%. As hSBA and anti-OMV IgG correlated, induction of anti-OMV antibodies could be beneficial for meningococcal vaccines for the Meningitis Belt.

S13.4

Investigating the 2D Immunoproteome of *Neisseria meningitidis*

Tom Mendum[1], Jane Newcombe[1], Chris Tang[2], Johnjoe McFadden[1]

[1]School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UK
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Several lines of evidence suggest that natural infection with the meningococcus confers cross-reactive protection; however the identity of the antigenic targets of natural immunity remains uncertain. Identification of such cross-protective targets, if indeed they exist, would greatly facilitate development of a vaccine capable of protecting against all strains of the meningococcus, including group B strains.

To identify likely protein candidates for use in a broad range neisserial vaccine, we adopted an immunoproteomics approach to identify the antigens that are recognised by patients who have recovered from meningococcal disease. Proteins from *Neisseria meningitidis* L91543 (group C) were extracted and separated on 2D gels. These gels were Western blotted and probed with individual acute and convalescent sera from a number of patients infected with a range of strains, including group B strains.

Individual patient responses tended to be very variable with most protein spots recognised by only a few patient serum samples. Immunoreactive spots were identified on a replicate gel, excised and identified by peptide mass fingerprinting and MALDI-TOF spectroscopy. Putative proteins sequences were cloned and expressed in *E. coli* and their immunoreactivity confirmed by further Western blotting against patient sera.

Approximately 450 protein spots were resolved on the 2D SDS-PAGE gels of which approximately 30 were found to be immunogenic with one or more of the sera. The proteins identified have diverse predicted functions, ranging from known surface immunogens such as RmpM, ribosomal components, to enzymes involved in central metabolism. Most of the identified proteins have not yet been investigated as potential vaccine components.

S14

Host Response: Part 1

Friday 15 September 9.00am

Plenary Hall (Halls A+B)

S14.1

A Novel Mechanism for Complement-mediated Killing of Encapsulated *Neisseria meningitidis* Elicited by Monoclonal Antibodies to Factor H-binding Protein (Genome-derived Neisserial antigen 1870)

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Genome-derived Neisserial antigen 1870 is a promising meningococcal vaccine candidate capable of eliciting complement-mediated bactericidal activity. Recently, we suggested that this lipoprotein be renamed factor H-binding protein (FHBP) to reflect its critical function in binding a key regulatory protein of the alternative complement pathway, which enables the organism to evade complement-mediated killing. As a vaccine target, FHBP is unique in that the density of surface-exposed epitopes is sparse. This may account for failure of some anti-FHBP mAbs to activate complement-mediated bacteriolysis. We identified two murine monoclonal antibodies (mAbs), JAR 4 (IgG2a) and JAR 5 (IgG2b) that recognize different FHBP epitopes. These mAbs are not bactericidal alone but are synergistically bactericidal. When JAR 4 and a non-immune human serum complement source were incubated with encapsulated *Neisseria meningitidis* there was C4 and C3 binding, which was insufficient to lead to C5 convertase formation, C5b-9 binding, or killing. JAR 5 but not JAR 4 inhibited binding of fH, but JAR 5 did not activate C4 or C3. The synergism of JAR 4 and JAR 5 enhanced C4, C3, C5 convertase, and C5b-9 binding, and resulted in efficient bacterial killing at mAb concentrations <0.5 µg/ml. Although the individual mAbs were unable to bind C1q, the first protein in the classical pathway, the combination of JAR 4 and 5 engaged and activated C1q. In addition, the ability of JAR 5 to block binding of fH would result in amplification of alternative pathway activation and more efficient killing of the bacteria. Thus FHBP may elicit two distinct mechanisms of antibody-mediated protective activity: antibodies that are bactericidal, and antibodies that inhibit binding of a complement down-regulator protein to the Neisserial surface, which may enhance the bactericidal activity of a second antibody. These data provide further support for inclusion of this molecule in a meningococcal vaccine.

S14.2

Differential Use of MAPKS by *N. Meningitidis* PorB and Los in Costimulatory Molecule Expression and Cytokine Production

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Activation of the innate immune system is partly attributed to the recognition of bacterial components by toll-like receptors (TLR), which aid in initiating an effective adaptive immune response. Porin from *Neisseria meningitidis* constitutes a large portion of the outer membrane protein content and has been demonstrated to activate antigen presenting cells (APC) via TLR2. LOS, also from the outer membrane, is capable of activating cells through TLR4. Macrophages, which express many TLRs, patrol tissues for invading pathogens and upon activation are capable of initiating an immune response by activating T cells and inducing cytokine production. T cell activation requires two signals, one from the T cell receptor and a second through the interaction of CD86 on the APC with CD28 on the T cell. Activation of APCs by PorB or LOS induces a series of signal transduction events, including mitogen activated protein kinase (MAPK) phosphorylation, and results in the upregulation of the costimulatory molecule CD86. However, little is known about the role of these signaling processes in CD86 expression, particularly in response to activation by TLRs. Using specific MAPKs inhibitors we set out to determine if any of the MAPKs are involved in CD86 expression induced by the TLR2 ligand PorB or the TLR4 ligand LOS on murine macrophages. Our data demonstrate that inhibition of Erk1/2 phosphorylation with the inhibitor U0126 has no effect on TLR2 or TLR4 induced CD86 surface upregulation. Interestingly, inhibition of p38 using SB203580 prevented LOS, but not PorB, induced CD86 expression. When SP600125 was used to inhibit JNK activity upregulation of CD86 was prevented on PorB, but not LOS treated cells. These data indicate that PorB and LOS induce activation of similar signaling pathways but utilize them differently for CD86 expression, thereby furthering our understanding of the initiation of the immune response to Neisseria.

S14.3

DC-SIGN (CD209) Recognition of *Neisseria gonorrhoeae* is Circumvented by Lipooligosaccharide Variation

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Neisseria gonorrhoeae (GC) expressing opacity (Opa) proteins adhere to human host cells and stimulate phagocytosis due to the interaction of certain Opa proteins to CEACAM1 (CD66a) receptors. However, our experiments show that the Opa-CEACAM1 interaction does not play a significant role in adherence between these bacteria and dendritic cells (DCs). Instead, phagocytosis of GC by DCs is mediated by the dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin, (DC-SIGN, CD209) receptor. DC-SIGN recognition and subsequent phagocytosis of GC is limited, however, to a lipooligosaccharide (LOS) mutant (*lgtB*) of GC. This conclusion is supported by experiments demonstrating that HeLa cells expressing human DC-SIGN (HeLa-DC-SIGN) bind exclusively to and engulf an *lgtB* mutant of GC and this interaction is specifically blocked by an anti-DC-SIGN antibody. The experiments suggest that LOS variation may have evolved as a mechanism for GC to avoid phagocytosis by DCs.

Furthermore, we attempted to map the sites within the core LOS that are directly involved in GC LOS-DC-SIGN interaction. We took advantage of four sets of well-defined core LPS mutants, which are derived from *E. coli*, *Salmonella enterica* serovar Typhimurium, *Neisseria gonorrhoeae* and *Haemophilus ducreyi* and determined interaction of each of these four sets with DC-SIGN. Our results demonstrated that N-acetylglucosamine (GlcNAc) sugar residues within the core LPS in these bacteria play an essential role in targeting the DC-SIGN receptor. Our results also imply that DC-SIGN is an innate immune receptor and the interaction of bacterial core LPS and DC-SIGN may represent a primeval interaction between Gram-negative bacteria and host phagocytic cells.

S14.4

Non-Lipoligosaccharide Mediated Signalling through TLR4 causes Meningococcal Sepsis via the MyD88 Dependent Pathway

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The Toll-Like Receptors (TLRs) have a crucial role in host innate immune defences against microbial infections, controlling a range of events including the induction of chemokines, proinflammatory cytokines, costimulatory molecules, and adhesion molecules. These events can be both beneficial, as well as potentially harmful, with uncontrolled proinflammatory responses leading to sepsis. Meningococcal lipooligosaccharide (LOS) has long been thought to be a major inflammatory mediator of fulminant meningococcal sepsis and meningitis with disease severity correlating with circulating concentrations of LOS and proinflammatory cytokines.

To investigate the role of TLR2 and TLR4 on the development of meningococcal sepsis we infected wild type C57BL/6 mice and mice with mutations in these receptors and in the adaptor molecule MyD88 with the serogroup C meningococcal strain FAM20 and an LOS-deficient isogenic strain, *lpxA,* and examined *in vivo* responses to infection.

Wild type C57BL/6 mice and $TLR2^{-/-}$ mice were highly susceptible to a high dose meningococcal infection. Induction of cytokines in response to the wild type strain and the LOS-deficient mutant was abolished in $TLR4^{-/-}$ mice and $MyD88^{-/-}$ mice, and both strains of meningococci failed to cause fatal sepsis in these mice. Fatality in the wild type and $TLR2^{-/-}$ mice correlated with high proinflammatory cytokine (IL-6 and TNF), chemokine (KC) and C5a levels in serum, and high neutrophil numbers in blood. The proinflammatory response to meningococci was independent of the expression of LOS since the isogenic LOS-deficient strain *lpxA* induced equivalent proinflammatory responses as the wild type strain FAM20, and caused fatal infection in both wild type C57BL/6 mice and $TLR2^{-/-}$ mice.

We conclude that both LOS and another neisserial TLR4 ligand cause meningococcal sepsis, and confirm that LOS can act via both the MyD88 dependent and independent pathways, whilst stimulation with the non-LOS TLR4 ligand occurs solely via the MyD88 dependent pathway.

S15

Host Response: Part 2

Friday 15 September 10.50am

Plenary Hall (Halls A+B)

S15.1

Role of H8 and LOS in Gonococcal Macrophage Interactions

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We studied the effects of two neisserial surface molecules, LOS and H.8 on the interactions of *N. gonorrhoeae* with human monocytes and macrophages. We constructed an isogenic set of strains that expressed genetically invariant LOS (expressing only the lacto-N-neotetraose α chain, lactosyl α chain) and/or the outer membrane protein H.8, and studied the interactions of these strains with human monocytes and/or macrophages, including their ability to induce cytokine expression. Our data demonstrate that alterations in the carbohydrate moiety of LOS do not directly impact the production of proinflammatory cytokines. However, when strains expressing sialyated LOS were used, an up regulation of the chemokine MCP-2 was observed. (Differential levels of cytokines such as IL-8, TNFα, and IL-12 were not seen.) When we compared cells expressing H.8 to those lacking this protein, we similarly observed no significant differences in monocytic cytokine production. This data seems to indicate a minor role for H.8 in eliciting the inflammatory responses of monocytes. However, H.8 does appear to play a significant role during the initial physical interactions between gonococci and macrophages. In comparison with the parent strain, most of the $H.8^-$ bacterial challenge population was observed to form large aggregates over time. This aggregation was shown to occur only upon bacterial contact with macrophages. These aggregates appeared to resist phagocytosis, while the parent strain was more readily taken up by macrophages and appeared to be distributed within multiple vacuoles. We propose that monocyte/ macrophage recognition of gonococcal surface structures, such as H.8, may promote efficient phagocytosis of a portion of the bacterial population. This scenario may explain why gonococci appear to be distributed throughout multiple vacuoles in macrophages and neutrophils during natural infection and may allow for the intracellular growth of some gonococci.

S15.2

Serum Resistance of *Neisseria gonorrhoeae* is Restricted to Humans; A Possible Explanation for the Species Specificity of Gonococcal Infections

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Neisseria gonorrhoeae is the causative agent of gonorrhea, a disease restricted to humans. Complement forms a key arm of the innate immune system that combats gonococcal infections. *N. gonorrhoeae* uses its outer membrane porin (Por) molecules to bind the alternative pathway of complement down-regulatory protein factor H (fH) to evade killing by human complement. Por1A gonococci that resisted killing by normal human serum (NHS) were killed by sera from rodent, lagomorph, and primate species, which cannot be readily infected experimentally with these organisms. We found that these gonococci did not bind fH in rhesus, baboon and chimpanzee sera; the addition of purified human fH to these sera at physiologic concentrations fully restored serum resistance. Sialylation of gonococci through lipooligosaccharide augments binding of human fH to Por1B gonococci and also results in serum resistance. In similar experiments, Por1B sialylated gonococci bound human fH, but did not bind fH from other primate species.

To locate the fH binding site(s) to Por1A and to Por1B gonococci, the latter sialylated, we genetically constructed fusion proteins consisting of different fH fragments and the Fc portion of mouse IgG2a (fH-Fc) to facilitate recognition of fH via the Fc tag. Using flow cytometry, we found that both Por1A gonococci and Por1B sialylated gonococci bound to short consensus repeats (SCRs) 18-20-Fc. Using constructs that deleted SCRs 18, 19 and 20 individually, we found that the three SCRs together were required for binding. We also confirmed that SCRs 18-20-Fc inhibited free fH binding to sialylated Por1B and resulted in killing by NHS, of otherwise serum resistant gonococci. Our findings may lead to the development of better animal models for gonorrhea and may also have implications in the choice of complement sources to evaluate neisserial vaccine candidates.

S15.3

Killing of *Neisseria gonorrhoeae* by Human Polymorphonuclear Leukocytes Occurs Non-Oxidatively

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Symptomatic infection by *Neisseria gonorrhoeae* (Gc) is characterized by an abundant purulent exudate consisting primarily of polymorphonuclear leukocytes (PMNs). Since the gonorrheal exudate contains viable, infectious bacteria, Gc must be resistant to some of the bactericidal factors of PMNs. To identify the Gc gene products that confer resistance to PMNs and the PMN factors they are directed against, we established an *in vitro* assay in which adherent, primed human PMNs are synchronously infected with Gc. Under these conditions approximately 40% of the viable Gc associated with PMNs at the start of the experiment are killed within the first hour of infection, after which the surviving Gc begin to replicate.

Activated PMNs generate antimicrobial reactive oxygen species like hydrogen peroxide (H_2O_2). We recently defined the transcriptional response of Gc to sublethal levels of H_2O_2 and identified several genes whose products confer resistance to H_2O_2, including those known to be important for H_2O_2 degradation (*kat*) and repair of damaged DNA (*recN*), as well as a previously uncharacterized gene product with similarity to zinc metalloproteases (*ngo1686*). Mutants in *ngo1686* and *recN* were more sensitive than the wild-type FA1090 parent to PMN killing. However, the *kat* mutant was killed as readily as the parental strain; furthermore, treatment of PMNs with inhibitors that prevent the generation of reactive oxygen species did not affect the survival of the *ngo1686* mutant or the wild-type parent. These results provide compelling evidence that PMN killing of Gc occurs by non-oxidative means and argue that Gc induction of the H_2O_2 transcriptional response to is dispensable for bacterial survival after PMN exposure. We are currently investigating which PMN non-oxidative antimicrobial factors have antigonococcal activity and why oxidative killing mechanisms are ineffective against Gc.

POSTER PRESENTATION ABSTRACTS

15th International Pathogenic Neisseria Conference 2006 (IPNC 2006 Australia)

As at 31 July 2006; Refer to the Addendum for any program updates

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P01
Antibiotic Resistance

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P1.1.01

Quinolone-Resistant *Neisseria gonorrhoeae* (QRNG) Surveillance: Integrating Molecular Resistance Testing into Clinical Care

Tukisa Smith[1], Julie Giles[2], Khalil Ghanem[1], Johan Melendez[1], Vincent Marsiglia[3], Florence Keane[4], Margaret Bash[4], Jonathan Zenilman[1]

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QRNG prevalence has increased dramatically. Traditional susceptibility testing cannot be performed when nucleic acid amplification tests (NAAT) are used. We have developed NAAT probes for QRNG in NG. To test the feasibility and outcome of using a NAAT-based resistance assay in an area not hyper-endemic for QRNG, we tested 716 NG clinical samples collected from March to October 2005 from patients attending Baltimore STD clinics. We identified mutations in gy*rA* and *parC* using Roche LightTyperTM fluorescence melt-curve analysis (confirmed by sequencing), and typed samples by *porB* variable region (VR) typing.

Mutations in *gyrA*, *parC*, or both were identified in 38/716 isolates (5.3%). 21/38 (55.3%) could be re- cultured for MICs: 6/21(29%) were CipR (MIC≥ 1µg/mL), and 8/21(38%) were CipI (MIC= 0.12-0.5µg/mL). By clinical records, 40% of CipR and 67% of CipI strains were isolated from patients not known to be part of a QRNG high-risk group. All CipR strains had had an identical *porB* VR type. This genotype was also identified in 7/17 samples that could not be re-cultured, suggesting endemic transmission of a CipR strain in 1.8% (13/716) of NG infections tested. *N. meningitidis* was identified by *porB* VR typing in 9 samples with *gyrA* or *parC* melt-curve changes.

Molecular-based resistance surveillance found a much higher prevalence of mutations than was clinically suspected. A large proportion of QRNG did not have CDC-defined risk factors. *porB* VR typing increased the specificity of molecular resistance testing and identified sustained transmission of a CipR strain indicating an early endemic pattern in an area with low QRNG by traditional surveillance.

Optimizing and integrating real-time high throughput non-culture based molecular assays into surveillance and clinical care may better define the local epidemiology of resistant organisms, help guide appropriate therapeutic interventions, and provide a potential solution to antimicrobial susceptibility testing in the NAAT era.

P1.1.02

The MtrE Outer Membrane Protein and Not the TolC-like Protein of *Neisseria meningitidis* Is Used by the *Mtr* Efflux System

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Our inspection of the *Neisseria meningitidis* MC58 and Z2491 genome sequences revealed the presence of an open reading frame (ORF) that would encode a protein bearing significant similarity to the TolC outer membrane protein (OMP) of *Escherichia coli.* Klee et al. reported that *tolC* and a second ORF, which they classified as a *hlyD* pseudogene, were linked within a genetic island in the chromosome of strain Z2491, which is absent in *N. gonorrhoeae* In *E. coli*, TolC serves as the OMP channel for both multi-drug efflux pumps and the secretion of the RTX toxin α-hemolysin. With respect to drug efflux, the amino acid sequence of TolC is similar (22.6% identity) within a 186 amino acid stretch to the MtrE OMP of *N. gonorrhoeae* strain FA19 and *N. meningitidis*. The MtrC-MtrD-MtrE efflux pump serves to export antimicrobial hydrophobic agents (HAs) that enter gonococci or meningococci, including those that bathe mucosal surfaces and are part of the innate host defense system (e.g., antimicrobial peptides, bile salts and progesterone). The documented role of TolC in export of antimicrobials from *E. coli* and the presence of a TolC homolog in meningococci but not gonococci led us to ascertain whether the predicted TolC protein in meningococci could substitute for MtrE in efflux of HA mediated by the *mtr* system. We report that a 2.3 kilobase pair locus in *Neisseria meningitidis* strain M7 was identified containing two transcriptionally linked open reading frames that encode TolC- and HlyD-like proteins. These transcriptionally linked genes were found to be maximally expressed during log phase. Through the use of insertional mutagenesis of *tolC* and *mtrE,* we found that the meningococcal TolC protein could not functionally replace the MtrE protein as the OMP channel in drug export mediated by the *mtr* efflux pump.

P1.1.03

Antimicrobial Susceptibility of *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* Isolates Causing Meningitis at the Far East of Russia in 2003-2004

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Epidemiology Department, State Vladivostok Medical University[1]

Despite of the measures of epidemiological surveillence, the Far East of Russia takes the first place in Russia on morbidity and mortality from bacterial meningitis..In addition of the difficulties of diagnosis, there is still a problem with etiological strucuture of the meningitis in our region because there is a lack data about epidemiological role of such a dangerous pathogen as *Neisseria meningitis*, and also there is no data what part take the meningitis caused by more common pathogens as *Streptococcus pneumoniae*

and *Haemophilus influenzae*. Aim was to define etiological strucutre of bacterial meningitis in the Far East of Russia in 2003-2004 years, to establish role of *Neisseria meningitis* in it and to study antimicrobial susupectibility pattern of these pathogens.Methods: we investigated 540 strains which were isolated from patients with meningitis in the Far East of Russia in 2003-2004 years, for antimicrobial resistance patterns they were tested by disk diffusion and microdilution methods, including beta-lactamase testing methods.RESULTS: Patients with culture-confirmed bacterial meningitis (540 patients) were identified including 205 (39%) patients with *H. influenzae*, 162 patients (30%) with *Streptococcus pneumoniae*, 70 patients (13%) with *N. meningitidis,* 97 patients (18%) with other bacteria. The susceptibility of *H.influenzae* strains to ampicillin was about 25,85% (53 strains) and 100% to ceftriaxone. Resistance of *S.pneumoniae* strains to penicilline was about 2,4% (4 strains) and 7,4 % (12 strains) to erythromycine, and in *Neisseria meningitis* strains there was revealed resistance to tetracycline and doxycycline in 17,1% (12 strains), and nine strains were resistant to rifampicine.Conclusion: so, we revealed that in addition of the ubiqitous pathogens the significant role in etiological structure of meningitis in the Far East of Russia plays *Neisseria meningitis*. The disinctive antimicrobial resistance pattern of this pathogen and epidemiological danger of it have us to optimize the routine schemes of treatment of aerosol infections.

P1.1.04

Vancomycin Reaches its Site of Action in the Periplasm by Diffusing Through the Gonococcal PilQ Pore.

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Vancomycin is a large glycopeptide antibiotic (1450 Da) that binds to the D-Ala-D-Ala C-terminus of disaccharide pentapeptide substrate during synthesis of the bacterial cell wall. Because it is too large to diffuse through porin channels, which have pore sizes of ≥500-600 Da, vancomycin is not effective at inhibiting growth of most Gram-negative bacteria (Minumum Inhibitory Concentrations (MICs) ≥ 125 μg/ml). Previous studies, however, have documented that vancomycin has a relatively low MIC (~4-32 μg/ml) against several strains of *Neisseria gonorrhoeae* when compared to other Gram-negative bacteria. Given the ability of PilQ to permit influx of penicillin and tetracycline into the periplasm, we reasoned that PilQ might allow influx of vancomycin and thus be responsible for the relatively low MICs of vancomycin against *N. gonorrhoeae*. In support of this hypothesis, mutation or deletion of PilQ in FA19 or FA19 *penA mtrR penB* (PR100) resulted in 4- to 6-fold increases in the MIC of vancomycin. The presence of the well-characterized *penA*, *mtr*, and *penB* resistance determinants had little to no effect on resistance. The *pilQ1* F595L mutation, which was shown previously to increase the diffusion of haem across the outer membrane, decreased the MIC for vancomycin 5-fold, suggesting that it increases either the size or the open time of the PilQ pore. In addition, deletion of *pilM*, *pilN*, or *pilO*, which we have shown increases antibiotic resistance in PR100 by preventing the opening of the PilQ pore, also increases resistance to vancomycin. Taken together, these data are consistent with a model in which the PilQ secretin forms a pore in the outer membrane that allows diffusion of antibiotics into the periplasm, and is a major site of entry for large antibiotics such as vancomycin.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P1.2.02

Cost-effective Sampling of Pharyngeal Meningococci to Predict Antimicrobial Resistance in Invasive *N. meningitidis*: a Ten-Year Study

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Background. Surveillance of antimicrobial resistance (AMR) in *N. meningitidis* from invasive disease in Australia and elsewhere revealed increasing numbers of meningococci with decreased penicillin susceptibility and instances of chloramphenicol, quinolone and rifampicin resistance. Some of this AMR originates in oro-pharyngeal commensals and is acquired by meningococci through transformation. Surveillance of pharyngeal meningococci may thus provide additional information on AMR in invasive isolates. We compared AMR in invasive and pharyngeal meningococci over a 10-year period in Sydney.

Methods. The serogroup and penicillin, ceftriaxone, rifampicin and ciprofloxacin MIC were determined for all invasive meningococci isolated in Sydney from 1994 onwards. These were compared with meningococci obtained from the pharynx of STD clinic attendees over the same period.

Results. The 1620 isolates examined comprised 1009 invasive and 611 pharyngeal meningococci. 1004 (99.5%) of the invasive, but 216 (35%) of the pharyngeal meningococci were encapsulated. The pharyngeal isolates were significantly less sensitive to penicillins than invasive meningococci and this differential trended higher over 10 years. Two (0.2%) invasive but 23 (3.7%) pharyngeal meningococci were penicillin-resistant. Beta-lactam use in Australia was high in the study period. One quinolone and two rifampicin resistant meningococci were in the invasive group but none in the pharyngeal meningococci.

Conclusions. The higher rate of decreased penicillin susceptibility in pharyngeal meningococci probably results from high beta-lactam exposure in the general community. STD clinics have not used penicillins for 15 years and clinic use of ceftriaxone is single-dose. Conversely, the low rates of resistance to other antibiotics in both pharyngeal and invasive meningococci reflect their restricted use as reserve agents. Longitudinal surveillance of AMR in pharyngeal meningococci may serve as an 'early warning' for AMR in invasive meningococci. STD clinic patients represent a stable sample with high rates of meningococcal carriage. Their meningococci are readily sampled by routine diagnostic practices, making this approach cost-effective.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

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P1.2.03

Total Variation in the *penA* Gene of *Neisseria meningitidis.* Correlation Between Susceptibility to β-lactam Antibiotics and *penA* Gene Heterogeneity

Sara Thulin, Per Olcén, Hans Fredlund and Magnus Unemo

National Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Introduction:
Recent decades, the prevalence of *Neisseria meningitidis* isolates with reduced susceptibility to penicillins has increased. Strains with intermediate resistance to penicillin (pen^I) are mainly due to mosaic structures in the *penA* gene, encoding the penicillin-binding protein 2.

Aims:
To explore the totally reported and presently identified variation in the *penA* gene and to describe the detailed association between *N. meningitidis penA* sequences and minimum inhibitory concentration (MIC) of different penicillins.

Materials and Methods:
The susceptibility to different β-lactam antibiotics was determined, using Etest, for 60 Swedish clinical *N. meningitidis* isolates and 17 reference strains. The *penA* gene of the isolates was sequenced and compared to 237 *penA* sequences published in GenBank.

Results:
The divergent mosaic alleles differed by 3% to 24% compared to the designated wild type *penA* gene. By studying the final 1143-1149 bp of *penA* in a sequence alignment, 130 sequence variants were identified. In a 402 bp alignment of the most variable regions, 84 variants were recognized. Good correlation between elevated MIC values and presence of *penA* mosaic structures was found for penicillin G and ampicillin. Based on our results, pen^I isolates comprise MIC>0.094 mg/L for penicillin G and MIC>0.064 mg/L for ampicillin. Ampicillin was the best antibiotic for precise categorization of *N. meningitidis* isolates as pen^S or pen^I. In comparison with wild type *penA*, two specific pen^I sites were altered in all except two mosaic *penA* sequences, which were published in Genbank and no MIC values of the corresponding isolates were described. Consequently, we have no evidence that these isolates comprise the pen^I phenotype.

Conclusions:
Monitoring the relationship between *penA* sequences and MIC values to penicillins is crucial for developing fast and objective methods for susceptibility determination. By studying the *penA* gene, genotypical determination of the susceptibility in also culture negative cases can be accomplished.

P1.2.04

Rapid and Sensitive Identification of Reduced Susceptibility to Penicillins in *Neisseria meningitidis* by Real-time PCR and Pyrosequencing technology

Sara Thulin, Per Olcén, Hans Fredlund and Magnus Unemo

National Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Introduction:
The prevalence of *Neisseria meningitidis* isolates with reduced susceptibility to penicillins (pen^I) has increased during recent decades. The pen^I phenotype is mainly due to mosaic structures in the *penA* gene, encoding the penicillin-binding protein 2. In a previous study, we identified two nucleotide sites in the *penA* gene, which in comparison with penicillin susceptible *penA* wild type isolates were altered in all pen^I isolates. Consequently, two specific nucleotide markers for *penA* mosaic alleles in *N. meningitidis* were identified.

Aims:
To develop a fast and sensitive method for diagnosis of reduced susceptibility to penicillins in *N. meningitidis* using real-time PCR and pyrosequencing.

Materials and Methods:
A 140 bp fragment of the *penA* gene was amplified using real-time PCR (LightCycler system with SYBR Green I fluorescence melting curve analysis). Subsequently, a smaller fragment (40 to 50 nucleotides), including the two previously identified pen^I specific sites, was studied using pyrosequencing technology. For evaluation of the developed method, 60 Swedish clinical isolates and 19 reference strains, previously *penA* sequenced with conventional Sanger sequencing, were examined.

Results:
All clinical isolates and reference strains were successfully amplified and pyrosequenced. The two pen^I sites were identified in all pen^I isolates, previously shown to comprise *penA* mosaic alleles and reduced susceptibility to penicillins, but not in any *penA* wild type isolates. Pyrosequencing allowed rapid (in approximately 1.5 hour) determination of 96 sequences. Overall, the results using pyrosequencing technology were in full concordance with the previously performed conventional Sanger sequencing.

Conclusions:
Real-time PCR and pyrosequencing for detection of *penA* mosaic alleles form a rapid, sensitive, specific and high throughput method for molecular detection of reduced susceptibility to penicillins in *N. meningitidis*. The method enables fast and sensitive genetic diagnosis of reduced susceptibility to penicillins in clinical *N. meningitidis* isolates as well as in culture negative *N. meningitidis* cases.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

The Discussion Session for the following posters is from: 9.30pm – 10.15pm Tuesday 12 September

P1.3.01

Real-Time Multiplex PCR Assay Detecting *penA* and *ponA* Genotypes In *Neisseria gonorrhoeae*

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In the gonococci, the chromosomally mediated expression of multiple resistance genes and the production of TEM-1 β lactamase (a penicillinase plasmid-mediated resistance) are the two independent mechanisms involved in a susceptible to resistant strain switch. Chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae* (CMRNG) arise, in part, through alterations in Penicillin-Binding Proteins (PBPs), the major targets of □-lactam antibiotics, notably by the production of altered forms of penicillin binding protein 2 (PBP 2) that have a decreased affinity for penicillin. This reduction is largely, although not exclusively, due to the insertion of an aspartic acid residue (Asp-345A) into the amino-acid sequence of PBP 2 *penA* gene. This alteration led to a mosaic structure in the transpeptidase region of this gene and was associated with a decreased susceptibility to penicillin in all NG strains with a minimum inhibitory concentration (MIC) ≥ 0.03 μg/ml. In the same way, a single amino-acid mutation, (Leu_{421}→ Pro), in the *ponA* gene which encodes PBP1 is due to a single base change and was reported in all CMRNG strains for which MICs of penicillin were ≥ 1 □g/ml. We developed a real-time multiplex PCR protocol based on the LightCycler® technology with a fluorometric hybridization probes system for rapid and specific detection of these mutations, predicting penicillin susceptibilities, notably in the absence of culture. Following an extensive evaluation involving 120 isolates of NG or direct clinical samples, melting curve analysis correctly evidenced a reproducible 5 °C Tm shift in all N. gonorrhoeae strains harbouring one of these mutation, as determined by conventional sequencing analysis. Moreover, the mutation profiles obtained with our assay showed a good correlation with the pattern of penicillin susceptibility generated with classical antibiograms. In conclusion, our protocol proved to be a good means for the CMRNG detection and useful to set up an effective antimicrobial strategy to gonococci.

P1.3.02

Is the Mosaic *penA* Sequence Solely Responsible for Decreased Ceftriaxone Susceptibility in *Neisseria gonorrhoeae*?

David M. Whiley[1], Athena Limnios[2], Sanghamitra Ray[2], Theo P. Sloots[1], John W. Tapsall[2]

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[2]Microbiology Department, South Eastern Area Laboratory Services, Sydney, NSW, Australia

Mutations in the *penA* gene encoding the penicillin-binding protein 2 (PBP2) are a major contributor to the emergence of chromosomally-mediated resistance to beta-lactam antibiotics in *Neisseria gonorrhoeae*. Japanese studies on untyped gonococci have identified mosaic *penA* genes associated with decreased susceptibilities to the later-generation cephalosporins cefixime and ceftriaxone, widely used for treatment of gonorrhoea. Danish investigators noted the 'clonal' nature of a cluster of ceftriaxone less-sensitive gonococci in Copenhagen. We observed increasing numbers of ceftriaxone less-sensitive gonococci in Sydney over several years and examined the complete *penA* sequences of 79 gonococci selected on the basis of geographical and temporal diversity and representing ceftriaxone MICs in the range <,/= 0.03 mg/l (n=25); 0.06 mg/l (n=43); and 0.125 mg/l (n=11). Phenotyping was by means of auxotyping, serotyping and antibiogram determination and genotyping by NG-MAST sequence-based analysis.

We identified 17 different PBP2 amino acid sequence patterns indicating considerable variation in the PBP2 proteins in this sample. Notably, 11 isolates had previously-reported mosaic *penA* sequences and all but one of these had a ceftriaxone MIC of 0.06 mg/l. A further five strains had near-identical sequences to the genbank reference sequence (M32091) and all had ceftriaxone MICs </= 0.008 mg/l. The same and different PBP2 sequence patterns were distributed amongst diverse pheno- and genotypes. Aside from these observations, there was no distinct association between PBP2 sequence and ceftriaxone susceptibility, with some of the amino acid sequence patterns providing ceftriaxone MICs ranging from 0.016 to 0.125 mg/l.

Overall, the results provide further evidence that the mosaic *penA* sequence is of considerable interest from an antibiotic susceptibility perspective and that further spread of this sequence should be monitored. However, most of our isolates with reduced susceptibility to ceftriaxone lacked the mosaic *penA* sequence. Thus, the results indicate that additional factors are contributing to the emergence of altered ceftriaxone susceptibility.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P1.3.03

Antimicrobial Susceptibility and Molecular Determinants of Quinolone Resistance in *Neisseria gonorrhoeae* Isolates from Shanghai

Yang Yang[3], Mingmin Liao[1], Wei-Ming Gu[3], Kelli Bell[1], Lei Wu[3], Nelson F. Eng[1], Chu-Guang Zhang[3], Yue Chen[4], Ann M. Jolly[4], and Jo-Anne R. Dillon[1,2]

[1]Vaccine and Infectious Disease Organization, and [2]Department of Biology, University of Saskatchewan, Saskatoon, Canada
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[4]Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Canada

Objectives: To determine the antimicrobial susceptibility of *Neisseria gonorrhoeae* from Shanghai and to type the quinolone resistance-determining regions (QRDRs) of ciprofloxacin resistant (Cip^R) isolates.

Methods: *N. gonorrhoeae* isolates (n=159) were consecutively collected from male patients in Shanghai and examined for their antimicrobial susceptibilities to penicillin, tetracycline, ciprofloxacin, spectinomycin and ceftriaxone. The mutation profiles of the QRDRs of *gyrA* and *parC* was determined for 103 isolates including 1 susceptible isolate and 1 isolate with intermediate levels (Cip^I) of susceptibility to ciprofloxacin.

Results: High percentages of the 159 isolates were resistant to ciprofloxacin (98.7%), penicillin (93.1%) and tetracycline (56.5%). PPNG (37.8%) or PP/TRNG (13.8%) accounted for 51.6% of the isolates. Chromosomal (CM) resistance (R) to pencillin was observed in 41.5% of the isolates. Tetracycline resistance was noted in 56.5% of the isolates with 20.1% carrying plasmid-mediated resistance and 36.4% being chromosomally resistant. All isolates were susceptible to ceftriaxone and spectinomycin, although a trend to decreased susceptibility was noted. QRDR mutations were observed in the 101 Cip^R and the 1 Cip^I isolate, in contrast to the ciprofloxacin susceptible isolate tested. Mutations in the QRDRs comprised 4 predominant (65.0% of the 102 isolates) patterns of a total of 19 patterns. Mutations in *parC* were significantly associated with higher MIC values to ciprofloxacin.

Conclusions: Spectinomycin and ceftriaxone are currently recommended for the treatment of gonorrhea in Shanghai. Although this study indicates that these antimicrobials should remain effective, the identification of isolates with decreased susceptibility underscores the importance of on-going antimicrobial susceptibility surveillance to monitor and respond to the emergence of resistant isolates.

P02
Bacterial Genetics, Physiology & Metabolism

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P2.1.01

Gonococcal AHU Strains Establish a Nitric Oxide Steady State Level Within an Anti-inflammatory Range

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N. gonorrhoeae can grow by anaerobic respiration by using nitrite as an alternative electron acceptor. Under these growth conditions, *N. gonorrhoeae* produce and degrade nitric oxide (NO), an important host defense molecule. These bacteria have been shown to establish and maintain NO at a steady state level, which within laboratory strain F62 is a function of the nitrite reductase/nitric oxide reductase ratio. Using a free radical analyzer, we examined if the parameters of NO metabolism within clinical isolates were similar to those previously measured in F62. In addition, we studied their ability to effectively control and detoxify NO levels, illustrating a potential role in modulating NO to anti-inflammatory concentrations within a host.

The nitrite reductase activities (122-197 nmoles NO_2 reduced/ min-OD) and nitric oxide reductase activities (88-155 nmoles NO reduced/ min-OD) were similar to the specific activities seen in F62 (241 nmoles NO_2 reduced/ min-OD and 88 nmoles NO reduced/ min-OD, respectively). In 7 gonococcal strains, the NO steady state levels established were similar to that of F62 (801-4236nM NO), while 2 of the strains, identified as AHU auxotrophs which cause asymptomatic infection in men, had 100-fold lower NO steady states (13-24nM NO). All tested strains in the presence of a NO-donor, DETA/NO, quickly lowered and maintained NO levels far into the anti-inflammatory range of NO (<300nM).

Sequencing of the two reductases involved in denitrification showed no major amino acid substitutions in the AHU strains. In presence of a gratuitous electron donor, methyl viologen, NO steady states were similar between F62 and the AHU strains. A double mutant in two potential electron donors, Laz and ccp, showed a 25% decrease in NO levels when steady state was reached, compared to F62. Thus the lower NO steady states with nitrite in AHU strains is likely due to differences in electron donors.

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P2.1.02

The Gonococcal Genetic Island Provides a Bypass Mechanism to TonB-Dependent Intracellular Survival of *Neisseria gonorrhoeae* within Cervical Epithelial Cells

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Neisseriae gonorrhoeae has evolved a repertoire of iron acquisition systems that facilitate essential iron uptake in the human host. Acquisition of iron requires both the energy-harnessing cytoplasmic membrane protein, TonB, as well as specific outer membrane TonB-dependent transporters (TdTs) As the iron sources accessed by intracellular gonococci are currently unknown and intracellular residence may contribute to persistence of infection, it is important to characterize the mechanisms the gonococcus uses to thrive within epithelial cells. In this study, we investigated the survival of gonococcal strains FA1090 and MS11 within ME180 human cervical epithelial cells and the contribution that high affinity iron acquisition plays in this process. Gonococcal intracellular survival was monitored over 24 hours by use of a gentamicin protection assay. Survival was dependent upon iron sources supplied by the host cell since treatment of infected cultures with Desferal attenuated the intracellular viability of both gonococcal strains. With respect to the role of the Ton system, this protein complex provided a substantial benefit to survival of gonococcal strain FA1090 within ME180 cells. Furthermore, expression of the putative outer membrane transporter TdfF was critical to the intracellular replication of this strain, most likely by providing a means of high affinity iron acquisition. However, infection with a TonB-mutant strain of MS11 yielded a similar number of gentamicin-resistant colonies compared to the corresponding wild-type parent. The Ton system defect was bypassed in MS11 by the presence of the gonococcal genetic island (GGI). More specifically, inactivation of a structural protein, TraN, within the GGI in combination with the TonB mutation resulted in the inability to replicate within cervical cells. We are currently investigating the detailed molecular basis by which the Ton system defect in MS11 can be bypassed by expression of type IV secretion components.

P2.1.03

Identification and Characterization of Three *Neisseria gonorrhoeae*-Specific Gene Clusters that are of Putative Bacteriophage Origin

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Global gene expression analysis of *Neisseria gonorrhoeae* has shown that a subset of genes upregulated after treatment with sublethal concentrations of ciprofloxacin is annotated as hypothetical proteins of putative bacteriophage origin in the sequenced *N. gonorrhoeae* strain FA1090 genome. These genes cluster into three regions on the gonococcal chromosome. The *Neisseria* Uptake Sequence (NUS, necessary for gonococcal DNA uptake), occurs less frequently in the three gene clusters than in the entire genome, at one per 2683 bp, 5639 bp and 9307 bp; compared to one per 1069 bp in FA1090. The GC content of each of the clusters is comparable to the entire FA1090 genome (54.6%, 56.3%, 51.8% vs 54.0%). BLAST searches indicate that these clusters are not present in the sequences of *N. meningiditis* serogroup B strain MC58, *N. menigiditis* serogroup A strain Z2491 and *N. lactamica* ST-640 (University of Chicago).

The three clusters are similar in that many of the genes homologous to known bacteriophage genes are present in two or all three of the clusters, with an organization reminiscent of the temperate bacteriophage phage λ. However, there are several genes that are unique to each cluster, some of which have homology to genes implicated in virulence in other pathogenic bacteria.

Preliminary experiments show that one such gene is present in *N. gonorrhoeae* strain MS11 as well as in 20 tested recent clinical isolates from Michigan. This gene is not present in the eight commensal *Neisseria* species examined or in a serogroup C *N. menigitidis* strain. We hypothesize that the genes unique to *N. gonorrhoeae* may have been retained in the genome over time as an adaptation of the gonococcus to the human host. The characterization of gonococcal-specific genes will enhance our understanding of the gonococcal disease process and the distinction of *N. gonorrhoeae* as a human pathogen.

P2.1.04

A TlpA Homologue from *Neisseria gonorrhoeae* Plays a Role in Defense against Oxidative Stress

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TlpA is a protein belonging to the family of thiol-disulfide oxido-reductases. TlpA proteins are small, soluble, redox-active proteins of low molecular mass (~12kDa) that contain the conserved active site motif, CxxC within a domain that contains a thioredoxin-fold. Many bacterial genes encoding periplasmic thiol:disulfide oxidoreductase proteins have been identified. Enzymes of this type have previously been reported to been involved in protein folding/disulfide bond formation (DsbA) as well as the biogenesis of c-type cytochromes (CcmG) and maturation of cytochrome c oxidases of the aa_3-type (TlpA).

Although *N. gonorrhoeae* does not contain a cytochrome c oxidase of the aa_3-type it does possess a *tlpA* gene. By insertion of an antibiotic resistance selection marker into the *tlpA* gene of *N. gonorrhoeae* 1291, a knock out mutant was generated. Phenotypic analysis was carried out on the *tlpA* mutant to determine the role of the TlpA protein in pathogenic Neisseria. *N. gonorrhoeae* strain

1291 *tlpA* mutant was found to be highly sensitive to oxidative killing by paraquat and hydrogen peroxide, indicating that the TlpA protein of *N. gonorrhoeae* strain 1291 is involved in protection against oxidative stress which suggests a new antioxidant role for TlpA proteins. In addition, the TlpA protein of *N. gonorrhoeae* strain 1291 was over-expressed as a water-soluble protein in *Escherichia coli.* The protein was purified and used to generate polyclonal antibody to confirm the phenotype of the *tlpA* mutant and sub-cellular location of the TlpA protein. A thioredoxin activity assay was also used to confirm the activity of the TlpA protein.

P2.1.05

Detection of Two Genetic Elements Associated with Increased Incidence of Serogroup C *Neisseria meningitidis* Infection and Antigenic Shift in the United States during the 1990s by Representational Difference Analysis (RDA)

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Background. The increased incidence of *Neisseria meningitidis* serogroup C invasive disease in the United States during the 1990s was attributed primarily to strains belonging to the ST11 clonal complex. Subcapsular genotyping of isolates from Maryland identified distinct “early” and “late” clones defined by antigenic shift at FetA. Whether this antigenic change was responsible for the increased incidence or a marker for other genetic events is unclear. This study uses RDA to identify additional genetic differences that may have contributed to the emergence of the late clone.

Methods. *Rsa*I-digested tester DNA (late clone) was ligated to specific adaptors followed by two rounds of subtractive hybridization with *Rsa*I-digested driver DNA (early clone). PCR amplification of subtracted tester DNA was performed with adaptor specific primers. PCR products were confirmed to be tester specific by Southern Blot analysis. The identity of tester specific products was determined by BLAST homology search. PCR amplification with primers specific to identified homologous sequences was performed on DNA from ST11 isolates belonging to either the early or late clone to confirm late clone specificity.

Results. PCR amplification of subtracted tester DNA with adaptor specific primers generated bands ranging in size from ~600 bp to ~1500 bp. Southern Blot analysis of ~200 cloned RDA generated fragments identified 17 tester specific clones. A BLAST search of all late clone-specific sequences identified homology to either IS1301 or pJS-B plasmid sequences from *N. meningitidis*. PCR amplification of IS1301 or pJS-B plasmid sequences detected these elements only in genomic DNA from ST11 isolates belonging to the late clone.

Conclusions. Two unique genetic elements were identified by RDA from an emergent *N. meningitidis* serogroup C ST11 clone that had undergone antigenic shift at FetA. Further investigation is required to determine the role of IS1301 and the pJS-B plasmid in clonal emergence and *N. meningitidis* pathogenesis.

P2.1.06

PilQ Missense Mutations Distinguish Multimer Stability, DNA Transformation, and Pilus Expression from Host-Cell Adherence and Colony Morphology in *N. gonorrhoeae*

R. Allen Helm, Michelle M. Barnhart, and H. Steven Seifert

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The type IV pilus is a critical virulence factor in *N. gonorrhoeae* pathogenesis. Previous studies demonstrated that pili are involved in twitching motility, host cell adherence, and DNA transformation. While the detailed mechanisms allowing pili assembly and expression are not fully understood, it is thought that they assemble in the periplasm and are secreted through PilQ, a homo-dodecameric outer membrane protein that is a member of the secretin family of proteins.

To help probe the role of PilQ in pilus expression and function, error-prone PCR was performed on the middle one-third of *pilQ*, and the mutated gene returned to the gonococcal chromosome by transformation. A total of 19 missense mutants were isolated, each with a single point mutation. The 19 mutations spanned the length of the mutated region. All mutants produced colony morphologies similar to a *pilQ* null mutant. Mutants were tested for presence of pili via TEM, DNA transformation, PilQ multimer stability, and adherence to host epithelium and displayed a diverse array of phenotypes. Sixteen mutants had greatly reduced pilus expression while three showed piliation levels similar to the parental strain. Transformation efficiencies ranged from undetectable to parental levels. PilQ multimer stability in SDS-PAGE also varied from undetectable to parental levels. Neither transformation nor PilQ multimer stability correlated with piliation or adherence. Although several mutants had adherence levels higher than the null mutant, none approached the parental level. Taken together, these results indicate: Missense mutations in PilQ can drastically effect piliation and pilus-dependent functions, PilQ multimer stability in SDS-PAGE does not correlate with piliation or pilus-dependent functions, and there is no correlation between transformation, adherence, or colony morphology. Finally, the fact that two mutants have parental levels of detectable pili yet have adherence levels similar to the null mutant indicates that PilQ may directly influence host-cell adherence.

P2.1.07

Natural transformation in *Neisseria lactamica*

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DNA exchange plays an important role in the generation of diversity and so aids the evolution of pathogenic Neisseria. Sequence analysis has revealed that several meningococcal genes have a mosaic structure and this, together with the acquisition of genes from certain commensal Neisseria species, indicates that interspecies transfer of DNA contributes to diversity in

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meningococci. In a recent study we were intrigued to find all members of a diverse bank of *N. lactamica* strains (53 in total) wholly resistant to conjugative transfer of plasmid pMIDG100, in contrast to *N. meningitidis* and a wide range of commensal Neisseria -*N. flavescens, N. cinerea, N. subflava, N. flava* and *N. sicca* (O' Dwyer *et al.* (2004) I&I 72:6511-6518). A similar result was described by Genco *et al*., who also failed to identify a *N. lactamica* strain that could be induced to take up the conjugative plasmid pLE2451 (Genco *et al*. (1984) J Infect Dis 150:397-401).

Neisserial strains were tested for their ability to take up an antibiotic resistance marker located in equivalent chromosomal loci in *N. meningitidis* and *N. lactamica*. While meningococci were as readily transformed with DNA from *N. lactamica* as from other meningococcal strains, the converse was not the case. *N. lactamica* strains are significantly less transformable than *N. meningitidis* ($p<0.05$, Fisher's exact test). We speculate that the difference in specificity found between *N. lactamica* and *N. meningitidis* transformation, not explained by disparity in the presence of the neisserial uptake sequence, divergence of DNA sequence, potential *N. lactamica/N. meningitidis* intermediates or *dam/drg* phenotype, is due to more discriminant restriction/modification system(s) operating in *N. lactamica* than in *N. meningitidis*.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P2.2.01

Construction and characterisation of a nitrite reductase-deficient mutant of *Neisseria meningitidis*

Jay Laver[1], Tania Stevanin[1], James W.B. Moir[2], Robert C. Read[1]

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Neisseria meningitidis, the causative agent of meningococcal disease in humans, is likely to be exposed to nitrosative stress during natural colonisation and disease. Monocyte-derived macrophages (MDMs) produce nitric oxide (NO) in response to infection via the inducible NO synthase (iNOS). The genome of *N. meningitidis* includes the genes *aniA* and *norB,* which encode nitrite reductase and nitric oxide reductase, respectively. Previous work has shown that expression of the *norB* gene enhances survival of *N. meningitidis* within MDMs. A mutant containing an insertionally-inactivated *aniA* gene was generated by homologous recombination of extracted *aniA*$^-$ DNA with the wild type chromosome. PCR analysis has shown that it contains the insertionally-inactivated copy of the *aniA* gene; and Western blotting indicates there is no expression of AniA protein. Under microaerobic conditions, the wild type reduces nitrite to NO over 24hrs. In contrast, the *aniA* mutant does not clear nitrite from the system. We also show that binding, internalisation and killing by MDMs is similar for both strains.

P2.2.02

Nucleotide Excision Repair in *Neisseria Gonorrhoeae*

Brian LeCuyer and H. Steven Seifert

Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago IL, USA

Nucleotide excision repair is a system used by eukaryotes and prokaryotes to repair many types of DNA damage. In bacteria, the nucleotide excision repair system consists of UvrA, UvrB, UvrC, and the UvrD helicase, DNA polymerase I, and ligase. When DNA damage stalls the transcriptional machinery, transcription-repair coupling factor (TRCF), the product of the *mfd* gene, recognizes the stalled RNA polymerase and recruits the Uvr complex. TRCF then facilitates the release of RNA polymerase from the DNA.

In order to examine the role of UvrABCD and TRCF in DNA repair in *N. gonorrhoeae*, each gene was disrupted by a transposon insertion and introduced into strain FA1090. Similar to reports in *E.coli*, the *uvr* mutant strains were highly sensitive to UV. A *recA* null strain had an intermediate level of survival, similar to that of the *mfd* mutant. Complementation of *uvrB* and *uvrD* restored survival to wild type levels. Disruption of *uvrD* additionally resulted in a much greater frequency of PilC-mediated pilus phase variation. Furthermore, the *uvrD* mutant had a higher frequency of spontaneous mutations. The nucleotide excision repair genes did not play a role in pilin antigenic variation or in DNA transformation. These results shed new light on the role of nucleotide excision repair in this human-specific pathogen.

P2.2.03

The MntABC Transport System of *Neisseria gonorrhoeae*

Karen H.L Lim[1], Rachel N vanden Hoven[1], Michael P Jennings[1], Alastair G McEwan[1]

School of Molecular and Microbial Sciences, The University of Queensland, St Lucia 4072, Australia.

Manganese can play a variety of roles in cellular processes and is increasingly recognised as a key ion in the regulation of gene expression in response to oxidative stress in a number of pathogenic bacteria. There is evidence that in *Neisseria gonorrhoeae*, the accumulation of Mn(II) occurs via an ABC cassette transporter, MntABC. *mntC* encodes a periplasmic binding protein while *mntAB* encode subunits of a transmembrane ATP-dependent transporter. Mutational analysis has shown that *mntABC* genes are required to confer Mn-dependent protection against superoxide and hydrogen peroxide. In contrast to wild-type *N. gonorrhoeae* 1291, *mntAB* and *mntC* mutants grew very poorly on BHI medium. Growth of *mntC* mutants was restored by addition of 5 μM Mn^{2+} but addition of high concentrations of Mn^{2+} (100 μM) was unable to restore growth of the *mntAB* mutant. However, growth of the *mntAB* mutant could be restored by addition of mannitol, a quencher of hydroxyl radicals, consistent with the view that the loss of *mntAB* results in increased oxidative stress. These data also suggest that the periplasmic MntC protein is required for high affinity Mn^{2+} transport via the MntABC system and that the MntABC

transporter is the only sole Mn^{2+} transport system in *N. gonorrhoeae*. The biochemical properties of purified MntC were also investigated and it was shown that the protein had a high affinity for Mn^{2+} and Zn^{2+} ions with a K_d in the pM range.

P2.2.04

Gonococcal Biofilms and Membrane Blebbing

Brock Neil, Megan Falsetta, and Michael Apicella

University of Iowa, Department of Microbiology, Iowa City, Iowa, USA

We have previously shown that a biofilm phenotype exists for *Neisseria gonorrhoeae* in flow cells assembled with glass cover slips as well as on top of primary cervical epithelia in 4- and 8-day infection studies. To determine if there is clinical relevance of the biofilm phenotype, we obtained 10 cervical biopsies from culture-proven *N. gonorrhoeae* infection. Electron microscopy revealed biofilm formation in 3 out of 10 of these biopsies. The *ex vivo* biofilms showed similar characteristics to biofilms formed over glass cover slips or on extended infection of primary cervical tissue *in vitro*. We recently developed a flow chamber that can accommodate cover slips seeded with primary or transformed cervical tissue in an attempt to simulate natural infection. *N. gonorrhoeae* 1291 challenge studies performed with these chambers for 48 and 96 hours show similar biofilm formation to biofilm formation on glass alone. SEM analysis of biofilms grown over cultured cervical epithelial cells indicated that surface blebbing of the gonococci was extensive. Our analysis showed that these blebs fused to form mats over the surface of the organisms within the biofilm. These blebs combined with membrane fragments from dead organisms may be fusing to form at least a portion of the underlying matrix substance of the gonococcal biofilm. Biofilm studies with 1291*msbB* which shows markedly decreased ability to bleb forms a very immature biofilm over glass surfaces and cervical epithelial cells. Gonoccal biofilms as part of the cervical infection may explain mechanisms by which asymptomatic infections, persistence, and increased antibiotic resistance may occur.

P2.2.05

Identification of an autotoxin gene in *Neisseria meningitidis*

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Chromosomally encoded toxin-antitoxin systems (autotoxins) were first identified by homology to postsegregational killing genes of *Escherichia coli* plasmids. They consist of a stable toxin and a labile antitoxin. When their expression is inhibited, the labile toxin is degraded leaving active toxin that prevents protein synthesis. The first toxin-antitoxin system to be characterized was the MazEF system of *E. coli*. MazF encodes the stable toxin, which binds to, and is inactivated by, the labile antitoxin, MazE. When rates of transcription fall, relatively labile MazE is degraded by proteases leaving active toxin and causing the cells to become moribund. This MazEF system is activated by stresses that reduce rates of expression, such as amino acid starvation, heat shock and certain antibiotics. Although a part of the general stress response, the precise function of this system remains undefined. It has been proposed to be involved in the stringent response, translation quality control, global shifts in expression, a defense against phage or a mechanism of programmed cell death. Such systems are not confined to *E. coli* with similar genes being identified in a large number of other bacterial chromosomes, suggesting that such mechanisms are widely distributed amongst prokaryotes. We have cloned and expressed, in *E.coli,* a MazEF- type toxin of a putative toxin –antitoxin system. Over –expression of the toxin causes a large drop in culturable cell numbers. This neisserial toxin-antitoxin system may be involved in the response of *N. meningitidis* to clinically relevant antibiotics.

P2.2.06

Auxotype Classifications of *Neisseria gonorrhoeae* Represent the Overall Genetic Content of Individual Strains

Lai-King Ng, Paul Backhouse, Adam B. Olson, Joanne McCrea, Gary Van Domselaar and Matthew W. Gilmour

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB

DNA microarray technology was used to genotype clinically relevant auxotypes of *N. gonorrhoeae.* FA1090 and test DNA samples were differentially labelled and hybridized to a custom oligonucleotide microarray representing 2,184 FA1090 coding sequences (CDS), 8 plasmid CDS and 9 CDS belonging a genetic island found preferentially in isolates causing disseminated infections.

Cluster analysis revealed genetic relatedness between strains of *N. gonorrhoeae* belonging to the same auxotype and exhibiting similar antimicrobial resistance profiles. This included strains characterized as PCU, OUH, AHU, CO, NR and P auxotypes, and strains clustered according to auxotype. Several regions appeared to be auxotype-specific, and notably, a variable region unique to all PCU strains encoded the *carA* locus. The product of *carA* is required for carbamylation of ornithine to yield citrulline and its deficiency in the PCU strains studied could account for their citrulline auxotrophy.

An *in silico* comparative analysis between the complete genome sequence data for *N. meningitidis* strain MC58 and the FA1090 oligonucleotides using Smith-Waterman alignment allowed for validation of experimental CGH data and our post-hybridization analysis scheme. The computer-predicted outcome for a MC58 v. FA1090 CGH closely matched the actual CGH data set for these strains when the cut-off for hybridization was statically placed at 87.5 percent identity. These methods also indicated that there were >3 clusters in the FA1090 genome that were not present in the MC58 genome, and these were principally comprised of bacteriophage-related sequences.

These results suggest the patterns of divergence at the genome level correlated to traditional typing methods such as auxotype

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determination. Regions of putative divergence that are characteristic of a particular group of strains (or species) may prove useful as genetic markers that identify clinical manifestations of gonococcal infections in the future.

P2.2.07

Molecular Typing of *N. Gonorrhoeae* Using Clinical Specimens for PCR Diagnosis

Lai-King Ng [1], Allan Lau[1], Irene Martin[1], Richard Garceau[2] and Paul Van Caesele[3]

[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada
[2]Dr. G. L. Dumont Regional Hospital, Moncton, New Brunswick, Canada
[3] Cadham Provincial Laboratory, Manitoba Health, Winnipeg, Manitoba, Canada

Background: Gonorrhea is increasing in Canada but there are fewer isolates for identifying clusters of cases and antimicrobial susceptibilities because more clinical laboratories have shifted to molecular diagnostics. Therefore, we attempted to use DNA from clinical specimens to obtain DNA sequences of *por* and *tbp*B to determine genotypes using the *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) and *gyr*A and *par*C genes to determine ciprofloxacin resistance of *N. gonorrohoeae*.

Methods: 23 urine or swab specimens from New Brunswick, Canada and 15 urine specimens from Manitoba, Canada that showed detectable gonococcal DNA using either the Cobas Amplicor CtNg assay and the NG 16s rRNA PCR Roche kit or the BD ProbeTec were used in this study. PCR primers targeting *por, tbpB, gyr*A *and par*C directly from DNA extracted from the patient specimens were used to obtain amplicons for sequencing. NG-MAST (http://test3.mlst.net/) data was used to assign *por* and *tbp*B genotypes.

Results: Of the 38 specimens tested, 6 did not yield PCR products and the remaining 32 specimens yielded at least one product of the 4 genes tested. The *por* gene was detected in only 10 of 38 specimens, *tbp*B was detected in 16 of 38 specimens, *gyr*A was detected in 30 of 38 specimens and *par*C was detected in 29 of 38 specimens. Six different *por* sequences and 8 different *tbp*B sequences were identified using NG-MAST. Six specimens were found to have the *gyr*A and *par*C mutations associated with ciprofloxacin resistance.

Conclusions: The sensitivities of PCR to amplify targets from clinical specimens for the NG-MAST molecular typing method and those for ciprofloxacin resistance are less than commercial detection kits. The ability to detect mutations within the *gyr*A and *par*C may predict ciprofloxacin resistance in some specimens. The molecular typing method requires optimization with PCR primers and conditions as well as testing more clinical specimens for evaluating its value in epidemiological studies.

The Discussion Session for the following posters is from:
9.30pm – 10.15pm Tuesday 12 September

P2.3.01

The NmlR Defence Systems Against Oxidative Stress in *Neisseria gonorrhoeae* and *Neisseria meningitidis*

Adam Potter, Stephen Kidd, Michael Jennings, Alastair McEwan

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A novel redox responsive transcription factor has been described in *Neisseria gonorrhoeae*, the *Neisseria* MerR-like regulator (NmlR). NmlR has been shown to induce the expression of *adhC*, a gene adjacent to *nmlR* which is transcribed from the opposite DNA strand on the *N. gonorrhoeae* chromosome. *adhC* encodes a pyridine nucleotide-dependent oxidoreductase which is known to catalyse the NADH-dependent reduction of adducts of glutathione, including *S*-nitrosoglutathione. *adhC* was found to be expressed as part of an operon along with an esterase (*estD*). A similar arrangement of genes was found in *Neisseria meningitidis*, but a key difference is that while the *N. meningitidis adhC* is monocistronic, in *N. gonorrhoeae* the *adhC* gene appears to be interrupted by a stop codon. We have investigated the oxidoreductase activity of the AdhC from *N. meningitidis and N. gonorrhoeae* and our data suggest that the *adhC* of *N. gonorrhoeae* may be a pseudogene. In *N. gonorrhoeae*, NmlR also regulates the expression of *trxB*, which encodes an NADPH-dependent thioredoxin reductase and *copA*, which encodes a cation translocating P-type ATPase. A similar arrangement of genes is found in *N. meningitidis*. The results suggest that the two *Neisseria* species may have distinctive mechanisms for handling oxidative stress via the NmlR-dependent system.

P2.3.02

Characterisation of Periplasmic Oxidoreductase DsbA3 of *Neisseria meningitidis*

Jessica Scoullar.[1], Jason Paxman,[2] Tony Velkov [2], Martin Scanlon,[2] and Charlene Kahler[1].

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Oxidoreductases are responsible for the addition of disulphide bonds into unfolded polypeptides in the periplasm of gram-negative bacteria. Most gram negative bacteria, including *Escherichia coli*, possess a single oxidoreductase DsbA. *N. meningitidis* is unique as it contains three DsbA proteins, making it a useful system for examining substrate specificity and recognition by DsbA proteins. NMDsbA1 and NMDsbA2 show overlapping substrate specificity in *N. meningitidis*, both being able to oxidise disulphide bond formation in Type IV pilin, whilst there are no known substrates for NMDsbA3. Expression of these oxidoreductases in *E. coli* demonstrated that each enzyme had different substrate specificities

towards *E. coli* substrates. While NMDsbA3 has been shown to have oxidoreductase activity *in vivo* it is suspected to have a lower oxidative potential than other DsbAs due to a variation in the active site which may allow it to interact efficiently with specific substrates.

NM*dsbA3* was over-expressed in *E. coli* and purified using Fast Protein Liquid Chromatography (FPLC). The purified NMDsbA3 was proven to act as an oxidoreductase using the insulin reduction assay. In order to compare the oxidoreductase activity of NMDsbA3 with *E. coli* DsbA (ECDsbA), a peptide was synthesised based on the sequence of the *E. coli* outer membrane protein OmpA, a known substrate of ECDsbA. The peptide contains an aminobenzene (Abz) group at the N-terminus and a 2,4-dinitrophenol (Dnp) attached to the Nε-of a lysine at the C-terminus. Formation of the disulfide brings these two groups into proximity, where Dnp quenches the Abz fluorescence. A catalytic quantity of DsbA promotes oxidation of the substrate peptide and the DsbA is in turn reoxidised by glutathione which is also present in the reaction mixture. Our data show clear differences in the rate of oxidation of the OmpA peptide by its cognate oxidoreductase, ECDsbA and NMDsbA3

NMDsbA3 is an active oxidoreductase and functions as a DsbA protein. It shows differing levels of substrate specificity and oxidative efficiency compared to the DsbA protein from *E. coli* when exposed to *E. coli* based substrates.

P2.3.03

Regulation of Denitrification in *Neisseria meningitidis* by Nitric Oxide and Repressor NsrR

Melanie J. Thomson[1], Jonathan R. Rock[1], Robert C. Read[2] and James W. B. Moir[1]

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Neisseria meningitidis can grow using denitrification of nitrite to nitrous oxide under microaerobic conditions. The conversion of nitrite to nitric oxide (NO) is performed by nitrite reductase AniA and the subsequent conversion of NO to nitrous oxide is performed by NO reductase NorB. Here we show that in *N. meningitidis* MC58, *norB* gene expression is regulated by nitric oxide via the product of gene NMB0437 that encodes NsrR. NsrR is a repressor in the absence of NO, but de-repression occurs in the presence of NO. Growth studies show *nsrR* deficient mutants grow by denitrification more rapidly than the wild-type, and somewhat slower than wild-type under aerobic conditions. NO usage studies show the *nsrR* deficient strain to have up-regulated NOR activity, even under aerobic conditions in the absence of nitrite and NO. Exogenous addition of NO to these cultures did not induce further expression of NOR in the mutant strain in contrast to the wild type, which show an up-regulation of NOR activity by NO. The NsrR repressor is also reported to regulate *aniA* gene expression. The mutant strain had highly expressed AniA nitrite reductase - ten fold higher than the wildtype strain was seen in a promoter-LacZ fusion system. The addition of exogenous NO to aerobic cultures made little difference in the mutant strain and only a small increase of *aniA* expression in the wildtype. This increase of expression may be accounted for by using an equivalent amount of nitrite. Immunoblotting also showed an elevated expression of AniA in the *nsrR* deficient strain under aerobic conditions in the absence of nitrite or NO, which was not seen in the wildtype. From these studies, we conclude that NsrR acts as an NO-dependent repressor of *norB* gene expression, but an NO-independent repressor of *aniA* expression.

P2.3.04

Regulation of *c*-type Cytochromes of *Neisseria gonorrhoeae*

Nicholas Tovell, Tim Overton, Harry Smith and Jeff Cole

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Pathogenic *Neisseria* are a prolific source of *c*-type cytochromes. *Neisseria gonorrhoeae* expresses eight *c*-type cytochromes. On the basis of their similarity to cytochromes *c* in other bacteria, they have tentatively been identified as CcoP (Subunit III of the cbb_3 terminal oxidase), CCP (cytochrome *c* peroxidase), PetC (cytochrome c_1 of the cytochrome bc_1 complex), cytochrome c_4, CcoO (Subunit II of the cbb_3 terminal oxidase), cytochrome c_5, cytochrome *c*' and cytochrome c_{552}.

Cytochromes *c* are characterised by the covalent attachment of haem (iron protoporphyrin IX) to a polypeptide chain via two thioether bonds. The haem moiety is attached to the apo-cytochrome *c* in the periplasm by the cytochrome *c* assembly apparatus. In the case of *Neisseria gonorrhoeae*, assembly system II is used.

Previous work has shown that expression of one of the gonococcal *c*-type cytochromes, CCP, is dependent on a functional FNR protein. Within the related meningococcus data suggests that the *cycA* and *cycB* genes, encoding cytochrome c_4 and c_5, respectively, are part of the FNR regulon. Using SDS-PAGE gels stained for covalently bound haem, we demonstrate that levels of all of the gonococcal *c*-type cytochromes increase as growth becomes oxygen-limited. However, the same result was observed in an *fnr* mutant. Real-time PCR to investigate levels of cytochrome *c* transcripts showed that while gonococcal CCP is directly regulated by FNR, the other cytochromes *c* are not part of the FNR regulon. Their accumulation during oxygen-limited growth is therefore a post-transcriptional event that we propose is controlled via the cytochrome *c* assembly apparatus at a post-translational level. We also report that a mutant defective in cytochrome c_4 is more sensitive than the parent to oxygen inhibition during growth, consistent with the proposal that cytochrome c_4 transfers electrons from the quinol pool and the cytochrome bc_1 complex to cytochrome oxidase cbb_3.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P2.3.05

The Use of Microarray in Assessing the Role of IHF in *Neisseria meningitidis* Gene Regulation

Sally A Turner, Shauna Lyons-Schindler, Charlene Kahler and John K Davies

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Integration host factor (IHF) mediates a variety of genetic events in Gram-negative bacteria, including transcriptional regulation of specific genes. In *Neisseria gonorrhoeae*, IHF has been implicated in expression of at least one of the major virulence determinants, the pilin subunit PilE. Although much effort has been focussed upon understanding the role of IHF in Neisseria gene regulation, the generation of non-lethal *ihf* mutants has not been reported in either *N. gonorrhoeae* or *Neisseria meningitidis*. We have previously described the isolation of an *ihfA* mutant and a complemented mutant in *N. meningitidis* NMB.

Using the otherwise isogenic *N. meningitidis* wildtype, *ihfA* mutant and complemented mutant strains, we have employed microarray analysis to profile gene expression, and assess the role of IHF in gene regulation. We have identified over 400 genes that are differentially regulated 1.5 fold up or down in the presence or absence of IHF ($p<0.05$). Amongst these are genes encoding components of the translation machinery, and an ArsR-like transcriptional regulator. In an attempt to identify those genes that are directly regulated by IHF, a subset of these genes was further analysed by cloning promoter regions into a CAT-reporter system and assessing the level of promoter activity in an *E. coli* wildtype and *ihfA::Tn10* background. Initial results indicate some of these promoters may be directly regulated by IHF, and a variety of methods are being used to demonstrate direct interactions between IHF and these promoter sequences.

P2.3.06

The aerobic respiratory chain of *Neisseria gonorrhoeae*

Rachel vanden Hoven[1], XiuHua Lin[1], Erin Anderson[1], Stephen Kidd[1], Michael P. Jennings[1] & Alastair G. McEwan[1]

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The components of the aerobic respiratory chain of *Neisseria gonorrhoeae* have been predicted as a consequence of the sequencing of its genome. This annotation predicts that *N. gonorrhoeae* possesses a single cytochrome oxidase of the cbb_3-type which is connected to the ubiquinone pool via a cytochrome bc_1 complex.. We have shown that aerobic respiration in membranes from *N. gonorrhoeae* 1291 is blocked by myxothiozol and antimycin A, inhibitors of the cytochrome bc_1 complex. This confirms that, unusually, *N. gonorrhoeae* contains no additional oxidases that use ubiquinol as an electron donor. In contrast to the quinol-oxidising segment of the respiratory chain, the quinone-reducing segment contains a number of primary dehydrogenases, including NADH dehydrogenases (Nuo and Nqr), D- and L-lactate dehydrogenases as well as a malate-quinone oxidoreductase (MQR). Membranes from *N. gonorrhoeae* 1291 cells grown on BHI medium exhibit very high D- and L-lactate dehydrogenase activity and low NADH dehydrogenase activity. We suggest that the primary dehydrogenases of *N. gonorrhoeae* have evolved to allow this bacterium to maintain a high respiratory rate with lactate as electron donor, under conditions where iron levels may not be sufficient to support the biogenesis of an active NADH dehydrogenase (Nuo). Consistent with this observation we have observed that the *nuo* genes are only expressed at a high level under iron replete conditions. Under the low iron conditions the presence of a malate-quinone oxidoreductase (MQR) would also allow the TCA cycle to proceed without the need for a respiratory NADH consumer to 'pull' the thermodynamically unfavourable oxidation of malate to oxaloacetate with NAD^+ as electron acceptor (via the well-known malate dehydrogenase).

P2.3.07

Characterization of *N. meningitidis* (Serogroup B) Wild-Type and Isogenic Knock Out Mutants by Proteomic Analysis

D. Veggi, E. Cartocci, J. Adu-Bobie, L. Ciucchi, N. Norais, D. Serruto, S. Savino, R. Rappuoli and M. Pizza

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Neisseria meningitidis is the major cause of bacterial sepsis and meningitis worldwide. Serogroup B of *N. meningitidis* (meningococcus B) is a global killer of children and young adults, which has not been conquered by modern medicine and cannot be controlled by vaccination yet.

Using the reverse vaccinology approach, based on the analysis of the genome sequence for the identification of potential vaccine candidates, novel surface-exposed proteins have been identified in *N. meningitides.* In order to investigate the role of these novel antigens in neisserial physiology, we have generated isogenic knock-out mutants for the genes coding each selected protein and analyzed them in comparison with the wild-type strain, using several different approaches. One of these tools was the proteomic analysis performed either on wild-type or knock-out strains. To evaluate differences in expression and/or localization of these antigens, total lysate and sub-cellular compartments from both strains were produced and analyzed by 2D gels electrophoresis. 2D maps were compared in order to investigate possible differences and related spots were detected by MALDI-TOF. These analysis might allow us to comprehend the possible role of the novel antigens in microbial physiology and pathogenesis.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P03
Cellular Microbiology

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P3.1.01

***Neisseria gonorrhoeae* Escape from Cervical Epithelial Cells to Promote Bacterial Persistence**

Samuel Bish, Wenxia Song, Daniel Stein

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

The human pathogen *Neisseria gonorrhoeae* (gonococci) causes the sexually transmitted disease gonorrhea. In the female reproductive tract, gonococci can breach the mucosal epithelial barrier to initiate invasive disease, yet most women remain asymptomatic. Most of the important *Neisseria gonorrhoeae*-female reproductive tract interactions that allow gonococci to suppress symptoms and persist to cause disease are not defined. The purpose of this study is to determine how *Neisseria gonorrhoeae* interacts with cervical epithelial cells to avoid innate immune defenses and maintain infection.

The main hypothesis tested is that a subpopulation of gonococci manipulate distinct cellular processes to cycle in and out of cervical epithelial cells, thereby evading innate immune defenses. To demonstrate that gonococci invade cervical epithelial cells, we cloned and expressed beta-lactamase on the gonococcal cell surface. Confocal microscopy revealed that beta-lactamase expressing gonococci invade cervical epithelial cells loaded with a fluorescent beta-lactamase cleavable substrate. Using modified gentamicin protection assays, we show that, once inside cervical epithelial cells, most of the invading gonococcal population is cleared by intracellular killing mechanisms, but some viable gonococci escape into the extracellular milieu. Treatment of cervical epithelial cells with tannic acid after invasion inhibits gonococcal escape suggesting that *Neisseria gonorrhoeae* utilizes the host exocytosis pathways to escape cervical epithelial cells.

In the model of female gonococcal disease, persistent gonococci may undergo transient invasion-escape cycles without triggering a robust immune response. Invasion allows gonococci to avoid extracellular host defenses and escape lets gonococci evade intracellular killing and infect new susceptible host cells. In this investigation, we have uncovered a possible escape mechanism utilized by a subpopulation of *Neisseria gonorrhoeae* to avoid the innate immune response and establish long-term, low-level infection in the female reproductive tract.

P3.1.02

The genetic structure and diversity of human CEACAM genes: implications for Opa function and susceptibility to invasive meningococcal disease.

Martin J. Callaghan[1], Carly Banner[1], Kirk Rockett[2], Elene Haralambous[3], J. Simon Kroll[4], Michael Levin[4], Martin C.J. Maiden[5], Dominic Kwiatkowski[2], Andrew J. Pollard[1]

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Most invasive meningococcal disease is attributed to less than 10 hyperinvasive lineages and, since they are also carried asymptomatically, acquisition of these genotypes alone cannot explain why only some people develop disease. Intimate adhesion to the human host is mediated by interactions between meningococcal Opa proteins and human carcinoembryonic antigen cell adhesion molecules (CEACAMs). Differences in CEACAMs may result in some hosts being more easily colonised or invaded. The aim of this investigation was to determine whether susceptibility to meningococcal disease is influenced by genetic diversity in CEACAMs.

High-throughput genotyping was employed to compare the genetic diversity at 53 single nucleotide polymorphism sites across CEACAM1, 3, 5 and 6 in 384 DNA samples from patients and 190 samples from healthy individuals. CEACAM genes exhibited different levels of diversity relative to each other and a small number of high frequency haplotypes were observed. CEACAM1 exhibited less polymorphism than the other genes, which may explain why most Opa proteins tested bind to the CEACAM1 protein. No differences in the diversity of any CEACAM gene between the case and control cohorts was observed suggesting that diversity in CEACAM is not associated with susceptibility to meningococcal disease.

These data imply that a meningococcus with a particular Opa protein repertoire may exhibit the same binding specificities in different hosts with equivalent CEACAM genotypes. However, the participatory CEACAM variants alone would not determine the outcome of the infection. The relatively low level of diversity in CEACAM is not echoed by the diversity of the Opa proteins and meningococci with different Opa repertoires may interact differently with genotypically equivalent hosts. Future studies to understand the role of Opa proteins and other bacterial CEACAM-binding adhesins in pathogenesis may be aided by our observation that variation in their receptors is limited.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P3.2.01

Uncommon CEACAM Targets of Respiratory Pathogens

Natalie Griffiths[1], Darryl J. Hill[1], Kerry Setchfield, Ka Ling Mak, Richard B. Sessions[2], Bernhard B. Singer[3], Lothar Lucka[3] & Mumtaz Virji[1*].

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It has now been well established that several mucosal pathogens target CEACAMs via their N-terminal domains. Moreover, the centre of their target, as assessed by mutational analysis of CEACAM1, is the Ile91 residue of the CFG face of the molecule. Ile91 is conserved in the majority of CEACAMs but the surrounding residues that also affect bacterial binding may be highly variable, thus determining bacterial ability to recognize the receptor and therefore potentially its tissue tropism. Of the three opportunistic respiratory pathogens *Neisseria meningitidis*, *Haemophilus influenzae* and *Moraxella catarrhalis* that target the receptors, the latter two appear to be restricted to a smaller number of CEACAM targets. On the other hand, *N. meningitidis* and its closely related urogenital pathogen *N. gonorrhoeae*, were shown to bind to a relatively larger repertoire of CEACAMs but excluded CEACAM8 and in some situations CEACAM3. CEACAM4 has not yet been studied in detail.

In our recent survey on bacterial adhesion to CEACAM receptors, we have used HeLa and Balb/c cells expressing distinct CEACAMs as well as soluble receptor constructs. Our data shed new light on the repertoire of receptors targeted by distinct mucosal pathogens. Of the CEACAMs bound by bacteria, CEACAM4 has the most divergent structure, yet it is recognized by a number of neisserial and other strains. We have created a molecular model to study how CEACAM4 may accommodate bacterial ligands.

P3.2.02

The Roles of Meningococcal OPA, OPC and PILI in Modulating T Cell Proliferation and Function and the Effect of Recombinant CEACAM-Binding Bacterial Adhesion Blocking Peptides

Abdel-Rahman Youssef, Darryl J. Hill, Neil A. Williams and Mumtaz Virji

Department of Cellular and Molecular Medicine, School of Medical Sciences, University of Bristol, Bristol, UK.

A variety of neisserial outer membrane proteins have been shown to induce either stimulatory or inhibitory effect on T cells. Of these, pili and Opa proteins, which are major adhesins of *N. gonorrhoeae* and *N. meningitidis* have been shown to exert effects, at least when gonococcal/human T cell interactions were examined (Boulton and Gray-Owen, Nature Immunology, 2002; Plant and Jonsson, Infection and Immunity, 2006). The aim of our investigation was to study how Opa, Opc and pili of meningococci might affect human CD4+ T cell function, when expressed individually or in combination. Using well defined meningococcal isolates expressing a repertoire of adhesins and varying expression of surface sialic acids, we have investigated the direct effects of bacterial ligand interactions with receptors on T cells. In addition, comparative studies of commensal Neisseria, meningococci and gonococci appear to show similarities between the former two spp. in modulating proliferation and cytokine production of isolated CD4+ T cells as well as peripheral blood mononuclear cells. The precise molecular basis for the similarities and differences between different Neisseria spp. are being investigated.

We have also examined the effects of blocking bacterial receptors on T cells. While monoclonal and polyclonal antibodies to CEACAM1 inhibited T cell proliferation, such an effect did not occur with rD-7, a CEACAM-binding recombinant polypeptide that was produced in our laboratory (Hill *et al,* Molecular Microbiology, 2005). This reagent, by blocking the bacterial binding site on the N-domain of CEACAMs, prevents bacterial interactions with the receptor. Further cross-linking of rD-7 with anti- rD-7 produced some inhibition of T cell proliferation. The data suggest that blocking agents that have a low capacity for cross-linking of receptors may not have deleterious effect on T cell function.

The Discussion Session for the following posters is from: 9.30pm – 10.15pm Tuesday 12 September

P3.3.01

Opa Negative Gonococci Possess Enhanced Virulence

Daniel C. Stein, Adriana LeVan, Samuel Bish and Wenxia Song

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We constructed a derivative of *N. gonorrhoeae* strain MS11 that has the Opa-encoding genes deleted to allow us to study the role of Opa in gonococcal adherence to and invasion of human tissue culture cells/monolayers. The Opa-deficient strain was constructed using a PCR/replacement mutagenesis/deletion procedure, allowing us to construct a series of isogenic derivatives of MS11 that possessed different abilities to express a single defined Opa. One strain employed lacked the ability to make 10 of the 11 Opas (the remaining *opa* possessed sequences that suggested that it would bind to CEACAM, and was fully capable of phase variation). A second strain employed was genetically deficient in the ability to produce any Opa. The deletion of all *opa* genes was confirmed by Southern Hybridization experiments.

We studied the ability of the Opa-negative strains to transcytose across polarized T84 monolayers. We found that these strains efficiently transcytosed the monolayer in less than 6 hrs, whereas a wild type strain capable of expressing all 11 Opa proteins was unable to transcytose in this time period. A strain that could only variably express a single Opa could transcytose across the epithelial monolayer in 6 hrs, however, the phenotype of the

colonies that arose from the basolateral media were almost exclusively Opa negative.

The ability of Opa negative strains to adhere to T84 or ME180 cells was examined, and the data indicate that the presence or absence of Opa did not seem to alter the ability of the strain to adhere to these cells. Taken in toto, the data support a model of infection where Opa-expressing gonococci are better at invading into cells, while Opa negative gonococci are better at transmigrating across an epithelial barrier. This suggests that Opa expressing gonococci would be less likely to disseminate from the site of infection.

P3.3.02

Search for meningococcal DNA binding proteins involved in transformation

Afsaneh Vahdani-Benam, Emma Lång, Håvard Homberset, Burkhardt Fleckenstein, Tone Tønjum

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Neisseria meningitidis (the meningococcus) is the cause of meningitis and/or septicaemia worldwide. This bacterium is naturally competent for transformation of DNA throughout its entire life cycle. The binding and uptake of transforming DNA into the meningococcal cell can be divided into four stages: entry through an outer membrane pore, transit of the periplasm, transport across the inner membrane and genome integration. The transformation process in the *Neisseriae* is dependent on the presence of a 10 bp DNA uptake sequence (DUS) in the exogenous DNA, type IV pilus expression and RecA-dependent homologous recombination. A number of pilus biogenesis components required for transformation have been identified, and mutants with defects in these components are non-competent. However, the complete system processing DNA during transformation is not yet charcaterized.

In order to characterize the transformation process in more detail, we subjected meningococcal cellular fractions to DNA binding by South-Western analysis. In order to obtain improved resolution of DNA-binding components, 2D-South-Western analysis was developed. DNA-binding components were identified by using mass spectrometry (MS). Verified DNA-binding components were further characterized by band shift analysis with and without competing DNA, and the corresponding mutants were tested for competence. The goal is to define how DNA-binding components provide dynamic multi-site targeting and entry of DNA. In this way, we aim at identifying all DNA-binding components involved in the multi-step transformation process. The ultimate goal would be to recognize the DUS-specific receptor that mediates selective transmission of DNA into the meningococcal cell.

P3.3.03

Using CD46 Cyt1 and Cyt2 Monoclonal Antibodies to Study CD46 During Infection of Epithelial Cells with *Neisseria gonorrhoea*

Nathan J. Weyand[1], Shaun W. Lee[1,2], Dustin L. Higashi[1], Christine Calton[1], Daniel Cawley[3], Paul Yoshihara[3] and Magdalene So[1]

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CD46 (Membrane Cofactor Protein) is a family of complement regulatory proteins that participate in innate and acquired immunity. CD46 also serves as the receptor for a number of viral and bacterial pathogens. Type IV pili of pathogenic Neisseria can associate with CD46, and Neisseria infection triggers CD46 secretion and cellular downregulation. CD46 isoforms terminate in one of two cytoplasmic tails, Cyt1 or Cyt2, that differ in signaling and trafficking properties. Dissecting the functions of the two cytoplasmic tails in these cellular processes has been hampered by the absence of specific reagents. We report the construction of Cyt1- and Cyt2-specific monoclonal antibodies. These mAbs recognize unique epitopes within the tails, and can be used for immunofluorescence microscopy, immunoblotting and immunoprecipitation. Studies of *Neisseria gonorrhoeae* infected cells with the CD46 tail mAbs demonstrate the differential recruitment of Cyt1 and Cyt2 to the cortical plaque.

P3.3.04

T-cell Stimulating Protein A of *Neisseria meningitidis* is required for Optimal Adhesion to Human Cells

Neil Oldfield, Sarah Bland, Maria Taraktsoglou, Karen Robinson, Karl Wooldridge & Dlawer Ala'Aldeen

Molecular Bacteriology and Immunology Group, Institute of Infection, Immunity & Inflammation, School of Molecular Medical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK

T-cell stimulating protein A (TspA) is an 875 amino acid, immunogenic, T-cell and B-cell stimulating protein of *Neisseria meningitidis* that is expressed by meningococcal isolates but not by *N. gonorrhoeae* and *N. lactamica.* Sequence homology between TspA and FimV, a *Pseudomonas aeruginosa* protein required for twitching motility, suggested a link between TspA and the type IV pilus (Tfp). To determine the role of TspA in meningococcal pathogenesis, an isogenic deletion mutant was created in the group B meningococcal strain MC58. Subsurface twitching motility assays showed no difference between the wild type and isogenic deletion mutant suggesting that despite similarities, there are important differences between TspA and FimV. In addition to a role in twitching motility, Tfp are also involved in numerous other

phenotypes including adherence, auto-aggregation and natural transformation competence. Three independent methods were used to compare the capacity of the wild type and *tspA* mutant strain to adhere to either HEp-2 (human larynx carcinoma) or meningothelial (primary human cell lines derived from benign meningothelial tumors) cells. These methods included florescence microscopy, environmental scanning electron microscopy (ESEM) and viable counts of bacteria associated with homogenized infected monolayers. Loss of TspA led to a significant reduction in adherence of meningococci to human cells after only 30 min of incubation. However, piliation, auto-aggregation and natural transformation competence phenotypes were identical in the wild type and isogenic deletion mutant suggesting that the effect of *tspA* mutation on adhesion is unlikely to be directly linked to the function of the Tfp. In summary, TspA is a well-conserved antigen that is required for optimal adhesion to human cells, but is dispensable for most Tfp-mediated phenotypes. Future studies will be required to determine the mechanism by which TspA modulates adhesion to host cells.

P04
Epidemiology

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P4.1.01

Epidemiological Study of *Neisseria gonorrhoeae* Isolates by Auxotyping and Serotyping

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Background: The study of relationship between isolates of *N.gonorrhoeae* can contribute to the identification of outbreaks in sexual networks, reinfection, temporal and geographic changes, sexual abuse and monitoring of antibiotic resistance. In developing countries, the data on the incidence and epidemiology of *N.gonorrhoeae* is very scarce. Auxotyping and serotyping, two independent and unlinked characteristics, have been used together to enhance discrimination between strains.

Objective: The present study was carried out to study the relationship between gonococcal strains isolated in New Delhi, based on auxotyping and serotyping.

Methods: A total of 50 consecutive *N.gonorrhoeae* strains were studied. The strains were isolated from 52 males with urethritis, 22 females with endocervicitis, 10 their sexual contacts at Lok Nayak hospital, New Delhi from January 2004 to June 2005 . Auxotyping was performed in the basis of their requirements for arginine, proline, uracil, hypoxanthine, serine, isolocine, cysteine and cystine. Serotyping was performed using the Phadebact Monoclonal GC kit and the Phadebact GC serovar test (Boule Diagnostic AB, Hudding, Sweden), which consisted of five WI reagents (Ao, Ar, As, At, and Av) and nine WII/III reagents (Bo, Bp, Br, Bs, Bt, Bu, Bv, Bx, By).All isolates were classified into serogroups WI and WII/III and further subdivided into serovars.

Results: The strains belonged to 7 different auxotypes. The most common auxotypes were non- requiring (44 %), proline requiring (32%), and arginine requiring (12%) auxotypes. Out of the total 50 strains studied, 22 (44%) isolates belonged to WI (IA) serogroup, and 28 (56%)were of WII/III (IB) serogroup. Among the serogroup WI isolates, serovar Aost (50 %) was the most prevalent, followed by Arost (18.18 %) and Ast (18.18 %). The serogroup WII/WIII isolates were differentiated into 12 serovars, of which the most prevalent serovar was Boprt (39.3%) followed by Btuvy (14.3%), Bopty (7.1%), Bpr (7.1%) and Bsy (7.1%). Fifty isolates were subdivided in to 28 auxotype/serovar (A/S) classes. The main A/S classes were NR/Aost, NR/Arost, Pro/Aost and Pro/Boprt.

Conclusion: The results showed that serotyping in combination with auxotyping (A/S typing) provided greater discrimination between isolates than the use of only one of these techniques.

P4.1.02

Research up in a puff of smoke- are we asking the right questions?

Jane Jelfs[1], Robert Booy[1], Pietro Coen[2], Jo Tully[2], Russell Viner[2]

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[2]Institute of Child Health, University of London, United Kingdom

Cigarette smoking, passive and/or active, has been implicated as a risk factor for acquiring, carrying and contracting invasive diseases due to encapsulated bacteria including *Neisseria meningitidis*. We reviewed the literature to try to determine whether the key exposure was smoke or the smoker.

The literature suggests that it is the smoker and the likelihood that they are more predisposed to being a carrier of meningococci that poses the risk to those around them, irrespective of whether they actively smoke around other people or go outside to smoke. Yet many articles did not distinguish within 'passive smoke' exposure as a risk factor for disease as to the relative contributions of smoke v smoker exposure. There was no standardisation in the depth of questions asked regarding active or passive smoking. Hardly any study endeavored to unpick the relative contributions of exposure to smoke v smoker even where dose-response relationships were revealed. A recent factorial analysis by Coen et al however showed that exposure to smokers (not smoke) was an independent risk factor taking account of confounding variables such as intimate kissing, SES, prior viral infection, drug and alcohol use, attendance of parties.

The habit of parents smoking outside their homes to reduce the harmful effects may not be as protective as imagined. The population attributable fraction of 'passive smoking' has been estimated to be in excess of 30%. Many countries have recently legislated to ban smoking in bars, pubs, clubs, dance venues and

restaurants. The risk of passive smoking may now be minimised within these environments so it is imperative that future investigators ask the right questions of participants in their studies – questions which measure exposure to smokers, not just their cigarette smoke.
Many risk factors have been found for meningococcal disease but of them, cigarette smoking remains the most easily modifiable.

P4.1.03

Meningococcal Disease Epidemiology Update – United States, 2005

Thomas Clark[1], Kimberly Cushing[1], Sonal Pathak[1], Elizabeth Zell[2], Leonard Mayer[1], Nancy Messonnier[1], and the Active Bacterial Core Surveillance Team (ABCs)

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Background: *Neisseria meningitidis* is a leading cause of bacterial meningitis in the United States (US), and meningococcal disease continues to have a high case-fatality rate and cause substantial morbidity.

Methods: ABCs conducts active, population-based, laboratory-based surveillance for invasive meningococcal disease (IMD) in 10 geographic areas with a population >39 million. Confirmed cases are defined by isolation of *N. meningitidis* from a normally sterile body site from a person with a clinically compatible illness. All isolates are serogrouped by standard methods.

Results: In total, 171 IMD cases were reported in 2005; 77 (45%) were serogroup B, 37 (22%) C, 48 (28%) Y, and 9 (5%) other serogroups. The proportion of serogroup B isolates was highest in Oregon (69%), and ranged from 21% to 43% in other geographic areas. The incidence of IMD in 2005 was 0.35 cases per 100,000 persons, continuing a downward trend since 1991. The incidence among 11 to 17 year-olds and 18 to 22 year-olds, which includes the target populations for tetravalent meningococcal conjugate vaccine (MCV4), was 0.56 and 0.78, respectively. Fifty-nine percent and 33% of cases in these age groups, respectively, were potentially vaccine-preventable (C, Y, or W135). Since 1991, the peak incidence of serogroup C disease (0.5) occurred in 1994, Y disease (0.45) in 1997, and B disease (0.24) in 1991. Downward trends were observed for C and Y disease, but not B. Since 1991, the case fatality rate (overall 11%) was higher for pneumonia (12%) and bacteremia without focus (13%) than for meningitis (9%), and lower in persons <17 years than in persons ≥18 years.

Conclusions: The incidence of IMD has declined since 1991 in the US. Increasing use of MCV4 should further reduce the incidence of IMD caused by vaccine-preventable serogroups. Additional reductions could be achieved through novel serogroup B vaccines.

P4.1.04

Surveillance of Meningococcal Disease in New Zealand

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Surveillance of meningococcal disease in New Zealand is based on a combination of notification and laboratory data. Data on each case are sent to ESR and collated with the results of characterisation of patient specimens determined at ESR.

New Zealand has been experiencing an epidemic of meningococcal disease caused by group B meningococci expressing the PorA type P1.7-2,4 since 1991. The significant morbidity associated with many cases makes it one of the most important notifiable diseases in the country. Ongoing surveillance has allowed us to provide information against which changes in disease epidemiology, and the effectiveness of the recently introduced MeNZB™ vaccine can be evaluated.

In 2005 228 cases of meningococcal disease were notified, a rate of 6.1 per 100 000 population. This is a significant decrease ($p<0.0001$) on the rate for 2004 (9.2 per 100 000 population), but still approximately four times higher than the rate of 1.5 per 100 000 population in the immediate pre-epidemic years 1989-90. In 2005 there were 14 deaths (case-fatality rate 6.1%), with three cases aged less than 20 years; six had the epidemic strain type, with one aged less than 20 years. A correlation between high rates of disease in children aged less than five years and increasing deprivation has been observed over the period of the epidemic. Seasonality of meningococcal disease has tended to match that of the influenza season.

200 of the 228 cases (87.7%) were laboratory confirmed; the use of PCR to detect meningococcal DNA increased the confirmation rate by 35%. Of the 190 specimens characterised, 113 (59%) were the epidemic strain, significantly lower ($p<0.0027$) than in 2004 (184/252, 73%).

In the year January to May 2006 there have been 46 cases notified and no deaths, compared to 92 cases and 7 deaths for the same period in 2005.

P4.1.05

Antigenic expression of LPS from Brazilian meningococcal strains, production of Monoclonal Antibodies as Subsidy to epidemiological studies

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The profile of antigen expression among meningococci is important for epidemiology surveillance and vaccine development. To this end two new mouse monoclonal antibodies (MAbs) have been produced against *Neisseria meningitidis* lipopolysaccharide (LPS). The MAbs

were reactive against outer membrane antigens and present bactericidal activity. They were tested against different meningococcal strains 118 strains of serogroup A, 66 strains of serogroup C (1972 to 1974) and 293 strains of serogroup B (1992) meningococci by Dot-ELISA.

Our results demostrated that the expression of LPS (immunotypes) in the *N meningitidis* Brazilian strains studied is heterogeneous. The prototypes and subtypes of B:4:P1.15, B:4:P1.9, B:4:P1.7, B:4:P1.14, B:4:P1.16, B:4:NT and B:NT:NT were detected in *N.meningitis* B serogroups. The strains C:2a:P1.2 and A:4,21:P1.9 were dominant in the C and A serogroups respectively.
FACS analysis showed that the MAbs immunotypes recognized LPS immunotypes on the surface of *N.meningitidis*. The immunotype L_{379} was strongly expressed in 90% of *N.meningitidis* B compared with 67% in serogroup C and 18% in serogroup A, whereas L_1 and L_8 were weakly to moderatly expressed by 7% and 15% in serogroup B and 3% serogroup A and was not expressed in serogroup C.

The importance of establishment a broad set of immunotypes antigens caracteristics of the prevalent strain during one epidemic is important for vaccine preparation.

P4.1.06

Genotypic Characterisation of Non-invasive *Neisseria meningitidis* in Scotland 1997 – 2004

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The dynamics of meningococcal carriage and the relationship between carriage and invasive meningococcal disease remain poorly understood. Here we describe, the characterisation of non-invasive meningococci received by the SMPRL between 1970s and 2000s. The case definition of non-invasive for this study was "throat isolates collected from individuals without clinical manifestations of invasive disease and with no epidemiological link to an invasive meningococcal case clinically or laboratory confirmed". Serogrouping, Multi-locus Sequence Typing (MLST) and *porA* genosubtyping of variable regions VR1, VR2 and VR3 were carried out on all isolates.

In total 150 non-invasive meningococci received between 1974 and 2006 were used as a sample population for this presentation and precursor to a larger study using all non-invasive meningococci at the SMPRL over a 30 year period.

Non-invasive isolates were composed of serogroups A (4%), B (37.3%), C (16.6%), W135 (6%), Y (7.3%), Z (0.66%), and Z' (6%). Twenty four percent of isolates were non-groupable and *siaD* PCR resolved a further 5% of meningococci pertaining to serogroup B (4%) and C (1%). The most common VR1, VR2, and VR3 variants were P1.5 (25.2%), P1.2 (18.1%) and 38 (48.4%) respectively.

Non-invasive strains were compared to invasive strains previously sequenced for MLST. Just over 45% and 35% of sequence types (ST) were found to be unique to non-invasive isolates and invasive isolates respectively, while nearly 20% of STs were common to both. Non-invasive isolates include global lineages such as ST11/ET37, ST269 and ST41-44/Lineage 3 and all been associated with invasive disease.

These results will provide a unique insight into the evolution of meningococci over a 30 year period and provide much needed long-term epidemiological data which is essential for understanding its evolution. In addition this data may help determine future vaccine policy and provide important information on the population dynamics of this pathogen.

P4.1.07

Global Incidence and Case Fatality Rates of Meningococcal Disease due to Different *Neisseria meningitidis* Serogroups

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The epidemiology of invasive meningococcal disease (IMD) varies based on the distribution of the five main *N. meningitidis* serogroups – A, B, C, Y and W-135. The clinical course of IMD also varies by serogroup. We examined incidence, case fatality rates (CFR) and serogroup distribution in 19 countries worldwide. Analysis of CFR by serogroup using publicly available data for the time period 1994-2005 for 9 countries was performed and is presented.

Reported IMD incidence varied by country and by serogroup. Ireland reported the highest incidence of IMD (7.53 overall, 5.87 serogroup B, 1.33 serogroup C). Overall IMD incidence was lower in Canada, the U.S. and Australia compared to the reported incidence in Europe.

Reported CFRs ranged from 6% to 21%. Differences in CFRs were observed by serogroup for all countries, and were statistically significantly higher for serogroup C disease compared to B disease in the majority of countries. Data from the U.S. indicate significantly increased overall CFRs resulting from disease associated with outbreaks compared to sporadic disease. For serogroup C this difference was statistically significant. The CFR for serogroup Y in the U.S. was 11.2% overall but increased to 22% for outbreak associated disease. In Canada, the CFR for serogroup Y disease was 7.3% versus 19.1% for C and 11.1% for B disease. The highest CFRs for serogroup B disease were reported in Canada (11.1%), Norway (9.2%), France (8.8%) and Denmark (7.7%). The highest CFRs for serogroup C were reported in Canada (19%), France (17.0%) and England (15.3%).

Conclusion: There are distinct geographic differences in the incidence and serogroup distribution of IMD. The clinical impact of these differences is evident in the overall high CFR and in the variable serogroup-specific CFRs. Implementation of quadravalent conjugate vaccination programs will serve to significantly reduce the mortality associated with A,C,Y and W-135 IMD.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P4.1.08

Investigation of the Basis for Persistent *Neisseria gonorrhoeae* Porin VR Types in Community Infections

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Epidemiological studies that utilize variable region (VR) typing, a porin-based typing method, show that certain VR types of the *por*BIA allele persist within communities. Here we showed that some porin types may have a functional advantage and that certain VR types are not merely a marker of more successful clones. We analyzed a set of 26 PIA isolates collected over a 10 year period in Baltimore, Maryland by pulsed field gel electrophoresis (PFGE). Isolates of two successful VR types (1;1;1;1,4;1 and 1;2;1;1;1), which were recovered for 5 or 10 years of the 10 year study, respectively, belonged to six different PFGE clusters and thus were not clonal. As some gonococcal porins confer serum resistance (SR), we performed bactericidal assays to determine if there is a link between VR type and evasion of host complement-mediated protection. Fifteen of the 17 (88%) isolates with the two most common VR types were SR in contrast to 5 of the 9 (55%) isolates of less common VR types. Ten of 26 (38%) isolates could use lactoferrin (LF) as an iron source (LF^+), which is a phenotype unrelated to porin, and unlike SR, LF phenotype did not correlate with VR type. LF phenotype did correlate closely with PFGE cluster, however, which confirms the PFGE analysis. Isolates of VR type (2;4;3;3;3), which were recovered only the first 3 years of the 10 year study were > 85% related, LF^-, and less often SR than strains of the more persistent VR types. The persistence of certain VR types among PIA strains of different ancestral backgrounds is evidence that some porin sequences may confer a survival or transmission advantage perhaps via increased resistance to complement. These studies may provide insight into the success of certain strains within communities and valuable information for the design of porin-based vaccines.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P4.2.01

Risk Factors for Meningococcal Disease in U.S. High School Students

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Background: Adolescents and young adults in the United States are at high risk for severe invasive meningococcal disease (IMD). Risk factors for IMD among U.S. college students have been studied but little is known about factors contributing to the high risk of IMD in high school students.

Methods: We conducted surveillance for IMD in high school students in 10 sites throughout the U.S. from April 2002-December 2005. Cases had a normally sterile body fluid positive for *Neisseria meningitidis* by culture, PCR, and/or a positive cerebrospinal fluid gram stain. For each IMD case occurring in a high school student, up to 4 classroom-matched controls were selected. Case patients (or surrogate informants) and controls were interviewed about demographic, behavioral, and health risk factors for IMD. Conditional logistic regression analysis was performed to identify factors independently associated with IMD.

Results: There were 70 patients who met our case definition, 49 (70%) were enrolled and had at least one control. Isolates were available for 59 (85%) cases. In logistic regression analysis, attending at least 1 barbeque or picnic (odds ratio 0.27, p value = 0.003) or school dance (OR 0.27, p=0.03) were associated with a decreased risk of IMD. In contrast, male gender (OR 2.74, p=0.01), marijuana use (OR 4.69, p=0.003), and nightclub/disco attendance (OR 3.24, p=0.04) were all associated with an increased risk. Among 45 isolates not from Oregon (where serogroup B predominates), 34 (75.5%) were potentially vaccine preventable: 15 (33.3%) serogroup C, 18 (40.0%) serogroup Y, and 1 (2.2%) serogroup W-135.

Conclusions: Accumulating evidence suggests that there are behaviors that increase the risk of meningococcal infection, whereas others are associated with a decreased risk. The majority of IMD cases in high school students are preventable if Advisory

Committee on Immunization Practices recommendations on meningococcal conjugate vaccine are fully implemented.

P4.2.02

Integrated Bacteriophage and Invasive and Carriage Isolates of *Neisseria meningitidis*

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Recently, an integrated bacteriophage termed "meningococcal disease associated island" was reported and is proposed to be associated with the ability of the bacteria to cause meningococcal disease. The purpose of this study was to investigate the occurrence of this bacteriophage in a large number of diverse isolates of *N. meningitidis* causing invasive disease or isolated from asymptomatic carriers in the United States. We screened 403 meningococcal isolates (211 invasive and 192 carriage) for the presence of the integrated bacteriophage by polymerase chain reaction and confirmed the presence of the bacteriophage by Southern hybridization in selected isolates. Fifty-two percent (52%) of invasive and 34% of carriage strains were found to possess the 8 Kb bacteriophage (Odds Ratio [OR]=2.13; 95% Confidence Interval [CI] 1.42-3.19). In univariate analysis, a significant association was also seen between the presence of the phage and serogroup. Serogroup B strains were 7.73 times (95% CI 1.79-33.31) and serogroup C strains were 4.17 times more likely to have the phage than non-groupable strains, while serogroup Y strains were 7.46 times less likely to have the phage (95% CI 1.76-31.25). Among all isolates, the bacteriophage was present in 77% of serogroup C strains (*n*=78) and 86% of serogroup B (*n*=79) strains, while the bacteriophage was absent in 91% of the invasive serogroup Y strains analysed (*n*=55). After controlling for serogroup, the presence of the bacteriophage was no longer associated with isolate source (OR=1.13; 95% CI 0.62-2.08). We propose that the integrated bacteriophage represents a genetic element acquired by certain clonal complexes prior to their global spread. The lack of this element among many invasive isolates, including almost all serogroup Y strains in the United States, reinforces the need for continued investigation into factors related to invasive meningococcal disease.

P4.2.03

The Epidemiology and Surveillance of Meningococcal Disease in England and Wales since 1995

Edward Kaczmarski[1], Stephen Gray[1], Manosree Chandra[2], Andrew Fox[1], Anthony Carr[1], John Marsh[1], Lynne Newbold[1], Richard Mallard[1] and Mary Ramsay[2].

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The HPA Meningococcal Reference Unit (MRU) has been carrying out surveillance of invasive meningococcal disease for England and Wales since 1984. Microbiology laboratories in England and Wales have been actively encouraged to submit isolates from all clinical cases to the MRU. In October 1996 a national service for meningococcal DNA detection by PCR was introduced thereby improving epidemiology and case ascertainment. This currently confirms over 50% cases.

The number of laboratory confirmed cases rose from 1,443 in 1995 to peak at 2,785 in 1999, subsequently falling to 1,413 in 2005. The observed increase in serogroup C cases in the late 1990s, which at its maximal incidence caused 40% meningococcal cases, prompted the introduction of serogroup C conjugate (MenC) vaccine into the UK infant schedule commencing in November 1999 accompanied by a catchup programme targeting the entire under 18 year population.

The proportion of disease attributed to serogroups B, C, Y or W135 has altered, largely as a result of MenC introduction. Currently the respective proportion of cases attributable to serogroup B is 89%, C=3%, W135=2% and Y=3%. The 2% ungrouped cases are due to low numbers of genome copies in PCR only positive cases.

The age distribution has shifted with higher proportions in children under five years where serogroup B predominates and in adults aged over 20 who have not received MenC vaccine. A transient increase of W135 infections in the UK following the introduction of a strain by pilgrims attending the Hajj was seen in 2000 and 2001. This was ameliorated by an amended travel vaccination policy.

During 1995-2005 phenotypic and genotypic shifts have been observed, specifically the relative contribution of MLST clonal complexes ST-41/44, ST-269, ST-32, ST-213 and ST-11. The first four complexes comprise predominantly serogroup B strains and demonstrate the natural fluctuation over time.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

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P4.2.04

Surveillance of Serogroup Y Meningococcal Infection in England and Wales – Characterising a Recent Increase in Cases

Edward Kaczmarski, Lynne Newbold, Stephen Gray, Anthony Carr, Andrew Fox

Health Protection Agency (HPA) Meningococcal Reference Unit, NW Regional HPA Laboratory, Manchester Royal Infirmary, Manchester, UK.

Serogroup Y meningococci account for a small proportion (typically 2-3%) of invasive meningococcal infections in England and Wales but are more prevalent in other parts of the world, notably the USA where in recent years they have caused up to 30% cases. In 2005, 38 cases were seen, a 50% increase on the average 25 cases seen annually in the previous 3 years. More than three quarters of cases have occurred in patients aged over 20 years. Only 10-15% have been confirmed by PCR alone compared with about 50% cases caused by other serogroups reflecting the greater use of non-culture diagnostics in paediatric practice.

Multilocus sequence typing (MLST) is now the gold standard for meningococcal strain characterisation allowing historical comparisons between isolates and phylogenetic analysis. A collection of serogroup Y isolates from invasive infections in 2005 was characterised phenotypically and by MLST and porA sequence typing. The sequence typing data was compared with data from a historical collection of serogroup Y isolates from the last ten years.

Amongst the 2005 isolates, two clonal complexes were prevalent, ST-23 and ST-174. Whereas ST-23 is the endemic serogroup Y lineage in England and Wales and has predominated during the last ten years, isolates belonging to ST-174 complex were relatively rare but in the last two years have been responsible for a third of cases. A wide variety of porA variants has been seen. About 50% strains were VR1 5.1 while a further quarter were VR1 21. There was more diversity among VR2 sequence types the commonest being VR2 16, 10-4, 10-1 and 10-22.

Early analysis of cases seen in 2006 suggests that the increased incidence rate has been maintained. A prospective study of presenting clinical features has been initiated to identify the proportion with pneumonic symptoms.

P4.2.05

Clonal Analysis of *Neisseria meningitidis* Serogroup C Responsible for Invasive Disease in Poland

Anna Skoczynska[1], Marcin Kadlubowski[1], Iza Wasko[2], Anna Klarowicz[1], Waleria Hryniewicz[1]

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Introduction. Since 2002 the National Reference Centre for Bacterial Meningitis (NRCBM) noticed the increase in the number of cases caused by *Neisseria meningitidis* of serogroup C (MenC) in Poland. Therefore, the aim of the study was to establish the clonality of MenC isolates related to this rise.

Methods. The study was performed on all invasive MenC isolates collected between 1997 and 2004. Serogroups were determined by slide agglutination, serotypes and serosubtypes were evaluated by whole-cell ELISA method. The relatedness among isolates was evaluated by multilocus sequence typing (MLST).

Results. Out of 466 invasive meningococci, 347 (74.5%) belonged to serogroup B, 103 (22.1%) to serogroup C, and 10, 4 and 2 to W135, Y and non-groupable, respectively. Until 2002 the percentage of MenC had an average value of 11.2, but since then has maintained above 30. The most common serotypes among MenC were 2b, 2a and 4. The MLST analysis revealed in the studied group 45 sequence types (STs) including 21 new STs. In spite of such heterogeneity, 54.4% of isolates belonged to five STs: ST-8 (20.4%), ST-5133 (13.6%), ST-11 (9.7%), ST-5199 (5.8%) and ST-3698 (4.9%). The highest number of representatives had the ST-8 complex/Cluster A4 (21.4%), followed by ST-103 complex (16.5%), ST-11 complex (9.7%), ST-116 complex (6.8%) and ST-41/44 complex/Lineage 3 (3.9%).

Conclusion. Previous analysis of Polish MenC using PFGE did not reveal any dissemination of new epidemic clone related to the increase in MenC infections in 2002. However, the MLST typing showed that for the first time two STs, ST-8 and ST-5133 emerged in Poland in 2002, which are presently the most common amongst Polish MenC. Therefore, a common appearance of two clones, especially in a country with a small number of cases, may be difficult to notice in short time analysis without proper tools.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

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P4.2.06

Epidemiological study of gonococcal isolates based on diversity of the *opa* gene

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Background: Gonorrhea still remains one of the most common sexually transmitted disease worldwide. The study of relationship between isolates of *N.gonorrhoeae* can contribute to the identification of outbreaks in sexual networks, reinfection, temporal and geographic changes, sexual abuse and monitoring of antibiotic resistance. Newly developed molecular typing methods have contributed significantly to recent advances in the efficient monitoring trends in the epidemiology of bacterial infections. Opa typing is one of the most extensively tested and discriminatory of the molecular genetics techniques for studying the epidemiology of gonorrhea.

Objective: The aim of this study was to analyses the molecular epidemiology of the gonococcal isolates based on opa gene typing method.

Methods: 50 gonococcal isolates were examined. The strains were isolates from men with urethritis, women with endocervicitis, and their sexual contacts who attended at STD clinic of Lok Nayak hospital, New Delhi from January 2004 to June 2005. The diversity of the opa gene in gonococcal isolates were determined by digestion with two restriction enzymes TaqI and Hpa II. Discriminatory power was calculated applying Simpson's index.

Results: 43 opa types were detected among 50 gonococcal isolates. 37 isolates had unique opa type while 6 opa types were found in more than one patient. The discriminatory power of opa typing by using TaqI, Hpa II, and combination of both enzymes were 0.990,0.994and 0.994 respectively. All isolates were typeable. The method was reproducible and stable. The opa types from isolates that were epidemiologically unrelated were distinct, while those from the sexual contacts were identical.

Conclusion: The results demonstrate that opa typing, even with a single restriction enzyme, has a high level of discrimination between epidemiologically unrelated isolates. Opa typing method is highly useful to confirm self-reported sexual contacts and to identify short chains of transmission. In conclusion, the opa typing can be used for epidemiological characterization of *N. gonorrhoeae* strains.

P4.2.08

Genetic Diversity of *porB for* 174 *Neisseria gonorrhoeae* Isolates from Shanghai: Impact on Molecular Epidemiology and Antimicrobial Resistance

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Chu-Guang Zhang[3], Yue Chen[4], Ann M. Jolly[4], and Jo-Anne R. Dillon[1,2]

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DNA sequence analysis of *porB* genes (*porB1a* and *porB1b,* encoding PIA and PIB proteins, respectively) provides a high index of discrimination for isolates of *Neisseria gonorrhoeae*. Complete *porB* sequences of 174 consecutive clinical isolates of *N. gonorrhoeae* collected in Shanghai were determined to ascertain isolate clusters and to investigate the association of *porB* sequences with antimicrobial susceptibility. *porB*1a genotypes comprised 27.0% of the isolates and included 15 unique sequences, while 73.0% of the isolates carried *porB*1b genotypes with 64 unique sequences. Phylogenetic tree analysis indicated that *porB*1a isolates were divided into 2 clusters and *porB*1b isolates into 3 clusters. The association of antimicrobial susceptibility and *porB* sequences indicated that *porB*1b isolates had a significantly higher percentage of chromosomally mediated tetracycline resistant isolates as well as higher MIC_{50}s to penicillin, ciprofloxacin, spectinomycin and ceftriaxone. Mutations at residues G120 and A121 of PIB proteins have been associated with resistance to penicillin and/or tetracycline. In the present study, a high percentage of PIB isolates were resistant to penicillin (94%) or tetracycline (59%); 85% of the PIB isolates carried a G120K mutation (including 70.0% with G120K/A121D and 15.0% with A121G/N or H mutations). In addition, other PIB isolates carried G120N/A121N or G120R/A121D mutations (1.6%), or a G120D (11.0%) mutation, while 2.4% of the PIB isolates did not carry any mutation. 91% of PIA isolates were resistant to penicillin and 36% were resistant to tetracycline with 83.0% having G120D/ A121G mutations and 17% had a single A121G mutation. A simultaneous deletion/insertion in loops V and VI was identified in 18 PIB but not in PIA isolates, and isolates with these mutations had significantly higher MICs to spectinomycin and ceftriaxone. Clusters of gonocoocal isolates in Shanghai have been identified through *porB* sequence analysis and certain mutations have been correlated with decreased susceptibility to antimicrobial agents.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

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P4.2.09

Epidemiology of *Neisserria meningitis* Infection at the Far East of Russia

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Despite of the intent study meningitis caused by *Neisseria meningitidis* take the significant place at the morbidity and mortality at the Far East of Russia. For the 2003-2004 years at the Far East of Russia and Siberia there were registered 2,8 and 3,0 morbidity cases per 100,000 of population accordingly. The morbidity among children younger of the 14 years was another main problem of meningococcus infections in our region and it was charecterized as 10,7 cases (2003 year) and 11,7 (2004 year) per 100,000 of population. Morbidity of *Neisseria meningitis* infection in a group of children younger 1 year was 55,5 (2003 year) and 59,0 (2004 year) cases per 100,000 of population. Rates of mortality was established as high and there were as 11,1% in 2003 and 11,9% in 2004. So, the significance of problem is obvious and the aim of our research was to define serogroups and antimicrobial agents susceptibility of the *Neisseria meningitis* strains isolated in patients of our Primorskyi region. Methods: we defined serogroups of the 70 isolates of *Neisseria meningitis* strains isolated at the territory of Primorskyi region in 2003-2004 years, and the susceptibility to antimicrobial agents was determined by disko-diffusion and method of microdilution on NCCLS standards, serotyping was performed on common procedure.Results: There were revealed prevalence of strains of B serotype 68,57% (48 strains), C serotype 25,7% (18 strains), 5,7% strains were of A serogroup. Elevated penicillin and ampicillin MICs (> or =0.12 microg/ml and > or =0.25 microg/ml, respectively) occurred in 14,2% (10 strains) and 8,6% (6 strains).Resistance in tetracycline and doxycycline was revealed in 17,1% (12 strains), nine strains were resistant to rifampicin 12,8%. Conclusion:epidemiological situation on meningococccal infection remains the threatening factor for development of epidemy at the Far Eastern regions of Russia and requires careful planning of prevention.

P4.2.10

Invasive Meningococcal Disease in Italian Children and Adolescents

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Meningococcal invasive disease is a life threatening infection that mostly affects children and adolescents. In Italy, the incidence of the disease is low (0.4 - 0.5 x100.000 inhabitants per year), but it increases to 3.5 and 1.2 x 100.000 when considering cases only in children (0 - 4 years) and adolescents (15-19 years), respectively.
This study was carried out to compare phenotypic characteristics in meningococcal strains isolated in Italy from children and adolescents with those from the other age groups to assess whether strategies for treatment and prevention elsewhere implemented can be applied to invasive meningococcal disease in Italy too.

From 2003 through 2005, the National Reference Laboratory analysed 433 meningococcal strains responsible for invasive disease. In particular, 192 were isolated from cases which occurred in children and in adolescents.
Since 2004 serogroup C has replaced B as prevalent serogroup, representing 61% of all meningococci isolated with C:2b:P1.5,2 as predominant phenotype. No predominant phenotype was detected among serogroup B strains, with the most frequent subtypes P1.4 and P1.13 representing less than 30% of the total, underlining the dispersion of B phenotypes in the country. As far as the antimicrobial susceptibility patterns of the isolates are concerned, in the three year period, a dramatic increase of strains with reduced susceptibility to penicillin has been observed accounting for 87.4% of serogroup C meningococci in the year 2005. The genotypic characterization by MLST showed that C:2b:P1.5,2 meningococci belonged to ST8/A4 whereas the phenotype C:2b:P1.5, which disappeared in 2005, but was prevalent in previous years, was in part ST8/A4 and in part ST1860/ET37.

In a public health perspective, these data are necessary to evaluate the cost-effectiveness of anti-meningococcus C vaccination, since routine vaccination programs at national level have not yet been defined.

The Discussion Session for the following posters is from:
9.30pm – 10.15pm Tuesday 12 September

P4.3.01

Distribution of multi-locus sequence types of *N. meningitidis* serogroup B in the US—2000-2005.

Anne Whitney[1], Leonard Mayer[1], Elizabeth Mothershed[1], Claudio Sacchi[1], Susanna Schmink[1], Jordan Theodore[1], Jane Marsh[2], Kathleen Shutt[2], Nancy Messonier[1], Patricia Wilkins[1], Lee Harrison[2] and the Active Bacterial Core Surveillance Team

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Subtyping of *Neisseria meningitidis* using multi-locus sequence typing (MLST) has been widely used for studying the epidemiology of meningococcal disease and the evolutionary biology of the meningococcus. The aim of this study was to identify the major MLST complexes of *N. meningitidis* serogroup B (MenB) causing invasive disease in the US during the period 2000 through 2005. Representative strains could then be used for further antigen characterization for development of non-polysaccharide vaccines against MenB. *N. meningitidis* isolates from invasive meningococcal disease are submitted through the population- and laboratory-based Active Bacterial Core Surveillance program (ABCs) supported by the Emerging Infections Program at CDC. ABCs is conducted in 9 sites throughout the United States representing a population of 36 million. A total of 463 MenB isolates were submitted during 2000-2005, and the STs of 444 (96%) of these were determined. 220 (49%) were in the ST-32/ET-

5 complex including 189 (43%) that were ST-32; 116 (26%) were in the ST-41/44 complex / lineage 3 and 22 of those were ST-136. Forty percent of MenB isolates subtyped were from Oregon (OR), a state which has had an ongoing MenB epidemic since 1993. A total of 74% of MenB from OR were of the ST-32 complex, demonstrating that invasive meningococcal disease in OR continues to be caused by a meningococcal clone. When OR isolates were excluded from analysis, the proportions of the two major complexes changed: isolates of the ST-32 complex were reduced from 49% to 31% and ST-41/44 complex increased from 26% to 33%. In summary, approximately 76% of all US MenB isolates were in 2 clonal complexes (ST-32 and ST-41/44), and 86% were associated with only 4 clonal complexes. Further characterization of potential vaccine targets in these isolates will be performed to determine the optimal formulation of future MenB vaccines in the US.

P4.3.02

Neisseria meningitidis PorA Subtype Distribution in the South of Brasil

Luciana Weidlich[1,3], Ludmila Baethgen[1,3], Camile de Moraes[3], Cecilia Klein[2,3], Luciana Nunes[3], Maria Rossetti[3], Arnaldo Zaha[2], Leonard Mayer[4]

[1]Departmento de Bioquímica, [2]Departmento de Biologia Molecular e Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil
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[4]Centers for Disease Control and Prevention, Atlanta, GA, USA

Neisseria meningitidis (N.men) is an important cause of meningitis and septicemia in Rio Grande do Sul (RS), a state in the extreme south of Brasil, as it is in the whole country. RS has a population of approximately 11 million people and a meningococcal disease incidence around 1.5 cases per 100,000 inhabitants. Serogroup B (MenB) remains the most prevalent serogroup in Brasil but an effective vaccine is not widely available.

We evaluated the prevalence of subtypes of N.men isolated in RS by determining the PorA variable region (VR) sequences. A total of 79 isolates from patients with confirmed meningococcal meningitis from 2003 and 2004 in RS were analyzed. All the isolates had two fragments of *porA* gene amplified and sequenced, encoding VR1 and VR2 regions, and each nucleotide sequence was submitted to N.men PorA variable region database (http://www.neisseria.org/nm/typing/porA) to assign the corresponding subtype..

Of the total of 79 isolates, 54 (68%) were MenB, 16 (20%) were MenC and 9 (11%) were MenW135. The strains were grouped into 19 different subtypes, including 3 new subtypes. The most prevalent subtype was P1.19,15 (27%), followed by P1.7,16 (19%), P1.5,2 (16%), P1.18,1-3 (9%) and P1.22,14-6 (8%). The other subtypes were represented by 1-3 isolates each. All P1.5,2 strains belong to MenW135 and P1.22,14-6 to MenC. The majority of other subtypes correspond to MenB strains. RS seems to have a different distribution of subtypes compared to other states of Brasil with a wide diversity of subtypes in MenB. The proposed Outer Membrane Vesicle (OMV) bivalent vaccine containing P1.19,15 and P1.7,1, would theoretically protect against about 70% of MenB circulating in north, northeast and southeast regions of Brasil, but only 30% in RS. To provide about 70% coverage for MenB in RS, an OMV based vaccine needs to also include P1.19,15, P1.7,16, and P1.18-1,3.

P4.3.03

Carriage of serogroup A meningococci on the peak of an NmA epidemic in Burkina Faso

Njanpop-Lafourcade BM [1], Yaro S [2], Mueller JE [1], Sangaré L [3], Drabo A [2], Diagbouga SP [2], Traoré Y [2], Nicolas P [4], Borrow R [5], Gessner BD [1]

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Background/Objective
Information on the age distribution of Neisseria meningitidis serogroup A (NmA) carriage during NmA epidemics is needed to plan the use of future serogroup A conjugate vaccines, which have an indirect effect through carriage reduction. This study describes the age distribution of genogroup-specific Nm carriage in a rural population of Burkina Faso during an NmA epidemic.

Methods
In this cross-sectional study, we took nasopharyngeal swabs from a representative sample of 1- to 39-year-old residents in three villages in rural western Burkina Faso from March 12 to 28, 2006. The swabs were analysed by culture and all isolated Nm are currently being confirmed and genogrouped by PCR using *crgA*, *siaD*, and *mynB* oligonucleotides, and serogrouped by immune serum agglutination. Exhaustive PCR-based surveillance of bacterial meningitis was conducted in parallel to the carriage study.

Results
From the 625 study participants, 132 meningococci were isolated. Overall Nm carriage prevalence was lowest in the 1- to 4-year old population (11%), and constant at 24% for age groups 5-9, 10-19 and 20-39 years. Genogroup-specific carriage prevalences will be presented. During the study period, the PCR-confirmed weekly incidence rate of NmA meningitis in the study population was around 75 per 100,000.

Conclusion
During this NmA epidemic, overall Nm carriage prevalence was substantially higher than in previous studies in Burkina Faso, and was not vary by age in the 5- to 39-year-old population. Following genogroup determination, we will interpret how these data may impact the development of NmA conjugate vaccine schedules for Sub-Saharan Africa.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P4.3.04

Variability in Meningococcal Serogroups Causing Invasive Infection in Children in Canada, 2002-2005

Julie Bettinger[1], David Scheifele[1], Nicole Le Saux[2], Scott Halperin[3], Raymond Tsang[4], for Members of the Canadian Immunization Monitoring Program, Active (IMPACT)

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Background: The epidemiology of meningococcal infections is central to the design of vaccination programs but substantial variability exists with these infections. Within a nationwide surveillance project we examined the influences of age, serogroup and geographic locale on childhood infections.

Methods: Active surveillance for invasive meningococcal infections in children age 0-19 years was conducted by the 12 academic centers of the Canadian Immunization Monitoring Program, Active (IMPACT). Surveillance included all pediatric units within defined urban areas containing half of the Canadian population (4.2 million children) and was laboratory-based. *Neisseria meningitidis* was isolated from normally sterile sites or detected by PCR assay, in 2002-2005. Isolates were serogrouped at a reference laboratory. Population census data were used for incidence rate calculations.

Results: 179 cases were detected, mainly in those 0-4 years (52%) and 15-19 years (31%). Incidence rate was highest for those ≤ 1 year old, ranging from 3.7 to 6.8 per 100,000 annually. Serogroup B disease predominated (104 cases, 58%), at a near-constant rate of 0.6 per 100,000/yr. The proportion of isolates that were serogroup B decreased from 69% among 0-4 to 41% among 15-19 year olds, while serogroup C isolates did the opposite (12% and 30% rates, respectively). Group Y disease was constant at 0.1 per 100,000/yr, occurring mainly in 10-19 year olds. W-135 isolates were encountered only in 2003, reaching a rate of 0.2 per 100,000. The fraction of cases with C-Y-W135 ranged from 19% (n = 72) in Quebec (after widespread use of C conjugate vaccine in 2001) to 57% in Western Canada (n = 38).

Conclusions: Serogroup B disease predominates in Canadian children but C & Y contribute significantly among 10-19 year olds. W-135 infections occur only sporadically but exceeded Y cases one year. Regional differences are noteworthy, allowing for the limited case totals, complicating vaccine policy-making.

P4.3.05

Changes in Serogroup Distribution of Agents of Invasive Meningococcal Disease in Australia Following a Selective Publicly-Funded Programme of Vaccination with Serogroup C Conjugate Vaccine

Helen Smith[1] for the Australian Meningococcal Surveillance Programme

[1]Queensland Health Scientific Services, Coopers Plains, Brisbane, Queensland, Australia

A publicly-funded programme of serogroup C conjugate vaccination of Australian children and adolescents was completed between 2002 and 2004. The Australian Meningococcal Surveillance Programme has analysed the age and serogroup of laboratory-confirmed cases of invasive meningococcal disease (IMD) nationally and by jurisdiction each year since 1994. We report results of analyses of 2227 cases of IMD from 2001 to 2005.

Serogroup B and C meningococci were responsible for most IMD in Australia, but with substantial jurisdictional and age differences noted prior to and after the vaccination period. Nationally in 2001, of 447 confirmed cases, 258 (58%) were B and 153 (34%) C meningococci and in 2002 of 580 cases 282 (49%) were B and 213 (37%) C. By 2005, all cases numbered 345 with 251 (73%) B and 50 (14%) C, meningococci.

Nationally by age in 2001, there were 106 B and 27 C meningococci in those aged < 4 years and 57 B and 56 C in those aged 15-24 years. In 2002, there were 84 B and 20 C in those < 4 and 60 B and 73 C in those 15-24. By 2005 there were 99 B and 6 C in those < 4 and 61 B and 12 C in those 15 –24.

Jurisdictional serogroup differences were noted in 2001 and 2002 with Victoria, Tasmania and the Australian Capital Territory having high rates of serogroup C infections in adolescents. Serogroup C disease decreased markedly by 2005 in these regions whereas serogroup B IMD remained prominent in all age groups in South and Western Australia and the Northern Territory.

Laboratory data indicated a significant shift in serogroup distribution following the use of conjugate serogroup C vaccines with the reduction most notably in adolescents in South-Eastern Australia, but also in infants and in other jurisdictions.

P4.3.06

***Neisseria meningitidis:* Carriage in Africa**

Montse Soriano-Gabarró[1], Joanne Wolter[2]

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In Africa, meningococcal disease causes a major disease burden and is heavily influenced by climate and season. Disease affects children and young adults, with highest incidence rates among infants and young children. Major epidemics are largely confined to the “meningitis belt” encompassing 400 million people from 21

countries. Epidemics peak during dry seasons and are rare in regions with continuous high humidity or extensive forestation. In contrast, carriage appears to be environmentally but not seasonally influenced.

Meningococcal disease in Africa is primarily caused by serogroup A *Neisseria meningitidis* and to some extent to serogroup C. More recently, serogroup W-135 has been identified as a cause of disease, after a major W-135 epidemic in Burkina Faso during 2002. Serogroup W-135 continues to cause disease as observed by ongoing surveillance in some African countries and carriage of W-135 has been observed in Burkina Faso and Ghana. Serogroup X meningococcal disease and carriage have also been reported in countries such as Ghana and Niger. Carriage of non-virulent strains also occurs.

Meningococcal carriage is dynamic. During epidemics, high levels of carriage are observed that fall many months after epidemic activity ceases. Prevailing strains change over time and regional variation exists. Seroprevalence data constitutes a marker of recent carriage. Highest carriage rates are observed in African children <10 years of age compared to 15-24 year olds in non-African settings.

Risk factors for meningococcal carriage include crowding, smoking, respiratory infection, and low socio-economic status. Carriage induces a protective antibody response in some individuals, while it results in invasive disease in others.

For the majority of countries within the meningitis belt, meningococcal carriage and its relationship to disease is poorly characterized. New meningococcal conjugate vaccines are expected to have an impact on carriage and disease transmission. Early vaccination during infancy will protect those at highest risk.

P4.3.07

Survival of Meningococci Outside of the Host: Implications for Acquisition

Claire Swain[1] and Diana Martin[1]

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There seems to be little doubt that the transfer of respiratory droplets shed from the upper respiratory tract is the means by which *Neisseria meningitidis* is spread. However, the exact mechanism is still unclear and, due to the assumption that meningococci are unlikely to survive outside of the human host, the mechanism of fomite transmission has been largely disregarded. To determine whether meningococci can survive in the environment, seven strains representing the three most common capsular groups B, C, and W135, were tested for their ability to survive on glass or plastic (common drinking vessel materials). A known number of colony forming units (cfu) of each strain were dried onto glass and onto plastic and tested for viability over time. Survival on glass was significantly better than on plastic (P<0.0001) with 9-20% of the cfu of the group B strains surviving desiccation on glass compared to 5-12% cfu on plastic. Isolates of the New Zealand epidemic strain, B:4:P1.7-2,4 , survived better on glass than all other strains tested (P=0.0013) with one strain surviving up to 168 hours. Recovered isolates were found to still express their capsules and outer membrane proteins. These findings raise the question of whether meningococci can be transferred from person to person via fomites such as drinking vessels contaminated with meningococcal-containing oropharyngeal secretions.

P4.3.08

Intact-cell MALDI-TOF Mass-spectrometry for Identification and Subtyping of Pathogenic *Neisseria*

Vladimir Vereshchagin[1], Elena Ilina[1], Nazar Al-khafaji[2], Tatiana Priputnevich[2], Anna Kubanova[2], Sergey Sidorenko[3] and Vadim Govorun[1]

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[3] National Research Centre for Antibiotics, Moscow, Russia

Mass-spectrometric analysis of bacterial cell was shown to be an alternative approach for the decision some of clinical microbiology problems - rapid and accurate identification and strain differentiation of microorganisms, overcoming the existing limitations of classical cultural methods.

We investigated the feasibility of intact-cell MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time Of Flight) mass spectrometry for identification and subtyping pathogenic *Neisseria*.

Details of analytical procedure and instrument's settings were worked through using the laboratory strain of *E.coli* DH5α. Sample preparation was very simple and included two steps: 1) a few of bacterial cells was removed from the culture plate using a 1mkl loop and dissolved in 50% acetonitrile, 2,5% trifluoroacetic acid solution; 2) supernatant from previous step was co-crystallized with the MALDI matrix (α-cyano-4-hydroxycinnamic acid, α-CHCA) at the steel target surface (MSP 96 target ground steel, Bruker Daltonics, Germany). Reproducible mass-spectra for laboratory and clinical strains of *N. gonorrhoeae*, clinical strains of *N. meningitidis* and some other nonpathogenic *Neisseria* species were collected by means of Microflex MALDI-TOF MS (Bruker Daltonics, Germany). The followed analysis using BioTyper 1.0 software (Bruker Daltonics, Germany) was shown significant interspecies and little intraspecies heterogeneity of bacterial mass-profiles. Based on that observation the application of intact-cell MALDI-TOF MS for identification of *N. gonorrhoeae* and *N. meningitidis* as well as distinctions between pathogenic and non-pathogenic *Neisseria* was suggested. For increase of identification reliability the additional number of related Neisseria species have to be included for further comparative analysis.

We concluded that suggested approach based on mass-spectrometric fingerprinting of *Neissseria* species have a great advantages such as the request of small amount of biological material, very easy sample preparation, high accurate measurement with low cost per analysis. It can be used as a rapid method for detection of pathogenic *Neisseria* and also be easily adopted for other microorganisms.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P4.3.09

W135 meningococccal disease: an emerging problem in Singapore related to the Hajj pilgrimage

Annelies Wilder-Smith, Nicholas Paton, Timothy Barkham

Travellers' Health & Vaccination Centre, Singapore

Introduction: *N. meningitidis* W135 meningococcal disease was hitherto a sporadic problem. In the years 2000 and 2001, an outbreak of W135 disease occurred during the Hajj pilgrimage in Saudi Arabia with clusters in Moslem communities all around the world, most likely related to returning pilgrims. In response to the outbreak, tetravalent meningococcal vaccine (covering W135) was introduced as mandatory vaccination for all Hajj pilgrims in the year 2002.
Methods: We obtained data on W135 meningococcal disease from the Ministry of Health in Singapore for the years 1991 to 2005. We also took pharyngeal swabs from Hajj pilgrims returning to Singapore in the years 2001 (n=214) and 2002 (n=198).

Results: W135 meningococcal disease in Singapore did not occur before the year 2000. The incidence in 2000 and 2001 was 30 and 25 per 100,000 respectively in pilgrims and their household contacts. No further cases of W135 occurred in the years 2002 to 2005, in parallel with the absence of further W135 outbreaks in Saudi Arabia. In 2001, 15% of returning pilgrims to Singapore were carriers of W135; in 2002 only 1%.

Conclusions: The small outbreak of W135 meningococcal disease in Singapore was associated with the clonal outbreak during the Hajj 2000 and 2001. Returning pilgrims in 2001 became carriers of W135 thereby putting their close contacts at risk. The introduction of mandatory vaccination that includes protection against W135 for all Hajj pilgrims in 2002 led to the control of W135 disease during the Hajj. In 2002, the proportion of of *N. meningitidis* W135 carriers in returning pilgrims to Singapore was significantly reduced and this was associated with an absence of further W135 cases since 2002.

P4.3.10

Meningococcal Meningitis in China: Status in 2005

Zundong Yin, Xiaofeng Liang, Yixing Li, Junfeng Yang, Junhong Li

Chinese Center for Disease Prevention and Control (Beijing, China: 100050)

A total 2318 cases of meningococcal meningitis were reported in 2005 from all provinces except Hainan, an incidence of $0.177/10^5$ population. There were 206 fatal cases, a case fatality rate (CFR) of 8.9%. The incidence rate decreased 15% from 2004, but CFR increased 45%. The five provinces with the most cases were: Anhui(260), Guizhou(213), Hebei(136), Zhejiang(129) and Sichuan(126), accounting for 37% to total. The five provinces with the highest incidence were: Qinghai($0.789/10^5$), Xinjiang($0.582/10^5$), Guizhou($0.541/10^5$), Beijing($0.525/10^5$) and Ningxia($0.488/10^5$). Incidence is higher in west than in east China. As in 2004, cases increased in January, peaked in March and April and decreased beginning in May. Age-specific incidence and CFR were higher for people < 20 years of age, accounting for 73% of total cases and 52% of total deaths, respectively. Distribution by occupation shows students accounted for 37% of cases, followed by 24%. in pre-school children. Farmers and rural workers accounted for 12% and 8% of cases respectively.

Strains Isolated: Serogroup A has been the dominant strain in China, with sporadic serogroup C cases in recent years. In 2005, some C group outbreaks occurred in Anhui province. Enhanced lab surveillance isolated C group strains in 17 of 31 provinces in mainland China.

Immunity among healthy people: In total 18 provinces tested the immunity of group A and C among healthy people. In general, a majority of people don't have immunity against group A and C, especially group C. Immunity against group A in north-west China is lower than east China, probably because of lower coverage of meningococcal group A polysaccharride vaccine in north-west China.

Control measures: Lab network and surveillance were enhanced, meningococcal group A polysaccharride vaccine was integrated into expanded program on immunization (EPI) in 11 provinces. Mass vaccinations were conducted in some areas to control outbreaks. Sentinel sites were established.

P05
Genomics & Gene Expression

The Discussion Session for the following posters is from:
8.00pm – 8.45pm Tuesday 12 September

P5.1.01

The Neisserial DNA Uptake Sequence Revisited: Locational Bias and Quantitation of Effect on Transformation

Ole Herman Ambur[1], Stephan A. Frye[1,2] and Tone Tønjum[1,2]

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The DNA-uptake sequence (DUS) 5'-GCCGTCTGAA-3', overrepresented in the genomes of the *Neisseria sp.*, is important for genetic exchange by means of DNA transformation. By using a combination of bioinformatics tools and molecular biology techniques we have quantitated and extended the knowledge on the role of DUS in transformation. Our investigations of *Neisseria* genome sequences showed that among the approximately 2000 DUS per genome, nearly 50 % exist as intergenic inverted repeats. We have predicted that many of these constitute parts of transcriptional terminators.

Plasmids containing the neisserial DUS in different arrangements, as well as sequential DUS variants, were tested for their ability to transform the human pathogens *N. gonorrhoeae* and *N. meningitidis*. We found elevated transformation rates with DNA

containing an inverted DUS-repeat relative to the single DUS and the direct repeat constructs. These results suggest that the orientation of the DUS signal is of importance for making sequence-specific associations with the receptor protein(s) in the receiving bacteria. DUS-negative donor-DNA yielded minute transformation rates, enforcing the importance of DUS in efficient neisserial transformation. Finally, we extended the established 10 nt DUS to include two semi-conserved nucleotides to become a 12 nt DUS, and found a strong increase in transformation rates with 12 nt DUS as compared to 10 nt DUS in all the *Neisseria* strains tested. Also DNA uptake studies with radiolabelled DNA showed preferential uptake of the 12 nt DUS DNA relative to the 10 nt DUS DNA. We thereby challenge the established DUS consisting of only 10 nucleotides.

P5.1.02

Overexpression of GNA1870, a conserved surface-exposed protein, in group B *Neisseria meningitidis* and in *E. coli*

Mikhail Donets, Boris Ionin, Valerian Pinto, Wendell D. Zollinger

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There is currently no comprehensive vaccine against serogroup B meningococci. A native outer membrane vesicle (NOMV) vaccine containing normal levels of lipooligosaccharide (LOS) and overexpressed, conserved, surface-exposed outer membrane proteins (OMPs) may provide a solution to this problem. We hypothesized that overexpression of the highly conserved *Neisseria meningitidis* (*Nm*) lipoprotein GNA1870 would increase the immunogenicity of the GNA1870 component of the NOMV and provide a more cross-reactive immune response.

We were able to overexpress variant 1 of GNA 1870 in *Nm* strain 8570 (which exhibits a high native level of expression of variant 1) and variant 2 of GNA1870 in *Nm* strain B16B6 (with low level of native expression of variant 2). Expression of variant 1 under the control of IPTG-inducible *E. coli* Ptac promoter in strain 8570 was about 4-fold higher than in the parental strain. Expression of variant 2 (using the same promoter) in strain B16B6 was about 32-fold higher than in the parental strain. The maximum expression level achieved in the two strains was about the same. The protein appeared properly processed, lipidated, and translocated to the surface of the outer membrane. An alternative expression system that utilized the PorA promoter was less effective in overexpression of GNA1870 in either strain. Remarkably, the *Neisseria* PorA promoter was able to induce high expression of GNA 1870 in *E. coli*.

The orthologous expression system that utilizes the Ptac promoter in *Nm* organisms has several advantages in comparison to *E. coli* recombinant technology: 1) the combination of overexpressed GNA1870 with additional antigens such as lipooligosaccharide (LOS) in the NOMV provides additional protective antigens in the vaccine; 2) LOS, which has adjuvant activity, can increase vaccine immunogenicity; 3) overexpression in *Neisseria* guarantees a native conformation and proper lipidation of GNA1870.

P5.1.03

Transcriptional Profiling of *Neisseria gonorrhoeae* Biofilm

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N. gonorrhoeae forms biofilm over glass surfaces and on primary cervical cells. In addition, our laboratory has evidence that biofilms occur during natural cervical infections. To study the mechanism of biofilm formation, transcriptional profiles of *N. gonorrhoeae* biofilm were compared to planktonic profiles. Biofilm RNA was extracted from *N. gonorrhoeae* 1291 grown for 48 hours in continuous flow chambers over immortalized cervical cells (TCX). Planktonic RNA was extracted from batch cultures grown to mid-log phase over TCX. The Genisphere 3DNA® 350MPX kit was used to synthesize and hybridize cDNA to printed *Neissera* microarrays, designed by TIGR. A reference was constructed from digested genomic DNA, treated identically to cDNA. Arrays were hybridized with a cy5 labeled reference and a cy3 labeled experimental sample. All spot intensities were lowess normalized and analyzed in Genespring®. Genes with a fold change ≥1.5 and a p-value ≤0.05 were identified as differentially regulated. We found that 181 genes were upregulated in TCX biofilm versus planktonic, comprising 8.3% of the genome. Of these 181 genes, 56 belonged to 23 operons, each containing 2-5 of these upregulated genes. Thus, upregulated genes belonging to operons with other upregulated genes made up 39% of all those upregulated, while 8.8% (16 genes) could not be evaluated due to incomplete annotations. Similarly, 142 genes were downregulated in TCX biofilm versus planktonic, comprising 6.5% of the genome. Of these 142 genes, 11 genes belonged to 5 operons, each containing 2-3 downregulated genes. Thus, downregulated genes belonging to operons with other downregulated genes made up 7.7% of all those downregulated, while 25% (35 genes) could not be evaluated due to incomplete annotations. Microarray analysis of gonoccocal biofilm demonstrates substantial transcriptional changes between biofilm and planktonic growth over TCX. Future studies will focus on the defining the genetic and biochemical basis of gonococcal biofilm formation.

P5.1.04

A mutation in the gonococcal *pilT* has a global influence on the Transcriptome of *Neisseria gonorrhoeae*

Alexandra Friedrich[1,4], Cindy G. Arvidson[2], William H. Shafer[3], Eun-Hee Lee[3], Magdalene So[4]

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Poster Discussion Session: Tuesday 12 September 7.30pm

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The *Neisseria gonorrhoeae* (GC) type IV pilus is a retractile structure that mediates twitching motility, DNA uptake, microcolony formation and host colonization. Recently, it has been shown that GC pilus retraction influences host responses, such as clustering of host proteins direct beneath microcolonies and activation of stress cascades. Furthermore, it has been shown that the mechanical force generated by pilus retraction activates epithelial cell gene expression.

In order to determine if GC also senses and responds to pilus retraction, we analyzed the transcriptome in the GC wild type strain MS11 and in the corresponding pilus retraction-deficient *pilT* mutant. Using a DNA microarray approach, we identified 27 genes, which were differentially regulated (9 up, 18 down) after growth on agar medium in the *pilT* mutant background. Using the same microarray approach, we examined the transcription profile of MS11 and the *pilT* mutant during infection of epithelial cells. 39 genes were identified as differentially regulated in the *pilT* mutant (12 up, 27 down). As in the absence of epithelial cells, genes encoding two putative ABC transporters and *mtrF,* which encodes a protein involved in resistance to antimicrobial compounds, were found to be more highly transcribed in the *pilT* mutant background. Only in infection was *pilE*, encoding the major pilus subunit, upregulated, and *farR*, encoding a transcriptional repressor, downregulated in the *pilT* mutant. Our data show that PilT has a global influence on the gonococcal transcriptome, most likely through its effect on pilus retraction, and that epithelial cells have an impact on the *pilT*-dependent regulation.

P5.1.05

Transcriptome of *Neisseria meningitidis* serogroup B grown in Human Plasma - an *Ex Vivo* Model of Infection

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In the absence of a satisfactory animal model of meningococcal septicaemia, we have chosen to explore the repertoire of meningococcal gene expression in the course of prolonged incubation in human plasma and blood.

We are using microarray technology to identify meningococcal genes that are progressively upregulated on incubation in anticoagulated human plasma. We speculate that any surface exposed gene products that are expressed more strongly in the bloodstream than on the mucosal surface, where immune selection pressure drives antigenic diversity, should be relatively less variable, and so attractive as cross-protective vaccine candidates.

To investigate the transcriptional profile of serogroup B strain MO1-240185, a member of the "hypervirulent" ST-11 complex, bacteria were incubated for up to four hours in plasma and in RPMI 1640 + 10% FBS. Experiments were replicated in plasma and RPMI, to control for alterations in gene expression arising simply as a consequence of growth in nutrient liquid medium. ~10 µg bacterial RNA was isolated from one and from four hour experiments, cDNA prepared by reverse transcription, and gene expression compared (plasma vs RPMI, early vs late time points) from five independent experiments using a Neisseria microarray. The array contained PCR products based on ORFs of all the annotated genes in the sequenced serogroup B strain MC58, plus extra genes from serogroup A and C strains and from *N. gonorrhoeae*.

135 genes were differentially expressed in plasma-grown organisms at the late versus early timepoint. 92/135 genes were upregulated, including genes associated with energy metabolism, regulatory functions, hypothetical/unknown function, and six genes encoding probable outer membrane proteins. These include NMB0663, encoding NspA, an established candidate vaccine antigen. 54 genes responded differently to prolonged growth in plasma compared to growth in conventional laboratory media. 25/54 genes were upregulated, including NspA, which was 3-fold upregulated over a four hour experiment.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P5.2.01

MtrA Regulation of Gene Expression in Neisseria gonorrhoeae

Paul Johnson[1], Jason Folster[1], Jennifer Rocco[1], William Shafer[1,2]

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Neisseria gonorrhoeae, the causative agent of gonorrhea, continues to be an important international public health concern. As with many pathogens, once successful antibiotic treatments for gonorrhea have become less effective over time. One way Neisseria gonorrhoeae can resist the action of antimicrobial agents is through the expression of an inducible, energy-dependent efflux pump encoded by the mtrCDE locus. We have previously shown that MtrA, an AraC-like transcriptional regulatory protein, positively regulates the inducible expression, mediated by the presence of a sub-lethal concentration of a hydrophobic antimicrobial, of the mtrCDE locus resulting in higher levels of antimicrobial resistance and increased mtrC transcription; this efflux pump system contributes to gonococcal virulence because loss of expression results in enhanced susceptibility to host-derived antimicrobials. In this study, we examined whether MtrA also regulates the mtrCDE locus under non-inducing conditions. We examined regulation of the mtrCDE locus by constructing lacZ fusions to the promoter of mtrC in mtrA+ and mtrA- isogenic transformants of strain FA19. Using these fusions, we determined that while mtrA expression is not auto-regulated, expression of mtrC is MtrA-dependent. MtrA appears to have a more global regulatory property because we have determined that the expression of other genes that are involved in peptidoglycan biosynthesis (ponA, encoding penicillin-binding protein 1) or pilin secretion (the pilMNOPQ operon) are de-repressed in the absence of MtrA. Thus, in gonococci, MtrA can function to both transcriptionally activate and repress genes involved in virulence or bacterial cell envelope structure.

Poster Discussion Session: Tuesday 12 September 7.30pm

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P5.2.02

The Attenuation of Meningococcal NMB1966 Mutant and its Transcriptome analysis

Noel Chow[1], Ming Shi Li[2], Sunita Sinha[2], Denise Halliwell[3], Michelle Finney[3], Andrew Gorringe[3], Rita d'Mello[2], Mark Watson[4], Simon Kroll[2], Paul Langford[2] and Steven Webb[1]

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NMB1966 of *Neisseria meningitidis* serogroup B strain MC58 encodes a putative component of an ABC transporter of unknown specificity and whose gene expression was found to be up-regulated in growth in whole blood compared to rich growth media. To further characterize the function of the gene an NMB1966 knockout mutant was constructed. The NMB1966 knockout was significantly attenuated, after intraperitoneal challenge, in the mouse model of infection compared to its isogenic parent. In-vitro study showed that the NMB1966 mutant was slightly impaired in growth in complex liquid media, less invasive to human epithelial cells and rapidly killed in human blood. To explore the mechanism of NMB1966 function in relation to the phenotypic changes observed, the transcriptome of the mutant was compared with wild type MC58. The down-regulation of the entire *atp* operon (ATP synthesis), the *nuo* operon (NADH synthesis), and the cytochrome operon (oxdative phosphorylation) correlated with the impairment of growth in liquid media. Down-regulation of the genes encoding major adhesins including *pilE* for pilin, *pilQ* for pilus secretin and *opcA* for opacity protein, which are most important factors for meningococcal adherence and invasion, may explain the invasion defect of the NMB1966 mutant. The down-regulation of these genes was further validated by TaqMan real time PCR. The transcriptome anaylsis has provided important clues for further experiments to divulge the functional mechanism for the attenuation of NMB1966 knockout mutant.

P5.2.03

Regulatory network involved in the human nasopharyngeal colonization by Neisseria meningitidis

Patricia Martin, Clotilde Rousseau and Xavier Nassif

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The molecular mechanism responsible for efficient interaction between *Neisseria meningitidis* and the host cell remains mostly unknown. Identification of the function of the meningococcal genes differentially expressed upon initial interaction with host cells and of the regulation pathways controlling the expression of these genes are prerequisites to the understanding of how efficient colonization can be achieved.

A putative regulatory network has been identified in *N. meningitidis* as the first system that potentially controls the co-ordinated gene expression during the initial contact with the host cells (Morelle *et al.,* 2003). The expression of genes, which display the same promoter region, was indeed shown to be induced during the attachment of *N. meningitidis* to the host cells.

The genes of this putative regulon are likely to encode functions that are potentially crucial for the establishment of the nasopharyngeal colonization. We have started to investigate one of them, the *parC* gene, which encodes a topoisomerase IV subunit. We showed that the level of supercoiling of a plasmid DNA replicating in *N. meningitidis* was related to the level of expression of the *parC* gene. Because the transcription of many genes is known to be affected by the level of DNA supercoiling (Peter *et al*., 2004), a transcriptomic analysis will be performed to identify genes whose expression is modified by the level of *parC* transcription.

We aimed at identifying the regulation pathway controlling the expression of the regulon by constructing sequential deletions in the promoter region. The influence of the deletions on the inducibility of the transcription of the associated gene is under investigation.

By using a reporter gene and a quasi-exhaustive library of mutants (Geoffroy *et al*., 2003), a genome-wide search is in progress to identify the bacterial transcription factors involved in the control of the expression of the genes of the regulon.

P5.2.04

The Gonococcal Ferric Uptake Regulator (Fur) and Fur-dependent Genes are Induced During Experimental Gonococcal Genital Tract Infection of Female Mice

Mathanraj Packiam and Ann E. Jerse

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Iron is an essential element for microbial growth and free iron is limiting in mammalian hosts. Under iron limiting conditions *Neisseria gonorrhoeae* (*Ng*) controls the expression of several important genes via the ferric uptake regulator (Fur) protein. Fur induction has been reported in clinical human male urethral samples. We previously reported the development of the 17β-estradiol-treated mouse model for studying adaptation of *Ng* to the female host. Here we examined Fur and Fur-dependent gene expression during experimental murine infection to assess environmental conditions prevalent in the murine genital tract and to study *Ng* gene expression *in vivo*.

Test mice (n=3) and control mice (n=2) were injected with 0.5 mg/ml of estradiol intradermally on days (-2, 0, +2) before, on, and after intravaginal inoculation with *Ng* (3 x 10^5 cfu/mice). RNA was extracted from vaginal mucus collected with a sterile swab on days 1, 3 and 5 post-inoculation. A portion of the sample was serially

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diluted to enumerate the number of bacteria in each sample. The total RNA extracted was used to synthesize cDNA and gene expression patterns of *fur* and Fur-dependent genes were examined using realtime PCR analysis.

Realtime PCR results showed that *fur* was induced on day 1 (~3-4 fold more than *in vitro* levels), peaked at day 3 (~6-8 fold), and repressed at day 5 (~3-4 fold). Induction of Fur-dependent genes (*tonB*, *tbpA*, *fbpA*) was also observed and closely mirrored the kinetics of *fur* induction. The expression of bacterioferritin subunit A (*bfrA*), which functions as an iron storage protein and protects *Ng* from oxidative stress was unchanged on day 1 and day 3, but decreased by ~3-4 fold on day 5. In conclusion, these results suggest the murine genital tract is iron-limited. Additionally, gene expression during murine infection reflects the pattern reported for human urethral infections.

P5.2.05

A Leading/Lagging Strand Bias in Homopolymeric Tracts and the Development of a Green Fluorescent Protein Reporter Construct for the Analysis of Phase Variation

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The rate of phase variation in *Neisseria meningitidis* is dependent upon various aspects of DNA metabolism. Mononucleotide and longer units in repeat tracts are repaired and replicated with different fidelity by different parts of the DNA metabolism and repair machinery. Replication of genomic DNA starts at the origin of replication and travels in both directions away from the origin with a strand of continuous replication (the leading strand) and a discontinuous strand of replication (lagging strand). These different methods of replication favour different types of mutations and rates of mutation. Bioinformatic analysis of the Neisserial genome sequences suggests that a leading and lagging strand bias exists in homopolymeric G/C tracts. These tracts mostly occur on the leading strand (27/33 homopolymeric G tracts are on the leading strand). The means of measuring phase variation rates include phenotypic observation, LacZ reporters and antibody reactivity. We report the development of a green fluorescent protein (GFP) reporter construct for the measurement of phase variation rates. This reporter system should enable the observation of phase variation events between individual bacteria and allow better analysis of the *cis*- and *trans*- acting factors that influence phase variation. Thus, this reporter construct may enable a better understanding of the phase variation events that are postulated to be important in pathogenic Neisseria's adaptation to host environments and immune challenges.

The Discussion Session for the following posters is from: 9.30pm – 10.15pm Tuesday 12 September

P5.3.01

Transcriptional Regulation of the *pilE* Gene of *Neisseria gonorrhoeae*

Catherine Ryan[1], James Whisstock[2,3], Jackie Cheung[1], Sally Turner[1] & John Davies[1]

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The *pilE* gene in *Neisseria gonorrhoeae* encodes the subunit of type 4 pili. Understanding the regulatory processes associated with expression of *pilE* may provide an insight into the progression of infection. The control of expression of *pilE* is complex with two potential UP elements, found on either side of a confirmed IHF binding site (IHFBS) (Hill *et al*., 1998) having been shown to bind the RpoA subunits of RNA polymerase (RNAP). The interaction of the UP elements with RpoA appears to stabilise the RNAP interaction with the promoter resulting in elevated levels of transcription. We have proposed that binding of IHF may allow RNAP to shift from using a relatively weak UP element adjacent to the -35 element to a stronger UP element upstream of the IHFBS, resulting in increased expression levels. This was tested by making mutants of the *pilE* upstream region where the position of the IHFBS or putative upstream UP element with respect to the –35 element was altered. Transcription from mutant promoter regions fused to promoterless *cat* genes was assayed by measuring CAT levels. Molecular modelling using known X-ray crystal structures and atomic force microscopy (AFM) was used to investigate the interactions between IHF, RNAP and the *pilE* promoter. *In vitro* transcription investigated transcription from wild type and mutant *pilE* promoter regions in the presence or absence of IHF and/or RNAP. The data do not support our original suggestion but indicate: 1) the AT-rich region upstream of the IHFBS is not an UP element but is part of the IHFBS; 2) IHF binds to bend the upstream DNA back toward the RNAP where we postulate it forms additional contacts with the enzyme resulting in increased transcription and 3) the AT-rich region immediately adjacent to the –35 element is an UP element that interacts with RpoA.

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P5.3.02

Comparative Genomics of Pathogenic and Apathogenic Meningococci

Christoph Schoen[1], Heike Claus[1], Alexander Goesmann[2], Petra Brandt[3], Tobias Müller[4], Anja Schramm-Glück[1], Ines Otto-Karg[1], Ulrich Vogel[1], Matthias Frosch[1]

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Neisseria meningitidis (the meningococcus) colonises the human nasopharynx of up to 30% of the population. However, only few pathogenic meningococcal lineages cause human disease, whereas most carrier isolates are considered as commensals.

The genomes of the two meningococcal strains Z2491 (Sanger Centre) and MC58 (TIGR) have already been published and the genome sequence of strain FAM18 (Sanger Centre) is annotated and awaits publication. As all three meningococcal strains are disease isolates belonging to hyper-virulent clonal lineages it was the aim of the present study to obtain genome sequences of apathogenic meningococcal carrier isolates for comparison with the genomes of these pathogenic strains to unravel the genetic basis of their different pathogenic potential.

To this end, the genome of the unencapsulated strain α14 has been sequenced and annotated and the genome of the non-pathogenic serogroup B strain α710 is in the cap closure phase with 4 contigs the largest being 1,5 Mb in size. Surprisingly, *in silico* whole genome comparisons with the genomes of the three hypervirulent meningococcal isolates showed that with the exception of genes involved in capsule synthesis almost all other genes so far considered to be virulence associated were also present in the avirulent strain α14. In addition, whole genome comparisons identified an unexpected number of mobile genetic elements such as IS elements, restriction-modification systems and prophages mostly coding for proteins of unknown function which are differently distributed between the pathogenic and apathogenic strains.

In conclusion, these data illustrate that our current understanding of the meningococcal patho-genome is still very limited and requires further analysis.

P5.3.03

Defining the Phasevarion of Pathogenic Neisseria

Yogitha N. Srikhanta, Kate L. Fox, Stefanie J. Dowideit and Michael P. Jennings

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We previously showed that the random switching of a type III Restriction-Modification (R-M) system of *Haemophilus influenza* strain Rd resulted in coordinated phase variation of a "regulon" of genes (phasevarion). A number of which were potential virulence factors or vaccine candidates. To further define the role of the phasevarion in other host adapted pathogenic bacteria, we made a mutant strain lacking the R-M methyltransferase (*mod*) in both *Neisseria meningitidis* and *Neisseria gonorrhoae* and analyzed its phenotype. Microarray analysis identified a number of genes that were either up- or down-regulated in the *mod* mutant strains some of which were known virulence factors or vaccine candidates. In addition, phylogenetic studies revealed that all *N. meningitidis* and *N. gonorrhoae* strains have 2 different *mod* genes - *mod*1 and *mod*2. Furthermore, there are 3 distinct *mod*1 alleles (*mod*1A, B and C) and 2 distinct *mod*2 alleles (*mod*2A and B). These alleles differ in their DNA recognition domain, suggesting that different phasevarions exist for each allele. Our work presented here has characterized the phasevarions controlled by two of these alleles. Further work is underway to define the phasevarions controlled by the remaining alleles. Our data confirms that the phasevarion is a commonly used system mediating coordinated phase variation of multiple genes in bacterial pathogens and may have major implications for vaccine development and host:pathogen interactions.

P5.3.04

A Type I Protein Secretion System Is Controlled by the MisR/MisS Two-Component Regulatory System in *Neisseria meningitidis*

Xinjian Zhang and Yih-Ling Tzeng

Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

Two-component regulatory systems control processes important for bacterial pathogenesis. The *misR*/*misS* (*phoP*/*phoQ*) system is one of only four two-component regulatory systems encoded in the *Neisseria meningitidis* genome. This system has been shown to influence LOS inner core phosphorylation, while the response regulator mutant was avirulent in a mouse model of meningococcal infection. Meningococci are known to secrete proteins with sequence similarity to the repeat-in Toxins (RTX) family of cytotoxin. The RTX proteins are secreted via the type I secretion system and the expression of these proteins is iron repressible. Unlike the homologous *hlyB*/*hlyD*/*tolC* hemolysin secretion proteins of *E. coli,* which are transcribed as an operon, the meningococcal type I protein secretion system, which is composed of NMB1400 (*hlyB*) and NMB1738/1737 (*hlyD*/*tolC*), is encoded at two distant genetic loci and has been characterized to mediate the secretion of

RTX proteins, FrpC and FrpC2. In addition, this system was shown to be pathogen specific and is absent in all commensal Neisseria species. Here we reported that the MisR/MisS two component regulatory system directly regulates the expression of *hlyB* and *hlyD* as shown by electrophoretic mobility shift assays using MisR-6xHis protein. *HlyD::lacZ* and *hlyB::lacZ* reporter strains were constructed in the wild type, the *misR* and the *misRS* mutants, and β-galactosidase assays demonstrated that transcription of *hlyD* and *hlyB* in the mutants was higher than that of the wild type strain. Further, multiple MisR binding sites were identified by DNase I protection assays in both *hlyB* and *hlyD* promoter regions and were overlapped with the promoter elements, thus repressing gene expression. This is the first example of the type I secretion system demonstrated to be under the control of a two-component regulatory system.

P5.3.05

Mutation of MtrR and MtrA, Transcriptional Regulators of the MtrCDE Efflux Pump System, Alters the Fitness of Neisseria gonorrhoeae for Experimental Genital Tract Infection

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The MtrCDE efflux system may play an important role in defending *N. gonorrhoeae* against innate defenses of the genital tract based on studies with experimentally infected mice. Known substrates of the MtrCDE efflux pump system include certain antibiotics, hydrophobic substances, and antimicrobial peptides. The *mtrCDE* operon is under negative and positive control by transcriptional factors MtrR and MtrA, respectively. Here we tested the effect of derepressing the MtrCDE pump via mutation of *mtrR* on survival in vivo. In competitive infections, derepressed mutant JF1 (Δ*mtrR*) demonstrated a 100- to 1000-fold increase in recovery relative to the wild type strain. The fitness advantage conferred by mutation in *mtrR* was not accompanied by increased infectivity based on dose response studies with stain KH-15, a second derepressed *mtrR* mutant. Mutant JF1 showed no advantage over an *mtrR,mtrE* double mutant in vivo. We conclude that derepression of the *mtrCDE* operon is the primary reason that mutation in *mtrR* causes increased fitness and that derepression of the pump has an effect after infection is established. We also examined MtrA-deficient mutants for survival in vivo. MtrA belongs to the AraC family of global regulators, and therefore mutations in *mtrA* may profoundly affect adaptation in vivo. An *mtrA*::*aphA3* mutant of FA19 displayed a 100- to 1000-fold decrease in fitness when co-infected with the wild type strain. Interestingly, selection of *mtrR* mutants of an MtrA-deficient strain occurred in vivo, which suggests derepression of the *mtrCDE* operon can compensate for fitness loss conferred by *mtrA* mutation. Competitive infection studies with an *mtrA* mutant and *mtrE, mtrA* double mutant further localized the primary effect of MtrA-based activation to the *mtrCDE* operon since one mutant had no advantage over the other in vivo. This study illustrates how transcriptional regulators co-evolved with the MtrCDE system to promote *N. gonorrhoeae* adaptation to the host.

P5.3.06

Proteomic Analysis of Manganese Regulation of *Neisseria gonorrhoeae*

Hsing-Ju Wu[1], Kuan-Tin Pan[1], He-Hsuan Hsiao[1], Chen-Wen Yao[3], Alastair G. McEwan[2], Michael P. Jennings[2] and Andrew H-J. Wang[1]

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Neisseria gonorrhoeae is an important human pathogen which causes gonorrhoea and pelvic inflammatory disease. It is a facultative aerobe with a high iron requirement and a highly active aerobic respiratory chain. These factors would suggest that this bacterium would require defense systems to respond to toxic oxygen species. In the previous studies, we have shown that the accumulation of manganese (Mn) and Mn(II) uptake system, MntABC, in *N. gonorrhoeae* protected against killing by superoxide anion, and was independent of superoxide dismutase activity. Also, investigation of a regulatory role for Mn(II) in *N. gonorrhoeae* has revealed that a key virulence factor, pili, is repressed by Mn via a PerR-independent post-transcriptional mechanism. To provide a more comprehensive view of the regulatory network and its molecular mechanism, the shotgun proteomic approach, i.e. one dimensional (1D) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D-SDS-PAGE) coupled with 1D liquid chromatography (LC) - tandem mass spectrometry (MS/MS) and the quantitative method, i.e., isotope coded affinity tag (ICAT) were performed.

N. gonorrhoeae cells were grown in the presence and absence of Mn and cell lysates were fractionated into cytoplasmic, inner membrane and outer membrane components. These results revealed that 109 proteins were differentially regulated at the post transcriptional level under conditions of increased Mn. The Mn-regulated proteins have a broad range of functions including oxidative stress defence (i.e. superoxide dismutase (SodB), azurin, bacterioferritin), cellular metabolism, protein synthesis, RNA processing, cell division, pilin and the proteins involved in the pilus assembly, such as PilC1 and PilQ. This confirms our previous study and may explain how the expression of pili was downregulated when cells were grown in the Mn supplement. Taken together, these data give us a proteomic view of Mn regulation and provided us with leads to correlate protection against oxidative stress with pilus formation and surface protein expression.

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P06
Host Response

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P6.1.01

Macrophage responses to C-reactive protein-opsonised *Neisseria meningitidis*

Rosalyn Casey, Jane Newcombe, JohnJoe McFadden & Katherine Bodman-Smith.

University of Surrey, Guildford, Surrey, GU2 7XH

Pathogenic strains of *Neisseria meningitidis* express phosphorylcholine (PC) on their pili in a phase-variable fashion, which appears to be critical in the colonisation of the human respiratory tract and may enhance complement–mediated killing in human serum. The expression of PC also facilitates interactions with other molecules of host immunity such as the acute phase reactant C-reactive protein (CRP).

CRP and CRP-opsonisation have been shown to affect numerous host cell responses including increased cytokine secretion and intracellular signalling *via* cross linked Fcγ receptors. CRP-binding has been reported to increase phagocytosis of PC expressing bacteria by professional phagocytes and to cause increased Tumour Necrosis Factor alpha expression during *Leishmania* infection. We have demonstrated that a piliated PC expressing strain of *N. meningitidis* can bind to CRP in a calcium dependent manner and current research is focused on the cellular consequences of this binding.

Experiments using phorbyl myristate acetate–differentiated THP1 cells indicate that CRP-opsonised *N. meningitidis* exhibit increased uptake into macrophages and that this interaction causes changes in the expression profile of surface molecules such as complement receptor proteins and Fcγ Receptors differently from treatment with CRP or *N. meningitidis* alone. Cytokine expression profiles of macrophages encountering CRP-opsonised *N. meningitidis* will also be presented and the biological significance of these findings will be explored.

P6.1.02

Glial and Neuronal Responses to Meningococcal Meningitis

Tonje Davidsen[1,2], Cesilie G. Castellanos[2], Erlend Nagelhus[1], Jan Bjaalie[1], Ole P. Ottersen[1], Tone Tønjum[1,2]

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Meningococcal disease is a leading cause of meningitis worldwide. Among the serious consequences of meningitis are brain edema and disturbed neuronal excitability. These effects are often the direct cause of death or neurological sequelae. Despite the serious complications, the molecular basis for these events is not known and virtually nothing is known about the trafficking of membrane proteins governing water homeostasis during the infection process. Other local cellular responses to meningococcal meningitis are also poorly characterized; neither the cytokine profile nor the effects on neurotransmitter transporters and receptor activities in glial cells or neurons and consequent changes in excitatory pulse control and neuron excitability during meningitis are characterized to date.

We are studying the interaction of meningococci with host cells in cellular assays and mice models to determine which components in glial and neuronal cells are affected during the infection process. Mice are subjected to intra-cerebrospinal fluid injection of wildtype and mutant meningococci. Ultrastructural analysis by EM is performed on mice that are fixated by perfusion before ultrathin sections of the brain are subjected to immunogold labelling. In addition, we are employing a microPET CTI-Concorde Focus 120 imaging system to assess the infection process quantitatively in real time. The combination of meningococcal virulence and fitness mutants with murine models and microPET imaging represents a novel approach to study the meningitis process. The results are expected to yield new information on meningococcal meningitis and brain edema, with significant potential for discoveries that can directly influence and inspire new strategies for prevention and treatment of this serious disease.

P6.1.03

The inflammatory response of human meningeal cells to Neisseriae and the role of neuropeptides

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Introduction We have demonstrated that *Neisseria meningitidis* and other bacteria causing meningitis were different in the dynamics of their association and invasion of human meningeal cells and in their ability to induce inflammation and cell death. These pathogens normally enter the cerebrospinal fluid (CSF) by the haematogenous route, but meningeal irritation can occur following the entry of other bacteria by non-haematogenous routes and interestingly, *Neisseria lactamica* (Nlac), which is closely related to meningococci, is a rare cause of meningitis.

Initially, we investigated how the innate response of meningeal cells to Nlac compared to that observed following meningococcal infection. In addition, we investigated the influence of neuropeptides, such as α–melanocyte-stimulating hormone (MSH), which is elevated during meningitis, on the response of meningeal cells to meningococci.

Results Nlac adhered to meningeal cells and induced a similar profile of cytokine secretion as meningococci. Both species induced similar amounts of IL-8, MCP1 and GM-CSF. However, Nlac induced much lower levels of IL-6 than meningococci and high concentrations of the latter, but not the former, significantly down-regulated secretion of RANTES. Notably, the two bacteria showed

large differences in pathogenic potential. Whereas meningeal cells remained viable on prolonged challenge with Nlac, death was induced by meningococci and the mechanism was overwhelming necrosis with no significant apoptosis.

We also demonstrate that during infection with meningococci, the presence of α–MSH enhanced the inflammatory response of meningeal cells, which correlated with its ability to promote increased early bacterial association.

Conclusion When Neisseria species enter the CSF the innate response of the human meninges is largely conserved and elevation of α-MSH neuropeptide during meningitis is a pro-inflammatory event aimed at increasing clearance of bacteria from the CSF. The differential expression of modulins between Nlac and meningococci accounts for the observed differences in pathogenic potential.

P6.1.04

Role of nitric oxide in cellular damage to human Fallopian tubes following infection with *Neisseria gonorrhoeae*

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Introduction
Following infection with *Neisseria gonorrhoeae*, bacteria may ascend into the Fallopian tubes (FT) and induce salpingitis, a major cause of infertility. Gonococcal infection results in mucosal damage that is primarily mediated by TNF-$\tilde{\alpha}$ However, Gram-negative bacteria have also been reported to induce cellular damage in other epithelia by means of stimulating the release of nitric oxide (NO). It is known that the FT can produce NO as a key regulator of paracrine function. The purpose of our study was to investigate the hypothesis that infection of FT explants with *Neisseria gonorrhoeae* stimulates the release of NO and that this mechanism contributes to the observed cellular damage.

Methods
FT explants were infected with *Neisseria gonorrhoeae* Pil^+ Opa^+ and cellular damage was quantified by measuring the release of lactate dehydrogenase (LDH) enzyme into culture medium. Nitric oxide synthase (NOS) enzyme activity was measured using the L-[^{3}H]citrulline assay and the effects of NOS inhibitors N-nitro-L-arginine (L-NAME) and 1-(2-trifluoromethylphenyl)-imidazole (TRIM) and the role of NO donor S-Nitroso-N-penicillamine (SNAP) in cellular damage was also investigated.

Results
Release of LDH from FT explants was proportional to the bacterial concentration, with approximately 30% of the cells showing cellular damage by 24h. Normal FT explants showed increases in physiological NOS enzymatic activity over time but this rise was inhibited by infection with gonococci. Significantly, the inhibition of NOS activity with L-NAME and TRIM did not prevent cellular damage induced by gonococci. Furthermore, administration of an exogenous NO donor failed to produce any cellular damage to FT explants when compared to explants in basal conditions.

Conclusions
Nitric oxide does not contribute to cellular damage to FT induced by gonococcal challenge. Moreover, the ability of gonococci to prevent NO production in FT explants suggests that the pathogen alters the physiological environment during infection, possibly as a mechanism to aid colonization.

P6.1.05

Gonococcal Lipooligosaccharide Phosphoethanolamine Substitutions Modulate Serum Resistance and C4bp Binding

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Complement forms a key arm of the innate immune system that combats gonococcal infections. In serum sensitive gonococci we have identified lipooligosaccharide (LOS), the opacity proteins and porin as targets for C4b deposition. Further, we have demonstrated that phosphoethanolamine (PEA) substitutions on the second heptose (HepII) of neisserial LOS form amide linkages with C4b. To evade complement mediated killing many serum resistant strains of *N. gonorrhoeae* (including strain FA19) down regulate the classical pathway by binding to the complement regulatory protein C4b-binding protein (C4bp). C4bp binds gonococcal porin, which results in C4b inactivation.

To further explore the role of LOS PEA substitutions in a serum resistant strain we constructed isogenic mutants in FA19 that lacked PEA on lipid A or at the 6-position of HepII or both.

Loss of HepII 6-PEA alone did not notably affect serum resistance, however, FA19 mutants lacking lipid A PEA were sensitive (12% survival) to killing by 50% normal human serum when compared to the parent (70% survival). The combined loss of both of these PEA residues resulted in enhanced sensitivity (0% survival). Complement C4b remained unprocessed and therefore active in strains lacking lipid A PEA or lacking both lipid A PEA and HepII 6-PEA. Additional C4b targets evident in the PEA mutants, but not

detected in the FA19 parent, were identified as amide linked C4b-porin. FA19 strains lacking lipid A PEA or lipid A PEA and HepII 6-PEA were impaired in their ability to bind C4bp.

Together these data suggest a role for LOS PEA, notably that found on lipid A, in C4bp binding and serum resistance. Strains lacking lipid A PEA were impaired in their ability to bind C4bp and thus not able to effectively regulate complement. Complement deposition was increased in these strains and correlated with increased serum sensitivity.

P6.1.06

Neisserial Lipooligosaccharide and Complement Activation: Contrasting Effects of Heptose II Substitutions in Gonococci and Meningococci

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The classical pathway of complement initiates direct killing of pathogenic Neisseriae. C4b binds to meningococcal lipooligosaccharide (LOS); the location of phosphoethanolamine (PEA) substitutions determines the type of C4b linkage. Most meningococci possess PEA at the 3-position of heptose II; most gonococci possess a lactose substitution at this position. Differential substitution of hexose(s) and PEA on heptose II may modify C4b binding and impart distinct survival advantages to each species.

To compare the differential role of heptose II substitutions, we constructed isogenic mutants where the 6-positions of heptose II had PEA or were unsubstituted, while the 3-positions were substituted with hexose(s), PEA or were unsubstituted. The type of C4b –LOS linkage was examined by western blots. In gonococci, we detected amide linked C4b-LOS complexes in strains that possessed either a 3- or 6-PEA. Only ester linkages were detected in gonococci that lacked PEA. Hexose(s) substitution of heptose II resulted in a decrease in C4b deposition. In meningococci, amide linked C4b-LOS was detected only in strains with 6-PEA; C4b deposition was diminished in the absence of 6-PEA. Hexose substitution of heptose II did not affect C4b deposition.

Gonococci with heptose II hexose(s) were more serum-resistant than their isogenic hexose negative counterparts. Serum-resistance could not be explained by the loss of 3-PEA (a C4b target); strains that lacked 3-hexose(s), with or without 3-PEA, were more serum-sensitive than strains with 3-hexose(s). Meningococci with 3-hexose and no PEA were highly serum-sensitive highlighting the impact of the 3-hexose substitution. Meningococci that lacked 3-hexose, 3-PEA and 6-PEA were the most serum resistant. 3- and 6-PEA are targets for C4b binding; their removal increases survival.

Our data highlight the contrasting roles of heptose II substitutions in gonococci and meningococci and provide a better understanding of how complement binding is regulated on different neisserial surfaces.

P6.1.07

Induction of an Inflammatory Response but not a Humoral Response in Experimental Gonococcal Genital Tract Infection of Estradiol-treated MIce

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Gonococcal genital tract infection in women can be asymptomatic or inflammatory with numerous polymorphonuclear leukocytes (PMNs) at the infection site. The adaptive immune response is less well studied although some studies suggest the antibody response to gonorrhea in women is poor. Currently the only in vivo infection model for studying the immune response to *N. gonorrhoeae* in females is a mouse model in which 17-β estradiol is given to promote an estrous-like state, the most susceptible stage of the murine reproductive cycle for *N. gonorrhoeae* infection. Here we tested mice treated with a lower total dose of 17-β estradiol than used previously to reduce the influence of exogenous estrogen on the host response and to facilitate re-infection studies. Mice were colonized by strain FA1090 for an average of 10 days and a vaginal PMN influx, which was higher than that observed in placebo controls occurred in 80% of mice. Gonococci persisted during periods of inflammation and in some mice, transient influxes of PMNs occurred, which coincided with periods of lower bacterial recovery. No antibody response was detected in serum or vaginal washes collected on days 5 and 10 post-infection. Consistent with the lack of an adaptive response, mice were susceptible to reinfection by the homologous strain. There was no evidence of a memory response to reinfection based on serum and vaginal antibody titers measured 10 days after the second bacterial challenge. Intranasal or subcutaneous immunization of uninfected, estradiol-treated mice with gonococcal outer membrane vesicles resulted in gonococcal-specific serum IgG, but not intravaginal immunization as assessed by western immunoblot. These studies demonstrate that gonococcal infection of estradiol-treated female mice results primarily in an inflammatory response with little or no adaptive immune response. Additionally, this work shows that the site of infection may contribute to the poor immune response to gonococcal infection.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P6.1.08

***Neisseria gonorrhoeae* Induces Cyclin Down Regulation and Early G1 arrest in Human Epithelial Cells**

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Pathogenic bacteria can modulate and interfere with the human cell cycle progression. Here we study the human pathogen, *Neisseria gonorrhoeae*, and its ability to influence and affect the cell cycle in two human target cell lines. We found that bacteria adhere equally well to cells synchronized into the different cell cycle phases of G1, S and G2. We also present the novel finding that bacteria cannot adhere to mitotic cells or non-cycling cells in GO. In addition, we demonstrate that bacterial infection for 24 h results in decreased levels of the cell cycle regulatory proteins Cyclin B1, Cyclin D1, and Cyclin E, as determined by Western blot analysis and flow cytometry. Further studies in *N. gonorrhoeae* infected epithelial cells involving analysis of DNA content, BrdU incorporation, quantification of late mitotic cells and analysis of nuclear phenotype provide compelling evidence that a 24 h gonococcal infection arrests the cells in the early G1 phase of the cell cycle. In summary, we present data showing that MS11 P^+ strain of *N. gonorrhoeae* can down regulate cyclins, important regulators of the cell cycle and result in a G1 arrest.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P6.2.01

Differential Responses of Human Dendritic Cells to Live and Killed *Neisseria meningitidis*

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Introduction: The development of effective vaccines to serogroup B *Neisseria meningitidis* (Nm) remains elusive. One strategy to achieve this goal is by identifying the optimal way to target meningococcal antigens to dendritic cells (DC), which are key regulators of adaptive immunity. Recent evidence has highlighted differences in DC responses to live and killed microbes, which have important implications for the effectiveness of immune responses to these organisms. We therefore compared maturation, cytokine production, and phagocytosis of viable and killed serogroup B meningococci by human monocyte derived DC's.

Results: Live Nm consistently failed to induce up-regulation of co-stimulatory molecules CD40 and CD86 and DC maturation markers HLA-DR and Class I MHC, in contrast to the efficient maturation seen in response to both killed Nm and LPS. However, when live meningococci were pretreated with bacteristatic doses of chloramphenicol, good DC maturation was observed, but not when antibiotic treatment was delayed by just 2 hours. Uptake of live Nm was significantly impaired compared to killed Nm by DC. Live Nm induced significantly more IL-12 whereas killed Nm induced higher levels of IL-10 than viable organisms.

Conclusion: This study is the first to demonstrate that live Nm induces distinct DC responses compared to killed Nm. The failure of DC maturation in response to live Nm may be important, since semi-mature DC's may induce ineffective immune responses after microbial encounter. Since phagocytosis is required for IL-10 and IL-12 production, differences in kinetics of uptake of bacteria and the differential cytokine responses seen in this study may be linked. The consequences of these findings upon the effectiveness of antigen presentation and T-cell responses to live Nm maybe biologically important and are currently under investigation.

P6.2.02

Use of Monoclonal Antibodies on the Lipopolysaccharide (LPS) antigen selection from epidemic strain of *Neisseria meningitidis* B: Nasal immunization in rabbits

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Unlike others serogroups to which the capsular polysaccharydes constitute efficacious vaccines, the serogroup B capsule is poorly immunogenic in humans. In modern vaccine development, strong emphasis has been laid on mucosal immunization system. In particular, the intranasal (i.n) route holds promise for a potencial induction of protective immune responses, since its able to elicit both local and strong systemic immune response.

Intranasal vaccination may therefore be of particular interest againt respiratory tract infections, such as those caused by *N.meningitidis*. Lipopolysaccharide (LPS) are complex macromolecules which are part of outer membrane, of Gram-negative bacteria.

The New Zealand white rabbits was employed as model for representative by intranasal immunization in humans with regards to vaccine disposition. Immunizing with native outer membrane vesicles (NOMV) would be an excellent way to prevent meningococcal antigens in their native conformations ;however, NOMV does contain native endotoxin that would cause unwanted local and systemic reactions if given intramuscularly (i.m) administrated in humans.

In this present study the immunogenicity of NOMV administerd in rabbits divided in groups of rabbits immunized at 7-days intervals. Antigens used included: NOMV of the strains B:4:P1.15.L3,7,9,8 NOMV of the strain B:4:P1.15.L8,3,7,9↑or B:4:P1.15.L3,7,9,8↑ selected by colony blot using monoclonal antibody. The antigens preparations were administered to nonanesthetized intranasally rabbits i.n with 1000 µg protein in a 0.5 mL volume. Nasal lavage samples were collected prior to initial immunization and 35° days later by instilling 2.5mL of sterile PBS into nostril. The rabbits head directed in "aslight downward" direction, the nasal lavage was collected from contralateral nostril into a sterile petri dish . Kinetics

of immune response using different concentration of NOMV administered i.n was also analysed.

Rabbits immunized i.n four times with NOMV from strain B:4:P1.15.L3,7,9,8 had lower bactericidal titers and IgG levels in sera compared with the same strain selected with MAb for the immunotypes L3,7,9↑or 8↑. Also difference in specificity of produced the IgG antibodies on immunoblot were detected. Immunoblot showed that on day 35° sera reacted with a wide variety of immunoreactive bands, including, Por A, Por B, class 4, 50 kDa, as well as L8 LOS, NspA and NadA. Marked increases of serum IgG and nasal lavage (IgA) to *N.meningitidis* was detected by ELISA.

These data emphasize the importance of establishing of the LPS immunotypes in order to produce a future meningococcal vaccine able of generating long lasting immunity in all population.

P6.2.03

Use of monoclonal antibodies in the selection of LPS from the epidemic strain B:4:P1.15 of *Neisseria meningitidis* B: Nasal immunization in mice

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The present study analyzed the reactivity of the antibodies (IgM, IgG and IgA) found in the serum and in the saliva of mice who were immunized IN or IM, as well as the presence in the serum of antibodies with bactericidal activity. In the serum of the IN immunized mice, antibodies found four months and one year after immunization and with cross-reactivity against a whole-cell suspension antigens of heterologous strains of *N. meningitidis* bacteria (B:4:P1.9, B:4:NT, B:14:NT and C:2a:P1.2) were also tested.

The results of the ELISA test shows that IM immunized mice produced higher levels of antibodies of the IgG isotypes and the IN immunized ones produced higher levels of antibodies of the IgG and IgA isotypes, specific against whole-cells of homologous and heterologous strains of *N. meningitidis*. The analysis of specificity through the Immunoblotting of the antibodies found in the serum of the IM and IN immunized mice revealed that the response was directed mainly against the proteins of classes 1, 2 and 5, and also against proteins 50, 65 and 70 kDa of the whole-cell suspension bacteria and the NOMC of strains B:4:P1:15 L3,7,9↑, B:4:P1:15L3,7, 9↓,B:4:P1.15L8↑ and B:4:P1:15 L8↓. The specificity of antibodies of isotypes IgG and IgA (obtained from the serum of intranasally immunized mice) against strains B:4:P1.9, B:14:NT, B:4:NT and C.2a.P1.2 revealed that the response was directed mainly against proteins of molecular weight higher than 50 kDa. In the saliva of IN immunized mice the reaction of antibodies of the IgA isotype was also observed by ELISA and Immunoblotting. When the antigen preparations obtained from the strains with the highest and lowest expressions of immunotypes L3,7,9 and L8 were compared, no significant differences were found by ELISA. Immunoblotting analysis, however, showed differences in the specificity of the response, particularly for peptides of high molecular mass. The titers obtained by the assay of bactericidal assay were higher for the mice IN immunized with strains selected for LPS L3, 7, 9 and L8. The results of antibodie's specificity indicate that the selection of LPS may be important to elicit systemic as well as local muccosal effective immune response, when used in an intranasal vaccine preparation.

P6.2.04

The Anti-apoptotic Effect of Meningococcal PorB Does Not Require TLR2-mediated NF-κB Activation

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Meningococcal PorB is known to inhibit cellular apoptosis mediated by the intrinsic apoptotic pathway in several cell types. This anti-apoptotic effect is correlated with PorB-induced protection from mitochondrial depolarization, blockade of cytochrome c release and inhibition of caspase 9 and 3 cleavage and activation. Consequently, DNA degradation is also prevented and the cells are protected from apoptotic cell death.

NF-kB nuclear translocation is known to induce activation of anti-apoptotic genes. In this work we examine the involvement of NF-kB in the anti-apoptotic effect of PorB. Upon incubation of PorB with HeLa cells, NF-kB activation was detected as early as 30 minutes after stimulation and it was maintained up to 24h. However, PorB-mediated protection from apoptotic mitochondrial depolarization induced by staurosporine was only detected after a 24h pre-incubation of the cells with PorB. Furthermore, no effect of PorB has been detected on the expression of the anti-apoptotic protein Bcl-2 or on the translocation of the pro-apoptotic protein Bax into the mitochondria (required for activation of the intrinsic apoptotic pathway). Since PorB is a TLR2 ligand, and HeLa cells lack TLR2 expression, we hypothesize the existence of aTLR2-independent NF-kB cell activation pathway in HeLa cells, but its contribution to the anti-apoptotic effect of PorB has not been defined yet.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P6.2.05

A Novel Interaction between Factor H SCR 6 and the Meningococcal Vaccine Candidate GNA1870: Implications for Meningococcal Pathogenesis and Vaccine Development

Jutamas Ngampasutadol[1], Jo Anne Welsch[2], Lisa A. Lewis[1], Hanna Jarva[3], Guillermo Madico[4], Ulrich Vogel[5], Alberto Visintin[1] and Sanjay Ram[1].

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We recently identified GNA1870, a lipoprotein currently under evaluation as a broad spectrum meningococcal vaccine antigen, as the ligand for the alternative pathway regulator, factor H (fH). Surface-bound fH regulated complement and enhanced meningococcal serum resistance. To reflect its critical function, GNA1870 was renamed factor H-binding protein (fHBP). Based on amino acid sequence, fHBP can be classified into 3 variant families, all of which bind fH. Recombinant fHBP fragments were used to localize the binding site for fH to a conserved region spanned by amino acids 58-100. Using recombinant fH fragments-murine Fc fusion proteins, we observed that only fusion proteins containing fH short consensus repeat (SCR) 6 bound to meningococci. Kinetics of fH binding to meningococci revealed that maximal binding occurred after 1 min of incubation and that the bond was resistant to 1M NaCl, suggesting a hydrophobic interaction between the two molecules.

We also observed that only human fH, not fH from other primates or lower animals, bound meningococci. Similarly, resistance of meningococci to complement-mediated killing was restricted to human serum. Restriction of fH binding to humans may at least partly explain the limitation of meningococcal disease to humans, may prove useful in developing suitable animal models for this disease and guide the choice of complement sources in evaluating meningococcal vaccine candidates.

To our knowledge, this is the first ligand for SCR 6 of fH and the first demonstration of a hydrophobic bond involving fH. Our findings support the hypothesis that inhibiting the binding of a complement down-regulator protein to the neisserial surface by specific anti-fHBP antibody may enhance intrinsic bactericidal activity of the antibody, resulting in an additional mechanism of antibody-mediated vaccine efficacy. These data provide further support for inclusion of fHBP in a meningococcal vaccine.

P6.2.06

Differential Gene Expression After Infection of *Neisseria meningitidis* in Host Cerebrovascular Endothelial and Respiratory Epithelial Cell Lines

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Meningococcal cellular association and invasion has the ability to potentiate bacterial pathogenesis through the use of specialised surface-exposed adhesins, such as type IV pili and Class 5 opacity proteins. Physiologically relevant cells derived from the cerebrovascular and respiratory systems would provide a suitable model of potential contact sites of *Neisseria meningitidis* during infection.

Using functional *in vitro* assays, including confocal microscopy and transmission electron microscopy, meningococcal cells have been show to effectively associate and invade physiologically relevant cell lines derived from the cerebrovascular region (HBMEC and meningothelial), as well as cells derived from the respiratory epithelium (BEAS-2B and HEp-2).

After meningococcal infection (8hrs), RNA was extracted from these host cells and hybridised to 30K-gene human microarray slides. The profile of gene expression after meningococcal infection was investigated and compared across the cell lines used, for example in terms of origin specificity, as well as significantly expressed genes. Analysis has identified generic as well as cell line-specific differential expression of key genes involved in inflammation and apoptosis. The possible significance of cell lineage-specific differential gene expression will be discussed.

P6.2.07

Different forms of penta-acylated meningococcal LPS have distinct biological activity

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Mutants in *Neisseria meningitidis* with inactivated *lpxL1* and *lpxL2* genes lack one or both of the secondary C12 acyl chains of lipid A. Previously, we demonstrated that an *lpxL1* mutant of strain H44/76 makes penta-acylated lipid A lacking the secondary fatty acid at the 2' position, while an *lpxL2* mutant was mostly without any secondary C12, and only a minor penta-acylated component was still present. However, these mutants were constructed in different oligosaccharide backgrounds, wildtype L3 and *galE* respectively, due to lack of viability of the *lpxL2* L3 combination. We have now constructed *lpxL1* and *lpxL2* single and double mutants in an immunotype L8 variant of H44/76. Mass spectrometry analysis of

their lipid A reveiled the following structures. The *lpxL1* L8 mutant retained a secondary C12 at the 2-position, while the *lpxL2* L8 mutant retained the secondary C12 at the 2'-position. Lipid A without any secondary acyl chains was only found in the *lpxL1 lpxL2* double mutant L8 strain. The *lpxL2* mutant was the most impaired in growth. As the *lpxL2* mutation has different phenotypes in combination with different oligosaccharides, these results reveal a biosynthetic interaction between the oligosaccharide and lipid A moieties. A third type of penta-acylated lipid A could be obtained by expressing a *Bordetella*-derived *pagL* gene in *N.meningitidis*. This gene encodes an outer membrane enzyme which specifically deacylates lipid A at the 3-position, resulting in penta-acylated lipid A lacking one primary C12-3OH but still carrying both secondary C12. Biological activity of whole cells and purified LPS of the three different penta-acylated mutants was compared by measuring IL-6 induction in the human monocytoid cell line MM6. The order of activity was wildtype>*lpxL2*>*pagL*>*lpxL1,lpxL1+2*. Our results demonstrate how the biological activity of LPS is determined not only by the number of fatty acyl chains but also by their specific positions.

P6.2.08

Unravelling the Human CD4+ T cell Response to Neisserial PorA Antigens: Selective Processing and Presentation of Epitopes caused by Serosubtype Variation

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Introduction. PorA of *Neisseria meningitidis* is a β-barrel outer membrane protein with eight surface-exposed loops interspersed with membrane-embedded stretches. Antigenic variation, a hallmark of this molecule, is mainly confined to surface-exposed loops I and IV. While hypervariation of these loops is essential for protective antibody responses, the significance of PorA strain variation for processing and presentation of CD4+ T cell epitopes has not been studied.

Methods. Natural display of specific HLA-DR epitopes on antigen pulsed human dendritic cells was studied by nano-scale mass spectrometry of peptide samples after use of metabolically labeled PorA variants (P1.5-2,10 and P1.7-2,4). Immunogenicity of the HLA-DR eluted, predicted as well as known epitopes, was studied in functional T cell assays using PBMC from an HLA typed donor panel and synthetic peptides. Efficiency of functional epitope presentation was compared for PorA variants using established CD4+ T cell clones and recombinant PorA proteins.

Results. Naturally processed epitopes were presented at widely diverging densities, and originated from 8 distinct regions, located in membrane-embedded parts of PorA. Epitope display of 4 regions was serosubtype conserved, whereas for 4 other regions it occurred in a serosubtype-dependent manner. Three regions contained natural microvariation. The epitope repertoires of different HLA-DR alleles partially overlapped. Eluted PorA sequences appeared to represent *immunodominant* human CD4+ T cell specificities and included earlier identified CD4 T cell epitopes. The efficiency of PorA proteins to stimulate specific CD4+ T cell clones was serosubtype dependent, not only for variable but also for conserved epitopes.

Conclusion. This extensive epitope survey indicates that MHC class II processing and presentation of PorA is nonrandom, and that natural polymorphisms inside and outside epitope regions play a role in the efficiency of processing and presentation of PorA to CD4+ T cells. This may influence the level of immunogenicity of PorA serosubtypes.

P6.2.09

Different Regulation of B Cell Responses against Strongly and Weakly Immunogenic PorA antigens

Cécile van Els[1], Thomas Luijkx[1], Jacqueline van Gaans-van den Brink[1], Floor Pietersma[1], Claire Boog[1], Paul Roholl[2], Germie van den Dobbelsteen[1]

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Introduction. High-affinity bactericidal antibody titers against the variable outer membrane protein PorA are thought to protect against *Neisseria meningitidis* pathology, and develop through germinal center (GC) reactions. Currently it is unknown why vaccine-induced bactericidal IgG titers against the serosubtype P1.5-1,2-2 are consistently higher than those against the highly homologous P1.7-2,4, both in humans and in mice.

Methods. To understand the basis for this differential immunogenicity, early specific B cell responses were studied in Balb/c mice longitudinally after immunization with P1.5-1,2-2 or P1.7-2,4 outer membrane vesicle vaccines. Specific splenic memory B cells and bone marrow plasma cells were quantitated in a B cell ELISPOT assay. Changes in white pulp areas in lymphoid organs were monitored by immunohistology. Cellular findings were related to levels, avidity and bactericidal activity of PorA specific IgG in sera.

Results. All serological parameters were higher for P1.5-1,2-2 than for P1.7-2,4 at 2, 4 and 6 weeks after immunization, pointing at a different pace of antibody production and maturation. In contrast, equal numbers of plasma cells were involved in either response during the first 4 weeks. Boosting at week 4 did not alter numbers of plasma cells nor memory B cells specific for P1.5-1,2-2, while those specific for P1.7-2,4 increased dramatically, yet without serological effect.

Conclusion. These data, supported by immunohistological findings, indicate that weak immunogenicity of P1.7-2,4 is not associated with a deficiency in priming or expansion of specific B cells, but instead, surprisingly, with ultimate B cell hyperproliferation. Congestion of B cell responses also occurs in the absence of regular somatic IgG hypermutation or terminal B cell differentiation. Therefore, factors involved in poor affinity maturation

of P1.7-2,4 specific B cells could be i) suboptimal T cell help, ii) limited antigen persistence. Understanding how subtle antigen variations may affect GC reactions is of importance in vaccine development.

The Discussion Session for the following posters is from: 9.30pm – 10.15pm Tuesday 12 September

P6.3.01

Mucosal Immunity to *Neisseria lactamica* and the Development of Cross-reactive T-cell Memory to *Neisseria meningitidis*

Andrew Vaughan[1], Victoria Davenport[2], Andrew Gorringe[3], Neil Williams[1], Robert Heyderman[1]

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The palatine tonsils (PT) are situated at the entrance to the nasopharynx and may be the first site of contact between colonising *Neisseria* and the host immune system. Epidemiological evidence suggests that carriage of *N. lactamica* is associated with a lower incidence of invasive meningococcal disease, possibly due to the induction of cross-protective immunity. In this study we have investigated the possibility that colonisation by *N. lactamica* in early childhood results in the generation of specific CD4 T-cell memory in the PT and have assessed the potential cross-reactivity with *N. meningitidis*.

Tonsillar mononuclear cell preparations were produced from the PT of 20 patients aged 3-31 years undergoing routine tonsillectomy. CD19+ B cells and CD45RA+ naïve T-cells were depleted by MACS to measure the specific T-cell memory response to outer membrane vesicles (OMVs) generated from *N. meningitidis* strain H44/76 and *N. lactamica* strain 1009. Samples were taken from cultures on days 2-9 and proliferation was measured by incorporation of tritiated thymidine.

Four of 20 individuals displayed a CD4 T-cell memory response to OMVs from both *N. meningitidis* and *N. lactamica*. Five individuals demonstrated a memory response to either N. *meningitidis* or *N. lactamica* and 11 failed to show any CD4 T-cell memory to *Neisseria* OMVs. Surprisingly, most of the individuals that failed to exhibit a memory CD4 T-cell response to *N. lactamica* were under 5 years of age, when nasopharyngeal carriage is highest.

We conclude that potentially cross-reactive mucosal CD4 T-cell memory is uncommon in young children and is not widespread in older children and adults. These data suggest that any protection offered by natural carriage of *N. lactamica* may be mediated by thymus independent processes. This may explain the ongoing susceptibility to meningococcal carriage and invasion in childhood despite frequent colonisation with *N. lactamica.*

P6.3.02

Effect of Meningococcal Carriage on Human Humoral Immune Response

Jeannette N. Williams, Myron Christodoulides, and John E. Heckels

Molecular Microbiology Group, University of Southampton Medical School, UK.

Understanding the basis of protective immunity is a key requirement for the development of an effective vaccine against infection with serogroup B meningococci. We have conducted a longitudinal study into the dynamics of meningococcal acquisition and carriage in first year university students. Throat swabs, and serum samples were collected at four time-points, over a 31 week period. One student who was colonised by two distinct meningococcal strains was studied in detail. Carriage of the first strain (B:NT:P1.4,7b:L3) was detected at week 18 and was still present at week 31 when the individual was also colonised with the second strain (B:1:P1.14:L3). This subject had non-detectable serum bactericidal activity (SBA titre <1:4) prior to detection of carriage, but subsequently developed a significant increase in SBA to the homologous strains. In addition, SBA against heterologous strains also developed over the period of colonisation. 1D SDS PAGE followed by western blotting showed that bactericidal activity was associated with the presence of antibodies reacting against PorA and PorB proteins. In order to improve protein resolution, western blotting was carried out following 2D electrophoresis. Comparison of the results with a 2D map of the proteome of outer membrane vesicles permitted the identification of antigens capable of inducing cross-reacting antibodies.

Identification of such antigens with the ability to induce cross reactive SBA to a range of meningococcal strains may be a major step in the production of an effective vaccine against infections caused by serogroup B meningococci.

P6.3.03

Dynamics of Human Humoral Immune Response to Serogroup B *Neisseria meningitidis* Infection: Analysis of Pre-infection, Acute and Convalescent Sera from the same individual

Jeannette N. Williams, Myron Christodoulides, and John E. Heckels

Molecular Microbiology Group, University of Southampton Medical School, UK.

Studies of outbreaks of serogroup C meningococcal infection in the 1960's identified serum bactericidal activity (SBA) as the correlate of protection against disease. It is also generally accepted that bactericidal activity is the best correlate of protection against serogroup B infections although the evidence for this is less clear due to the difficulty of obtaining serum samples from individuals who subsequently become infected.

A 26 year old student was hospitalised with suspected meningococcal septicaemia, antibiotics were prescribed and the diagnosis was confirmed by isolation of serogroup B meningococci from blood cultures. The patient went on to make a full clinical recovery. Serum samples predating the infection, taken for other clinical reasons, were available from this patient, allowing investigation into levels of humoral immunity to meningococci before, during and after meningococcal infection. Assays for SBA showed that although the patient had protective bactericidal antibody levels (titre of 1:16) to the homologous strain three years prior to infection, this level had dropped to a non-protective level (titre of <1:4) one month prior to infection and at admission. A similar decline in the presence of antibodies to outer membrane proteins was demonstrated by western blotting. Convalescent sera showed raised SBA titres and increased antibody reactivity on immunoblots.

In this study, the low antibody and SBA levels in the patient prior to infection coincided with a period of personal stress. Other investigators have noted an association between stress and a reduced immune response. Specifically, a correlation has been noted between stress and depressed SBA titres following immunisation with the meningitis C conjugate vaccine. The results from this study suggest that depressed SBA, resulting from personal stress, may be an important factor in increasing susceptibility to meningococcal disease.

P6.3.04

Effect of Mannose-binding lectin on epithelial cell responses to *Neisseria gonorrhoeae*

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Introduction: Mannose Binding Lectin (MBL) is one of the first components of the innate-immune system to come into contact with invading pathogens. MBL is present within the genitourinary tract but its role in the interaction between pathogenic *Neisseria* and epithelial cells is undetermined. Toll-like receptor 2 (TLR-2) is important in the response of epithelial cells to *N.gonorrhoeae*. Gonorrhoea is primarily an inflammatory disease. We examined the effects of MBL on the interactions between *N.gonorrhoeae*, strain MS11, and Chang epithelial cells. Also the role of Toll-like Receptor (TLR)-2 was investigated using expression vectors.

Materials and methods: Bacteria were incubated with and without MBL (5µg/ml) prior to inoculation onto Chang cells. Binding was measured by fluorescence microscopy. Cell responses were measured by ELISA and protein array. Cells were transfected by standards techniques.

Results: MBL had no effect on the binding of MS11 to Chang cells. Protein arrays identified constitutive expression of IL-6, IL-8 and TIMP-2 by Chang cells. MBL did not influence the release of IL-8 after infection of unmodified Chang cells. However in TLR-2 over-expressing Chang cells, MBL caused a significantly greater IL-8 response (59% at 4hrs : p= 0.024, n=6; and 35% at 6hrs : p = 0.011, n=6). No effect was seen on the IL-6 response.

Conclusions: MBL modulates IL-8 production by epithelial cells during contact with *N.gonorrhoeae*. This effect is conditional on TLR2 expression. MBL does not enhance binding of *N.gonorrhoeae* to cells.

P6.3.05

Contribution of Catalase, Cytochrome C Peroxidase, Methionine Sulfoxide Reductase, and Manganese Transport in Survival of *Neisseria gonorrhoeae* during Lower Genital Tract Infection of Female Mice

Hong Wu and Ann E. Jerse

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A redundancy of anti-oxidant factors is hypothesized to protect *Neisseria gonorrhoeae* from phagocytic killing. The extent to which phagocyte-generated reactive oxygen species challenge the gonococcus *in vivo* is not known, however, and may be minimal in the relatively anaerobic environment of the female genital tract. Here we tested genetically defined mutants in catalase (*kat*), cytochrome c peroxidase (*ccp*), methionine sulfoxide reductase (*msrA*), and the substrate-binding gene (*mntC*) of the manganese transporter for the capacity to survive killing by murine polymorphonuclear leukocytes (PMNs) and to infect the lower genital tract of female mice. Insertional mutants were created in strain MS11 by allelic exchange. H_2O_2 sensitivity was used to confirm the resultant phenotypes. The *ccp*, *msrA*, and *mntC* mutants were more susceptible to H_2O_2 than the wild type strain ($p < 0.05$); mutations in *kat*, *kat,ccp*, *kat,msrA*, *kat,mntC*, and *kat,ccp,mntC* conferred higher levels of H_2O_2 sensitivity ($p < 0.01$). None of the single mutants showed increased susceptibility to PMN killing in suspension assays performed with aeration. In competitive infections with the wild type strain, the *mntC* mutant was reproducibly attenuated early in infection with average competitive indices showing a 60-fold reduction in fitness by day 4 post-inoculation. The *msrA* mutant was attenuated late in infection with a 30- to 250-fold reduction in fitness detected 10 - 14 days post-inoculation. The MS11 *kat* mutant, *ccp* mutant and a *kat,ccp* double mutant were not attenuated *in vivo*. In contrast, and as reported previously, a *kat* mutant of strain FA1090 was dramatically attenuated *in vivo* with a 10^2 -10^4 -fold decrease in fitness detected 2-6 days post-infection. A vaginal PMN influx occurred in ca. 50% of mice. These results suggest that manganese uptake and MsrA play a protective role during gonococcal genital tract infection and that the importance of catalase *in vivo* may be strain-specific.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P07
Surface Structures

The Discussion Session for the following posters is from: 8.00pm – 8.45pm Tuesday 12 September

P7.1.01

Effect of Pilin Glycosylation Alterations on Adhesion Invasion and Transmigration of *N. gonorrhoeae*

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Neisseria gonorrhoeae (gonococcus, GC), causes the (Uncomplicated Gonorrhoea, UG), as well as Pelvic Inflammatory Disease (PID) and Disseminated Gonococcal Infection (DGI). Pili of GC, are made up of multimers of pilin glycoprotein subunits – the pilin polypeptides are O-glycosylated at the Ser-63 residue.

This study was focused on understanding the pathogenic roles of pilin glycosylation (*pgl*) activities. Various isogenic *pgl* mutants were constructed through genetic strategies. Wild type (WT) and mutant pilin proteins were studied by various techniques like SDS-PAGE, Silver Staining, Western blotting, High-pH Anion Exchange chromatography in conjunction with Pulse Amperometric Detection (HPAE-PAD), Mass Spectrometry (MS) and gas chromatography in conjunction with Mass Spectrometry (GC-MS) analysis. Adherence and invasion assays were performed using infection models employing HEC-1-B endometrial cells, and ME-180 cervical cells. Monolayers of T84 cells were mainly used for transmigration assays. Through these experiments, we evaluated adhesion, invasion and transmigration abilities of isogenic *pgl* mutants of several GC strains. Overall, GC pilin glycan mutants with shorter glycoforms showed increased invasion (consequently proportional increase in transmigration) compared to their corresponding WT. Thus, some of the pilin glycoform switching (via phase variation of certain *pgl* genes) likely helps GC to invade faster and deeper inside the host body in order to cause complications like PID and DGI. Interestingly, our results show that these aforementioned GC pilin glycan alterations produced minor or negligible effects on the relative adhesive ability of this bacterium.

P7.1.02

***Neisseria meningitidis* NhhA is a multifunctional trimeric autotransporter adhesin**

Maria Scarselli, Davide Serruto, Paolo Montanari, Barbara Capecchi, Jeannette Adu-Bobie, Daniele Veggi, Rino Rappuoli, Mariagrazia Pizza and Beatrice Aricò

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NhhA, *Neisseria* hia/hsf homologue, or GNA0992, is an oligomeric outer membrane antigen of *Neisseria meningitidis* that was recently included in the family of trimeric autotransporter adhesins. Members of this family have a conserved C-terminal short translocation domain that is able to trimerize and form a complete □-barrel that directs passenger secretion, however proteins are heterogeneous in their N-terminal domains, which are involved in adherence to a variety of substrates.

In this study we present the structural and functional characterization of NhhA. By expressing the full-length gene, deletion mutants and chimeric proteins of NhhA in *Escherichia coli*, we demonstrated that the last 72 carboxyl-terminal residues are involved in trimerization and localization of the N-terminal protein domain to the bacterial surface.

In silico analysis of the NhhA amino acid sequence assisted us in the understanding of its possible role during meningococcal infection. The sequence similarity to Hia/Hsf, responsible of the formation of adhesive fibrils in *H. influenzae*, suggested the ability of NhhA to mediate adherence to epithelial cells. Moreover, the presence of a repeated amino acid motif common to eukaryotic adhesion molecules led to the prediction of putative binding sites to heparan sulfate. We show that *E. coli* expressing trimeric NhhA was able to adhere to two different epithelial cell lines. Furthermore we assessed the ability of recombinant purified NhhA to bind human epithelial cells as well as to laminin and heparan sulphate *in vitro*. We also observed a significant reduction in adherence capability with a capsulated meningococcal isogenic MC58dNhhA mutant. Collectively, our structural and functional results indicate that NhhA belongs to the emerging group of bacterial autotransporter adhesins with trimeric architecture, and underline its putative role in meningococcal infection providing additional insight into the mechanisms by which *N. meningitidis* colonizes the human respiratory tract.

P7.1.03

Human lactoferrin cleaves and inactivates *Neisseria meningitidis* surface antigens

Davide Serruto*, Silvana Savino*, Laura Santini*, Vega Masignani*, Marzia Giuliani*, Elena Cartocci*, Jiazhou Qiu‡, Andrew G Plaut‡, Rino Rappuoli*,Mariagrazia Pizza*, and Beatrice Aricò*

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Lactoferrin is a member of non-haem, iron-binding glycoproteins of the transferrin family that was first recognized in milk and was later

found in all human secretions. Its physiological role is still unclear, but it has been suggested to be responsible for primary defence against pathogenic organisms. Early studies suggested that lactoferrin has antimicrobial activity and contributes to innate immunity by limiting the availablility of iron to pathogenic organisms. Several groups have reported that lactoferrin has bactericidal activity against selected Gram-positive and Gram-negative bacteria. More recently it has been shown that human lactoferrin is a serine protease able to cleave and remove two surface virulence proteins of *Haemophilus influenzae* at arginine-rich sequences, thus accounting in part for its long-recognized antimicrobial properties.

In this study we have investigated whether lactoferrin is able to cleave the *Neisseria meningitidis* App and GNA2132 antigens, both containing conserved arginine-rich motifs. App is a recently described adhesin which promotes interaction with human epithelial cells and that presumably plays a fundamental role in mediating meningococcal colonization. GNA2132 is a novel outer membrane lipoprotein with a low similarity to transferrin binding proteins. This protein is able to induce high levels of bactericidal antibodies in mice and was therefore selected as a vaccine candidate. We observed that purified human milk lactoferrin specifically degrades App adhesin and GNA2132 antigen on the bacterial surface, that lactoferrin cleaves GNA2132 at the arginine-rich site and that two different serine protease inhibitors inhibit this cleavage. In addition, lactoferrin specifically interferes with GNA 2132-mediated bactericidal activity. These results suggest that human lactoferrin may attenuate colonization of *N. meningitidis* by selectively inactivating App, and they raise intriguing questions on the role played by the GNA2132 antigen in meningococcal infection. Further investigations are ongoing in order to understand whether this cleavage is necessary for the bacteria to escape the immune system.

P7.1.04

A Novel Serogroup W135 Strain Causing Disease in New Zealand

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Neisseria meningitidis W135 is an under-represented serogroup in meningococcal disease in New Zealand, resulting in zero to two cases annually or less than 1% of total cases. Since 2003, twelve isolates with the strain-type W:2a:P1.7-2,4 have been collected from disease cases in New Zealand, contributing to a ten-fold increase in the number of meningococcal W135 disease cases. Of greater interest is the presence of the subtype P1.7-2,4, which is associated with the current epidemic strain B:4:P1.7-2,4.

Genetic characterisation of the W:2a:P1.7-2,4 isolates and 92 other isolates with similar characteristics (eg: serogroup, serosubtype, or MLST type) revealed that the W:2a:P1.7-2,4 strain shares the same PorA, FetA, and MLST type as a C:2a:P1.7-2,4 strain first identified in New Zealand three years before the emergence of the W:2a:P1.7-2,4. Furthermore, sequencing of the *fumC* in both strains indicated that both belong to the ET-15 subgroup, a group within the hyperinvasive ST-11 complex, which has been responsible for outbreaks and hyperendemic rates of disease worldwide. To verify the results, the isolates were also subjected to whole genome RFLP, which again showed a close relationship between the W:2a:P1.7-2,4 and C:2a:P1.7-2,4 isolates. These results indicate that the W:2a:P1.7-2,4 strain has most likely come about by a capsule switch from a C:2a:P1.7-2,4 strain.

Using DNA sequence analysis, we have been able to show that at least 20kb of DNA has been transferred to bring about this switch. The upstream site of recombination has not been identified at the time of submission, but extends into the capsule transport operon. The downstream site is located within a 2kb conserved region of DNA encompassing *dnaJ*, thereby including both the capsule biosynthesis and LOS biosynthesis operons in the switch. This result demonstrates that a larger amount of DNA than previously thought can be involved in capsule switching.

P7.1.05

Primary Human Nasopharyngeal Epithelial Cell Receptor Expression and Interactions with Phenotypic Variants of *Neisseria meningitidis*

Claudia Sa e Cunha[1], Natalie Griffiths[1], Emma Hendy[1,2], Darryl J Hill[1], Michel Erlewyn-Lajeunesse [2], Adam Finn[2] and Mumtaz Virji[1]

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In order to understand the fundamental processes by which meningococci colonise and infect target tissues, investigations to date have used immortalised human cell lines to identify bacterial adhesins and their cognate human receptors. A number of adhesin-receptor pairs have now been well characterised or strongly implicated in meningococcal interactions with human cells. However, the *in vivo* importance of these potential interactions is yet to be established. Several laboratories have investigated meningococcal interactions with human respiratory organ cultures and cells derived from them[(1-4)]. These studies have implicated pili, Opa and Opc but the identities of receptors involved are not established. Studies on gonococcal interactions with human primary urogenital cultures have shown the involvement of novel receptors not previously found on transformed epithelial cell lines[5]. Our aim in the current studies is to characterise the receptors for the major meningococcal adhesins, Opa, Opc and pili, expressed on primary epithelial cells isolated from human nasopharynx (HNECs). Using flow cytometry and immunofluorescence microscopy, to date, we have assessed the expression of CEACAMs, CD46 and integrins on several primary cell lines (derived by culturing epithelial cells from nasal brushings from children). In addition, we have assessed changes in the receptor profiles following stimulation with pro-inflammatory cytokines. The pre-treatment of HNECs with IFN-γ and TNF-αaltered receptor-expression in the majority of cell lines studied, with $\alpha5\beta1$ integrin and CD46 showing the most consistent upregulation. However, the pattern and the level of receptor modulation varied between different sources of epithelial cells and

according to their differentiation status. In some cases, dramatic upregulation of CEACAMs was also observed. Bacterial interactions with stimulated and unstimulated cells have shown the involvement of multiple receptors for Opa proteins. Further involvement of various receptors is being assessed.

P7.1.06

Molecular Interactions of the Meningococcal Opc Further Explored - Identification of α-actinin as an Opc-Binding Protein

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Interactions of Opc-expressing *Neisseria meningitidis* (Nm) with endothelial cells have been shown to occur primarily via integrins. Previous studies have also demonstrated the presence of receptors for Nm other than integrins on the basolateral surface of human endothelial cells[1]. In addition, a 100kDa protein in whole cell lysates of human endothelial cells was identified that bound to Opc-expressing bacteria. Therefore, we set out to identify this potential receptor for Opc protein. By Far Western blots, we screened different endothelial and epithelial cell lines for the presence of this Opc-binding molecule, and observed that it was present in all cells screened and interacted with Opc-expressing Nm but not with variants that lack Opc expression. Using mass spectrometry, the 100kDa protein present on cell lines of different origins was identified as human α-actinin. The involvement of α-actinin was also demonstrated by antibody inhibition experiments and Western overlays. In addition, α-actinin was the only protein that could be co-precipitated with Opc from lysates of human cells using anti-Opc antibodies, also supporting the above evidence.

α-actinin, a cytoskeletal protein, can be found close to the plasma membrane in a variety of tissues and cells. There are also reports in the literature on a level of α-actinin exposure on some cell types[2,3]. However, studies on various cells using anti-α-actinin antibodies to assess surface exposure (studied both by flow cytometry and immunofluorescence microscopy), produced no evidence of surface expression of the protein. Antibody inhibition studies of bacterial binding were also in accordance with this.

Binding of neisserial adhesins to intracellular proteins has been observed previously[4]. The exact implications of the Opc- α-actinin molecular interactions in meningococcal pathogenesis remain to be defined.

P7.1.07

Investigation of the Role of Iron Acquisition Genes in Meningococcal Colonisation of Nasopharyngeal Tissue

Rachel Exley[1], Megan Winterbotham[1], Muriel Schneider[1], Richard Sim[2], Linda Goodwin[3], Robert Read[3] and Chris Tang[1]

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Neisseria meningitidis is a major cause of septicaemia and meningitis. Although systemic disease is often fulminant and fatal in up to 20% of cases, the most frequent outcome of infection with this bacterium is asymptomatic carriage. Our current understanding of the colonisation process is limited. Although carriage is asymptomatic, studies have demonstrated that colonising bacteria can be found deep within the nasopharyngeal tissue, indicating that passage through the epithelial layer is not restricted to invasive disease. The key structures involved in initial adhesion to epithelial cells have been identified but the mechanisms which enable the bacteria to survive within the tissue are unknown.

By screening a library of signature-tagged mutants of serogroup B *N. meningitidis* for their ability to survive in explanted human nasopharyngeal tissue we identified several genes that are necessary for colonisation. One of these is predicted to encode a TonB-dependent receptor, a member of a family of proteins implicated in scavenging iron from the human host. NMB1829 is one of a number of genes predicted to encode uncharacterised TonB-dependent receptors in *N. meningitidis*. The gene product shares amino acid similarity (30% identity, 50% similarity) with siderophore receptor FhuE from *E. coli* and is therefore proposed to be important for iron uptake.

In order to determine the precise role of NMB1829 in colonisation of the upper airway, we have examined the ability of this mutant to adhere to and replicate within epithelial cells. In addition, we have performed growth promotion assays using a range of iron loaded siderophores to gain insight into the nature of the NMB1829 ligand. Finally, the resistance of a strain lacking NMB1829 and other TonB-dependent receptors to upper airway antimicrobial peptides is under investigation.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P7.2.01

Meningococcal secretin PilQ – Actions and Interactions

Stephan A. Frye[1,2], Seetha Balasingham[1,2], Reza Assalkhou[1,2], Håvard Homberset[1], Richard F. Collins[3], Jeremy P. Derrick [3] and Tone Tønjum[1,2]

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Neisseria meningitidis is throughout its entire life-cycle constitutively competent for transformation. This is particularly important for genetic exchange and is dependent on the expression of type IV pili. Type IV pili are present on the cell surface as bundled filamentous appendages and are assembled, extruded and retracted by pilus biogenesis components.

The uptake of transforming DNA into the meningococcal cell can be divided into four stages: entry through an outer membrane pore, transit of the periplasm, transport across the inner membrane and genome integration. We propose that the early stage of DNA uptake is coupled to pilus retraction by non-specific attachment to the pili. The secretin PilQ is a macromolecular complex required for pilus expression that is located in the outer membrane, with its main N-terminal domain in the periplasm. We have shown that pili directly interact with PilQ and that the pilotin PilP in the inner membrane interacts with PilQ. We have evidence that DNA is introduced into the periplasm through the channel formed by the PilQ complex and that other components process the DNA during the transfer trough the membrane.

The secretin PilQ and other DNA binding candidates are currently being assessed for their structure-function relationships to define how they act and interact with each other and with DNA. The goal is to define how these components provide dynamic multi-site targeting of DNA during the steps of the transformation process.

P7.2.02

Analysis of Murine Immune Responses to Conserved- and Hypervariable-regions of *Neisseria gonorrhoeae* Pilin

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PilE is the primary subunit of type IV pili, and contains a surface exposed hypervariable region that has prevented development of a pilin-based cross–reactive vaccine. We have created a subunit protein from PilE containing only conserved regions in an effort to determine if this hypervariable deletion protein (HV-del) could be a vaccine candidate. The deleted hypervariable region was replaced with a two amino acid Asp-Gln linker chosen based on the sequences of the type IV pilin from *P. aeruginosa*. We then characterized the murine immune responses to this novel protein. As expected, a control recombinant PilE (re-Pilin) protein is able to elicit strong IgG responses, and these IgGs are specific to epitopes in both the subunit and re-Pilin proteins. However, HV-del is unable to elicit an antibody response in mice, suggesting that the conserved regions of PilE are not sufficient for T helper cell and subsequent B cell activation necessary for antibody production *even in the absence of the hypervariable region*. Further analysis of the HV-del and the re-Pilin proteins with suppressor cell assays shows that these proteins are not actively suppressing the immune system, and flow cytometry experiments suggest that they are not exerting suppressor effects indirectly by activating T regulatory cells. Currently, we are examining the processing and presentation of HV-del to murine immune cells by isolating MHC II-peptide complexes from bone marrow dendritic cells. Our hypothesis is that the hypervariable region and not the conserved regions of *N. gonorrhoeae* PilE is presented to the immune system, activating T and B cells and resulting in a strong antibody response.

P7.2.03

Meningococcal Autotransporter Proteins (App and MspA) mediate Adherence to Human Brain Microvascular Endothelial Cells

Sherko Omer, Karl Wooldridge David Turner and Dlawer Ala'Aldeen

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Autotransporter proteins are a family of secreted virulence-related proteins. Adhesion and penetration protein (App) and meningococcal serine protease A (MspA), which share 33% overall homology at the amino acid level, are among several autotransporters produced by *Neisseria meningitidis* that may play a role in the pathogenesis of meningococcal disease.

App has previously been demonstrated to mediate adherence to Chang epithelial cells but not human umbilical vein endothelial cells. We have recently demonstrated that *E. coli* expressing either recombinant App or MspA adhere to human bronchial epithelial cells and human brain microvascular cells (HBMECs) (Turner *et al*., Infect Immun 2006; (5): 2957-64).

In an attempt to further evaluate App and MspA mediated adhesion to HBMECs, polystyrene fluorescent micro-beads were coated with purified recombinant App or MspA, and used in adherence assays.

App, and to a lesser extent MspA, coated beads showed significant adhesion to HBMECs compared to control beads coated with BSA. Work is currently in progress to identify the host cell ligand(s) for App and MspA.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P7.2.04

O-acetylation of the terminal *N*-acetylglucosamine of the lipooligosaccharide inner core in *Neisseria meningitidis*: influence on inner core structure and assembly.

Charlene M. Kahler [1*], Shauna Lyons Schindler[1], Biswa Choudhury[2], John Glushka[2], Russell W. Carlson [2] and David S. Stephens [3,4].

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Departments of [3]Medicine and [4]Microbiology and Immunology and Laboratories of Microbial Pathogenesis, Emory University School of Medicine and VA Medical Center, Atlanta, GA

O-acetylation is a common decoration on endotoxins derived from many Gram-negative bacterial species, and has been shown to be instrumental *(e.g. Salmonella typhimurium)* in determining the final tertiary structure of the endotoxin, and the immunogenicity of the molecule. Structural heterogeneity of endotoxins produced by mucosal pathogens such as *Neisseria meningitidis* is determined by decorations on the heptose inner core, including O-acetylation of the terminal *N*-acetylglucosamine (GlcNAc) attached to HepII. In this report, we show that O-acetylation of the meningococcal LOS inner core has an important role in determining inner core assembly and immunotype expression. The gene encoding the lipoologosaccharide O-acetyltransferase, *lot3*, was identified by homology to NodX from *Rhizobium leguminosarum.* Inactivation of *lot3* in strain NMB resulted in the loss of the O-acetyl group located at the C-3 position of the terminal GlcNAc of the LOS inner core. While inactivation of *lot3* or *lgtG* encoding the HepII glucosyltransferase alone did not result in the appearance of the O-3 linked phosphoethanolamine (PEA) groups on the L2 immunotype LOS inner core, construction of a double mutant in which both *lot3* and *lgtG* were inactivated resulted in the appearance of O-3 linked PEA groups. In conclusion, O-acetylation status of the terminal GlcNAc of the □-chain of the meningococcal LOS inner core is an important determinant for the appearance or exclusion of the O-3 linked PEA group on the LOS inner core, and contributes to LOS structural diversity. O-acetylation is predicted to influence resistance to complement mediated lysis and may be important in LOS conjugate vaccine design.

P7.2.05

Hemagglutinin related genes encode novel adhesins of *Neisseria meningitides*

Corinna Schmitt, Maria Bösl, Heike Claus, Ulrich Vogel, Matthias Frosch, Oliver Kurzai

Institute Of Hygiene And Microbiology, Wuerzburg, Germany

The genome of *N. meningitidis* MC58 (serogroup B, ST-32 complex) contains five ORFs encoding large proteins annotated as hemagglutinin/hemolysin related proteins (hrp). By conserved domain search of the predicted protein sequences hrp share a hemagglutinin activity domain with the *Bordetella pertussis* filamentous hemagglutinin (fhaB), a well-known adhesion factor and main component of the pertussis vaccine. We investigated the presence of *hrp* genes in a panel of 822 carrier strains isolated from healthy individuals in Bavaria. At least one gene homologous to one of the five MC58 *hrp* genes is present in the majority of strains. Among those strains that did not hybridize with the probes specific to the MC58 hrp genes, 82% belong to four of five clonal complexes harboring the capsule null locus. Infection experiments with epithelial cells were performed to investigate whether *hrp* contribute to meningococcal adherence. No difference could be detected in the proportion of cell associated bacteria between *hrp* mutants of strain MC58 and their parental strains, indicating that adherence to epithelial cells is not dependent on a single *hrp* in MC58. In contrast, in strain 2120 (serogroup C, ST-11) carrying only one hrp gene, deletion of hrp lead to a significant decrease in the number of cell-associated bacteria in an unencapsulated and LPS truncated mutant, indicating that *hrp* can contribute to meningococcal adherence to epithelial cells and might be functionally redundant in strains carrying more than one allele.

P7.2.06

Simultaneous Mutations in Two Meningococcal *dsbA* Genes Compromise Transformation

Sunita Sinha[1], O. Herman Ambur[2], Paul R. Langford[1], Tone Tønjum[2] and J. Simon Kroll[1]

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Neisseria meningitidis is naturally competent for DNA uptake by transformation throughout its life cycle, and horizontal gene transfer contributes significantly to its variable phenotype. DNA uptake is dependent on the expression of type IV pili (T4P). T4P comprise ordered arrays of pilin subunits, assembled through the outer membrane secretin PilQ. T4P expression is associated with properties such as agglutination, twitching motility and adherence, as well as competence for transformation.

The thiol-disulphide oxidoreductase DsbA ensures the correct folding of many bacterial extra-cytoplasmic proteins by catalysing the formation of intramolecular disulphide bonds. DsbA has been implicated in the virulence of various bacterial pathogens including *N. meningitidis*, through its function in the folding of outer membrane and secreted proteins. *N. meningitidis* is unusual in possessing three *dsbA* genes, encoding one periplasmic (DsbA3) and two inner-membrane (DsbA1 and DsbA2) proteins.

Quantitative transformation assays using marked chromosomal DNA revealed that a meningococcal strain lacking DsbA1 and DsbA2 (strain M1M2) is impaired in natural transformation compared to the wildtype strain. The possibility that this reflected an inability to produce T4P was ruled out by electron microscopy, which demonstrated wildtype levels of piliation. It is likely therefore that the functionality of the pilus fibres or other components involved in transformation is compromised in the absence of DsbA1

and DsbA2. In order to establish which step(s) of the DNA uptake process are affected, DNA binding and uptake assays with radiolabelled donor DNA were performed. These showed that while DNA binding is unaffected by the lack of DsbA1 or DsbA2, M1M2 is reduced in its ability to take up DNA. DsbA was shown to bind PilQ at the disulphide bond-containing C-terminus, suggesting one possible step in the transformation process that might be compromised by a lack of DsbA. Further consequences of defects in DsbA in transformation are being explored.

P7.2.07

Liquid Chromatography MS/MS Analysis of the Protein Content of Outer Membranes and Vesicles Derived from MC58 and a Lipopolysaccharide-Deficient Mutant

Jeannette N. Williams[1], Paul J. Skipp[2], Holly E. Humphries[1], Myron Christodoulides[1], C. David O'Connor[2] and John E. Heckels[1]

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[2] Centre for Proteomic Research, University of Southampton School of Biological Sciences, UK

Current experimental and newly licensed vaccines against serogroup B meningococcal infection are based on meningococcal outer membrane (OM) proteins obtained by extraction with deoxycholate to produce vesicles (OMV) lacking toxic lipopolysaccharide (LPS). Outer membranes isolated from mutants lacking LPS represent an alternative source of OM proteins for vaccine use. Studies of such vaccines have shown that the immune responses to different components vary between individuals and only a proportion of the antibodies induced are protective. However, detailed understanding of the immune response to such preparations is hampered by lack of detailed knowledge of their composition.

SDS-PAGE and nanocapillary liquid chromatography- tandem mass spectrometry (GeLC-MS/MS) is an alternative approach, which involves 1D electrophoresis followed by liquid chromatography and tandem MS and can significantly increase the number of peptides that are identified by conventional 2D gel electrophoresis. GeLC-MS/MS was used to investigate the proteome of OM and OMV from meningococcal strain MC58, and OM from an LPS-deficient mutant of the same strain. Analysis of OM from wild-type MC58 revealed a much more complex composition than has previously been reported, with a total of 236 proteins being identified. Of these, only 6.4% were predicted to be located in the outer membrane. However, the predominant proteins detected were those that are well established as the major outer membrane proteins and these were also present in OMV. Several proteins had markedly increased abundances in the OM preparation from the LPS-deficient mutant, including enzymes that contribute to the TCA cycle, perhaps as a consequence of enhanced membrane permeability. Several proteins that have previously been identified as potential vaccine candidates were not detected in either OM preparation. These results show that GeLC-MS/MS is particularly suited to identifying the complex mixture of hydrophobic proteins found in OM preparations and have important implications for the development OM based vaccines.

The Discussion Session for the following posters is from: 9.30pm – 10.15pm Tuesday 12 September

P7.3.01

Crystal Structure of the N-Terminal Domain of Human CEACAM1: Binding Target of the Opacity Proteins During Invasion of *Neisseria meningitidis* and *N. gonorrhoeae*

Alena Fedarovich[1], Joshua Tomberg[2], Robert A. Nicholas[2], & Christopher Davies[1]

[1]Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, USA and [2]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC USA

CEACAM1 is a cellular adhesion molecule whose protein expression is down-regulated in several carcinomas and which also contributes to the pathogenicity of *Neisseria* by acting as a receptor for Opa proteins. We have determined the crystal structure of the N-terminal [D1] domain of human CEACAM1 at 2.2 Å resolution. The structure shows several differences with a lower resolution model of the same domain from mouse solved previously, especially in the functional regions. Mapping of the sites of mutations that lower or abolish the binding of CEACAM1 to Opa proteins shows a distinct clustering of residues on the GFCC'C" face of the molecule. Prominent amongst these are residues in the C, C' and F strands and the CC' loop. A similar analysis show that the region responsible for homophilic or heterophilic interactions of CEACAM1 is also on the GFCC'C" face and overlaps partially with the Opa-binding region. This higher resolution structure of CEACAM1 facilitates a more precise dissection of its functional regions in the context of *Neisseria* pathogenesis, cellular adhesion and immune evasion.

P7.3.02

The *Neisseria meningitidis* Outer Membrane Lipoprotein FrpD Binds the RTX Protein FrpC

Katerina Prochazkova[1], Radim Osicka[1], Irena Linhartova[1] and Peter Sebo[1]

[1]Institute of Microbiology of Academy of Sciences of the Czech Republic, Prague, Czech Republic

The opportunistic pathogenic bacterium *Neisseria meningitidis* produces FrpC (Ferrum Regulated) protein, the biological function of which remains unknown. Partial homology of FrpC to known RTX (Repeat in ToXin) virulence factors of other pathogenic bacteria, however, points a potential role of FrpC in meningococcal virulence and/or lifestyle.

Genes encoding the RTX proteins are commonly located in operons with genes of their secretion apparatus and/or genes for proteins required for RTX protein function. However, *frpC* is located in an iron-regulated operon with only one open reading frame, *frpD*, not exhibiting any similarity to other known sequences.

We found that the *frpD* alleles are present almost in all clinical isolates of *N. meningitidis* and are highly conserved in a set of

meningococcal strains representative of all main serogroups. Subcellular localization and [^{3}H]-palmitic acid labeling revealed that recombinant FrpD is synthesized in *E. coli* either as a cytoplasmic protein, or as a lipoprotein that is sorted to the outer bacterial membrane. Both forms of FrpD were found to bind the amino-proximal portion of FrpC with very high affinity (apparent K_d = 0.2 nM).

Interestingly, the RTX protein FrpC exhibits an intriguing biochemical activity, in that it undergoes a unique type of cleavage between residues Asp^{414} and Pro^{415} in the presence of calcium ions. Moreover, a portion of newly generated amino-terminal fragment of FrpC covalently links to another FrpC molecule. In the calcium-rich environments colonized by these bacteria, this activity is likely to be of biological importance. No effect of FrpD on the autoprocessing and linking activity of FrpC was observed but surprisingly the processed N-terminal FrpC fragment formed a covalently linked complex also with FrpD.

The results suggest that FrpD represents a novel type of *rtx* loci-encoded accessory protein. Its biological function appears to be linked to that of the RTX protein FrpC.

P7.3.03

Cross Reactivity of Lacto-N-neotretraose Epitopes on *N. meningitidis* LPS and Human Blood Cells

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In efforts to design a suitable *N. meninigitidis* group B vaccine, a number of strategies utilize complete or partial LPS structures (LPS epitopes) either as a major antigen or minor antigen (e.g., outer membrane vesicle vaccines). A potential concern is the spectre of autoimmune induction, due to the presence of the lacto-N-neotretraose epitope on both the alpha chain of LPS immunotypes commonly found on case isolates and on human glycosphingolipids. Though no evidence from human vaccination trials or natural infections supports this concern, the consequences of this structural similarity bears closer examination. We have used mouse monoclonal antibodies specific for epitopes on LNnT-containing L3,7,9 to examine whether they will bind to surface components of human blood cells. Four monoclonal antibodies generated against meningococci were found to bind L3,7,9 LPS with different specificity. These meningococcal monoclonal antibodies were tested in an ELISA assay for binding to human red blood cells, granulocytes, and monocytes at 4^{o}C, room temperature, and 37^{o}C. All four of the anti-L3,7,9 LPS monoclonal antibodies bound the granulocytes well with or without neuraminidase pretreatment of the cells. Two of the four antibodies demonstrated slightly better binding to granulocytes at 4^{o}C compared to higher temperature. In contrast, only one anti-L3,7,9 LPS antibody bound human RBC well, and only if the cells were pretreated with neuraminidase. Similarly, a monoclonal antibody specific for LNnT bound granulocytes and neuraminidase treated RBC. Monocytes were not bound by any of the antibodies. These results demonstrated that mice generate antibodies to meningococcal LPS which bind to human cells. Additional experiments are underway to address the functional significance of this cross reactivity, such as whether human blood cells can be agglutinated or lysed by these antibodies.

P7.3.04

Characterization of the Interaction Between Transferrin and Transferrin Binding Protein B

Stephen R. Shouldice[1], Collin Shima[1], Jessmi Ling[1], Jean-Nicholas P. Brouillard[1], David Schriemer[2], Michael J. Eggerston[2], Leslie W. Tari[3] and Anthony B. Schryvers[1,2]

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[3]ActiveSight, San Diego, CA, USA

Direct acquisition of iron from host transferrin through bacterial surface receptors has been shown to be critical for survival of pathogenic *Neisseria* in humans. Thus it is a logical pathway to target for development of vaccines and therapeutics. Delineation of the mechanism of iron transport and development of vaccines has been hampered by our limited knowledge of the structure of the receptor proteins and the details of their interaction with transferrin. In this study we report on two approaches adopted for acquiring this information: (1) protein crystallography and (2) hydrogen-deuterium exchange mass spectrometry.

The initial focus for protein crystallography has been on preparation of complexes of transferrin and transferrin binding protein B (TbpB). Complexes were prepared from enzymatically deglycosylated commercial transferrin and recombinant tagged TbpB. Affinity purified complexes were treated with cross-linked enzyme crystals (CLEC) of protease that resulted in generation of stable subfragments of recombinant TbpB. CLEC treated samples were screened for crystallization conditions and several different crystal forms were obtained. None of the crystal forms generated provided adequate diffraction data for solving the 3D structure of the complex. Current efforts are directed at preparation of complexes with recombinant non-glycosylated transferrin and a variety of different recombinant forms of TbpB.

Hydrogen-deuterium exchange coupled with mass spectrometry can be used to identify solvent-exposed regions of a protein, and more specifically, surface regions of a protein involved in protein-protein interactions. Peptides that demonstrate a loss in deuteration in a protein complex relative to the individual proteins are implicated in the region of protein-protein interaction. This approach has been used to identify peptides on transferrin that are involved in binding to TbpB. Current efforts are being directed towards identifying regions of TbpB involved in binding to transferrin and localizing regions of transferrin protected by various TbpB subfragments

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P7.3.05

The Role of Gonococcal PilC in Pathogenesis: Preparation for Experimental Human Challenge

Christopher Thomas[1], James Anderson[1] and P. Frederick Sparling[1,2]

Departments of Medicine, Division of Infectious Diseases[1] and of Microbiology and Immunology[2], School of Medicine, University of North Carolina, Chapel Hill

PilC is a 100kDa surface exposed pilus accessory protein expressed by most isolates of both *Neisseria gonorrhoeae* and *Neisseria meningititdis*. PilC is thought to be one of the initial points of direct interaction between the bacteria and the epithelial cell surface. It is our central hypothesis that antibodies directed against the right parts of PilC could render it non-adherent and thereby reduce infectiousness or render the bacteria non-infectious. Additionally we hypothesize that PilC expression is required to establish gonococcal infection in the human male urethral challenge model of infection. It has been observed by J. G. Cannon and co-workers that a *pilE* deletion strain of FA1090 retained the ability to initiate infection in this model (personal communication) and we contend that this could be due to it retaining the ability to express PilC. To test this we have created isogenic mutants of FA1090 varying only in their ability to express PilC and PilE. These strains have been constructed without leaving any antibiotic resistance markers in the chromosome so as to make them appropriate for use in human challenge. Strains expressing a pilus have had their *pilE* coding sequence restored to an exact match of the original sequence of the human challenge strain, FA1090A23a to eliminate any phenotypic effects of changes in the PilE sequence due to antigenic variation. Strains expressing either PilC1 or PilC2 have been locked in the "on" phase by alteration of their "g" string to prevent slip-strand miss-pairing. To test the effect of a loss of PilC mediated adherence without altering the ability of the gonococcus to express a pilus we have created a derivative of FA1090 expressing a non-adherent meningococcal PilC2 protein from it's *pilC1* locus (*ΔpilC1::mcpilC2*). In this poster we will present the construction of these strains and their preliminary in-vitro phenotypes.

P7.3.06

Involvement of NMB0065 in Translocation of Serogroup B Capsule Polysaccharide in *Neisseria meningitidis*

Rhonda I. Hobb[1,3] and David S. Stephens[1,2,3]

Division of Infectious Diseases, Department of Medicine[1], and Department of Microbiology and Immunology[2], Emory University School of Medicine and Veterans Affairs Medical Center[3], Atlanta, Georgia, USA.

The gene NMB0065, located immediately upstream of *syn*D, has been implicated as a member of the capsular polysaccharide (*cps*) locus based on decreased capsular expression and inability to cause bacteremia in an infant rat model of transposon mutants. NMB0065 shares homology (25% identity) with the proposed polysialytransferase NeuE, of *Escherichia coli* K1. A polyisoprenyl recognition sequence (PIRS) has been identified in NeuE that binds undecaprenyl phosphate (C_{55}), the anchor for polymer elongation. NMB0065 contains many of the residues of this motif suggesting a role for NMB0065 in binding C_{55}. In order to further understand the role of NMB0065 in capsule biosynthesis, a non-polar mutation was created in NMB0065 in the serogroup B strain, NMB. While the mutant by whole cell ELISA produced similar levels of capsule compared to wild type, this mutant was as sensitive as an unencapsulated (*syn*A) mutant to killing by normal human serum. Furthermore, the cell surface of the NMB0065 mutant was shown to be hydrophilic indicating that the capsule was not expressed on the surface of the bacterium. To determine whether this defect was due to changes in capsule structure, capsular polysaccharide was purified from the wild type and the mutant. Phospholipase digestion and NMR analyses revealed that the capsule of the parent and mutant was a non-acetylated sialic acid polymer in an ($\alpha 2 \rightarrow 8$) linkage and that identical lipid anchors were present in both strains. This suggested that NMB0065 was not involved in biosynthesis or lipidation of the capsule polymer. Immunogold electron microscopy analysis revealed little capsule on the bacterial surface but the presence of large vacuoles containing capsular polysaccharide within the cell. Thus, NMB0065 encodes a protein required for proper translocation and surface expression of the capsular polymer. NMB0065 is conserved among other sialic acid capsule expressing serogroups of *Neisseria meningitidis* (C; 97%, Y; 77% and W-135; 77%). A non-polar mutation in serogroup C (FAM18) results in an identical phenotype as the NMB mutant. These data support the hypothesis that NMB0065 may bind an undecaprenyl phosphate carrier and facilitates transfer of the polymer to the export apparatus.

P7.3.07

Characterization of pIII protein of Neisseria gonorrhoeae

Rosanna Leuzzi, Laura Ciucchi, Elisabetta Monaci, Barbara Nesta, Elena Cartocci, Laura Serino, Maria Rita Fontana, Rino Rappuoli and Mariagrazia Pizza.

Novartis Vaccines, Via Fiorentina n1, 53100 Siena, Italy.

Protein pIII is one of the most abundant surface-exposed outer membrane proteins of *Neisseria gonorrhoeae* (Ng). It has been reported that antibodies against pIII act as "blocking antibodies", inhibiting the bactericidal activity of antibodies raised against other surface antigens. However, the mechanism leading to this function has never been clarified and the role of pIII, both in the pathogenesis and in the physiology of the organism, remains unknown.

Homology searches revealed a significant similarity of the C-terminal region of pIII with members of the OmpA family, proteins known to be involved in structural integrity of outer membranes and in pathogenesis of various bacteria.

The abundant amount of pIII in outer membranes of Ng and its high conserved nature support the possibility of a role of pIII as a structural protein. To test this hypothesis, we studied the □pIII knock-out mutant strain to evaluate the possible effects expected after the loss of a structural protein. We observed that the □pIII

knock-out mutant strain has an affected growth rate, whereas it is unchanged in bacterial shape, morphology and sensitivity to detergents.

To evaluate whether the absence of pIII causes a reorganization of the Ng outer membrane, we examined the expression of the surface proteins in the pIII-deficient strain by 2D electrophoresis and Western blot. Our data are consistent with the evidence that pIII does not have a structural role.

Moreover, we studied the involvement of pIII in the interaction with epithelial cells. The pIII knockout mutant strain showed a significant reduction in adhesion to human immortalized cervical and urethral cells. In addition, the recombinant protein was able to specifically bind epithelial cells in an *in vitro* binding assay.

P7.3.08

Variation in the Repertoire and Sequence of Lipopolysaccharide Genes of *Neisseria meningitidis* and the Implications for Vaccine Development

J. Claire Wright[1], Derek W. Hood[1], Katherine Makepeace[1] Andrew D. Cox[2], James C. Richards[2] and E. Richard Moxon[1]

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Our laboratory has been investigating the surface exposed antigen, lipopolysaccharide (LPS) of *Neisseria meningitidis* (*Nm*) as a vaccine component. The LPS of *Nm* is composed of two genetically and structurally distinct regions: the core oligosaccharide and a lipophilic portion termed lipid A. The structure of the inner core oligosaccharide is relatively conserved and is based upon a di-heptose backbone. Additions occur to the proximal heptose (Hep I) and extension past the first glucose is classed as the outer core region, whilst the second heptose (Hep II) can be variably substituted with phosphoethanolamine (PEtn) and/or glucose. Initial studies investigating the immunobiology of LPS in this organism have shown the immunodominance of the PEtn on Hep II.

The genes responsible for the addition of all known sugars to *Nm* LPS have been identified, including those required for the addition of PEtn[1,2,3]. A number of the LPS genes are phase variably expressed due to the presence of homopolymeric tracts[4]. The majority of LPS genes are found clustered together in three regions of the genome, termed Lgt-1, Lgt-2 and Lgt-3[5], however individual strains have different combinations of genes present, contributing significantly to the heterogeneous nature of the LPS expressed by these strains.

Collections of disease and carriage strains have been screened for the presence of the genes encoding the transferases responsible for the addition of PEtn, *lpt3* and *lpt6* and both genes were shown to be present in 36% of all strains screened. Previous studies with monoclonal antibodies which specifically recognise PEtn at the alternative positions had indicated that only three strains produce di-PEtn structures[6,7]. We are currently investigating factors influencing incorporation of one or both PEtn in *Nm* strains where both genes are present and how this pattern of PEtn in the LPS alters the biology of the organism.

P08
Vaccinology

The Discussion Session for the following posters is from:
8.00pm – 8.45pm Tuesday 12 September

P8.1.01

Secreted Proteins of *Neisseria meningitidis* Protect Mice against Infection

Yanwen Li[1], Karl Wooldridge[2], Muhammad Javed[2], Christopher Tang[1], Dlawer Ala'Aldeen[2]

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[2]The Centre for Molecular Microbiology and Infection, Department of Infectious Diseases, Faculty of Medicine, Imperial College London, Nottingham NG7 2UH

In previous studies we demonstrated that meningococcal secreted proteins (MSPs) contain virulence-related proteins that play roles in the pathogenesis of disease. Here, we addressed the hypothesis that MSPs contribute to protective immunity against meningococcal disease. Endotoxin-depleted MSPs were prepared and used to immunise a group of 15 six week-old Balb/c mice (25 µg MSPs/dose mixed with Freund's incomplete adjuvant) on days 0, 14 and 21. The mice were challenged two weeks later with 10^7 colony forming units of live *Neisseria meningitidis* strain MC58 (serogroup B, ET-5). A negative and a positive control group of 15 mice each were injected with adjuvant only and a live attenuated strain of MC58, respectively. Seven out of 15 (46.7%) mice from the negative control group died after 72 h of challenge, whereas none of test and positive control group died. The protection afforded by anti-MSP antibodies can be at least partly attributed to complement-mediated bacterial lysis, detectable *in vitro* using the serum of immunised mice. The murine anti-MSP sera were bactericidal against homologous and five unrelated ET-5 serogroup B strains. However, they failed to kill strains from other hypervirulent clonal lineages belonging to the same or different serogroups, despite the presence of cross-reactive antibodies detectable by immunoblotting. FACS analysis of intact bacteria treated with anti-MSPs confirmed the surface-binding of antibodies. Additional mechanisms of host protective immunity, and broader cross-protection in the human host, could not be ruled out. MSPs have the potential to induce protective immunity against disease, and are worthy of consideration as vaccine candidates.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

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P8.1.02

Characterization of LP2086 Expression in *Neisseria meningitidis.*

Karita Ambrose, Deb Dilts, Kristin Alexander, Han-Qing Jiang, Jim Fulginiti, Susan Hoiseth, and Gary Zlotnick

Wyeth Vaccines Research, Pearl River, NY 10965, USA

LP2086 is a novel outer membrane lipoprotein and is a promising meningococcal vaccine candidate. Antibodies raised against rLP2086 are bactericidal against a broad range of meningococcal isolates and are protective *in vivo* in an infant rat model. Two subfamilies exist for LP2086, and protective antibodies are largely subfamily specific. Based on flow cytometry, electron microscopy, and whole cell ELISA, LP2086 has been localized to the surface of *N. meningitidis*. However, the amount of surface reactivity of polyclonal antisera to LP2086 varies among meningococcal isolates, and is independent of allelic sequences. Strains with identical LP2086 sequences may have different levels of reactivity when analyzed by FACS or whole cell ELISA, suggesting differences in either amount of total protein expressed, surface localization, or accessibility of the protein. In the present study, we correlated surface reactivity with relative quantities of LP2086 in meningococcal isolates and investigated regulatory mechanisms responsible for variation of protein expression. Using whole cell ELISA, over 100 meningococcal clinical isolates were examined for surface reactivity of LP2086 with polyclonal antisera. A continuum of high to low reactivity was observed among strains. A trend towards low surface reactivity along with lower levels of total LP2086 protein was observed when bacterial lysates were examined by Western analyses. Transcriptional analyses by quantitative real-time PCR showed high levels of LP2086 message in a strain that makes large amounts of LP2086 protein, and much lower message levels in strains which had lower whole cell ELISA and Western blot reactivity. Differences in growth media also affected LP2086 protein expression in some strains. Ongoing experiments are aimed at identifying the mechanism behind the differential expression of LP2086.

P8.1.03

Postmarketing Safety of MCV-4

Roger Baxter[1], Steven Black[1], Edwin Lewis[1], John Hansen[1], Greg Gilmet[2], Daniel Gordon[2], Michael Decker[2]

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Introduction: Menactra® ACWY conjugate meningococcal vaccine (MCV-4), was recently licensed in the United States for use in persons aged 11 to 55 years. We present initial postmarketing safety data collected in a large health maintenance organization.

Methods: Recipients of MCV-4 in the Kaiser Permanente database were monitored for adverse events (AEs) beginning with vaccine licensure through December 31, 2005. Rates of AEs at 0-30 days (exposed) and 31-60 days (control) were compared.

Results: Of 26,208 vaccine recipients, 18.005(68.7%) were 11 to 16 years of age. Overall, MCV-4 was well-tolerated, with no new or unexpected AEs reported. Significant more emergency room (ER) visits for abdominal pain were reported at 0-30 days versus 31-60 days (25 vs. 12, RR=2.049 CI 1.039-4.219, p=0.038). Although rates for ER visits for headache, cellulitis and suicide attempts were also higher on days 0-30 versus days 31-60, the number of cases was small. The rate of all cause hospitalization was increased in the 0-30 day period compared to 31-60 days (p=0.048) although this was not attributable to any particular diagnosis. No cases of Guillain-Barré syndrome (GBS) were observed in the observation windows in this population.

Conclusion: MCV-4 was generally well-tolerated, with no cases of GBS in 26,208 vaccine recipients.

P8.1.04

NMR-Based Assay for Tracking the Industrial Process and the Structural Characterization of Meningococcal A, C, W_{135} and Y Conjugate Vaccines

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We are developing a tetravalent polysaccharide-protein conjugate vaccine against *Neisseria meninigitidis* group A, C, W_{135} and Y. Capsular polysaccharides extracted from bacteria are covalently attached to CRM_{197} (a non-toxic diphtheria mutant) to provide T-cell dependent immonogenicity against the saccharide haptens.
High-field NMR spectroscopy has been found to be an extremely and robust tool for tracking the industrial process to manufacture carbohydrate-based vaccines. Qualitative and quantitative NMR methods have been developed for [a] the determination of the identity of the isolated polysaccharide (NMR spectrum provides a fingerprint characteristic of the molecular structure), [b] the quantification of labile groups which may be crucial for immunogenicity (e.g. O-acetyl content), [c] the identification of end groups as markers of depolymerisation of the polysaccharide chains (e.g. phosphomonoester group at the non-reducing residue of meningococcal group A oligosaccharide), [d] the characterization of activated intermediates in vaccine manufacture (e.g. reducing end activation by adipic acid diester; oxidation of suitable diol systems along the saccharide chain), [e] the monitoring of the conjugation process by analyzing the polysaccharide-protein ratio and the polysaccharide degradation to determine the product stability, [f] the quantification of NMR-sensitive residual process contaminants to estimate the product purity.
Although there is little published data in the field of glycoconjugate vaccines, in accordance with regulatory requirements NMR spectroscopy may already provides an appropriate commercial option as a routine release test, in addition to other applications usually performable in the discovery and development phase. For several purposes of quality control used in the manufacture of carbohydrate-based vaccines, these methods provide preferable alternatives to the currently colorimetric and chromatographic assay due their simplicity, specificity and reproducibility.

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The structural characterization by NMR is also important for predicting and evaluating the quality of the vaccines in alternative to immunonolgical experiments in animal models, and particularly useful to apply product comparability studies.

P8.1.05

The B Cell Response to a Booster Dose of MenC Vaccine at 1 Year of Age After Three Dose Priming in Infancy

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Introduction: A 3-dose primary series of immunisations with MenC vaccine induces a rise in MenC antibody but does not result in sustained serum bactericidal activity, and, at the population level, effectiveness of the vaccine wanes after a year. Despite these observations a booster response to further antigenic exposure persists for some years after primary immunisation, indicating the existence of immunologic memory.

In order to describe the phenotype and kinetics of the MenC-specific B cell compartments involved in the immune response in infants, we assessed the persistence of MenC specific memory B cells in children at one year of age, after priming with three doses of MenC at 2, 3 and 4 months of age. Furthermore, we evaluated the kinetics of the MenC-specific memory B cell and plasma cell response to a booster dose of vaccine at a year of age.

Materials and Methods: 34 healthy children primed with MenC vaccine at 2, 3 and 4 months of age received a booster dose of MenC vaccine at one year of age. Blood samples were obtained before the booster dose and then at various days (2, 4, 6, 8, or 9) after the immunisation and then finally at day 30. The number of MenC specific plasma and memory B cells were estimated using a B cell ELISpot at each time point.

Results: We found limited evidence of the presence of memory B cells in peripheral blood at the age of one year, prior to administration of a booster dose of vaccine. However a rapid rise in both plasma cells and memory B cells was detected after the booster dose with a peak plasma cell response in the first week after immunisation.

Conclusion: Polysaccharide-specific memory B cells persist after primary immunisation, even if they are not evident in peripheral blood. After a booster dose of vaccine they rapidly appear in the blood along with antibody-producing plasma cells.

P8.1.06

A Phase I, Double Blind, Randomized Study to Evaluate a New Meningococcal Group A Conjugate Vaccine in Healthy Indian Adults.

Ray Borrow,[1] George Carlone,[2] Nilima Kshirsagar,[3] Naidu Mur,[4] Urmila Thatte,[5] Varsha Parulekar,[6] Brian Plikaytis,[2] Prasad Kulkarni,[7] Nathalie Imbault,[8] Cheryl Elie,[2] Helen Findlow,[1] Julie Chatt,[2] Paul Balmer,[1] Joseph Martinez,[2] Helen Swift,[1] Scott Johnson,[2] Nancy Rosenstein Messonnier,[2] Elisa Marchetti,[8] Marie-Pierre Preziosi,[8] Simonetta Viviani,[8] Marc LaForce[8]

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Background: The annual incidence of disease during meningococcal group A epidemics in sub-Saharan Africa can exceed 1000 per 100,000 population. A vaccine that confers durable protective immunity after 1 dose, induces herd immunity and is affordable for widespread use in Africa is required.

Methods: A Phase I, double blind, randomized study was conducted to evaluate the safety and immunogenicity of a new *meningococcal* group A conjugate vaccine (PsA-TT; 0.5 ml contains 10µg Ps, 10-20µg TT, and adjuvant [$AlPO_4$]) versus a *meningococcal polysaccharide A+C vaccine* (reference) and a *Tetanus Toxoid-adsorbed vaccine* (control). Single intramuscular injections were administered to 74 healthy Indian adults from 18 to 35 years of age. The primary objective was to evaluate safety of a single injection of PsA-TT during 4 weeks post–vaccination, with comparison to a reference vaccine and to a control vaccine. The secondary objectives were to assess the week 4 immune responses in terms of *serum bactericidal antibody (SBA)* activity and anti–polysaccharide group A (anti-PsA) IgG responses and to determine the persistence of responses at weeks 24 and 48; antibody persistence testing is ongoing. Safety was assessed using well established criteria and metrics.

Results: All local and systemic solicited adverse events and unsolicited adverse events associated with PsA-TT were mild and transient in duration. No serious adverse events were reported up to 10-month follow-up. High but similar sero conversion rates were observed for the Meningococcal group A conjugate vaccine and Meningococcal A+C polysaccharide reference vaccine as measured by SBA and group A-specific IgG ELISA.

Conclusions: The meningococcal group A conjugate vaccine was safe and immunogenic in Indian adults and allows for progression to phase II studies.

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P8.1.07

Immunogenicity and Reactogenicity of a Combined *Haemophilus influenzae* type b and *Neisseria meningitidis* Serogroup C and Y-Tetanus Toxoid Conjugate (Hib-MenCY-TT) Vaccine Administered at 2, 3 and 4 Months and as a Booster dose at Second Year of Life

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Background and aims: To assess the immunogenicity and reactogenicity of primary (792014/003) and booster (100381) vaccination with three different formulations of a novel Hib-MenCY-TT conjugate vaccine co-administered with DTPa-HBV-IPV (*Infanrix penta*™).

Methods: In this phase II study, 76 infants were primed with Hib(2.5µg)–MenC-TT(5µg)–MenY-TT(5µg), 73 with Hib(5µg)-MenC-TT(10µg)–MenY-TT(10µg) and 77 with Hib(5µg)-MenC-TT(5µg)–MenY-TT(5µg) coadministered with DTPa-HBV-IPV. As control, 76 infants were primed with *Menjugate*™ coadministered with DTPa-HBV-IPV/Hib (*Infanrix hexa*™). After priming (2-3-4-months-schedule), a booster dose of the same vaccines was given in a subset at 12-18 months of age in each group (45,42,44,43 subjects respectively). Antibody concentrations/titres were measured one month post-dose_3, prior to and one month after booster dose. SAEs, solicited (local/general) and unsolicited symptoms were recorded.

Results: Post-dose_3, antibodies against Hib (anti-PRP≥1µg/ml), were observed in ≥98.5% of Hib-MenCY-TT-treated infants; a significantly higher percentage than in the Menjugate control group (80.3%); rSBA-MenC ≥1:128, observed in 94.0-95.7% of Hib-MenCY recipients, was statistically similar to control subjects for the Hib(2.5µg)–MenC-TT(5µg)–MenY-TT(5µg) formulation (difference between groups:4.3%(95%CI:**-1.0**,11.9)); 86.4%-95.8% of Hib-MenCY subjects had rSBA-MenY≥1:128. Prior to booster administration, 95.6%-100% and 90.5%-97.4% of toddlers had persistent protective anti-PRP antibodies (≥0.15µg/ml) and rSBA-MenC titres(≥1:8), respectively (similar to *Menjugate*™). In all three Hib-MenCY groups, 79.5%-85.7% of subjects presented SBA-MenY ≥1:8.

The booster dose induced a robust increase in anti-PRP concentration and rSBA-MenC/Y titres with anti-PRP≥1µg/ml and rSBA-MenC≥1:128 similar to that of the control group (100% each).

No SAE related to vaccination, as judged by the investigator, was reported. The incidence of solicited symptoms with each Hib-MenCY conjugate formulation was similar to or lower than that in the control group, with the Hib(2.5µg)–MenC-TT(5µg)–MenY-TT(5µg) tending to be the least reactogenic.

Conclusion: The new Hib-MenCY-TT formulations given as 3 doses primary vaccination at a 2-3-4-months-schedule with a booster dose in the second year of life were highly immunogenic and well-tolerated.

P8.1.08

A novel DTPw-HBV/Hib-MenAC Conjugate Combination Vaccine Administered to Infants in Northern Ghana is Safe and Induces Immune Memory

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*Background:*New meningococcal conjugate vaccines including serogroup A could reduce the burden of the disease in the meningitis belt in Africa. Combining meningococcal conjugates with vaccines routinely administered in infants will improve coverage and cost effectiveness.

*Methods:*A double blind, randomized (1:1), controlled study (studyID:104430; ISRCTN:35754083) to evaluate persistence of the immune response, immune memory and safety in 254 toddlers aged 12 months (127 per group) primarily vaccinated (6,10,14-weeks schedule) with DTPw-HBV/Hib-MenAC (study vaccine) or DTPw-HBV/Hib (control vaccine), was carried out in Northern Ghana, following the Expanded Programme of Immunization (EPI). Toddlers were evaluated for antibody persistence and immune memory (by giving a challenge dose of serogroup A and C polysaccharides).

*Results:*Prior to challenge dose, %subjects primed with DTPw-HBV/Hib-MenAC with persistent SBA-MenA titers≥1:8 (47.3%) and with SBA-MenC≥1:8 (56.8%) were significantly higher than in the control group (25.7 and 5.1% respectively). Persistence of the other vaccine antigens was good (>85%).

One month after polysaccharide challenge, a robust increase in SBA-MenA and SBA-MenC GMT (≥14 fold) was observed in the vaccine group, higher than in the control group (≤3.8 fold), with %subjects with SBA titers≥1:8 (MenA:93.5%; MenC:88.5%) higher than in the control group (MenA:51.4%; MenC:18.2%).

59 SAEs including 8 deaths (37 SAEs, 5 deaths in DTPw-HBV/Hib-MenAC group; 22 SAEs, 3 deaths in controls) were reported from study entry at 6 weeks of age up to end of the challenge phase, none of them reported as related to vaccination.

*Conclusions:*Three-dose primary vaccination with DTPw-HBV/Hib-MenAC induced similar persistent immune response as the routinely used DTPw-HBV/Hib vaccine; the MenA and MenC antigens induced persistent antibodies in approximatory half of the infants, an excellent memory response and a booster with these antigens is recommended for long-term protection. This vaccine provides protection against 7 important childhood diseases (including meningococcal A and C) in a single injection.

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P8.1.09

Preparation and characterization of murine monoclonal antibodies against three antigens of Group B *Neisseria meningitidis*

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Background Genome sequence of MC58, a *Neisseria meningitidis* serogroup B (MenB) strain, allowed the identification of novel surface-exposed antigens able to induce bactericidal antibodies. Five of them have been selected for developing a vaccine against Meningococcus B, a bacterium for which no vaccine is available.
To characterize the antigenic and protective epitope of the selected proteins, a program for raising murine monoclonal antibodies against each antigen is ongoing. In the present work we describe the Mabs, which have been selected against the proteins: Nad A, GNA2091, GNA1030.

Methods The selection of the Mabs was based on their different behaviour in different assays in order to identify Mabs directed against different epitopes of the antigens. The mAbs have been characterized by ELISA, Fluorescence-activated cell sorter (FACS), western blotting on the purified protein and on total cell extracts from different Neisseria strains, and for bactericidal activity, using baby rabbit as complement source.

Results Five monoclonal antibodies against NadA have been selected. Western blot analysis on purified deletion domains of NadA suggested that they recognize epitopes located on different portions of the protein. Two of them have bactericidal activity. Two mAbs against each GNA2091 and GNA1030 have been also selected and used in comparison with the polyclonal antibodies to evaluate the expression of the antigens in a panel of MenB strains.

Conclusion The use of monoclonal antibodies to identify immunogenic epitopes of the antigens can allow to better define the immunological properties and give us indications about the structure of this vaccine antigens.

P8.1.10

Development of a Multiplex Assay using X-MAP Technology for the Evaluation of Protein-Based Meningococcal Vaccines.

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In the absence of a group B vaccine component, there is currently no vaccine offering comprehensive protection against meningococcal disease. However, vaccine candidates based on outer membrane vesicles (OMVs) or combinations of protein antigens at least have the potential to protect against the prevalent virulent meningococcal clones. The evaluation of immune responses to individual antigens in such vaccines is a complex task, requiring multiple assays and more serum than for simpler vaccines. To overcome this problem, a multiplex antibody binding assay, based on x-MAP technology (Luminex Corp.), has been developed. The assay offers distinct advantages over ELISA: it allows the detection of multiple analytes simultaneously, requires less serum, and is potentially more sensitive because of the wider dynamic range that can be achieved.

This poster describes the development of an x-MAP assay to measure the antibody response to between different antigenic variants of PorA. Seven different recombinant, His-tagged PorA proteins have been produced as inclusion bodies in *E. coli*. These proteins were refolded, further purified by nickel affinity chromatography and then used to coat different Ni-NTA conjugated microspheres, each of which is distinguishable by its unique fluorescent signature. The resulting 7-plex antibody assay proved to be specific and reproducible. It has been used to compare the anti-PorA antibody responses to two OMV vaccines, MenBvac® and MeNZB®. The former is directed exclusively to the homologous P1.7,16 variant, whereas the latter is cross-reactive with a number of heterologous PorA variants.

The multiplex assay described here is currently being extended to include FetA variants and could be further extended to incorporate other vaccine antigens, including those developed from genome mining projects.

P8.1.11

Protection by Immunization with Alphavirus Replicon Particles against Gonorrhea in a female Mouse Model

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Sexually transmitted infections caused by antibiotic resistant *Neisseria gonorrhoeae* are on the rise. While vaccination represents the most effective control measure of infectious diseases, there is no vaccine for gonorrhea. We tested immunization regimens utilizing gonococcal outer membrane protein, PorB, for protection against lower genital tract infection in the estradiol-treated female mouse model. The regimens included re-natured recombinant (rr) PorB delivered subcutaneously in the dorsal area or the hind footpad with Ribi adjuvant; PorB-expressing Venezuelan equine encephalitis (VEE) virus replicon particles (PorB-VRP) delivered in footpad, with and without a boost of rrPorB; and VRP expressing an irrelevant influenza hemaglutinin peptide (HA-VRP), boosted once with rrPorB.

Female BALB/c mice were challenged with piliated, transparent FA1090 after being immunized three times. Protection was defined as shortening of gonococcal colonization, compared to the duration in the phosphate buffer saline immunized controls. Mice immunized with rrPorB, dorsal or footpad route, were not protected. Mice immunized three times with PorB-VRP exhibited a trend toward protection (*P*=0.105). A group vaccinated twice with PorB-VRP and boosted with rrPorB showed significant protection (*P*=0.003), but a repeat trial resulted only a trend to shortened colonization (*P*=0.09). Unexpectedly, the group vaccinated with HA-VRP and boosted with rrPorB showed strong protection (*P*=0.015).

Our results demonstrated a correlation of protection with the use of VRP vector and the resulting increased IFN-γ production by splenocytes. Protection was not correlated to levels of PorB antibodies, although PorB-VRP biased serum IgG1/IgG2a ratio toward Th1. We conclude that a non-PorB specific protective effect was generated by regimens that induced an IFN-γ, Th1-biased inflammatory response. Work by others has demonstrated a mucosal and systemic adjuvant activity of VRPs. VEE derived virus replicon particles offer promise as a platform for delivering vaccines for gonorrhea.

P8.1.12

Investigation of the Protective Potential of Antibodies Directed against Surface-Exposed Opacity Protein Loops of *Neisseria gonorrhoeae*

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The design of an effective gonorrhea vaccine is challenged by gonococcal surface variability and a lack of information regarding correlates of protection. The neisserial opacity (Opa) proteins are antigenically distinct outer membrane proteins with four surface-exposed loops, including a semi-variable loop (SV), hypervariable loops (HV_1 and HV_2), and a conserved loop (4L). Here we hypothesized that antibodies (Abs) against the more conserved loops might protect against gonococcal colonization. Affinity-purified polyclonal rabbit Abs against linear peptides that correspond to the SV, 4L, and HV_2 loops of strain FA1090 were tested for activity against *N. gonorrhoeae*. Ab_{sv} and Ab_{4L} were not bactericidal and did not bind the surface of Opa-expressing gonococci as determined by indirect fluorescent antibody staining. In contrast, Abs specific for the HV_2 loops of strain FA1090 (HV_{2A}, $HV_{2B/D}$, HV_{2C}, HV_{2F}) bound the bacterial surface and were bactericidal against homologous Opa variants (bactericidal$_{50}$ titers 1:25-1:70). In preliminary experiments designed to test the capacity of loop-specific Abs to prevent colonization, increasing numbers of OpaB-expressing gonococci were incubated with saline, Ab_{SV}, or $Ab_{HV2B/D}$ [10^2, 10^4, and 10^5 CFU/µg immunoglobulin (Ig)] prior to intravaginal inoculation of mice. Although the number of mice was too low to assess significance, fewer mice (3/8) were colonized in the $Ab_{HV2B/D}$ -treated group compared to the Ab_{sv}-treated group (7/8) when 10^2 CFU/µg Ig was used. The average colonization load in the $Abs_{HV2B/D}$ -treated groups was also lower than that of the PBS or Ab_{SV}-treated groups when 10^2 or 10^4 CFU/ µg Ig were used [average log_{10}CFU/vaginal swab suspension: 2.95 (Ab_{sv}) and 0.72 ($Ab_{HV2B/D}$); 4.2 (Ab_{sv}) and 2.0 ($Ab_{HV2B/D}$), for 10^2 and 10^4 CFU/µg Ig, respectively]. This difference was not apparent in the 10^5 CFU/µg Ig groups [3.7 (Ab_{sv}) and 3.9 ($Ab_{HV2B/D}$)]. The suggestion that HV_2-specific Abs may be partially protective at high concentrations warrants further investigation.

P8.1.13

Mucosal immunization against Meningococci: Induction of Bactericidal Antibodies and Cellular immunity in C3H/HeJ and C3H/He Pasteur mice following Intranasal immunization with Native Outer Membrane Vesicles

Aline Seneme Ferraz[1] , Monamaris M Borges[2], Mariana L Teixeira [1] ,Marta de Almeida[1] ,Claudia F Tunes [1], Andréia M dos Santos Carmo[1], André Yoshio Ito[1], Simone Néri[1], Verônica dos Santos[1], Ligia Bozzoli[1], Tatiane Ferreira[1],Túlio N Cunha[1], Elizabeth De Gaspari[1]

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Our goal is to develop a vaccine to prevent *Neisseria meningitidis*. We used native outer membrane vesicles (NOMV) of one selected epidemic strain that express L3,7,9 and L8 immunotype intranasally in mice. A response difference to bacterial lipopolysaccharides (LPS) has been reported in strains of C3H mice. In ours studies we used C3H/HeJ mice, low LPS responder and C3H/He Pasteur designated as high LPS responder. We measured the level of the antibodies produced the specificity and the pattern of cytokines.

NOMV of the Brazilian epidemic strain was extracted with Tris-saline EDTA buffer at pH 7,5 followed by differential centrifugation and ultrafiltration. The antigen preparation was analysed for composition by chemical assays, SDS-PAGE and Immunoblot with a panel of monoclonal antibody. Immunogenicity with or without aluminium hydroxide adjuvant was tested in C3H/HeJ and C3H/He Pasteur by i.m (20µg or i.n. with 100µg), purified L3,7,9 or L8 (2µg) by i.n. The secretion of Interferon γ, interleukin-2 (IL-2), IL-4,IL-5, IL-6, IL-10 and IL-12 by specific antigen stimulated splenic cells were determined at week 12 after immunization. IgG, IgM and IgA antibodies were determined by ELISA . The specificities of these antibodies were analysed by Immunoblot against NOMV. Per milligram of protein the NOMV contained 47µg LOS, and 17µg scialic acid. Analysis by SDS-PAGE showed the presence of Por A, PorB, Rmpm, Opa, Opc 50 kDa and iron regulated protein as a major components. Bactericidal antibodies titers were generated. The mixed Th1/Th2 responses were consistent with the detection of both IgG1 and IgG2a antibodies in the sera of mice immunized with NOMv of *N.meningitidis* .

The induction of humoral and cellular immunogenicity in C3H mice lead to undertimation of vaccine responses against the strain of meningococcal selected for the present study.

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P8.1.14

Immunogenicity of intranasally administered class 5C protein of *Neisseria meningitidis* in mice with different Adjuvants

Andréia M dos Santos Carmo [1], Marta de Almeida[1] ,Claudia F Tunes[1], André Yoshio Ito[1], Aline Seneme Ferraz[1], Simone Néri[1], Mariana L Teixeira[1], Verônica dos Santos[1]. Ligia Bozzoli[1], Tatiane Ferreira[1], Túlio N Cunha[1], Elizabeth N De Gaspari[1]

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The anti meningococcal vaccines produced from the meningococcal capsular polysaccharides are effective against the serogroups A and C, however they are few immmunogenic in children below two years and, in other ages, the response is of short duration.

Colonization of the human nasopharyngeal region by Neisseria meningitidis is believed to lead to natural immunity.This study investigated the immunogenicity of intranasally administered class 5C protein of *Neisseria meningitidis* B employing purified LPS (L3,7,9 or L8), cholera toxin and whole cells of *Bordetella pertussis* as adjuvants followed by a intramuscular booster in the presence of aluminum hydroxide. Sera of immunized mice were evaluated by ELISA and Immunoblot to detect the presence of specific IgG, IgM and IgA antibodies.

We also verified the avidity index and bactericidal activity of them. Expressive titers of IgG and IgM were detected in the serum of mice after the immunization, with avidity indexes that varied from intermediary to high which were correlated with the bactericidal activity. All adjuvants were capable to increase the immune response against the class 5C protein; however, LPS as adjuvant presented the best results.

The intranasal route was capable to sensitize the cells of the immune system which were quickly stimulated by the intramuscular route. Data suggest that the class 5C protein is important in the induction of mucosal immunity to *N. meningitidis* B and the quality and magnitude of the immune responses generated by mucosal vaccines are influenced by the antigen as well as the adjuvant. These results indicate that i.n. delivery of meningococcal 5C protein in mice is highly effective in eliciting the production of both a mucosal immune response and a systemic bactericidal antibody response.This report describes for the first time the study of the immunogenicity of the purified 5C protein administered intranasally.

P8.1.15

Identification of a surface protein in Neisseria commensal strains and evaluation of its immunogenic and protective capacity

Marta De Almeida, Claudia Tunes, Mariana Teixeira, Andréia Carmo, Tatiane Ferreira, André Ito, Simone Néri, Tulio Cunha, Ligia Bozzoli, Elizabeth De Gaspari

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N. meningitidis and *N. lactamica* are related gram-negative bacteria, which colonize the human nasopharynx, but differ in the final outcome. Immunological and epidemiological evidences suggest that the development of natural immunity to meningococcal disease results from colonization of the nasopharynx by commensal *Neisseria* sp., as *N. lactamica*. We have conducted studies using an intranasal mouse model to examine the immunogenicity of *N. lactamica* delivered via intranasal route. Live or heat inactivated *N lactamica* isolated from the oropharynx were delivered intranasally (i.n) to BALB/c mice to an OD of 0.5 at 650 nm in a 20-µl volume. Mice were immunized four times at 7-day intervals. In addition, strains of *N. lactamica* were isolated from the oropharynx of health carriers and *N. lactamica*, *N. subflava, N. elongata, N. sicca, N.perflava, N.mucosa* were isolated from spinal fluid or blood. We have established an adult mouse intranasal challenge model for group B *N. meningitidis* to evaluate potential vaccine candidates through active immunization. BALB/c mice were inoculated intranasally with meningococci. This model has been utilized to evaluate the potential of *N.lactamica* protection to group B meningococcal epidemic strain. MAb was analyzed by ELISA with whole cells of a homologous *N. meningitidis* strain and commensal strains. Mice developed levels of specific reactivity serum IgG antibodies as determined by ELISA using whole cells of homologous and heterologous strains. Immunoblot analysis of mice sera immunized with live or heat inactivated *N. lactamica* showed IgG antibodies that responded to peptides in the 30-65 kDa range. The antibodies present in the sera of mice immunized with live or heat inactivated *N. lactamica* fail to induce bactericidal activity against *N. meningitidis* strains, however, activity could be observed with antibodies of mice immunized intranasally with (NOMVs) of *N. meningitidis* or *N.lactamica* after booster. The 8C7Br1 MAb recognized a 50-65 kDa peptide by immunoblot using NOMV, with the commensal strains.

P8.1.16

Improved Purification of Native meningococcal Porin (Por B) and Studies using Intranasal route in Neonates mouse

Mariana L Teixeira [1] ,Marta de Almeida[1] ,Claudia F Tunes[1], Andréia M dos Santos Carmo[1], André Yoshio Ito[1], Aline Seneme Ferraz[1], Simone Néri[1], Mariana L Teixeira[1], Verônica dos Santos[1], Ligia Bozzoli[1], Tatiane Ferreira[1],Túlio N Cunha[1], Elizabeth N De Gaspari[1]

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Porins are the major proteins in the outer membrane of Gram-negative bacteria, such as *N. meningitidis*.The meningococcal porins from serogroups B have been pointed as candidates for vaccines composition.The present study aimed to investigate the use of highly purified PorB and *Bordetella pertussis* (Bp) as adjuvant, in intranasal (i.n.) immunization of neonates mouse.

Gel-filtration chromatography on Sephacryl 300 resin and Sepharose 4B-monoclonal antibody were used to purify PorB (serotype15).These chromatographies efficiently separe PorB from the majority of residual protein contaminants,as lipoprotein and LPS.A 38 KDa peptide was analyzed by SDS-PAGE,and 5µg of PorB plus $1X10^4$ Bp were administered i.n into the pup's nare of neonates mouse. A final volume of 5µl was gradually introduced with a micropipette in four groups of animals that received 1,2,3 and 4 doses of antigen in 3,5,7 and 9 days after birth.

At the 35th days the animals were immunized intramuscullary (i.m) with Por B and [Al(OH)3]. IgG,IgM and IgA specific antibodies were detected in group of mice after received two doses of antigen in 3,5 days.The ELISA conducted in sera from i.n. immunized neonates mice (median of optical density) showed an increase in the titer of IgG(1,2% above the value of normal serum),IgM(7,6%),IgA(0,1%), contrasting with the i.m booster that resulted in high levels of IgG (32,2%),IgM (8,6%),IgA(0,2%).It was not detected IF-γ in the spleens of mice stimulated with PorB protein,even in the presence of Bp used as adjuvant.

The prime-boost immunization shemes employing a Por B protein and Bp as adjuvant at priming and booster i.m is a competent immunization protocol to induce humoral immune responses against PorB protein in neonates model.This study shows for the first time that intranasal immunization of newborn mice with PorB protein and BP as adjuvant induce IgG and IgM antibodies in neonatal mice.

P8.1.17

Antibody Response in Rabbits Intranasally Aministered with Neisseria commensal strains

Claudia F Tunes [1] Marta de Almeida [1], Andréia M dos Santos Carmo[1], André Yoshio Ito[1], Aline Seneme Ferraz[1], Simone Néri[1], Mariana L Teixeira[1], Verônica dos Santos[1]. Ligia Bozzoli[1], Tatiane Ferreira[1], Túlio N Cunha,T[1], Elizabeth N De Gaspari[1].

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Neisseria lactamica, a commensal bacterium non-pathogenic to human beings and usually found in the upper respiratory tract of children, is closely related to pathogenic *Neisseria meningitidis*. Colonization with *N. lactamica* can be responsible for evolving natural immunity to meningococcal infection in childhood, when rates of meningococcus carriers are low. These features lead to suggest that *N. lactamica* components can be key-elements in the production of a new vaccine for *N. meningitides.* As little is known about dynamic carriers and *N. lactamica* population diversity in children, it has been difficult choosing a representative for preparing an adequate immunogenic product.

A protocol was proposed to study immunogenicity of *whole cells* of *N. lactamica*, *N. meningitidis*, *N. sicca* or N. *meningitides c* (carrier-isolated) by i.n. immunization in rabbits considering the natural pathogen entry route. Oropharinx-isolated *N. lactamica*, *N. meningitidis*, *N. sicca,* or *N. meningitides c* were i.n. inoculated into adult rabbits, in a concentration of optical density 1.0 at 650nm in a volume of 500 µL.

The rabbits were immunized four times at seven-day intervals. *N. subflava, N. elongata, N. sicca, N. perflava, N. mucosa* strains isolated from CSF and blood from patients were also used. The rabbits developed levels of specific IgG antibodies in serum, as determined by ELISA using *whole cells* of homologous and heterologous strains. Serum from rabbits immunized with *N. lactamica*, *N. meningitidis*, and *N. sicca* or *N. meningitides c,* presented IgG antibodies reactive to 5 to 130 kDa antigens on immunoblot. Antibodies in serum from rabbits immunized with *N. lactamica* failed to induce high concentration of antibodies with bactericidal activity against *N. meningitidis*; however, this activity could be observed with antibodies produced by rabbits i.n. immunized with *N. meningitidis.*

High avidity IgG antibodies were produced. Intranasal immunization of *N.lactamica whole cells* was suitable to efficiently sensitize mucosal immune system in rabbit model.

P8.1.18

MenA Natural Immunity and SBA Tests with A:L10 and A:L11 Target Strains

Isabel De Vleeschauwer, Vincent Weynants, Nathalie Durant, Dominique Boutriau, Jan Poolman

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In a clinical study performed in the Philippines infants were primed with a heptavalent DTPw-HBV/Hib-MenAC conjugate vaccine at 6, 10, 14 weeks of age, while 2 non-primed groups were added as a control. At 10 months of age, before the administration of the polysaccharide booster doses, 76.5% of the subjects had rSBA-MenA titres ≥ 1:8 in the control groups suggesting the development of naturally induced bactericidal antibodies. However, only 6.7% had anti-PSA IgG ≥ 0.3 µg/ml. It can be hypothesized that the difference is due to the presence of non-PSA antibodies induced by carriage of non-pathogenic *Neisseria*. The F8238 strain as recommended for *N. meningitidis* serogroup A was employed in the MenA rSBA test. Review of the literature seems to indicate that the majority of carriage strains express at least the L11 immunotype while most invasive strains express the L10 immunotype. Surprisingly, using the SGIP-Ouchterlony immunodiffusion technique performed by C. Hopman (University of Amsterdam, department of Medical Microbiology, Amsterdam, Netherlands), the F8238 strain appeared to be of the L11 immunotype and hence not representative of invasive strains. Therefore a MenA strain of L10 immunotype, more specifically the 3125 strain, was used in the MenA rSBA assay. 56 samples from the control groups were retested (n=23 in the DTPw-HBV + Hib primed group, n=33 in the DTPw-HBV/Hib + MenC group), 66% and 14% of the subjects had MenA rSBA titres ≥ 1:8, using the F8238 and the 3125 strain, respectively. These results suggest that the use of an assay strain with an L10 immunotype may be helpful to avoid the measurement of the A:L11 specific natural immunity and to focus on the probably more clinically relevant A:L10.

POSTER PRESENTATION ABSTRACTS

15th International Pathogenic Neisseria Conference 2006 (IPNC 2006 Australia)

As at 31 July 2006; Refer to the Addendum for any program updates

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P8.1.19

Natural Immunity to *Neisseria meningitidis* Serogroup C during the First Year of Life - Placental Transfer of Naturally Acquired Antibodies to Meningococcal Serogroups from Mother to Child

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Introduction
Vaccination against *Neisseria meningitidis* serogroup C (MenC) was introduced into the Dutch Vaccine Program in 2002 as a single dose at the age of 14 months. In addition, in a catch-up campaign all children and adolescents up to 18 years were vaccinated (vaccine coverage of 94%). This vaccination campaign has been very effective; no MenC cases are reported since among the vaccinated population. Due to this vaccination strategy current protection in the first year of life is based on: maternal Immunity, herd immunity and/or natural immunity.

Aim
Characterization of maternal antibody transfer of anti-meningococcal antibodies can give insight into the role of immunity in the protection of infants to meningococcal disease. Besides the antibody responses to MenC, the responses to the other serogroups (A, Y and W-135), to *N. lactamica* and to Diphtheria and Tetanus toxins are also measured in order to investigate differences in the maternal transfer of antibodies directed against polysaccharides or proteins. In addition, studying these antibody levels at 3, 11 and 12 months of age will provide information about naturally acquired immunity to Meningococci in the first year of life, in particular MenC.

Methods
Measurement of specific IgG antibodies to the Meningococcal polysaccharides and the IgG subclass distribution are performed using Luminex technology or ELISA. Responses to the OMPs of MenC or cross-reactive antibody responses are measured with whole cell ELISA using *N. meningitidis* en *N. lactamica* strains and functional antibodies with SBA.

Results
Preliminary results show that variable, but in general low levels of MenC-specific IgG antibodies are present in maternal delivery serum and in cord blood. At 11 months of age no or almost no MenC-specific antibody levels can be detected anymore. These data would suggest that there is no natural exposure to *N. meningitidis* or other commensal *Neisseria* species or that exposure does not contribute substantially to development of naturally acquired immunity during the first year of life.

The Discussion Session for the following posters is from: 8.45pm – 9.30pm Tuesday 12 September

P8.2.01

Comparing the Avidity of the IgG response to *Neisseria meningitidis* Group C Polysaccharide Conjugate Vaccine by Inhibition ELISA or Chaotropic ELISA

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Antibody avidity, which is the strength of the multivalent interaction between antibodies and their antigens, is an important characteristic of protective immune responses. We have developed an inhibition ELISA to measure antibody avidity to the capsular polysaccharide (PS) of *Neisseria meningitidis* group C (MnC) and determined the avidity constants (K_D values) for 100 sera from children immunized with a MnC PS conjugate vaccine. The avidity constants were compared to the avidity indices (AI) obtained for these same sera using a chaotropic ELISA protocol. After the primary immunization series, the geometric mean (GM) K_D value was 674 nM and did not change in the months following immunization. However, avidity did increase after the booster dose (GM K_D 414 nM one month after booster immunization). In contrast, the GM AI increased from an initial value of 118 after the primary immunization series to 147 seven months after the completion of the primary immunization series and then further increased to 178 after booster immunization. At the individual subject level the avidity constant and avidity index correlated after the primary immunization series and after booster immunization. There was no correlation seven months after the primary immunization series prior to booster immunization. This work suggests that the AI, as measured by the chaotropic ELISA, in contrast to the K_D, detects changes that render antibody populations less susceptible to disruption by chaotropic agents without directly affecting the strength of the binding interactions.

P8.2.02

Outer Membrane Vesicles Expressing L3,7 or *lgtB* LOS but not *galE* LOS Induce Cross-Protection in Mice

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Human convalescent sera have shown the presence of bactericidal antibodies directed against the lipooligosaccharide (LOS) of *Neisseria meningitidis* (Nmen). In infants anti-LOS IgG has also been detected suggesting that in vivo LOS is an immunogenic component.

Classically, outer membrane vesicles (OMVs) are purified using detergents such as deoxycholate (DOC). Varying the amount of DOC used in the preparation of OMVs, we are able to control the

content of LOS. Different preparations of OMVs were produced from genetically modified serogroup B H44/76 Nmen strains expressing either the L3,7 LOS or truncated versions such as *lgtB* and *galE* LOS.

Mice were immunized with these different OMV preparations and sera were analyzed by ELISA and by serum bactericidal assay (SBA) using baby rabbit complement. ELISA data have demonstrated that *galE*, *lgtB* and L3,7 LOS were immunogenic. The SBA results have shown that *galE* OMVs did not induce the production of bactericidal antibodies whatever the concentration of LOS in OMVs. Absence of bactericidal antibodies was also observed in the sera of mice immunized with OMVs containing low concentration of either L3,7 LOS or *lgtB* LOS. Only OMVs with high content of L3,7 or *lgtB* LOS elicited the production of bactericidal antibodies able to mediate the complement killing of not only the parental wild type strain H44/76 but also all other L3 strains tested.

We have also demonstrated that the level of LOS sialylation of the strains used in SBA did not impact the capacity of anti-L3,7 and anti-*lgtB* LOS sera to induce complement mediated killing of the serogroup B Nmen strains.

P8.2.03

Three cases of invasive meningococcal disease in Burkina Faso caused by a capsule null locus strain circulating among healthy carriers

Helen Findlow[1], Ulrich Vogel[2], Judith Mueller[3], Alan Curry[5], Berthe-Marie Njanpop-Lafourcade[3], Heike Claus[2], Steve Gray[4], Seydou Yaro [6], Yves Traoré[7], Lassana Sangaré[8], Pierre Nicolas[9], Brad Gessner[3], Ray Borrow[1,4]

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[8]Centre Hospitalier Universitaire Yalgado Ouédraogo, Ouagadougou, Burkina Faso
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During reinforced surveillance of acute bacterial meningitis in Bobo-Dioulasso, Burkina Faso, three meningococcal strains of phenotype NG:NT:NST by conventional serological typing were isolated from the CSF samples from 2 patients in 2003 and 1 patient in 2004. The strains were negative for the *ctrA* gene conserved among encapsulated meningococci, but positive for the *crgA* gene. Further molecular typing revealed that the strains harboured the capsule null locus (*cnl*) and belonged to the multilocus sequence type (ST)-192, previously not associated to *cnl*. PorA sequencing showed that all strains were P1.18,42 or P1.18,42-1 and negative for the FetA receptor gene. Serum killing assays showed these strains to be resistant to normal human serum comparable to a fully capsular serogroup B strain, MC58. Lipooligosaccharide (LOS) studies showed that LOS sialylation did not contribute significantly to the serum resistance of these strains. The same strains were found in 15 healthy carriers in the general population (age 4-29 years) of Bobo-Dioulasso in 2003 (100% of ST-192 isolates tested for *cnl*). All vaccines currently available in sub-Saharan Africa are based on capsular immunity. Thus, the presence of *cnl* meningococci that can escape serum killing and cause invasive disease, and occur in association with substantial carriage in the same population, is of concern for future vaccination strategies and should promote rigorous surveillance of *cnl*-meningococcal disease. Further studies on the attack rate in relation to asymptomatic carriage and the association with complement deficiency are required.

P8.2.04

Characterisation of the Antigenic Components and Immune Responses to a Vaccine Based on *Neisseria lactamica* Outer Membrane Vesicles

Michelle Finney[1], Jun Wheeler[2], Caroline Vipond[2], Ian Feavers[2], Chris Jones[2], Steve Taylor[1], Charlotte Heaton[1], Emma Boxer[1], Denise Halliwell[1], Jamie Findlow[3], Ray Borrow[3], Andrew Gorringe[1]

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Immunological and epidemiological evidence suggests that carriage of *Neisseria lactamica* is involved in the development of natural immunity against meningococcal disease probably due to the development of antibodies against the many surface structures in common with N. meningitidis. We have used methods developed for the production of meningococcal outer membrane vesicle (OMV) vaccines to develop an *N. lactamica*-based vaccine prepared from a strain belonging to the ST-613 clonal complex.

The major component antigens of the vaccine have been characterised by separation of the OMVs by 1 dimensional SDS-PAGE and excision of the visible bands. Each band was subjected to trypsin digestion and resulting peptides were analysed by LC MS/MS. The vaccine was shown to consist of proteins with the following meningococcal homologues: adhesion penetration protein, TbpA, LbpA, HpuA, Omp85, FetA, PorB, RmpM and NspA. Immunoblots of OMVs from various meningococcal strains separated by SDS-PAGE showed antibodies in mouse sera raised against *N. lactamica* OMVs principally reacting with Omp85, PorB, RmpM and NspA.

Mouse and rabbit sera have also been assayed for bactericidal antibody activity against a panel of five prevalent UK serogroup B meningococcal strains and for the ability to mediate opsonophagocytosis (OP). Mouse and rabbit serum bactericidal titres for the meningococcal OMV and *N. lactamica* OMV vaccine

were low for against the panel of strains. However, a meningococcal OMV vaccine only elicited a high SBA titre against the homologous meningococcal strain. Cross-reactive antibodies that mediate OP were elicited by an *N. lactamica* OMV vaccine in rabbits. Cross-reactive OP responses were observed against 44/76-SL and the panel of serogroup B meningococcal strains.
This vaccine commenced a Phase I safety and immunogencity trial in adult male volunteers in February 2006.

P8.2.05

The Role of PorA and PorB in the Antibody Response to Outer Membrane Vesicle Vaccines Determined by Antibody Binding, Complement Deposition and Opsonophagocytosis assays

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To assess the contribution of key antigenic components to the immunogenicity of meningococcal OMV vaccines, we have used insertion mutagenesis to produce a panel of mutants from the Norwegian OMV vaccine strain 44/76 and from five strains that represent the current diversity of UK meningococcal disease isolates. Mutants have been produced which are deficient in each of the key surface antigens PorA, PorB, OpC, NspA and FetA. It is known that PorA is a key antigen for bactericidal responses to OMV vaccines, with porA serosubtype-specific responses observed in laboratory animals and human vaccinees. This was confirmed in previous studies with this mutant panel. In this present study, PorA and PorB mutants have been used to determine the role of these proteins in responses to OMV vaccines in mice and rabbits.

We have tested sera raised against wild-type and mutant OMVs in three flow cytometry-based assays that determine antibody binding to intact meningococci (surface labelling assay, SLA), antibody-mediated complement C3c deposition onto intact meningococci (CDA), and an opsonophagocytosis assay (OPA) using fluorescently-labelled meningococci and differentiated HL60 monocytic cells. The importance of PorA was clearly seen using SLA, CDA and OPA for all strains. As expected, the loss of PorA had no effect on the SLA, CDA or OPA activity of anti-*Neisseria lactamica* sera. The loss of PorB had little effect in SLA and OPA with anti-meningococcal OMV sera, whilst anti-*N. lactamica* OMV sera showed a marked decrease in OPA, suggesting the importance of PorB in the protection afforded by *N. lactamica* OMVs in mice. Complement deposition was reduced in the PorB mutant strains with both meningococcal and *N. lactamica* OMV antisera.

These studies confirm this panel of knockout mutants to be a valuable tool for investigating the immunological activity of key antigens in the evaluation of new meningococcal vaccines.

P8.2.06

Human Monoclonal Antibodies with Specificities against Several Epitopes on the Surface of *Neisseria Meningitides* can be Isolated from Vaccinees using a Novel Recombinant Approach

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A novel recombinant approach was applied to screen volunteers receiving the outer membrane vesicle (OMV) group B meningococcal vaccine developed in Norway (MenBvac, strain 44/76, B:15:P1.7,16) for production of cross-reacting human monoclonal antibodies. More than 10^6 fully intact human monoclonal antibodies were isolated from the volunteers and screened in ELISA. ELISA plates were coated with the MenBvac OMV vaccine, and incubated with the human monoclonal antibodies isolated from the volunteers. More than 70% of the wells were positive, indicating the presence of specific monoclonal antibodies present, binding to the MenBvac OMV vaccine. To address the degree of cross-binding to a another group B strain, a parallel ELISA assay were performed using the OMV vaccine based on strain NZ98/254, B:4:P1.7b,4 (MeNZB**)**. More than 60% of the wells positive for the MenBvac OMV vaccine contained cross-reactive antibodies binding to the MeNZB OMV vaccine.
A parallel ELISA assay were performed using OMV from a group A strain (Mk 686/02, A:4/21:P1.20,9). More than 20% of the wells positive in ELISA for the MenBvac and MeNZB OMV vaccines were cross-reactive against OMV from the group A strain.
Further investigation of some of the monoclonal antibodies, revealed antibodies binding to linear epitopes on Omp85, PorA and PorB surface proteins. Also a number of monoclonal antibodies exhibiting cross-binding was found to bind non-linear epitopes presented by unknown surface proteins.The isolation and use of fully human monoclonal antibodies from vaccinees could show promise in a reverse vaccinology approach, identifying common epitopes on different strains of meningococci. Such epitopes could be interesting components in a common meningococcal vaccine.

P8.2.07

Sialylation Plays a Detectable Role in Protecting *Neisseria gonorrhoeae* from Bactericidal Antibodies Induced by Immunization *in vitro* but not *in vivo*.

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The addition of host sialic acid to lipooligosaccharide (LOS) may facilitate *Neisseria gonorrhoeae* evasion of bactericidal antibodies induced by immunization based on reports that the rabbit antisera against gonococcal surface antigens are less bactericidal when sialylated gonococci are used. Our laboratory has tested a variety

of antigens for the capacity to protect against experimental gonococcal genital tract infection of female mice with limited success. Since sialylation of *N. gonorrhoeae* occurs within one day of experimental murine infection, here we assessed the extent to which sialylation *in vivo* reduces the effectiveness of a candidate outer membrane vesicle (OMV) vaccine. Mice were immunized intranasally with 20 ug of OMV isolated from wild type *N. gonorrhoeae* strain F62, followed by subcutaneous and intranasal boosts. Immunization induced high titers of specific serum IgG and vaginal IgG and IgA as assessed by ELISA and western blot. Serum and vaginal washes from immunized mice demonstrated bactericidal activity against the homologous strain unless sialylated gonococci were used. Based on these results, we predicted an α2,3-sialyltransferase (Lst) mutant would be less able to infect immunized mice than the wild type strain due to increased susceptibility to the bactericidal activity of gonococcal-specific antibodies. Immunized and unimmunized control mice were challenged with wild type F62 or mutant F62(*lst::aphA3*) bacteria. There was no difference in the colonization load or duration of infection for any group. [F62: immunized, 9.7 days (range 7-11), unimmunized, 6.9 days (range 0-11); F62(*lst*): immunized, 6.9 days (range 0-11), unimmunized, 7.4 days (range 0-11)]. Similar results were obtained when wild type and Lst-deficient MS11 gonococci were tested in mice immunized with MS11 OMV. We conclude that sialylation does not detectably reduce the efficacy of an experimental OMV vaccine against gonorrhea infection and that other more formidable obstacles may exist in conferring protection against *N. gonorrhoeae*.

P8.2.08

Serum IgG Response Induced by a Bivalent Recombinant LP2086 Provides Broad Protection against Serogroup B *Neisseria meningitidis*

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Elena Novikova, Michael Hagen, Duzhang Zhu, and Gary Zlotnick

Department of Vaccines Discovery Research, Wyeth Research, Pearl River, New York 10965

The conserved, neisserial-specific outer membrane lipoprotein, LP2086, is a vaccine candidate for the prevention of meningococcal disease caused by serogroup B *Neisseria meningitidis*. Based on amino acid sequence variation, the LP2086 family of proteins can be divided into two subfamilies (A and B). Individual recombinant LP2086 proteins (rLP2086) from each subfamily were chosen and monovalent and bivalent vaccines were formulated for immunogenicity studies in mice and rabbits. Over a wide dose range, monovalent and bivalent vaccines demonstrated a trend towards an increased antibody response with increasing dosage. The IgG responses to each monovalent vaccine showed distinct subfamily specificity in both protein ELISA and whole cell ELISA. In mice, the bivalent rLP2086 vaccine formulated with RC529-SE elicited serum IgG responses with broad bacterial surface reactivity and bactericidal activity against serogroup B strains of both subfamilies. The bactericidal activity was associated with substantially enhanced levels of LP2086 specific IgG_{2a} and IgG_{2b}, but not IgG_1 and IgG_3. The bivalent rLP2086 vaccine formulated with $AlPO_4$ induced serum IgG responses in rabbits, which reacted very well in protein and whole cell ELISAs. The rabbit immune sera killed strains from both LP2086 subfamilies by a complement-mediated and anti-rLP2086 antibody dependent mechanism. Our studies provide additional support for the use of a bivalent rLP2086 vaccine for human clinical trials.

P8.2.09

Development of a Luminex-based Meningococcal rLP2086-specific Human IgG Assay

Shekema Hodge[1], Kimberly Schermerhorn[1], Jonathan Skinner[2], Ashoni Arora[3], Philip Fernsten[1], and Thomas Jones[1]

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A meningococcal group B candidate vaccine containing outer membrane lipoporoteins, designated LP2086-A and LP2086-B, is in development. A Luminex-based single well assay was developed to quantify the relative amounts of LP2086 subfamily A and B specific human IgG in serum to support candidate vaccine clinical studies. Recombinant lipidated proteins representing LP2086-A and -B were each coupled to spectrally distinct carboxylated microspheres. After incubation with test serum, captured human IgG are detected with phycoerythrin-coupled anti-human IgG and quantified relative to a standard reference serum (100 units) using 5-PL data reduction. This assay performed well when evaluated by ICH guideline parameters, including specificity, accuracy, linearity, and precision. The quantitation range of the assay was assessed using the method of Findlay *et al*. (J. Pharm. Biomed. Anal. 21:1249-1273 [2000]), which is based on accuracy and precision. Accurate and precise quantitation of the A and B assays occurred over a 2.7- and 3.0-log concentration range, respectively. These results, in conjunction with excellent dilutional linearity (between dilution coefficient of variation ≤8.1%), indicate that very few dilutions of subject sera need be tested to obtain valid and accurate IgG levels. This assay was used to assess the baseline level of LP2086-specific IgG in serum from 38 healthy unvaccinated adults from whom specimens were obtained 2-4 times at approximately monthly intervals. The geometric mean baseline IgG levels were 10.2 units (95% CI: 7.4-14.1 units) and 5.3 units (95% CI: 3.8-7.2 units) in the A and B assays, respectively. Within subject, between bleed variability assessment indicated that at least a 2.85-fold and 3.11-fold increase in LP2086-A and –B IgG units, respectively, may indicate a humoral response to the vaccine.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

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P8.2.10

Expression of the meningococcal vaccine antigen NadA and the *Mycobacterium tuberculosis* Acr2 antigen in commensal *Neisseria*

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Carriage of the non-pathogenic commensal neisserial species *N. lactamica* is believed to be the basis for the development of natural immunity to meningococcal infection in young children. Like meningococci, *N. lactamica* and other non-pathogenic *Neisseria* spp. bleb to produce outer membrane vesicles (OMVs), but lacking an imunodominant and hypervariable PorA, such OMVs hold promise as cross-protective vaccines. We are seeking to enhance this potential by the strategic incorporation of heterologous proteins into commensal *Neisseria* OMVs. We previously exemplified this approach by expressing the meningococcal vaccine candidate NspA at the surface of recombinant strains of *N. flavescens* and demonstrated protective immune responses in mice (O' Dwyer *et al.* (2004) Infect. Immun. 72:6511-6518).

Nad A is another vaccine candidate, expressed from a conserved gene for which four alleles have been identified, *nadA*1, *nadA*2, *nadA*3 and *nadA*4 (Comanducci *et al.* (2002) J Exp Med 195:1445-1454; *ibid* (2004) Infect Immun 72:4217-4223). *NadA*3 and *nadA*4 present in pathogenic and carrier isolates respectively were cloned into the expression vector pMIDG201 and expressed in *N. flavescens*. Allele-specific antibody reactivity of *nadA*-expressing transformants was demonstrated by flow cytometry using a polyclonal antiserum and also by RT-PCR. Recombinant OMVs containing NadA3 or NadA4 have been used to immunise mice and sera absorbed with WT bacteria. Antibodies in these sera bound only to the surface of meningococci expressing the homologous nadA allele and fixed complement to these bacteria. NadA4-specific sera provided protection against challenge with a NadA4-containing meningococcal strain in a mouse model of meningococcal disease, confirming appropriate conformation using this expression system.

pMIDG201 has also been modified to include a meningococcal leader sequence and neisserial fragment upstream of the *Mycobacterium tuberculosis* cytoplasmic antigen *acr*2, a novel member of the alpha-crystallin family of molecular chaperones. Outer membrane protein preparations and OMVs prepared from this construct demonstrate reactivity with anti-Acr2 polyclonal sera, indicating surface expression of this protein. Recombinant OMVs containing Acr2 have been used to immunise mice and the characteristics of the immune responses induced will be presented.

P8.2.11

Preclinical evaluation of a recombinant protein MenB vaccine with various adjuvants

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Background The reverse vaccinology approach allowed the identification of novel surface-exposed and bactericidal proteins. In the present work we describe the strain coverage induced by a combination of five of these antigens formulated with with aluminium hydroxide or other adjuvants suitable for human use.
Methods The bactericidal assay was used to evaluate the complement-mediated killing activity of antisera, since this property correlates with efficacy in humans. Binding of polyclonal antiserum to live encapsulated MenB strains was observed by FACS analysis.

Results The sera obtained by immunizing mice with the vaccine formulated with aluminium hydroxide, aluminium hydroxide plus CpG 1826, and MF59 were tested in a bactericidal assay against a panel of 85 *Neisseria meningitidis* strains, selected as representative of the genetic and geographic heterogeneity of Meningococcus population.

We found that 66/85 of the strains were killed at a serum dilution equal or greater than 1/128 by the sera obtained from immunizations the aluminium formulation, the coverage increases to 78/85 and 80/85 with aluminium plus CpG and MF59, respectively. FACS data against a panel of strains support this result.

Conclusion These preclinical results show that a protein–based vaccine can be developed against Meningococcus B. The coverage induced by this multicomponent vaccine could be optimized using different adjuvants.

P8.2.12

The Diversity of Meningococcal Vaccine Components in a Worldwide Strain Panel

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Methods
We applied a strain classification procedure using the allelic sequences of genes *gna1870, 2132, 1994*, encoding for vaccine components. Each allelic gene variant was given a numeric identifier, like in MLST. The number of variation in the allelic profile was used as a strain distance index. Statistical correlations and non-random association values of each allele with clonal complexes (cpx) and sequence types (ST) were assessed. UPGMA

dendrograms from diverse allelic profiles and from concatenated genes were compared with MLST dendrograms.
Aims:
1- Strain relationships analysis on the basis of vaccine candidate diversity.
2- Vaccine coverage prediction.

Results
The three loci diversity was determined in a 85 meningococcus pathogenic strain panel representative of the global non-serogroup A epidemiology, and used for coverage evaluation of a new meningococcal vaccine. The three genes showed different allele numbers, however the allele combinations were less than expected from random association. ST-32 cpx was the most homogeneous cluster, as all ST-32cpx strains harboured the same allele of whatever antigen. *gna1870* and *gna2132* genes diversified in a higher number of alleles clustering to different degrees with different clonal complexes. Most relevant non-random associations were between allelic variants and clonal complexes. Dendrograms were drawn for each gene, both as topological views and for representing genetic distances, and compared with MLST dendrograms.

Conclusions
Molecular epidemiology studies with genes other than MLST ones are important for both clonal complex characterisation and vaccine coverage prediction. The three genes showed a different spreading in the strain panel analysed, also within the same clonal complex. By analyzing strain relationships, the comparison of UPGMA dendrograms indicates that ST-32 complex is the most homogeneous complex. A few combinations of the vaccine components were found in a representative worldwide strain panel, indicating that a multicomponent vaccine could include a limited number of antigenic variants.

P8.2.13

Systemic and Mucosal Immunogenicity in Healthy Humans Immunized with Meningococcal Serogroup C Conjugate Vaccine MenC/P64k using P64k Recombinant Protein Carrier.

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In the present work, we evaluate the immune response generated by the MenC/P64k conjugate vaccine candidate in healthy humans as part of a Phase I Clinical Trial . The study group was immunized with a single dose of 5 µg of the MenC/P64k conjugate, while the control group received 50 µg of the plain C polysaccharide in Mengivac AC®. No statistical differences were observed in the anti-polysaccharide IgG response between both groups ($P>0.05$). The salivary anti-polysaccharide IgA and IgG responses showed a significant increase only in the MenC/P64k group ($P<0.001$). Besides, a significant anti-polysaccharide IgG avidity maturation was detected also only in the study group ($P<0.01$). No differences were detected neither in the serum bactericidal titers of both groups nor in the increments ($P>0.05$). However, the study group increased the serum bactericidal activity two fold 30 days and five-fold 180 days upon vaccination. Conjugation to P64k allowed generating a T-dependent response to the polysaccharide, suggesting that P64k is an excellent carrier candidate to be used in all those polysaccharide antigens needed for childhood vaccination.

P8.2.14

Molecular Mimetics of Meningococcal Serogroup A and C Capsular Polysaccharides Selected with Human Sera are Able to Elicit Bactericidal Activity against *Neisseria meningitidis.*

Tamara Menéndez[1], Yoelys Cruz-Leal[1], Edelgis Coizeau[1], Zurina Cinza[1], Evelyn Caballero[1], Anabel Alvarez[1], Nelson S Vispo[1], and Gerardo Guillén[1].

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An alternative approach to the polysaccharide-protein conjugates in the development of T-dependent vaccines against *Neisseria meningitidis* is the use of peptides that mimic the bacterial capsular polysaccharides (CPS). Mimotopes of CPS from *N. meningitidis* have been obtained by panning peptide libraries displayed on filamentous phages with monoclonal anti-CPS antibodies or by developing monoclonal anti-idiotypic antibodies that mimic the serogroup C CPS. In the present work we describe the selection of CPS mimotopes from phage libraries using whole serum samples. A clinical trial was conducted on healthy adults with the "vaccin méningococcique polyosidique A+C" from Pasteur Vaccins. Two sera, with the highest levels of IgG and the ability to kill *N. meningitidis* group A or C cells, were selected to screen a random peptide library displayed on filamentous phages. In separate experiments, each serum was used for panning the library and then for assaying by ELISA the resulting phage clones. Positive clones were tested by ELISA with several positive and control sera. Phages, displaying peptides reacting with antibodies present in positive sera and unable to bind antibodies from negative sera were purified and the DNA was sequenced.

Twelve unique sequences were identified and the hosting phages were purified and used to immunize Balb/c mice. Specific antibodies were elicited against the peptides displayed in the phages. Serum bactericidal activities against *N. meningitidis* serogroup A and C were detected in the sera of mice immunized with four and two peptides, respectively.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

Refer to times listed below for individual poster Discussion Sessions

P8.2.15

Immunization with Molecular Mimetics of Meningococcal Serogroup B Capsular Polysaccharide induces Bactericidal Activity against *Neisseria meningitidis*

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Serum antibodies against capsular polysaccharide (CPS) from *Neisseria meningitidis* confer protection against disease. However, the immunogenicity of meningococcal group B CPS is poor. Further, a portion of the antibodies elicited has autoantibody activity due to the similarity of B CPS with human polysialic acid. The bactericidal and protective monoclonal antibody (mAb) 13D9, raised against N-propionylated B CPS, reacts specifically with B CPS, but not with human polysialic acid (J Exp Med, 1997, 185: 1929-38). Using this mAb we have identified, from a phage-displayed peptide library, four different peptides able to compete with purified B CPS for mAb 13D9 binding.

The study of binding of mAb 13D9 by overlapping peptides covering the most frequently isolated sequence allowed the identification of residues critical for mAb binding. Screening two synthetic libraries consisting of variations of the same frequently isolated sequence identified several additional structures, able to bind mAb 13D9. Three peptides with the highest affinity with the mAb 13D9 were selected for immunological studies.

The four peptides identified from the original library and the three peptides consisting of variations of the most frequently isolated peptide were used to immunize Balb/c mice, either displayed on filamentous phages or as multi-antigen peptides. Specific anti-peptide antibodies were elicited after immunization with almost all immunogens. Some of these immunogens elicited antibodies with bactericidal activity. The peptides identified in this work could serve as a basis for a safe and effective vaccine against *Neisseria meningitidis* serogroup B.

P8.2.16

Transmission of Maternal Antibodies to Offspring in Response to Immunisation of Rabbits with MenACWY Glycoconjugate Vaccines

Elena Mori, Cristiana Balocchi, Francesca Meini, Giada Buffi, Giuseppe Del Giudice, Ali Alloueche

Novartis Vaccines & Diagnostics, Siena, ITALY

The assessment of safety and immunogenicity of meningococcal conjugate vaccines in mothers during pregnancy and the effect of maternal antibodies transmitted to the offspring is particularly interesting for the impact that this may have on the immune system of newborns. Much remains to be learned about the maternal antibody transmission and utilisation in the offspring early in life and its effects on the immunological responses to primary vaccination and/or infections.

In the present work we evaluated the antibody response to Novartis tetravalent meningococcal glycoconjugate vaccine MenACWY in rabbit and the effect of maternal immunisation on the vaccine specific response in the offspring. This preclinical study, facing the important issue of the interference of maternal immunisation on the infant immune response, may shed light on some aspects of the influence of maternally derived antibodies on the immune system development of offsprings and also may give some information for implementation of vaccination programmes in newborns.

Adult female rabbits were immunised with multiple doses of MenACWY vaccine prior to and during gestation. Vaccine-specific antibodies were measured in sera of mothers and their offspring by ELISA. Transmission of potentially protective antibodies from mothers to their offspring was demonstrated by measuring bactericidal antibodies with Serum Bactericidal Antibody (SBAb) assay. Antibody titres measured in dams were higher in response to MenACWY vaccine formulated with alum as adjuvant compared to the non-adjuvanted formulation; these responses increased with the number of doses administered. ELISA and SBAb titres measured in foetuses were equal or even higher than in dams. These results indicate the presence of maternally derived and MenACWY vaccine-specific antibodies in the foetal bloodstream of rabbits and their protective potential.

P8.2.17

Immunogenicity of Fractional Dose Tetravalent A/C/Y/W135 Meningococcal Polysaccharide Vaccine: Results from a Non-inferiority Trial in Uganda

Philippe J. Guerin[1,2,3], Lisbeth M. Næss[2], Carole Fogg[1], Einar Rosenqvist[2,3], Francis Bajunirwe[1,4], Rogers Twesigye[1], Helen Findlow[5], Ray Borrow[5], Oddvar Frøholm[2], Vincent Batwala[1,4], Ingeborg S. Aaberge[2,3], John-Arne Røttingen[3,4,6], Patrice Piola[1], Dominique A. Caugant[2,3,7]

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Background

Since 2000, clusters of cases due to *Neisseria meningitidis* serogroup W135, or mixed outbreaks of serogroups A and W135, have posed serious challenges in vaccine choice for mass campaigns in Sub-Saharan Africa. Thus, we explored the use of fractional doses of a licensed polysaccharide vaccine in an African population.

Methodology

A randomised, single-blind, non-inferiority trial was performed in Mbarara, Uganda, to compare the immunological response of the

full dose (50µg) of the tetravalent Menomune® vaccine versus a fractional dose of 1/5 or 1/10 in healthy volunteers aged 2 to 19 years. Pre- and post-vaccination (4 weeks) sera were analyzed by serum bactericidal activity (SBA) using rabbit complement and IgG polysaccharide ELISA. In the non immune population prior vaccination, i.e. SBA titers<128, a responder was defined as showing ≥4-fold increase in SBA.

Results

Of 750 volunteers included, 291 received a full dose, 225 1/5 of the dose and 234 1/10 of the dose. For serogroup W135, 94% of the vaccinees in the 1/5 dose arm were responders and 97% in the 1/10 dose arm versus 94% in the full dose arm. For serogroup A, 92% of the vaccinees in the 1/5 dose arm were responders, 88% in the 1/10 dose arm versus 95% in the full dose arm. For both serogroup W135 and A, the non inferiority was demonstrated for 1/5 dose arm versus the full-dose group. For the 1/10 dose arm, the non inferiority was shown for serogroup W135, but we could not conclude for serogroup A. The ELISA results were variable for the different polysaccharides and low correlation was seen between IgG titers and SBA responses.

Conclusion

Our study indicates that 1/5 dose of the licensed A/C/Y/W135 polysaccharide vaccine can confer a similar functional immune response as a full dose and be equally protective against serogroup A and W135 meningococcal disease.

P8.2.18

Identification and Characterisation of a T-Cell and B- Cell-Stimulating protein B (TspB) of *N. meningitidis*.

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In recognition of the need for T-cell and B-cell memory-inducing components of future *Neisseria meningitidis* group B vaccines, the proteome of *N. meningitidis* was previously screened revealing T-cell stimulatory protein B (TspB). The *tspB* gene was cloned and expressed in *Escherichia coli* and the recombinant protein (rTspB) was affinity-purified and used to raise monospecific polyclonal rabbit antiserum. The antiserum detected proteins in all meningococcal isolates examined demonstrating that TspB expression was well conserved amongst meningococcal strains.

A major objective of the study was to investigate the cellular immune response induced by rTspB vaccination. T-cell responses in mice injected with rTspB and an equivalent negative control were compared to those of untreated negative control animals. T-cell proliferative responses to rTspB were detected in spleen cells (SPLCs) from mice immunised with rTspB but not with the control group indicating an antigen-specific cellular response. rTspB was used in ELISAs to quantify levels of TspB-specific IgG in 15 serum samples of vaccinated and control mice. Five sera from rTspB-vaccinated mice contained significant levels of TspB antigen-specific IgG while low responses were detected in the control vaccinated and unvaccinated groups. T-cell proliferative responses to rTspB were detected in the peripheral blood mononuclear cells (PBMCs) of convalescent patients and carriers, confirming that TspB-specific T-cell responses were stimulated by invasive disease. Purified TspB had strong stimulating activity for T-cells isolated from convalescent patients, carriers and healthy donors confirming that TspB is expressed *in vivo* during infection and is a B-cell immunogen. Levels of T-helper subset-associated cytokines were also measured following immunisation of mice with rTspB. IL-10, IL-2 and IFN-γ responses were elicited, confirming that TspB is a potent T-cell stimulating antigen. Future vaccine studies will determine whether TspB is capable of stimulating a T-cell-dependent response to other meningococcal antigens, when used as a carrier protein.

The Discussion Session for the following posters is from:
9.30pm – 10.15pm Tuesday 12 September

P8.3.01

A New Attractive Method for Refolding and Increasing the Immunogenicity of Recombinant antigens

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Bacterial inclusion bodies are protein aggregates of unfolded proteins, which are produced by transformed bacteria after the over-expression of the cloned genes. These inclusion bodies, even when they allow a high recovery and production of the recombinant protein, pose a threat on the final goal that is to obtain a properly folded, biologically active, protein product. The recovery process from these aggregates usually involves complex steps of refolding. In this work we are introducing a method for the incorporation of recombinant antigens into bacterial outer membrane vesicles (OMVs), where these antigens form, by co-folding, a complex with this preparation of outer membrane proteins. A new vaccine candidate CDG9995 against *Neisseria meningitidis* was incorporated into neisserial OMVs and four groups of mice were immunized with this complex. A broader crossreactivity with diverse meningococcal strains was found for the antibodies generated against the complex, compared to the response detected for the antisera elicited against the recombinant antigen alone. Moreover, this work demonstrates the feasibility of this strategy in eliciting significant bactericidal antibody levels and a protective response against homologous and heterologous neisserial strains.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)
Refer to times listed below for individual poster Discussion Sessions

P8.3.02

The Antibody Response to Recombinant Protein NMB1126 From *Neisseria meningitidis* with Different Adjuvants

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A new Neisserial vaccine candidate (NMB1126) identified in the published genome of MC58 serogroup B strain was cloned, and expressed as inclusion bodies in *E.coli* where it counted for as much as 40% of the total cellular protein. It was purified by immobilized metal ion affinity chromatography in one step after solubilization. As a result, the protein was easy to purify and high yields were achieved. Mice were immunized with this protein adjuvated with Freund, polysaccharide C, or encapsulated into liposomes. Elicited antisera were evaluated by ELISA and serum bactericidal assay (SBA). These sera reacted with recombinant NMB1126 and were positive by SBA against CU385 strain when the protein was included in liposome or mixed with polysaccharide C. According to these results NMB1126 protein can be considered an attractive vaccine candidate.

P8.3.03

Plasmid DNA as Adjuvant for Meningococcal Outer Membrane Vesicles

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The use of naked DNA represents a new approach to immunization with the major advantages that DNA vaccination stimulates both antibody and cell-mediated components of the immune system and that it also serves as adjuvant *per se*. Several recent reviews have covered the potent adjuvant activity of DNA in vaccination with various antigens and with DNA vaccines. The present study explores the possibility of efficiently codeliver plasmid DNA and meningococcal outer membrane vesicles (OMV) by formulation with or without aluminum hydroxide. Different eukaryotic expression plasmid were used for these combinations, one of them carring meningococcal PorA (P1.19.15) protein (pELI-PorA). As controls we used the empty vector pELI3.1 and a non-related DNA vaccine vector expressing an HCV antigen (pIDKE2). Mice immunized with the combination of plasmid DNA and OMV without aluminum adjuvant demonstrated the immune modulator effect of DNA for meningococcal OMV, and this adjuvant effect was independent of the plasmid used in the combination. The combined formulations increased the antibody titers against OMV in comparison with mice immunized with the OMV alone, but these responses were significant lower than that observed after adsorption of OMV onto aluminum. However the isotype profile of the humoral responses induced by the OMV/DNA combinations indicated balanced Th1/Th2 responses as that elicited after administration of OMV formulated with aluminum. The evaluation of serum bactericidal activity and active protection after the injection of all the formulations indicated that the addition of plasmid DNA to the OMV increased the functionality of the induced antibodies. Finally, the OMV mixed with plasmid DNA in the $Al(OH)_3$ formulation did not improve its immunogenicity probably because DNA bind tightly onto aluminum hydroxide and this event diminish its immunopotentiator properties. Our preliminary results indicate the possible potentialities of DNA molecules as an adjuvant for meningococcal OMV.

P8.3.04

Immunogenicity of Meningococcal Outer Membrane Vesicles in Neonatal Mice is Influenced by the Route and Schedule of Immunization

Sonia González[1], Aracelys Blanco[2], Yusleydis Pérez[1], Yordanka Soria[2], Evelin Caballero[1], Karem Cobas[1], Milaid Granadillo[1], Rolando Pajón[1]

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Murine models of early life immunization may reproduce the main known characteristics of neonatal and infant vaccine antibody responses. During the development of outer membrane vesicle (OMV) vaccines, the greatest amount of information about the preclinical performance of the immunogens is usually collected in adult mice. Little is known about the magnitude and quality of the immune response in animal models that resemble the immature immune state of human newborns. However, the greatest meningococcal disease burden is in infant and young children.

We have previously reported that the administration of two intraperitoneal (i.p.) doses of OMV absorbed onto Alum, given at 7 and 14 days after birth, induced a significant antibody response and was highly effective in conferring protection against bacteremia in 21 day-old mice challenged with meningococci. In the present work, we intended to reduce the immunization period, and observed that if the second dose is given 12 days after birth the antibody titers detected at day 18 are significantly diminished. Additionally, we studied the immunogenicity of OMV administered to neonatal mice by using different adjuvants, and compared OMV doses (ranged 2.5-10 µg/mouse) given i.p, in terms of antibody response and protection against bacteremia. All the doses assayed induced a similar level of antibody titers. We found that even when the antibody response does not differ in mice immunized without adjuvant, the levels of protection are severely affected when the OMV were administered alone.

Moreover, we analyzed the immunogenicity of this complex antigen when given subcutaneously, with Alum or Freund´s Adjuvant (FA).

By subcutaneous route, the antibody titers elicited with Alum were significantly higher that those obtained with FA. A memory response was demonstrated when mice intraperitoneally immunized as neonates, with two doses of OMV-Alum, were given a booster immunization at 6 weeks of age.

P8.3.05

Detection Of Beta-Barrel Outer Membrane Proteins In Gram-Negative Bacterial Proteomes

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Recent studies in genomics, and structural biology have revealed the versatility and ubiquity of the beta-barrel outer membrane proteins [1]. Their distribution extends well into many families of organisms, all of which have an estimated level of about 3% of beta-barrel membrane proteins in their respective proteomes [2]. While several methods are available [2-4] not all are public, or capable of working in genome wide screening projects. It is therefore urgent to develop methods capable of detecting this class of outer membrane proteins in large sequencing projects and use this information as a tool to aid in protein annotation, and test the identified proteins as targets for vaccine or drug design.

In this work, we defined two variables primarily based on the statistical bias in amino-acid composition, as predictors of the potential to form a beta-barrel structure, and we fitted Linear and Quadratic Discriminant models with a training set comprising 1001 positive and negative beta-barrel proteins for the analysis of microbial proteomes. Leave-one-out cross validation showed that Linear Discriminant Analysis correctly classified 98.2 % of the 1001 proteins in the training set while Quadratic Discriminant Analysis performed for a 97.5 %. A program called PROB, with this algorithm implemented, is running as a web service and is available at [http://www.biocomp.cigb.edu.cu/prob/php/form.php].

Application of these algorithms to several Gram-negative bacteria: *E. coli, N. meningitidis, H. pylori, H. influenzae,* and *P. aeruginosa* have revealed several potential beta-barrel membrane proteins that have not yet been annotated as such. The application of this method to the genome derived proteomes from *H. influenzae* and *N. meningitidis* allowed us the identification of 26 novel vaccine targets to be explored.

P8.3.06

From current OMV-based vaccines to broadly cross-reactive Recombinant Protein-based Vaccines: challenges and realities on the Cuban strategy

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The global impact of infections due to *Neisseria meningitidis,* and the lack of a protective vaccine against serogroup B disease*,* has prompted the scientific community to develop a preventive universal vaccine against this deadly pathogen.

While promising recombinant vaccines candidates are under evaluation, several issues must be taken into account for the development of a successful anti-meningococcal vaccine. In this direction, however, the Cuban experience can give essential clues. From the research point of view, issues like the study of probable vaccine failures and the associated questions on host susceptibilities, the rapid evolution of antigenic types amongst meningococcal populations after vaccine introduction, the impact of minor OMV components in the induction of a significant immune response in humans, the exploration of new ways for the identification of vaccine candidates like Expression Library Immunization (ELI), must not be overlooked and are firmly in place in our research plans.

Application of 2DE-MS and SCAPE technology to different OMV preparations, including three different batches of Cuban VA-MENGOC-BC® OMVs, resulted in the identification of 106 proteins. Of them, 44 were identified as characterized or new membrane proteins. Seven of these proteins joined a total of 26 additional proteins *in silico* predicted to be outer-membrane beta-barrels and all 33 corresponding genes were cloned, expressed and *E. coli* and their respective protein products were tested as vaccine candidates. Three of these proteins were able to induce a protective immune response against homologous and heterologous strains as measured by the reduction of bacteraemia in neonatal mice and infant rat models, and induced cross-reactive antibodies that are able to recognize a wide range of heterologous strains.

P8.3.07

The transferrin-supplemented mouse model of meningococcal infection revisited.

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Transferrin constitutes the major protein involved in the iron transport from the sites of absorption to the sites of storage and utilization. Despite the high affinity of transferrin for iron, most

bacterial pathogens such as the human restricted Neisseria meningitidis, have developed iron acquisition mechanisms. Several animal models of bacterial infection that include the exogenous supply of human transferrin have been implemented, and further more a transgenic mouse model expressing human transferring was obtained and tested. The levels of human transferrin in mice sera strongly correlated with CFU levels in the 72 h time window employed, however infection was attained only with extremely high levels of human transferring, largely surpassing those physiologically found in humans. The intraperitoneal injection of 8 mg of human transferrin allowed a sustained value of human transferrin in mouse sera in the range of 1-2 mg/ml over the first 24 hours, indicating that bacteria accessing to blood stream during this time would be exposed to levels of hTf found in normal human serum. The transgenic mouse expressing human transferrin showed increased susceptibility only when low levels of additional iron were administered. These results enforce the need of careful timing if neisserial replication in blood is to be mimicked within a physiological range and that more human molecules are strongly needed in order to fully replicate the disease in the murine model.

P8.3.08

Recombinant Antigen NMB0088 as Vaccine Candidate Against *Neisseria meningitidis*

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Neisseria meningitidis is an obligate human pathogen that inhabits the upper respiratory tract. Occasionally, it translocates to the bloodstream causing sepsis and from there it can cross the blood-brain barrier and cause meningitis. Epidemic disease has been reported from all continents in the last few years. While early diagnosis and antibiotic treatment greatly enhance survival, prevention through vaccination would appear the best way to limit meningococcal disease. The availability of the annoted genome sequence of the serogroup B *N. meningitidis* MC58 strain has exerted an influence on meningococcal vaccine research. Here, a novel antigen that induces cross-reactive bactericidal antibodies against a number of *N. meningitidis* strains is described. This antigen, a 51 kDa called NMB0088, was first identified as a putative beta-barrel outer membrane protein, and later observed as a component of OMPs complexes by proteomics. Approximately 6 different neisserial isolates tested positive by Western blotting and PCR screening methods for the presence of the protein and the gene encoding NMB0088. The strains tested included isolates of *N. meningitidis* serogroups A, B, C, W135, and Y, *Neisseria gonorrhoeae*, and *Neisseria lactamica*. To better understand the microheterogeneity of this protein, the *NMB0088* genes from 5 neisserial isolates were sequenced. A high degree of amino acid sequence similarity was recorded in this gene. Additionally, the gene codifying for NMB0088 protein from strain CU/385-83 was cloned and expressed, and the recombinant polypeptide was purified and its immunogenicity evaluated in animal models by different routes. Antiserum produced against the recombinant protein was capable of eliciting bactericidal activity against strains expressing different serosubtype antigens. According to our work, the rNMB0088 antigen is a candidate for further vaccine development.

P8.3.09

The Application Of Physicochemical Methods Of Analysis To The Development Of Conjugate Vaccines Against Meningococcal Group A Bacteria

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The meningococcal group A organism is responsible for 90% of the cases of endemic and epidemic meningitis caused by *Neisseria meningitidis* bacteria. Preventive immunisation should avoid a great number of deaths and be less expensive than mass immunisation campaigns performed after epidemics have begun. This is best achieved by vaccination with conjugate vaccines which, unlike polysaccharide vaccines, are immunogenic in the very young, induce immunological memory and are likely to give long-lasting protection. MVP (Meningitis Vaccine Programme) is developing an affordable monovalent meningococcal A conjugate vaccine for sub-Saharan Africa which is manufactured by Serum Institute of India Limited (SIIL) using aldehyde-hydrazide condensation chemistry developed at the US Center for Biologics Evaluation and Research. Phase I results showed that the conjugate vaccine is safe and immunogenic and Phase II material has been prepared for a clinical study to be conducted in The Gambia and Mali later this year. Manufacturers and regulatory authorities have had decades of experience with meningococcal capsular polysaccharide vaccines, but the production and control of conjugate vaccines are more complex and vaccine specific. Successful development of the conjugate vaccine requires control of the production process from starting polysaccharide, to the formation of activated intermediates and their conjugation to yield the conjugate vaccine, as well as demonstration of manufacturing consistency. Since group A meningococcal conjugate vaccines are manufactured from purified components by a clearly defined chemical process, the strategy for the control of the vaccine relies heavily on determination of the molecular characterization and purity of each vaccine lot and intermediates. Recent advances in bioanalytical methodology permit the detailed structural characterization of conjugate vaccines and intermediates to be achieved by the use of physicochemical techniques. The application of these techniques, including chromatography and nuclear magnetic resonance spectroscopy, to the development of the group A conjugate vaccine will be discussed.

Poster Discussion Session: Tuesday 12 September 7.30pm

Presenting Authors will be available for informal discussions in the Poster Display Hall (Halls C+D)

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P8.3.10

OxyR and Fur Regulate the Oxidative Stress Response in *Neisseria meningitidis*

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Mechanisms for coping with oxidative stress are crucial for the survival of *Neisseria meningitidis* in the human host. Reactive oxygen species such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) are encountered by pathogenic *Neisseria* during endogenous respiratory processes and interactions with host cells. In response to oxidative stress, bacteria activate expression of a number of genes, including those required for the detoxification of reactive molecules as well as for the repair and maintenance of homeostasis. The roles of two transcriptional regulatory proteins, OxyR and Fur, in the oxidative stress response in *N. meningitidis* strain MC58 have been investigated using knockout mutant strains.

In particular we studied the effects of hydrogen peroxide on the transcription of the catalase gene (*kat*) and results indicate that catalase is regulated by OxyR and Fur in *N. meningitidis*. The *kat* promoter is induced by H_2O_2 in an OxyR-dependent manner: expression of *kat* is constitutive in the *oxyR* mutant and H_2O_2 induction of expression is restored in a complemented strain, but not in a strain complemented with OxyR containing mutated key cysteine residues (C199S or C208A). OxyR also represses the *kat* promoter: the basal level of *kat* expression is much higher in the mutant strain. Catalase induction by H_2O_2 is also dependent on Fur: expression of *kat* is constitutively low in the *fur* mutant and H_2O_2 induction of expression is restored in a complemented strain.

The *oxyR* mutant strain is more resistant than the wild type in xanthine/xanthine oxidase (generates extracellular $O_2^{\cdot-}$ and H_2O_2) and hydrogen peroxide oxidative killing assays. On the other hand, the *fur* mutant strain was more sensitive than the wild type in these oxidative killing assays. These results are consistent with the altered expression of *kat* that is seen in these strains.

P8.3.11

A Sustained Increase in Meningococcal Serogroup C Specific IgG Following Glycoconjugate or Plain Polysaccharide Meningococcal Vaccines in Adolescents Previously Immunised With Serogroup C Meningococcal Glycoconjugate Vaccine

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Introduction

Adolescence is a period of increased risk for serogroup C meningococcal disease. A rapid waning of bactericidal antibodies is observed following a single early childhood dose of serogroup C meningococcal glycoconjugate (MenC) vaccine. One approach for protection throughout adolescence is an additional dose of a meningococcal vaccine in the early teenage years; however no data exist on the persistence of specific antibodies following such a dose.

Methods

In a previous study 304 adolescents received a plain polysaccharide meningococcal serogroup A and C vaccine (MenAC, 1/5 dose) or a booster dose of a MenC vaccine (Menjugate®). All had previously been immunised with Menjugate® 3 years previously. In this 'follow on' study meningococcal serogroup C specific IgG geometric mean concentrations (IgG GMCs) were determined at 1 year after the booster immunisation and from age matched Menjugate® recipients who received no booster(controls).

Results

Sera from 244 participants aged 14 -16 years were analysed (149 MenAC, 95 MenC and 100 controls). Mean years since routine MenC was 4.7(MenAC), 4.7 (MenC) and 5.2 (controls). Meningococcal serogroup C specific IgG GMCs were significantly higher following either MenC or MenAC than in controls (MenAC 16.2 ug/ml (95% C.I. 13.4 – 19.6), MenC 17.4 ug/ml (95% C.I. 13.7 – 22.1), controls 3.6 ug/ml (95% C.I. 2.4 – 5.5), p values both <0.001). There was no significant difference in IgG GMCs in MenC and MenAC recipients (p value = 0.655).

Discussion

In adolescents previously primed by MenC vaccine there is no significant difference in the specific IgG GMCs measured one year following an additional dose of MenAC or MenC. Both groups have elevated specific IgG GMCs when compared with controls. Persistence of serum antibody following booster immunisation may be dependent on B cell memory generated during priming (with the T-dependent stimulus of the conjugate vaccine), and does not require T cell involvement during booster stimulation.

Poster Discussion Session: Tuesday 12 September 7.30pm

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P8.3.12

The Diversity of Meningococcal Vaccine Components in a Worldwide Strain Panel

Stefania Bambini[1], Alessandro Muzzi[1], Francesca Morandi[1], Ilenia Gioachini[1], Rino Rappuoli[1], Maurizio Comanducci[1], and Mariagrazia Pizza[1]

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Methods

We applied a strain classification procedure using the allelic sequences of genes *gna1870, 2132, 1994*, encoding for vaccine components. Each allelic gene variant was given a numeric identifier, like in MLST. The number of variation in the allelic profile was used as a strain distance index. Statistical correlations and non-random association values of each allele with clonal complexes (cpx) and sequence types (ST) were assessed. UPGMA dendrograms from diverse allelic profiles and from concatenated genes were compared with MLST dendrograms.

Aims:

1- Strain relationships analysis on the basis of vaccine candidate diversity.
2- Vaccine coverage prediction.

Results

The three loci diversity was determined in a 85 meningococcus pathogenic strain panel representative of the global non-serogroup A epidemiology, and used for coverage evaluation of a new meningococcal vaccine. The three genes showed different allele numbers, however the allele combinations were less than expected from random association. ST-32 cpx was the most homogeneous cluster, as all ST-32cpx strains harboured the same allele of whatever antigen. *gna1870* and *gna2132* genes diversified in a higher number of alleles clustering to different degrees with different clonal complexes. Most relevant non-random associations were between allelic variants and clonal complexes. Dendrograms were drawn for each gene, both as topological views and for representing genetic distances, and compared with MLST dendrograms.

Conclusions

Molecular epidemiology studies with genes other than MLST ones are important for both clonal complex characterisation and vaccine coverage prediction. The three genes showed a different spreading in the strain panel analysed, also within the same clonal complex. By analyzing strain relationships, the comparison of UPGMA dendrograms indicates that ST-32 complex is the most homogeneous complex. A few combinations of the vaccine components were found in a representative worldwide strain panel, indicating that a multicomponent vaccine could include a limited number of antigenic variants.

P8.3.13

Construction of a *Neisseria gonorrhoeae* Vaccine by Fusion of a Peptide Mimic of a Lipooligosaccharide Derived Carbohydrate Epitope with Complement C3d

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We have identified a conserved carbohydrate epitope on *Neisseria gonorrhoeae* lipooligosaccharide (LOS) that is recognized by monoclonal antibody (mAb) 2C7. This eptope is present in 95% of clinical isolates of *N. gonorrhoeae* and may represent a potential gonococcal vaccine candidate. Because oligosaccharides (OSs) are poor immunogens, we identified a peptide mimic (PEP1) as a surrogate of the 2C7 epitope by screening a random peptide display library with mAb 2C7. PEP1 emulsified in Complete Freund's adjuvant (CFA) elicited a $\geq$ 4 fold increase in cross-reactive anti-LOS antibodies in 27 of 30 mice (90%). One third (11 out of 30) of the mice developed complement dependent bactericidal activity against gonococci bearing the 2C7 epitope (Vaccine 24: 157-70, 2006).

To enhance the specific and functional immune response directed against PEP1 and therefore to the 2C7 epitope, we constructed a fusion protein comprised of PEP1 and 3 copies of C3d (PEP1-3,C3d) to induce a better long term adjuvant effect than CFA. Similar constructs with other immunogens have been shown to improve IgG antibody responses, hypothesized to occur as a result of engaging membrane Ig and CR2 (CD19/CD21) receptors simultaneously on B cells and CR2 on follicular dendritic cells. This might result in the production of a higher specific IgG antibody titer possessing higher affinity and improved function.

A PEP1-3,C3d construct that contained a pre-pro trypsin leader sequence-FLAG tag and the PEP1 gene was linked in tandem with 3, C3d genes (2.802 kb) and used to express the gene product in CHO cells. A correctly sized product (MW ~ 100 kd) was further validated by western blot using mAb 2C7, which recognizes PEP1, and anti FLAG. Immune responses to this purified PEP1-3,C3d protein will be studied in mice.

Poster Discussion Session: Tuesday 12 September 7.30pm

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P8.3.14

Adjuvant activity of different TLR agonists in combination with LPS-deficient meningococcal outer membrane complexes

Floris Fransen[1,2], Jos van Putten[2] and Peter van der Ley[1]

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When outer membrane complexes (OMCs) isolated from an LPS-deficient *Neisseria meningitidis lpxA* mutant are used to immunize mice, the resulting antibody response is much lower compared to wildtype LPS-containing OMCs. This demonstrates that wildtype meningococcal LPS has strong adjuvant activity, presumably through its ability to activate TLR4. We now compared the adjuvant activity in mice of a panel of different TLR agonists when used in combination with LPS-deficient OMCs. These include the following: FSL-1 and Pam3CSK4 (TLR2+TLR6 resp.1); poly(I:C) (TLR3); different forms of LPS (TLR4), i.e. *N.meningitidis* wildtype L3 and mutant penta-acylated *lpxL1* LPS, *Bordetella pertussis* LPS and monophosphoryl lipid A (MPL); flagellin from *Bacillus subtilis* (TLR5); imiquimod (TLR7); loxoribine (TLR7); CpG DNA (TLR9); muramyl dipeptide (MDP) (NOD2). The resulting mouse sera after two immunizations with 5 µg of OMC protein plus adjuvant were analyzed for total IgG and IgG1/IgG2a subclasses against meningococcal outer membranes, and for bactericidal titers (SBA). The best adjuvants included wildtype L3 and the less toxic *lpxL1* LPS, *B.pertussis* LPS and CpG DNA. Poly(I:C) and imiquimod were also active at the highest concentration tested (100 µg). Remarkably, the FSL-1 and Pam3CSK4 lipopeptides had no activity, possibly because the OMCs already contain sufficient amounts of other TLR2 agonists. The highest IgG2a/IgG1 ratios were found for CpG DNA, indicating strong Th1 skewing. We also determined total IgE titers. These were higher for LPS-deficient as compared to wildtype OMCs, but again reduced by the most active adjuvants. To conclude, the adjuvant activity of wildtype meningoccal LPS can be replaced by different TLR3, TLR4, TLR7 and TLR9 agonists, some of which have a much better safety profile. However, none of the adjuvants was able to restore the antibody response to the level of wildtype OMCs with endogenous LPS, indicating that formulation of the antigen/adjuvant mixture is also an important parameter.

P8.3.15

***N. Meningitidis* PorB Induces an Antigen-Specific Eosinophil Recall Response: Potential Adjuvant for Helminth Vaccines?**

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Vaccines have been the most influential immunological advances of the 20th century. Yet obstacles remain in the development of immunotherapies for pathogens such as HIV, malaria, parasitic helminths, andmycobacterium, as well as for emerging pathogens, such as Ebola virus. Our laboratory has focused on characterizing the immunostimulatory TLR2 ligand, PorB, an outer membrane protein of the bacteria *Neisseria meningitdis* (Nme). PorB has been shown to induce the proliferation, activation, and costimulatory capacity of murine B cells, macrophages, and dendritic cells. The potent immunostimulatory capacity of PorB and its characterization as a TLR2 ligand, suggested that PorB could be effectively exploited for use as an adjuvant for a model antigen, in this case, Ovalbumin (Ova). We found that mice immunized with PorB/Ova had significantly higher levels of Ova-specific IgG1 than mice immunized with Ova alone. Given IgG1's known association with a Th2 response, we hypothesized that PorB could possibly be used as an adjuvant for pathogens which require a Th2 response for protection, such as parasitic helminths. Interestingly, we found that PorB was also able to stimulate an eosinophil recall response, with highly activated cells, in mice injected with PorB/Ova and challenged with Ova when compared to mice injected with Ova alone. As eosinophils have been shown to be cytotoxic to helminths and are often associated with a Th2 phenotype, these results indicate promise for a PorB-parasite antigen conjugate vaccine that may stimulate protective immunity against helminth parasites. Here, we demonstrate the possible mechanisms of PorB-mediated eosinophil response, including both TLR2 dependent and TLR2 independent inflammation.

P8.3.16

Combination of Minor Outer Membrane Proteins Inducing Bactericidal Antibodies in Mice

Vincent Weynants[1], Karine Goraj[1], Christiane Feron[1], Martine Bos[2], Philippe Denoel[1], Vincent Verlant[1], Jan Tommassen[2], Jan Poolman[1]

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Due to the dominant immune response mediated by PorA, outer membrane vesicles (OMVs) derived from serogroup B meningococcal wild-type strains of *Neisseria meningitidis* (MenB) induce only limited cross-protection against circulating wild-type strains. This partial cross-protection appears to be induced by some minor and well conserved outer membrane proteins (OMPs).

We have genetically modified strain H44/76 to down-regulate the expression of the major variable proteins PorA and to up-regulate the expression of some minor OMPs that might have potential as vaccine antigens.

The bactericidal response measured in sera form mice immunized with OMVs over-expressing only one minor OMP showed no or only limited increase in bactericidal antibodies even if the induction of anti-specific OMP was demonstrated by ELISA. However, by mixing the sera of animals immunized with OMVs over-expressing TbpA with anti-OMVs over-expressing Hsf sera, we have observed a positive interaction between "anti-TbpA" and "anti-Hsf" antibodies in the ability to induce complement mediated killing of strain H44/76. This finding has been confirmed by the immunization of mice with OMVs co-over-expressing these two minor OMPs. Two others minor OMPs, NspA and OMP85, showed comparable synergy.

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P8.3.17

Complement Regulatory Proteins Attenuate the Functional Effect of Human Antibody Elicited by an Outer Membrane Derived Vaccine Prototype that Contains 2C7 Epitope Expressing Lipooligosaccharide

Jutamas Ngampasutadol, Peter A. Rice and Sunita Gulati

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Previously, we have identified a conserved carbohydrate epitope on gonococcal lipooligosaccharide (LOS) that is recognized by a monoclonal antibody (mAb) 2C7. This structure is present in 95% of clinical gonococcal isolates and may represent a potential target for an anti-gonococcal vaccine. Antibodies against this epitope mediate direct complement-dependent bacterial killing and opsonophagocytosis. In humans, the 2C7 epitope elicited a significant immune response after immunization with an outer membrane derived vaccine prototype that contained 2C7 epitope expressing LOS (JID: 1223-1237, 1996). To evaluate the effect of complement down regulators, factor H and C4b-binding protein (C4bp), on the efficacy of bactericidal antibody elicited by the vaccine, we examined bactericidal activity of 7 vaccinee sera that exhibited high, low and mid-range concentrations (1.76 to 5.97µg/ml) of 2C7 antibody. We used two strains as targets for bactericidal activity present in the vaccinee sera: 2C7-positive gonococcal strain 15253, which is highly resistant to non-immune normal human serum (NHS) and binds factor H and C4b-binding protein (C4bp) and strain F62 Δ*lgtA*, ∇*lgtG,* which has been genetically modified to express the same 2C7 bearing LOS structure as strain 15253 but remains sensitive to NHS, binding factor H weakly and C4bp not at all. Strains 15253 and F62 Δ*lgtA*, ∇*lgtG* bound similar amounts of mAb 2C7. Isogenic mutants that did not express the 2C7 epitope, 15253 Δ*lgtG* and F62 Δ*lgtA*, were used as controls.

Vaccinee sera possessed 2C7 antibody dose-responsive direct complement dependent bactericidal activity against the 2C7 bearing strains but not against the non-2C7 bearing mutants. Strain 15253 required at least a 10-fold higher 2C7 antibody concentration to kill it compared to strain F62 Δ*lgtA*, ∇*lgtG*. These data indicate that human immune 2C7 antibody at higher concentrations, can overcome the down regulatory effects upon complement dependent killing (serum resistance) by the complement regulators, factor H and C4bp.

P8.3.18

Immunisation with live Neisseria lactamica elicits protective immunity against meningococcal challenge

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The development of natural immunity against meningococcal diseases occurs in all human populations. Protection is thought to be mediated by bactericidal antibodies in serum that are elicited following nasopharyngeal carriage of *N. meningitidis* and non-pathogenic species of *Neisseria* during childhood. *Neisseria lactamica* is closely related to the meningococcus, and carriage of this bacterium is frequent during infancy but declines with age. In longitudinal studies, carriage of *N. lactamica* has been shown to be associated with the development of bactericidal antibodies against *N. meningitidis*, suggesting that some strains of *N lactamica* or their antigens may be used as potential vaccines against *N meningitidis*.

We provide further evidence that *N. lactamica* contributes to protective immunity again meningococcal infection. Using a murine challenge model, we demonstrate that parental immunisation with strains of live *N. lactamica* can generate cross-protective immunity against live meningococcal challenge. In contrast to results obtained with *N. lactamica* outer membrane vesicles, immunisation with some but not all strains of the bacterium leads to the development of high levels of serum bactericidal activity against serogroup A, B, and C *N. meningitidis*. Furthermore the animals developed significant opsonophagocytic activity against serogroup B *N. meningitidis*. Further detailed analysis of the response in animals immunised with *N. lactamica* should help to define the cross protective antigens expressed by this harmless commensal.

P8.3.19

Specificity of Cross-Reactive Bactericidal Antibodies in Normal and Convalescent Human Sera

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Analysis of the antibody response induced by natural infections may provide insight helpful in the design of an effective group B vaccine. A small number of human sera including group C and group B convalescent sera and several normal sera with naturally occurring cross reactive bactericidal activity were analyzed to determine the specificity of the cross reactive bactericidal antibodies. The sera were analyzed for their ability to kill a panel of group B strains of different serotypes, serosubtypes and immunotypes, and those with bactericidal activity were further analyzed using a bactericidal antibody depletion assay. Analysis by this method demonstrated that most of the bactericidal activity could be removed by purified lipooligosaccharide (LOS). LOS homologous to that expressed on the target strain was the most effective, but the antibodies exhibited various degrees of cross reactivity with LOS of different immunotypes and core structures. The anti-L3,7 LOS bactericidal antibodies appeared sensitive to the core structure of the LOS, suggesting that epitopes recognized involved more than just the lacto-N-neotetraose structure. When the target strain was grown in the presence of CMP-NANA to increase LOS sialylation, the anti-LOS antibodies in convalescent sera retained bactericidal activity with only a 2 to 4-fold drop in titer. Natural infections caused by strains expressing L3,7 LOS induce bactericidal antibodies that are likely protective and appear to be directed against unique bacterial epitopes. Development of a vaccine which mimics natural immunization with respect to the specificity of the anti-LOS antibodies induced may be an effective approach to group B vaccine development.

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