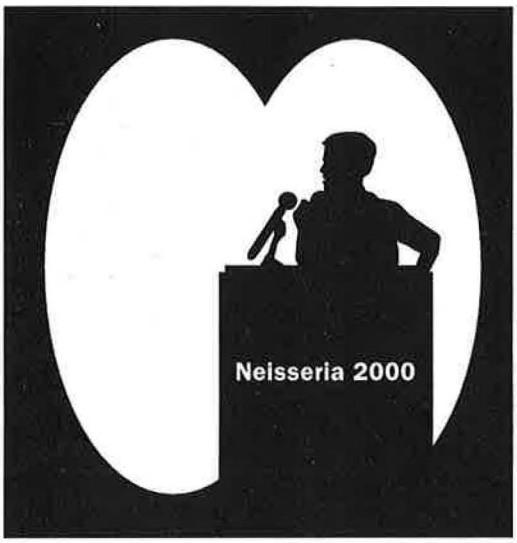


Twelfth International Pathogenic *Neisseria* Conference

November 12 - 17, 2000

Moody Gardens Hotel & Conference Center

Galveston, Texas

Abstract Guide

Abstracts of the Twelfth International Pathogenic *Neisseria* Conference

November 12 - 17, 2000

Moody Gardens Hotel & Conference Center

Galveston, Texas

Published by:
HCC
Health Care Communications, Inc.
[A Kendle Company]
One Bridge Plaza, Suite 350
Fort Lee, NJ 07024

HISTORY OF THE PATHOGENIC *NEISSERIA* MEETINGS

In the 1970s 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. But the first official conference was held in San Francisco, California, 1978.

First International Pathogenic *Neisseria* Conference
1978, San Francisco, California, USA.
Chair: G.F. Brooks.

Second International Pathogenic *Neisseria* Conference
1980, Hemavan, Sweden.
Chairs: S. Normark and D. Dannielson.

Third International Pathogenic *Neisseria* Conference
1982, Montreal, Canada.
Chair: I.W. De Voe.

Fourth International Pathogenic *Neisseria* Conference
1984, Asilomar, California, USA.
Chair: G.K. Schoolnik.

Fifth International Pathogenic *Neisseria* Conference
1986, Noordwijkerhout, The Netherlands.
Chair: J.T. Poolman.

Sixth International Pathogenic *Neisseria* Conference
1988, Pine Mountaine, Georgia, USA.
Chair: S.A. Morse.

Seventh International Pathogenic *Neisseria* Conference
1990, Berlin, Germany.
Chair: M. Achtman.

Eighth International Pathogenic *Neisseria* Conference
1992, Cuernavaca, Mexico.
Chair: C.J. Conde-Glez.

Ninth International Pathogenic *Neisseria* Conference
1994, Winchester, England.
Chair: M.C.J. Maiden.

Tenth International Pathogenic *Neisseria* Conference
1996, Baltimore, Maryland, USA.
Chair: C.E. Frasch.

Eleventh International Pathogenic *Neisseria* Conference
1998, Nice, France.
Chair: X. Nassif.

CONFERENCE ORGANIZING COMMITTEE

Peter Rice, M.D. ***(Co-Chair)***
Boston Medical Center
Boston, Massachusetts

Fred Sparling, M.D. ***(Co-Chair)***
University of North Carolina
Chapel Hill, North Carolina

William Shafer, Ph.D.
(Program Chair)
Emory University School of Medicine
Atlanta, Georgia

SCIENTIFIC COMMITTEE

Michael A. Apicella, M.D.
The University of Iowa
Iowa City, Iowa

Milan Blake, Ph.D.
Baxter Hyland Immuno
Beltsville, Maryland

Janne G. Cannon, Ph.D.
University of North Carolina
Chapel Hill, North Carolina

Myron Cohen, M.D.
University of North Carolina
Chapel Hill, North Carolina

John Davies, Ph.D.
Monash University
Victoria, Australia

Jo Anne R. Dillon, Ph.D.
University of Ottawa
Canada

David Dyer, Ph.D.
University of Oklahoma
Oklahoma City, Oklahoma

Carl Frasch, Ph.D.
National Institutes of Health
Rockville, Maryland

Caroline Genco, Ph.D.
Boston University Medical Center
Boston, Massachusetts

Bjorn Haneberg, M.D.
National Institute of Public Health
Oslo, Norway

John Heckels, Ph.D.
Southampton General Hospital
Southampton, England

Catherine Ison, Ph.D.
St. Mary's Hospital
Imperial College School of Medicine
London, England

Martin Maiden, Ph.D.
University of Oxford
Oxford, England

Thomas Meyer, Ph.D.
Max Planck Institute for Biology
Tübingen, Germany

Stephen Morse, Ph.D.
Centers for Disease Control
and Prevention
Atlanta, Georgia

Richard Moxon, M.D.
University of Oxford
Oxford, England

Xavier Nassif, M.D., Ph.D.
L'Hôpital Necker
Paris, France

Stella Nowicki, Ph.D.
University of Texas Medical Branch
Galveston, Texas

Brad Perkins, M.D.
Centers for Disease Control
and Prevention
Atlanta, Georgia

Maggie So, Ph.D.
Oregon Health Science University
Portland, Oregon

David Stephens, M.D.
Emory University
Atlanta, Georgia

Peter van der Ley, Ph.D.
National Institute of Public Health
Bilthoven, The Netherlands

Mumtaz Virji, Ph.D.
University of Bristol
Bristol, England

Lee Wetzler, M.D.
Boston University Medical Center
Boston, Massachusetts

Wendell Zollinger, Ph.D.
Walter Reed Army Institute
Washington, DC

Peter Rice, M.D. ***(Ex-Officio)***
Boston University Medical Center
Boston, Massachusetts

William Shafer, Ph.D. ***(Ex-Officio)***
Emory University School of Medicine
Atlanta, Georgia

Fred Sparling, M.D. ***(Ex-Officio)***
University of North Carolina
Chapel Hill, North Carolina

SPONSORS

The Organizing Committee thanks the following organizations for their generous financial support of the Conference.

Aventis Pasteur
Baxter Hyland Immuno
Binax, Inc.
Biomed Diagnostics
BioChem Pharma, Inc.
CDC (Centers for Disease Control)
Chiron
Eli Lilly and Company
Merck & Co., Inc.
NIH (National Institutes of Health)
Ortho-McNeil Pharmaceuticals
Pfizer, Inc.
SmithKline Beecham
University of Texas (Galveston)
Wyeth-Lederle Vaccines

MEETING SECRETARIAT

HCC
Health Care Communications, Inc.
(A Kendle Company)
One Bridge Plaza, Suite 350
Fort Lee, NJ 07024 USA
Telephone: (201) 947-5545
Fax: (201) 947-8406
E-mail: IPNC@healthcarec.com

CONTENTS - A QUICK OVERVIEW

CONTENTS

PLENARY SESSION I: TRACK G: NEISSERIAL SURFACE ANTIGENS

Oral Presentations

Poster Presentations

CONTENTS

PLENARY SESSION II: TRACK C: HOST DEFENSE & RESPONSES TO NEISSERIAL INFECTIONS

Oral Presentations

Poster Presentations

CONTENTS

CONTENTS

PLENARY SESSION III: TRACK E: VACCINE UPDATES

Oral Presentations

Poster Presentations

CONTENTS

PLENARY SESSION IV: TRACK D: MOLECULAR AND CELL BIOLOGY OF NEISSERIAL INFECTIONS

Oral Presentations

Poster Presentations

CONTENTS

CONTENTS

PLENARY SESSION V: TRACK H: PHYSIOLOGY AND METABOLISM

Oral Presentations

Poster Presentations

PLENARY SESSION VI: TRACK A-1: ANTIMICROBIAL RESISTANCE

Oral Presentations

CONTENTS

Poster Presentations

CONTENTS

CONTENTS

PLENARY SESSION VII: TRACK F: GENOMICS AND GENE REGULATION

Oral Presentations

Poster Presentations

CONTENTS

PLENARY SESSION VIII: TRACK A-2: EPIDEMIOLOGY OF NEISSERIAL INFECTION

Oral Presentations

Poster Presentations

CONTENTS

#001

THE MENINGOCOCCAL LACTOFERRIN RECEPTOR: STRUCTURE, FUNCTION AND VACCINE POTENTIAL

Tommassen J.#, Prinz T.#, Voet P.*, Pettersson A.#. Dept. Molecular Microbiology#, Utrecht University, The Netherlands, and SmithKline Beecham Biologicals*, Rixensart, Belgium.

Two components of the meningococcal lactoferrin receptor have been identified, i.e. the integral outer membrane protein LbpA and the surface-exposed lipoprotein LbpB. On the basis of homology with the siderophore receptors of *E. coli*, the crystal structure of which has been resolved, a topology model for LbpA is proposed. According to this model, LbpA forms a 22-stranded β-barrel, which is closed by an N-terminal plug. The model was partially confirmed in whole-cell ELISAs with antibodies directed against synthetic peptides, corresponding to the cell surface-exposed loops. Various electrophoretic techniques demonstrated that LbpA forms a dimer that is associated with LbpB. In addition, the RmpM protein is firmly associated with LbpA and appears to contribute to the stability of the lactoferrin receptor complex.

Uptake experiments with $^{55}Fe^{3+}$-loaded lactoferrin revealed that LbpA is essential for iron acquisition from lactoferrin, whereas LbpB is not. ELISAs revealed that a disulfide bond in loop 4 of LbpA is important for lactoferrin-binding, suggesting that this loop is part of the lactoferrin-binding site. Furthermore, LbpA appears to be able to discriminate between the apo- and the holo-form of lactoferrin. The N-terminal three amino acids of lactoferrin are not involved in binding to the receptor, but are essential for the subsequent release of iron from lactoferrin.

Screening of human convalescent antisera revealed the presence of antibodies reacting with LbpB of strain BNCV in most of them, demonstrating that LbpB is immunogenic in man and suggesting a considerable degree of immunological cross-reactivity. Furthermore, monoclonal antibodies were raised against purified LbpB. One of these mAbs reacted with all strains tested and was bactericidal. These results indicated that LbpB could be of great interest as a future vaccine candidate.

#002

IDENTIFICTION OF A FAMILY OF AUTOTRANSPORTER PROTEINS IN NEISSERIA MENINGITIDIS

Wooldridge KG, Abdel-Hadi H, Ait-Tahar K, Robinson K, Turner D, Ala'Aldeen DAA.

Division of Microbiology, University of Nottingham, UK.

By screening a meningococcal expression library for clones with potent T-cell stimulating activity we identified a protein (named AutA) with strong homology to the autotransporter family of proteins. A second gene with strong homology to *autA* was named *autB*. In a different screen using antisera raised against OMPs, we identified a third gene, *app*, which had extensive homology to the IgA protease gene of *N. meningitidis* as well as the adhesion and penetration protein *of H. influenzae*. Finally, two further genes (*map* and *ausP*) belonging to the autotransporter family were detected by searching the genome database with an autotransporter signature sequence.

Results: We have now cloned and expressed all of these autotransporter proteins and so far, we have raised antibodies to AutA, AutB and App. While *autB* appears to be a psuedogene, *autA* and *app* are widely expressed among meningococci of all major hypervirulent lineages and are recognised by convalescing by patients, suggesting that they are expressed during natural infection, and are immunogenic. In addition, they are both capable of stimulating T-cells of patients and healthy donors in vitro. Both AutA and App are processed and secreted by meningococci.

Conclusions: We have identified five new meningococcal autotransporter proteins, which are likely to be virulence factors. The expressed proteins are conserved, immunogenic,

Ref: Ait-Taha et al., 2000. Mol Microbiol. In Press.

#003

MODIFICATION OF FATTY ACID COMPOSITION OF MENINGOCOCCAL LIPID A

Steeghs L.*#, Berns M.#, van Alphen L.#, van der Ley P.#, Tommassen J.*
*Department of Molecular Microbiology, Utrecht University, Utrecht, The Netherlands # Laboratory of Vaccine Research, RIVM, Bilthoven, The Netherlands

Addition of the O-linked 3-OH fatty acids in lipid A is catalyzed by the LpxA acyltransferase. The fatty acyl specificity of LpxA differs among Gram-negative bacteria. To make novel *Neisseria meningitidis* mutants expressing less toxic LPS, we replaced the *lpxA* gene of strain H44/76 by the *E. coli* or *P. aeruginosa* homologues. This resulted in meningococcal strains in which the 3-OH C12 in lipid A has been partly replaced by 3-OH C14 (strain HA01E) or 3-OH C10 (strain HA25P), respectively, as was confirmed by GC-MS. Both strains, but more notably strain HA01E, had a reduced growth rate compared to the wild type strain. HA01E, but not HA25P, also had greatly reduced amounts of LPS as determined by Tricine-SDS-PAGE of whole cell extracts. The major outer membrane proteins were normally expressed in these mutants. Analysis of whole cells and outer membrane complexes (OMCs) in a LAL assay showed reduced endotoxic activity as compared with the wild type strain, which could partly be explained by the reduced amount of LPS present in these mutants. However, when quantified on the basis of the LPS content, a 10-fold reduction in TNF-α induction was seen for OMCs of both mutants. Preliminary data regarding the immunogenicity in BALB/c mice of wildtype and mutant OMCs indicated that there is no significant difference in bactericidal antibodies elicited by wild type and mutant OMCs. It can thus be concluded that replacement of 3-OH C12 by 3-OH C10 or 3-OH C14 in lipid A results in immunogenic *N. meningitidis* outer membranes with reduced endotoxic activity.

#004

OUTER MEMBRANE COMPOSITION OF A *NEISSERIA MENINGITIDIS* LPS-DEFICIENT MUTANT

van der Ley P.#, Steeghs L.*#, de Cock H.*, Tommassen J.*
*Department of Molecular Microbiology, Utrecht University, Utrecht, The Netherlands # Laboratory of Vaccine Research, RIVM, Bilthoven, The Netherlands

The insertional inactivation of the *lpxA* gene required for the first step in lipid A biosynthesis results in *N. meningitidis* strain H44/76 in a viable but LPS-deficient mutant still possessing an outer membrane. The outer membrane composition of this mutant was now studied in more detail. SDS-PAGE and Western blot analysis showed that PorA was partially degraded to a defined breakdown product. This proteolytic fragment as well as the mature PorA still formed trimers which is the normal physiological state of Gram-negative bacterial porins. The expression of the integral outer membrane components LbpA and TbpA of the lactoferrin and transferrin receptor, respectively, was similar to wild type levels, but expression of the lipoprotein components LbpB and TbpB was severely reduced. The most obvious candidates for replacing LPS in the outer membrane are phospholipids. The phospholipid composition of the LPS-deficient strain was different from that of the wild type strain with respect to the length and the saturation of the fatty acyl chains of phoshatidylethanolamine (PE). A preference for saturated PE with C14 and C16 fatty acyl chains was found in the LPS-deficient mutant. Of these phospholipids, saturated PE(C16C16) was unique to the LPS-deficient outer membrane. The viability of an LPS-deficient mutant seems to depend on the presence of the capsular polysaccharide (CPS), since mutants deficient in both LPS and CPS could not be isolated. By using a *lac* promoter-controlled *lpxA* we were able to confirm that LPS-depletion in a CPS-deficient background resulted in severe growth retardation on plates.

#005

STUDIES ON THE VACCINE CANDIDACY OF INNER-CORE LIPOPOLYSACCHARIDE (LPS) OF *NEISSERIA MENINGITIDIS* (*Nm*) GROUP B.

Plested J.S.[1,2], Makepeace K.[1], Coull P.A.[1,2], Mackinnon F.G.[1], Gidney, M.A.J [3], Lacelle, S.[3], Cox, A.D [3], Richards J.C.[3], and Moxon E.R.[1]. [1]Dept Paediatrics, University of Oxford. [2] Dept Clinical Immunology, Oxford [3]NRC, Ottawa, Canada.

Previously we described an inner core LPS epitope that was accessible and conserved in 70% of all major *Nm* serogroups [Plested *et al.*, 1999 IAI 67: 5417-5426]. The conserved epitope recognised by monoclonal antibody (mab) B5 (IgG_3) was identified in L1, L3, L7 to L12 LPS immunotypes, those that have phosphoethanolamine (PEtn) in the 3-position of β-chain heptose (HepII) of inner core LPS. The functional role of mab B5 was assessed in: 1) opsonophagocytosis assay; 2) serum bactericidal assay; 3) passive protection models. Mab B5 demonstrated opsonic but not bactericidal activity against *Nm* strains of immunotype L3. Mabs were obtained using *Nm* L4 *galE* organisms (PEtn in 6 position) and assessed for conservation and accessibility by: (i) dot blots of *Nm* whole-cell lysates; (ii) immunofluorescence microscopy. One of these mabs, A4 ($IgG2_a$), recognised all except three *Nm* B5 non-reactive strains. Together, mabs B5 and A4 recognised 97/100 *Nm* strains. In the presence of capsule, mab A4 accesses the inner core epitope of the *Nm* L4 *galE* mutant but not the core LPS of *Nm* L4 wild-type strain.
In conclusion, a minimum of three inner core LPS structures have been identified in *Nm*: (i) PEtn is present in the 3-position of HepII, (ii) the 6-position of HepII or (iii) PEtn is absent from the inner core. These findings support the potential of inner core LPS glycoforms as candidates for *Nm* Group B vaccine development.

#006

MENINGOCOCCAL-HOST CELL INTERACTIONS IN THE SKIN OF CHILDREN WITH PURPURA FULMINANS: VARIATION IN THE EXPRESSION OF CAPSULE AND TYPE IV PILI?

Harrison O.B[1], Faust S.N[2], Goldin R.D[3], Levin M[2], Robertson B.D[1], Heyderman R.S[4]. Depts of [1]Microbiology, [2]Paediatrics and [3]Histopathology, Imperial College School of Medicine, and [4]Dept of Pathology and Microbiology, University of Bristol.

Objective: To explore the hypothesis that following bloodstream invasion, variation in meningococcal surface structures such as capsule and type IV pili are key features of the interaction between *Neisseria meningitidis* and host cells in vivo.
Design: 3mm skin biopsies were taken from the edge of petechial/purpuric lesions of five children with purpura fulminans after informed consent. Consecutive 4μm formalin-fixed paraffin-embedded sections were immunostained for endothelial (CD31 and CD34), polymorphonuclear neutrophil (neutrophil elastase), monocyte (CD68) and meningococcal surface markers (PorA, capsule and pilin) using the immunoperoxidase technique with antigen retrieval where appropriate. Each section was assessed initially by OBH in comparison to control skin and then reviewed by RDG and RSH to ensure consistency and accuracy of the interpretation.
Results: The general tissue structure of all the biopsies was well preserved and showed evidence of thrombosis and frequently a perivascular inflammatory cell infiltrate consisting of neutrophils (neutrophil elastase positive) and monocytes (CD68 positive). Modified gram-stain revealed from 20 to over 100 gram-negative diplococci in each section. These were localised to neutrophils, small blood vessels (CD31 and CD34 positive) and the dermal interstitium. The specificity of the gram-stain for the invading meningococci was confirmed with sero-subtype specific anti-PorA antibodies. Immunostaining revealed that expression of the capsule and type IV pili by meningococci was not restricted to any particular location, and that meningococci positive for these antigens could be found associated with neutrophils, blood vessels and the dermal interstitium. Conventional co-localisation studies failed to exclude the possibility that there is some downregulation of meningococcal capsule or pilus expression within these different host environments. Further genetic studies are underway to explore this in greater detail.
Conclusions: In the skin lesions of children with purpura fulminans, meningococci express PorA, capsule and type IV pili in association with neutrophils, the vascular endothelium and the dermal interstitium. Whether there is variation in the expression of these molecules to modulate the interaction of meningococci with host cells within these environments remains to be determined.

#007

IDENTIFICTION AND CHARACTERISATION OF App: an Autotranporter Protein of *Neisseria meningitidis.*

Abdel-Hadi H, Wooldridge KG, and Ala'Aldeen DAA.

Division of Microbiology, University of Nottingham, UK.

The autotransporter family of proteins is a divergent family with a variety of biological functions that are thought to be secreted by a common defining mechanism. Here we report the identification, genetic cloning, sequencing and characterisation of a high-molecular weight autotransporter protein in *Neisseria meningitidis.*

Results: We screened a gene expression library using a murine polyclonal antibody raised against meningococcal OMPs. An antigenic recombinant protein was detected and its encoding DNA fragment sequenced. This led to the identification and reconstruction of a complete putative gene encoding a 158 kDa protein. We named the gene *a*dhesion and *p*enetration *p*rotein (*app*), because of its extensive homology to the *hap* gene of *Haemophilus influenzae*. Rabbit anti-App reacted with proteins in all isolates examined, which represented clonal groups responsible for the majority of meningococcal invasive disease. We demonstrated that App is cleaved and secreted by the meningococcus.

Conclusion The biological role of App is currently unknown but its close homology to the Hap protein of *H. influenzae* suggests that it may mediate meningococcal-host cell interactions. Further study including *in vitro* cell adhesion assays will be done to determine whether the App protein in meningococci has a similar biological role to the Hap protein of *H. influenzae.*

#008

INVESTIGATION OF THE POTENTIAL OF App AS A VACCINE CANDIDATE

Abdel-Hadi H, Wooldridge KG, Robinson K and Ala'Aldeen DAA.

Division of Microbiology, University of Nottingham, UK.

Background We have recently identified, cloned and expressed a 158 kDa autotransporter protein in *N. meningitidis.* This protein, named App (*a*dhesion *p*enetration *p*rotein), is highly conserved among different isolates and is secreted by the meningococcus. Here we examined the vaccine potential of App and determined its expression in-vivo, antigenicity and accessibility to immunoglobulins.

Results Immunogold labelling of whole meningococcal cells of strain MC58 demonstrated that App was localised to the cell surface before being secreted. Sera taken from patients convalescing from meningococcal disease contained cross-reactive anti-App antibodies, indicating that the protein is is both expressed during infection, and is a B-cell immunogen. Purified recombinant App strongly stimulated T cells in *in vitro* T-cell proliferation assays.

Conclusion App is conserved, expressed during infection, stimulates B-cells and T-cells and may, in addition, be a primary target for neutralisation of a potential role in pathogenesis. We propose that App is a worthy candidate for further study as a potential component of a future vaccine.

#009

CONSTRUCTION AND CHARACTERISATION OF ISOGENIC MUTANTS IN *N. MENINGITIDIS* SEROGROUP B.

Adu-Bobie J, Giuliani MM, Lupetti P#, Brunelli B, Santini L, Mercati D#, Dallai R#, Grandi G, Rappuoli R, Pizza M. IRIS, Chiron S.p.A., Siena, Italy, and #Unit of Electron Microscopy and Cryotechniques, Dipartimento Biologia Evolutiva, Università di Siena, Italy.

The genome of *N. meningitidis* serogroup B has enabled the identification of previously unknown surface exposed antigens. These genome derived Neisseria antigens (GNA) are highly conserved in sequence among different serogroup B strains, other meningitidis serogroups as well as gonococcus, and most of them induce antibodies with bactericidal activity. Some of these antigens show high sequence homology with proteins of known function expressed by different bacteria. To verify whether the function of these antigens is conserved also in meningococcus, and to define their putative role in pathogenesis and virulence, isogenic mutants were generated in serogroup B strain MC58. In particular, three different antigens were considered in this study: GNA1985 which shows 51% sequence identity to the hap protein, an adhesion and penetration protein factor of *Haemophilus influenzae,* GNA0992 which shows 42% and 45% identity respectively to the adhesins hia and hsf produced by *H. influenzae* and GNA2095 which shows 45% identity to a component protein of an adhesin complex of *Eikenella corrodens.* The mutants show no difference in colony morphology or growth rate as compared to wild type MC58. Further characterisation of these mutants is in progress.

#010

IDENTIFICATION AND CHARACTERISATION OF THE AUTO-TRANSPORTER PROTEIN A (AutA) OF NEISSERIA MENIGITIDIS : A POTENTIAL VACCINE CANDIDATE

Ait-Tahar K, Wooldridge KG, Todd I and Ala'Aldeen DAA. Division of Microbiology, University of Nottingham, UK.

Objective: to identify and characterise CD4+ T-cell and B-cell immunogenic proteins and assess their vaccine potential.

A meningococcal genomic expression library (in λZapII phage) was screened using peripheral blood lymphocytes (PBLs) derived from patients recovering from invasive meningococcal disease. Oligoclones derived from a representative population of Escherichia coli transformants containing recombinant meningococcal DNA were screened.

Results: A potent T- cell stimulating recombinant antigen was identified and its encoding gene isolated. This protein was designated AutA (Auto-transporter protein A) and its amino acid sequence showed significant homology at the C-terminal end with a family of proteins known as IgA-1 protease-like autotransporters. The complete autA gene expressed, as expected, a c. 69 kDa protein. The purified rAutA induced strong secondary T-cell responses in PBLs of patients and some healthy donors, and showed strong primary T-cell responses in all healthy donors examined. AutA is expressed during natural infection as the natively purified protein was recognised by sera from different convalescing patients. Rabbit polyclonal antibodies to rAutA demonstrated, on immunoblots the conserved nature, antigenicity and cross reactivity of AutA amongst meningococci of different serogroups and hypervirulent lineages.

Conclusion: we have identified a potent CD4+ T-cell and B-cell stimulating, auto-transporter protein of *N.meningitidis*.

Reference: Ait-Tahar K et al. Molecular Microbiology, in Press.

#011

BACTERIAL ANTIGEN EXPRESSION IN AN *EX VIVO* WHOLE BLOOD MODEL OF MENINGOCOCCAL BACTEREAMIA.

Anwar N, Levin M*, Ison CA, Dept , Dept Med Micro/*Academic Pediatrics, Imperial College, London, UK.

Objective: To investigate antigen expression by *N.meningitidis* in an *ex vivo* whole blood model of meningococcal bactereamia which mimics the invasive stage of meningococcal disease.

Design: A donor killing meningococci in the whole blood assay (donor A)[1], and one permitting survival (donor B) were selected for comparison of protein expression. Bacteria were recovered at various time points by lysis of the blood followed by neutralisation and centrifugation. Surface exposed epitopes of known proteins were detected by indirect immunofluorescence using mAb and measured by flow cytometry. Expression was also monitored on the level of mRNA synthesis by RT-PCR using primers specific for genes.

Results: Both populations expressed capsule, PorA, PorB, LPS. A decrease in viable bacteria was observed within 15mins in donor A, whereas a two hour lag phase was observed before the bacteria started growth in donor B. Almost 100% of the bacteria that had survived in blood from donor A expressed capsule, PorA, PorB and LPS however a more heterogeneous population was isolated from donor B. The amount of antigens expressed by bacteria grown in blood from donor B was also monitored. The antigens were relatively constant over time, however the total number of cells within the population that expressed a specific amount of antigen varied over time. The expression detected by flow cytometry was confirmed by RT-PCR.

Conclusion: We have shown that surface exposed antigens can be detected using flow cytometry on meningococci grown in an *ex-vivo* whole blood model of meningococcal bacteraemia. This technique has the potential to provide information regarding other antigens that may alter in levels of expression *in vivo* compared to growth *in vitro*. This may be relevant when evaluating known antigens for inclusion into an effective serogroup B vaccine.

#012

HETEROLOGOUS EXPRESSION IN GENOME ANALYSIS: PROPERTIES OF *Neisseria meningitidis* PROTEINS.

Aricò B, Capecchi B, Comanducci M, Serruto D, Bambini S, Jennings GT, Baldi L, Galeotti CL, Grandi G, Rappuoli R, Pizza M. IRIS, Chiron S.p.A., Siena, Italy.

The whole genome sequence of a *N. meningitidis* serogroup B strain was used to identify novel vaccine candidates. In a first approach, a total of 350 potential antigens were successfully expressed in *E. coli* as either His-tagged or GST-fusion proteins, purified and used to immunize mice. Analysis of the sera enabled the identification of proteins that are surface-exposed and able to induce a bactericidal antibody response.

To further characterize the newly identified surface proteins (comprising predicted outer and inner membrane proteins, periplasmic or secreted proteins and lipoproteins) we used an alternative approach by expressing 85 selected putative antigens in *E. coli* as "native" forms. These forms were obtained by cloning the ORFs as full length and without fusion to heterologous moieties. Our results provide evidence that most of the "native" proteins were correctly localised and post-transcriptionally modified (e.g. lipoproteins) in *E. coli*, suggesting that most of these proteins had the correct folding.

In addition, functional analysis of the "native" proteins expressed in the heterologous host is now being carried out. At present, one of the analysed proteins, the putative murein transglycosylase GNA33, purified as a lipoprotein from *E. coli* was shown to have the predicted enzymatic activity (Adu-Bobie J. et al, Abstract submitted).

In conclusion, large-scale screening of hypothetical ORFs by expression in a heterologous system can provide valuable information on the properties of predicted gene products and, at the same time, should hopefully bring some "rules" into the field of heterologous expression.

#013

ROLE OF PILIN GLYCAN PHASE-VARIATION IN GONOCOCCAL PATHOGENESIS

Banerjee A, Parker J, Wang R, Supernavage SL, Gulati S, Rice PA, Wang PG and Gotschlich EC. Department of Biology, The Catholic University of America, Washington, DC.

Background: The pilin glycoprotein (PilE) is the main building block of the pilus of *Neisseria gonorrhoeae* (gonococcus, GC). In GC pilin glycan, a Gal is attached by an α-1,3 glycosidic bond to a GlcNAc which is O-linked to the pilin polypeptide.

Results: A gene (pilin glycosyl transferase A, *pgtA*) has been cloned and characterized that codes for the galactosyl transferase catalyzing the synthesis of the Galα1-3GlcNAc bond of GC pilin glycan. A homopolymeric tract of Gs (poly-G) is present in the *pgtA* genes of many, but not all, GC. A screening of a collection of GC strains and clinical isolates revealed that the poly-G is present in the *pgtA* genes of all (27 out of 27) bacteria obtained from the patients with disseminated gonococcal infection (DGI). In contrast, no poly-G is found in the *pgtA*s of most (27 of 35) of the organisms originating from uncomplicated gonorrhea (UG). Additionally, the presence/absence of the poly-G in *pgtA* correlates strongly with the presence/absence of the β-chain of GC lipooligosaccharide (LOS). In all GC strains that react with the LOS β-chain specific mAb 2C7, a poly-G is present in *pgtA*. However, *pgtA*s of all 2C7-non-reactive GC lack the poly-G.

Conclusions: GC *pgtA* is the first gene discovered which can occur both in a phase-variable and in a phase-invariable form. Phase-variation of *pgtA* likely facilitates the conversion of UG to DGI. In contrast, the phase-invariable expression of *pgtA* may help GC to establish the initial infection of the genitourinary tract.

#014

A GENOTYPING SYSTEM FOR *NEISSERIA GONORRHOEAE* BASED ON BIOTINYLATED OLIGONUCLEOTIDE PROBES TO PIB AND PIA GENE VARIABLE REGIONS

Bash MC[1], Thompson DK[1], Lynn F[1], Ison CA[2], Wetzler LM[3], Deal CD[1], Zenilman JM[4]

[1]Division of Bacterial Products, CBER, FDA, Bethesda, MD; [2]Imperial College School of Medicine, London, UK; [3]Boston University School of Medicine, Boston, MA; [4]Johns Hopkins University School of Medicine, Baltimore, MD

The porin proteins PIA and PIB of *Neisseria gonorrhoeae* are serotyping antigens for the serovar classification system and candidates for gonococcal vaccines. Serotyping can be insensitive to critical sequence changes in the *por* gene, including those in surface-exposed variable regions (VRs). We have recently described a genotyping system for *N. gonorrhoeae* PIB strains that uses biotin-labeled oligonucleotide probes to PIB gene VRs corresponding to loops 1, 5, and 6. We have developed a similar typing system for PIA strains based on VR encoding loops 1, 2, 3, 6, and 7, and expanded the PIB system to include probes to regions encoding loops 3 and 7.

Results: The PIA VR type of 17 PIA strains representing 6 sexual partnerships and 5 geographically and temporally diverse strains was determined. Partner strains had identical *por* VR type. Among the 6 partner groups, 3 different por VR types were identified. All 5 diverse PIA strains were distinguished by *por* VR typing.

Por VR typing of 22 strains from Boston was performed in a blinded fashion. Eight PIA strains were identified, and the *por* VR type accurately distinguished four partner groups. The 14 PIB strains were from 7 partnerships, and all partners' strains had identical VR type. These strains were divided into two groups of two, one group of four and one group of six based on *por* VR type.

Conclusions: *Por* VR typing provides a useful degree of discrimination between strains as well as specific information about the molecular epidemiology of individual *por* VRs. A limitation of the current *por* VR typing method is that separate hybridization reactions are required for each probe. To address this, and to allow for eventual use of DNA microarrays, we are redesigning the PIA and PIB probes such that they can be used at a single hybridization temperature. We will use this panel of probes in checkerboard hybridizations, which will allow use of the genotyping system in large scale studies.

#015

LPS ACTS SYNERGISTICALLY WITH PorA AND PorB IN AFFECTING AN ANTI-PorA AND ANTI-PorB SPECIFIC IgG RESPONSE IN *NEISSERIA MENINGITIDIS.*

Bhasin N, Ho Y and Wetzler LM. Section of Infectious Diseases, Boston Medical Center and Boston University School of Medicine, Boston, MA.

The existing meningococcal polysaccharide vaccine is ineffective against serogroup B *Neisseria meningitidis* strains mainly because the serogroup B polysaccharide capsule cross-reacts with human brain antigens and is poorly immunogenic. As such, various outer membrane proteins are being evaluated for their potential use as vaccines. To this effect, we examined the levels of IgG elicited against PorA and PorB porins in mice immunized with group B *N. meningitidis* and the role of lipopolysaccahride in eliciting and modulating this response. This was achieved by evaluating the immunogenicity of wild type Group B meningococcal strain H44/76 and its recently derived LPS$^-$ mutant in LPS responsive (C3H/HeOuJ) and LPS hypo-responsive (C3H/HeJ) mice. IgG concentrations in the immune sera were quantitated by ELISA against purified PorA, PorB and LPS. The IgG concentrations elicited against PorB were about 1-2 logs higher than those against PorA in sera from mice immunized with *N. meningitidis*. The anti-PorB and anti-PorA IgG levels in mice immunized with the respective purified porins were almost equivalent. LPS appears to act synergistically with PorB in eliciting an anti-PorB response; higher anti-PorB IgG levels were detected in C3H/HeOuJ mice immunized with H44/76 as compared to mice immunized with its LPS$^-$ mutant, while the anti-PorB responses were similar using either bacterial strain in C3H/HeJ mice. The potential immunostimulatory activity of the *N. meningitidis* porins (which are currently being used as vaccine adjuvants) from either strain was assessed in an *in vitro* B cell activation assay. Outer membrane complexes prepared from H44/76 and the LPS$^-$ mutant induced a similar increase in expression of B7-2, B220 and class II MHC (Iak) cell surface markers in primary murine splenic B cells. This indicated that LPS is not necessary for the porins' immunostimulatory ability. However, as stated, LPS might have a greater (but not absolutely essential) role in inducing the anti-porin humoral responses.

#016

THE OPACITY PROTEINS OF THE NEISSERIAE

Callaghan M.J, Maiden M.C.J. Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK.

Objective: Opacity proteins have been identified as factors influencing the adhesion and invasion of host cells. Study of these proteins is, therefore, important in terms of understanding meningococcal infection and host-commensal interactions.
A multidisciplinary strategy including biochemical, bioinformatic and phylogenetic techniques is being used to investigate the *opa* repertoire among pathogenic and commensal *Neisseria*.

Design:
PCR and rapid automated DNA sequencing were used to define the opa genes from our collection of *N. meningitidis* and *N. lactamica* clinical isolates. A number of commensal isolates, for which the presence of these genes has not previously been documented, have also been probed. A range of phylogenetic approaches are being used to analyse the data. Two dimensional polyacrylamide gel electrophoresis (2D PAGE) is being used to investigate the expression patterns of *opa* genes and other outer membrane proteins in both the meningococcus and commensal strains.

Results:
opa sequences have been identified in all isolates from our commensal collection. This data, in addition to that generated from our collections of *N. meningitidis* and *N. lactamica* is being analysed by phylogenetic techniques such as split decomposition analysis.

#017

THE MEMBRANE PHOSPHOLIPIDS OF *NEISSERIA MENINGITIDIS* AND *NEISSERIA GONORRHOEAE* AS CHARACTERIZED BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY

MM Rahman[1], Kolli[1]VSK, Kahler[2]CM, Shih[2]G., Stephens[2]DS, and Carlson[1]RW. [1]Complex Carbohydrate Research Center, Univ. of Georgia, Athens, GA; and [2]Depts. of Med. and Microbiol. and Immunol., Emory Univ, School of Med., Atlanta, GA; and Dept. of Veterans Affairs Med. Center, Atlanta, GA.

The phospholipids (PLs) of *N. meningitidis (Nm)* and *N. gonorrhoeae (Ng)* were characterized by fast atom bombardment-mass spectrometry (FAB-MS) and GLC-MS. The major PLs were phosphatidylethanolamine (PE), followed by phosphatidylglycerol (PG), with minor amounts of phosphatidic acid (PA), and trace levels of cardiolipin (DPG). All PLs varied in their fatty acyl substituents which included C16:1, C16:0, C18:1, C14:0, C14:1, and C12:0. By MS/MS analysis, all PLs contained a saturated fatty acyl, and a saturated or unsaturated fatty acyl substituent in the *sn1* and *sn2* positions, respectively. Compared with enteric bacterial species, the phospholipids of *Nm* and *Ng* have increased levels of PLs with short chain fatty acyl residues (i.e., increases in C12:0, C14:1, and C14:0) and variable amounts of C18:1. The percentage of total PE and PG molecules with the shorter chain fatty acids ranges from 35-47% and 42-66%, respectively, for *Nm* while these respective values are <10% and <5% for *E. coli*. The variability and variety of *Nm* and *Ng* PLs suggests novel mechanisms of PL synthesis, which may be important for *Nm* and *Ng* pathogenesis. Supported in part by PHS grant AI-33517 to DSS and DOE grant DE-PG09-93ER20097 to the CCRC.

#018

STRUCTURALLY CONSTRAINED PEPTIDE MIMICS OF MENINGOCOCCAL LIPOOLIGOSACCHARIDE.

Paul J. Brett[1], Ian M. Feavers[2], and Bambos M. Charalambous[1]. [1]Department of Medical Microbiology, Royal Free & University College Medical School, Royal Free Campus, Rowland Hill St, London NW3 2PF. [2]Division of Bacteriology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Hertfordshire EN6 3QG.

Meningococcal lipooligosaccharide (LOS) is potentially an alternative vaccine candidate to capsular or cell surface expressed protein antigens, although its endotoxicity and mimicry of part of its structure to cell surface antigens poses serious concerns. However, there are epitopes within the LOS moiety that are immunogenic in man. Consequently, we are determining whether peptides which mimic these epitopes can elicit an immunogenic response that may also provide protection against group B disease. A variety of linear and conformational peptides from several phage display libraries have been screened against the murine monoclonal antibody, 9-2-L3,7,9. This Mab is bactericidal with a high affinity towards the LOS immunotype L379 most frequently associated with disease. Although linear and conformational peptides have been identified the latter show binding affinities from 10.9±0.95(SD) nM to 184.39±18.45 nM comparable to binding of LOS (7.5nM). These conformational peptides also competed effectively for binding of LOS to the mAb, indicating they bind to the same paratope as LOS. The structure of these conformational peptides is critical to their binding and ability to compete with LOS. Phage expressing a conformational peptide at the exposed N-terminus of the membrane-associated PIII protein elicit a weak antigenic response against LOS in mice. To enhance this response various conjugate structures are being developed and tested in mice.

#019

THE OLIGOMERIC STRUCTURE OF THE *NEISSERIA MENINGITIDIS* SECRETIN COMPLEX PILQ AND IMPLICATIONS FOR TYPE IV PILUS BIOGENESIS.

Collins[1], R.F., Davidsen[2], L. Derrick[1], J.P., Ford[1], R.C. & Tønjum[2], T.
[1]Department of Biomolecular Sciences, UMIST, P.O. Box 88, Manchester, M60 1QD, U.K.
[2]Institute of Microbiology, Section of Molecular Microbiology, Rikshospitalet, N-0027 Oslo, Norway

PilQ is a member of the secretin family of outer membrane proteins and is specifically involved in the secretion of type IV pili in *Neisseria meningitidis* and *N. gonorrhoeae*. The quaternary structure of PilQ from *N.meningitidis* was analysed by transmission electron microscopy on negatively stained material. Single particle averaging was carried out on 650 individual particles, producing a projection map at a determined resolution of 2.6nm. Oligomeric PilQ adopts a donut-like structure, with an outside diameter of 16.5nm surrounding a central cavity of 6.5nm diameter. Self rotation analysis demonstrated the presence of 12-fold rotational symmetry, suggesting that PilQ is organised as a dodecameric ring of 12 identical subunits. A model of the type IV meningococcal pilin fibre, based on the X-ray crystal structure of the highly similar *N.gonorhoeae* pilin subunit, fitted neatly into the cavity, suggesting that PilQ and the growing pilus fibre are in close contact during pilus biogenesis. These data demonstrate the precise structural complementarity between a secretin and its substrate protein.

#020

GENE VARIABILITY ANALYSIS ON MEN B VACCINE CANDIDATES

Comanducci M, Capecchi B, Bambini S, Masignani V, Grandi G, Rappuoli R, Pizza M. IRIS, Chiron SpA, Siena, Italy.

DESIGN: By genome analysis of MC58 strain, we identified novel surface exposed antigens, useful for the development of a vaccine against menB. To evaluate whether these antigens are produced also by other strains and to study their conservation, 34 different strains representing the diversity of the neisserial population have been analyzed.
RESULTS: Seven genes of *Neisseria meningitidis*, coding for proteins able to evoke antibodies with bactericidal activity, were sequenced in 34 different neisserial strains, spanning seven *N.meningitidis* serogroups and *N. cinerea*, *N. lactamica*, and *N. gonorrhoeae*. All the gene sequences were compared and analyzed for their diversity. The porA gene was used as a standard, as known to code for a hypervariable protein. Western blot analysis was performed to check the production of each protein in all strains. Unexpectedly, although surface exposed proteins are generally variable because prone to selective pressure by the immune system, most of the antigens under study were well conserved in sequence and not mosaic in structure. The frequency of recombination was calculated using the Homoplasy test: the final value resulted to be comparable with the one formerly obtained using a panel of housekeeping genes. A phylogenetic analysis was therefore performed with this set of genes, by complexing their sequences in a single "multigel": this allowed to cluster hypervirulent strains in agreement with the results previously obtained with Multi Locus Enzyme Electrophoresis and Multi Locus Sequence Typing techniques applied to housekeeping genes.
CONCLUSIONS: We show that the meningococcal antigens identified by genome analysis and shown to be able to induce bactericidal are expressed and conserved in all strains. Moreover, we show that these proteins could be used for a phylogenetic analysis of meningococcal population.

#021

STRUCTURAL ANALYSES OF LPS FROM *NEISSERIA MENINGITIDIS* STRAINS NON-REACTIVE WITH MONOCLONAL ANTIBODY B5.

Cox, A.D[1]., Li, J[1]., Gidney, M.A.J[1]., Lacelle, S[1]., Martin, A[1]., Plested, J.S[2]., MacKinnon, F.G[2]., Moxon, E.R[2]. and Richards, J.C[2]. [1]Inst. for Biological Sciences, NRC, Ottawa, Canada. [2]Inst. for Molecular Medicine, University of Oxford, UK.

A monoclonal antibody (MAb) B5 has previously been shown to recognise an epitope containing phosphoethanolamine (PEtn) at the 3–position of the β–chain heptose (HepII) in the inner core of *Neisseria meningitidis* (*Nm*) LPS (Plested *et al.*, 1999). This MAb was obtained following immunisation with meningococcal cells expressing immunotype L3 *galE* LPS.

The structural basis of MAb B5 reactivity was studied further by structural analyses of LPS from several MAb B5 non-reactive (B5-) strains, identifying additional structural features that preclude recognition with MAb B5. Structural analyses provided an excellent correlation between lack of B5 reactivity and absence of the PEtn moiety from the 3-position of HepII and will be described.

Interestingly, one strain of *Nm* designated BZ157, was shown to have two natural variants that were MAb B5 reactive (B5+) or non-reactive (B5-). Structural analysis of purified LPS extracted from the B5- BZ157 *galE* mutant revealed the presence of a glucose residue at the 3-position of HepII. Detailed analysis of the BZ157 *galE* B5+ LPS confirmed that the major glycoform contained PEtn and not a glucose residue at the 3-position of HepII. Additionally a subset of glycoforms that elaborated 2 PEtn moieties at this β-chain heptose residue were discovered. Evidence for this glycoform was obtained by ES-MS analyses, CE-MS-MS techniques and also NMR spectroscopy and will be described in detail.

#022

THE IDENTIFICATION OF AN INVASION ASSOCIATED OPACITY PROTEIN PRESENT IN MENINGOCOCCAL B STRAIN H44/76

De Jonge MI, Van Alphen L, Van der Ley P
Laboratory of Vaccine Research, RIVM Bilthoven, The Netherlands

Objective: To identify the previously described invasion associated 28 kDa opacity protein present in the meningococcal B strain H44/76.Design: An investigation of the different sequences encoding the opacity proteins present in *Neisseria meningitidis* serogroup B strain, H44/76 and the expression of Opa proteins in an invasive and a non-invasive variant. With the use of two monoclonals with known epitopes a distinction could be made between the invasive and non-invasive variant. This has been confirmed with OMC- and Whole cell ELISA and SDS-PAGE. The invasive variant of H44/76 was selected by colony-blot, a direct colony PCR was performed to determine the in- or out of frame state.
Results: Three of the four *opa* genes appeared to be identical with the *opa* genes present in another Norwegian strain, Nm B 190/87. In the recently sequenced genome of MC58 the same genes were found. Two of the four Opa proteins appeared to have an identical HV1 and HV2 region. The other two opacity proteins have an identical SV and HV1 region. The invasive variant found by De Vries et al. (1996) was obtained after three succesive cycles of infection and exposure to gentamicin. Monoclonal 15-1-P5.5 (W. Zollinger) is reacting with the opacity proteins expressed in the invasive variant while αD2 (B. Kuipers) is reacting with the Opa proteins in the non-invasive variant. The epitope of 15-1-P5.5 is present in two of the four Opa proteins present in H44/76. With the direct-colony PCR experiments only *opa J 129* appeared to be in-frame.
Conclusions: The previously described 28 kDa invasion associated Opa protein is likely to be Opa J 129, which is an identical sequence of strain Nm B 190/87 submitted to GenBank. Three out of four opacity proteins present in Nm B 190/87 and MC58 are 100 % homologous to Opa proteins in H44/76. The invasion associated Opa J 129 was expressed as PhoE-Opa fusion protein in *E. coli*. The Opa fusion protein was exported and assembled in the outer membrane of *E. coli* in a manner similar to that of Opa in *N. meningitidis*, as evaluated by antibody binding. The use of *E. coli* expressing Opa fusions can be useful in defining the pathogenic role of one particular Opa protein, excluding the influence of other meningococcal outer membrane components.

#023

MUTATION OF THE *lpxL* GENE IN NEISSERIA GONORRHOEAE

Ellis CD [1], Khan CMA[1], Lindner B[2], Zähringer U[2] and Demarco de Hormaeche R[1]. [1] Department of Microbiology and Immunology, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle, NE2 4HH, UK. [2] Borstel Research Centre, Institut für Experimentelle Biologie und Medizin, Borstel, Germany.

Like all Gram negative bacteria the outer membrane of the gonococcus contains lipopolysaccharide (LPS) which is a well known mediator of inflammation. It is generally accepted that the lipid component (lipid A), rather than the oligosaccharide chain is responsible for the inflammatory effect. We have attempted to investigate the role of a lipid A constituent in causing inflammation by cloning and disrupting the *lpxL (waaM, htrB)* gene from the strain MS11 *aroA*. The cloned gene was inserted into plasmid pBR322, inverse PCR was used to create unique sites within the gene which was then disrupted by insertion of an erythromycin gene cassette. After PCR amplification the disrupted gene was amplified by PCR and the product was used for transformation of MS11 *aroA*.

SDS-PAGE analysis showed that the resulting mutant bacteria had an altered LPS profile in comparison to the parent strain LPS. Mass spectrometric analysis of the crude lipid A fraction from the LPS of the *lpxL* mutant showed that it had lost a lauric acid residue from the (R)-3-hydroxyl myrisitic acid at the 2 position of the non reducing end of the lipid A while keeping a lauric acid at the 2 position of the reducing end. Both, parent and mutant LPSs were examined for their ability to induce cytokine production from the human histiocyctic cell line U937. The *lpxL* mutant LPS induced a reduced level of all cytokines investigated.

In conclusion, the presence of lauric acid at the 2 position of the non reducing end of the lipid A encoded by the *lpxL* gene is crucial for the induction of inflammatory cytokine production.

#024

IMMUNOGENICITY AND BACTERICIDAL ACTIVITY OF CONSERVED *N. MENINGITIDIS* SEROGROUP B ANTIGENS

Giuliani MM, Pizza M, Santini L, Brunelli B, Luzzi E, Adu-Bobie J, Granoff D.M[1], Grandi G, Rappuoli R. IRIS,Chiron S.p.A., Siena, Italy. [1]Children's Hospital Oakland Research Institute, Oakland, USA.

To prevent group B infections, the approach using polysaccharides in vaccine formulations cannot be used because of the cross-reactivity of the capsular polysaccharide with host antigens. In order to identify novel vaccine candidates we determined the genomic sequence of a virulent serogroup B strain MC58. 350 novel proteins were successfully expressed in *Escherichia coli* and used to immunize mice. Immune sera were then tested in Western blot on Men B total cell lysate and outer membrane vesicles (OMV), in enzyme-linked immunoabsorbent assay (ELISA) and Fluorescence Activated Cell Sorter (FACS) analyses on a Men B strain, and for bactericidal activity, a property known to correlate with efficacy in humans. The screening allowed the discovery of 85 new surface-exposed proteins, 25 of which induced antibodies with bactericidal activity. Some of these antigens are very well conserved in sequence for a set of 35 strains representing the sequence diversity of the *Neisseria* population strains. In the present study we describe the properties of nine novel proteins: GNA1946, GNA33, GNA1162, GNA992, GNA86, GNA2001, GNA1030, GNA 2095 and GNA1220. Most of these are lipoproteins and outer membrane proteins. We have evaluated the expression and bacterial surface exposure in the MenB population of these antigens analyzing the respective sera by ELISA using 32 capsulated bacteria strains to coat the plates. and by FACS on strains whose capsule had been permeabilized by treatment with ethanol. For several of these antigens, using different approaches, we confirmed that the bactericidal antibodies are specific for the protein analyzed.

#025

GONOCOCCI POSSESS AN ALTERNATIVE LIPOOLIGOSACCHARIDE SIALYLATION SITE NOT RECOGNIZED BY MAB 3F11

Gulati S, Ram S, Rice PA. Boston Medical Center, Boston, MA.

Gonococcal α(2.3)-sialytransferase can sialylate the terminal Gal of the 4.5 kDa lacto-N-neotetraose structure of lipooligosaccharide (LOS) when cytidinemonophosphate-N-acetylneuraminic acid (CMP-NANA) is added to growth media. Sialylation of gonococcal LOS confers a serum resistant phenotype to the organism. We identified a gonococcal strain, 398078 that did not bind mAb 3F11 (specific for terminal Galß1→4GlcNAc epitope in the unsialylated state), but did become serum resistant upon growth in CMP-NANA–containing media, suggesting the presence of an alternative sialylation site. SDS-PAGE demonstrated only a 3.6 kDa LOS band, which showed slower migration when bacteria were grown in CMP-NANA, confirming sialylation; neuraminidase treatment restored the 3.6 kDa band and incompletely reversed serum resistance. These data suggest that gonococci can potentially sialylate an LOS structure that lacks the conventional 3F11 sialylation epitope. LOS structures are known to vary considerably in vivo, and an alternative sialylation site may permit the organism to survive complement-mediated lysis during periods of LOS antigenic variation.

#026

IMMUNISATION WITH MULTIVALENT LIPOSOMES CONTAINING RECOMBINANT CLASS 1 PROTEINS FROM *NEISSERIA MENINGITIDIS* SEROGROUP B.

Humphries, H.E., Jolley, K., Pead, P.J., Christodoulides, M., and Heckels, J.E. Molecular Microbiology, Southampton University Medical School, Southampton, U.K.

The meningococcal Class 1 outer membrane protein (PorA) has been proposed as a potential vaccine candidate for serogroup B *N. meningitidis*. Given the extensive antigenic diversity of PorA a successful vaccine must contain multiple PorA serosubtypes to cover the majority of serogroup B strains in circulation. The aim of these studies is to develop a multivalent, Class 1 protein, liposome vaccine suitable for use in humans.

Recombinant PorA proteins, containing the four most common serosubtypes present in serogroup B outbreaks in the U.K (P1.4: 25%, P1.15: 12%, P1.16: 8%, and P1.10d: 6%); were expressed in *E. coli* using the pQE-30 (Qiagen) high expression vector and purified with histidine$_6$ tag technology. To regenerate their natural conformation, the proteins were incorporated into liposomes singly, divalently, and tetravalently. Mice were immunised with each of these liposome preparations, with mixtures of the individual Class 1 protein liposomes, and with Class 1 protein liposomes in combination with liposomes incorporating monophosphoryl lipid A (MPLA) as an adjuvant.

The serosubtype specificity of the antibodies induced was analysed by ELISA, and their ability to induce complement-mediated killing of the bacteria was determined. The various Class 1 protein liposomes were immunogenic, inducing high titres of antibodies against the respective homologous strain. Antibody titres were further increased when the immunising dose included adjuvant liposomes. These results indicate the potential for liposomes containing recombinant Class 1 proteins to be included in a multivalent vaccine against serogroup B meningococcal infection.

#027

IDENTIFICATION OF A NOVEL GENE INVOLVED IN PHOSPHORYLATION OF INNER CORE LPS IN NEISSERIA MENINGITIDIS.

Mackinnon FG[a], Cox AD[b], Tang CM[a], Kathy Makepeace[a], Plested JS[a], Hood DW[a], Chalmers RM[c], Richards JC[b], Moxon ER[a]. [a]Dept. of Paediatrics, IMM, University of Oxford, UK. [b]IBS-NRC, Ottawa, Canada. [c]Dept. of Biochemistry, University of Oxford, UK.

Monoclonal antibody (Mab) B5 specifically recognises inner core LPS structures of *Neisseria meningitidis* (*Nm*) that contain phosphoethanolamine (PEtn) attached at the 3-position on the β-chain heptose. Using *in vitro* mutagenesis, a putative LPS phosphoethanolamine transferase gene was identified in *Nm*. *Tn*10 was used to construct a library of kanamycin resistant mutants in a B5 reactive *Nm* strain (MC58). Introduction of genomic DNA from this library into a B5 non-reactive *Nm* strain (NGH15) identified a region of DNA (linked to the Tn10 insertion site) that conferred Mab B5 reactivity. Detailed examination of the region in strain MC58 revealed a series of open reading frames of unknown function. Insertional inactivation of one of these open reading frames, designated as *lpt* (LPS phosphoethanolamine transferase), resulted in abolition of Mab B5 binding in strain MC58. ES-MS analysis of the structure of LPS from the *lpt*- mutant indicated loss of the PEtn moiety from the inner core.
In a survey of neisserial strains, PCR amplification and Southern analysis revealed all B5 reactive strains possessed the *lpt* gene, further evidence that the *lpt* gene is required for PEtn substitution at the 3-position on the β-chain heptose of *Nm* inner core LPS.

#028

PATHOGEN ASSOCIATED *PIL* HOMOLOGUES IN COMMENSAL *NEISSERIA* : VIRULENCE GENE TRANSFER OR INTRA-SPECIES VARIATION?

Marshall ZV[1], Allison HE[1], Kerényi M[1], Hart CA[2] and Saunders JR[1]
[1] School of Biological Sciences
[2] Department of Medical Microbiology, University of Liverpool, Liverpool L69 7ZB, UK

The class II pili of *Neisseria meningitidis* shows greater structural and sequence homology to pili of commensal *Neisseria* species than to the meningococcal class I and gonococcal pilins [1]. In meningococci expressing class II pili, both non-expressed pilin gene copies (*pilS*) are homologous to the class I gene. These observations suggest a common ancestor for the class II meningococcal and commensal pilins that is distinct to that of the meningococcal class I and gonococcal pilins.
Evidence of inter-species gene transfer within the genus *Neisseria* is well documented. The transfer of commensal pilin sequences to *N. meningitidis* may be responsible for the evolutionary divergence of the two distinct pilin classes. To assess the potential for pilin gene exchange between commensal and pathogenic *Neisseria* species, sixty one commensal isolates from eight different species were screened for class I *pil* sequences using medium-low stringency Southern hybridization. Two isolates of commensal species *N. flavescens* and *N. lactamica*, identified by sugar fermentation pattern and 16S RNA gene sequencing, were found to harbour Class I meningococcal *pilE* like sequences. These putative class I *pil* homologues, together with the class II meningococcal *pilE* may represent products of a bi-directional transfer process in which virulence determinants such as *pil* genes and other genetic elements are exchanged. Thus pathogenic and commensal *Neisseria* may provide a reciprocal reservoir for the exchange of genetic information within the nasopharyngeal flora. These gene transfer events could lead to the formation of novel pathogenic strains with increased virulence.

1. Aho EL, Keating AM, McGillivray SM. A comparative analysis of pilin genes from pathogenic and nonpathogenic Neisseria species. Microbial Pathogenesis 2000; 28: 81-88

#029

GENETIC MECHANISMS RESPONSIBLE FOR NONGROUPABLE NASOPHARYNGEAL CARRIAGE ISOLATES OF *NEISSERIA MENINGITIDIS*

Miller, Y.K.[2], Dolan-Livengood, J.M.[2], Kellerman, S.E.[3], McCombs, K.[4], Ray, M.[4], Baughman, W.S.[1], and Stephens, D.S.[1,2]

[1]Department of Veterans Affairs Medical Center, Atlanta, GA, USA
[2]Emory University School of Medicine, Atlanta, GA, USA
[3]Centers for Disease Control and Prevention, Atlanta, GA, USA
[4]Division of Public Health, Georgia Department of Human Resources, Atlanta, USA.

Meningococci recovered from the nasopharynx are often nongroupable. Several genetic mechanisms (slipped-strand mispairing of *synD*, IS*1301*, IS*1106*, novel capsular serogroups, recombination events in the capsular biosynthesis locus) have been shown or proposed to explain nongroupable strains. However, the occurrence of these events in defined populations of meningococcal clinical isolates has not been determined. One hundred ninety-five meningococcal nasopharyngeal carriage strains were isolated from 2,730 asymptomatic high school students in two Georgia counties. Thirty-five of these strains were found by serologic screening to be nongroupable. We undertook to determine the mechanisms for nongroupability in this defined population of isolates. PCR was used to screen for the presence and size or absence of individual genes of the capsule biosynthesis operon. In 8.6% (3/35) of strains the biosynthesis operon was intact and contained the serogroup B polymerase *synD*. These strains are predicted to be nongroupable due to slipped-strand mispairing. Two (5.7%) of the nongroupable strains contained an insertion of IS*1301* in the *syn* operon. IS*1106* was not detected in any of these nongroupable strains. None of the strains contained *sacA*, the first gene in the synthesis operon of serogroup A *N. meningitidis*. The majority (51.4%, or 18/35) of the strains did not, by PCR, contain any genes of the sialic acid biosynthesis (*syn*) operon. In 25.7% (9/35) of the strains the sialic acid biosynthesis genes *synA-C* were present, but a serogroup B polymerase was absent; however, 2 of these strains contained a serogroup C polymerase, and 6 had the serogroup Y/W-135 polymerase. In conclusion, the majority of nongroupable strains did not contain a recognized meningococcal capsule biosythesis operon. The insertion of IS*1301* and slipped-strand mispairing in *synD* appear to be less-prevalent mechanisms of nongroupability for *N. meningitidis* in nasopharyngeal isolates.

#030

BACTERIAL SURFACE BINDING AND BACTERICIDAL ACTIVITY OF ANTI-NSPA MABS AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP B (NmB) STRAINS

G. R. Moe, P. Zuno, S. S. Lee, J. Wang, A. H. Lucas, and D. M. Granoff, Children's Hospital Oakland Research Institute, 5700 Martin Luther King, Jr. Way, Oakland, CA 94609

Background: Although Neisserial surface protein (NspA) is highly conserved, we reported that one-third of genetically diverse NmB strains were negative for surface binding by flow cytometry, and resisted bactericidal activity (BCA) of murine polyclonal antibodies against recombinant NspA (Infect Immun 1999;57:5664).
Objective: To prepare anti-NspA Mabs and determine surface binding by flow cytometry and susceptibility of genetically diverse NmB strains to complement-mediated bacteriolysis in relation to capsular (inhibition ELISA) and/or NspA (SDS PAGE) production.
Results: 5 mAbs were prepared to rNspA. One (12F2.1) bound to the bacterial surface of all 9 strains tested, including 4 strains that had been negative for surface binding with polyclonal antiserum. Relative binding of the mAb was inversely proportional to capsular production. The only exception, strain 8047, was positive for binding despite high capsular production but had increased NspA. For BCA, 4 strains were killed by $\leq$1µg/ml of the anti-NspA mAb, and 5 required $\geq$ 10 µg/ml but all 9 were killed by a mAb to the capsule. In contrast to anti-NspA surface binding, strain susceptibility to anti-NspA BCA was not related to capsule production (1/GMT: 513 vs. 600, respectively). Also, 3 of 6 strains with strong surface-binding by flow resisted BCA.
Conclusions: An anti-NspA mAb can bind to the surface of diverse NmB strains but the amount of bound mAb is affected by capsular production and/or strain differences in NspA production. Further, some strains with strong antibody binding to the surface resist complement-mediated lysis. These factors may limit the ability of a NspA-based vaccine to elicit broad protection against NmB strains.

#031

DEVELOPMENT OF STRUCTURAL MODELS OF MENINGOCOCCAL TRANSFERRIN BINDING PROTEIN A

J.S.Oakhill[1,2], A.R.Gorringe[2], C.L.Joannou[1], R.Pajon[3] and R.W.Evans[1].
[1]Metalloprotein Research Group, Division of Biomolecular Sciences, King's College London, London, U.K. [2]Centre for Applied Microbiology and Research, Salisbury, U.K. [3]Center for Genetic Engineering and Biotechnology, Havana, Cuba.

Introduction. *Neisseria meningitidis* expresses two transferrin-binding proteins, TbpA and TbpB. TbpA is thought to be a porin-like integral protein which functions as a channel for the passage of iron into the periplasm. A number of topology models for TbpA have been proposed comprising from 26 to 38 transmembrane ß-strands. The crystal structures of FepA and FhuA, 2 *E.coli* siderophore receptors that exhibit approximately 20% homology with TbpA, both show 22-stranded ß-barrels and a globular N-terminal domain that folds into the barrel to create a plug.
Results. A 2-D model of TbpA was generated using sequence alignments with FepA and FhuA. 24 putative transmembrane regions were identified using interstrain sequence comparisons and a number of structure prediction programs. These form a barrel occluded by a N-terminal plug region of 162 residues containing a TonB interacting region. A number of peptides representing surface exposed loops and membrane spanning regions were synthesised and several have been shown to have transferrin-binding activity. A 3-D model based on FepA using MODELLER 4 has also been developed.
Conclusions. 2-D and 3-D models of TbpA have been developed based on homology with FepA and FhuA. These indicate fewer ß-strands than proposed in previous models and the presence of a N-terminal plug region.

#032

REGIONS OF MENINGOCOCCAL TBPS RESPONSIBLE OF RECOGNITION BY HUMAN SERA.

Pajón R. [1];. Pérez, L.[1]; Nazábal C [1]; Silva, R. [1]; Reyes, O.[1]; Rodríguez, A. [1]; Gorringe A.[2]; Borrow R.[3]; Oakhill J.[3]
[1]CIGB,Apartado Postal 6162, La Habana-10600, Cuba. [2]Centre for Applied Microbiology and Research, Salisbury SP4 0JG, UK. [3]Manchester Public Health Laboratory, Withington Hospital, Manchester M20 2LR, UK.

Pathogenic Neisseria express two transferrin binding proteins (Tbps,TbpA and TbpB)[1] .Cross-reactive antibodies to meningococcal TbpA+B complexes and to the isolated proteins have been detected in both patients and earners [2]. In this study we have characterized the human response against linear determinants in both Tbps from B385 strain, and TbpB from B16B6 strain, by using overlapping peptides (18-mer long). Their ability to bind human sera, initially tested for 5 patients, was extended to 17 survivors of meningococcal disease.
Human response was found to be directed against TbpA peptides 7-8, 21-23, 27-24, 30-31, 42, 50,55,59, 67 83-84; and peptides 5, 12, 17, 25, 39, 44, 48, 55, 56 for B385 TbpB. The response against B16B6 TbpB was directed against the peptides 4-5, 9-10, and 19 with most cross-reactivity showed in the Nt region. Human response, from the 17 patients, was clearly broad and comprised conserved and variable regions, with the previously reported TbpA VR1 [3], and its flanking regions, as the most notable. In the case of TbpB, the human antibody response was different in the number and distribution of the immunoreactive regions identified for each strain, standing for the existence of a cross-reactive response against the Nt terminal region.

References
1. Rokbi, B., *et al* 1993 FEMS Microbiol Lett 110:51-58
2. Johnson, A. S *et al*1997 FEMS Immunol Mod Microbiol 19:159-167
3. Pajón R, *et al*. Microbial Path. 1997; 23:71-84.

#033

NHHA, A NOVEL SURFACE ANTIGEN AND VACCINE CANDIDATE FOR *NEISSERIA MENINGITIDIS*

Peak, I.R.A.[1], Srikhanta, Y., Dieckelmann, M.[1], Moxon, E.R.[2], & Jennings, M.P.[1]
[1]Department of Microbiology and Parasitology, University of Queensland, Brisbane, Australia, [2]Department of Paediatrics, University of Oxford, Oxford, England

In previous studies we identified homologues of the adhesin AIDA-I of *Escherichia coli*. Here we report the identification and characterisation of a further homologue of AIDA-I. This gene has been designated *nhhA* (*Neisseria hia* homologue) as analysis of the complete coding sequence revealed that it is more closely related to the adhesins Hia and Hsf of *Haemophilus influenzae*. These similarities to adhesins indicate that NhhA may represent a novel adhesin of *N. meningitidis*. The *nhhA* gene is highly conserved, being present in 85/85 strains of *N. meningitidis* of all the major serogroups. It has not been detected in *N. gonorrhoeae*, but is detectable by Southern blot and PCR in *N. lactamica*. Sequence comparison of the gene from eleven strains demonstrated that sequence variation is largely confined to the NH_2 domain of the mature protein.

Its sequence characteristics place it in the Autotransporter family of outer membrane proteins. Western immunoblot and immunogold electron microscopy analysis confirmed that NhhA is localised to the outer membrane. NhhA represents an interesting new outer membrane protein of *N. meningitidis*. Its function is as yet undetermined, but it may prove to be a useful target for future vaccines.

#034

TOWARDS THE STRUCTURE OF AN OUTER MEMBRANE ADHESIN: REFOLDING AND CRYSTALLIZATION OF RECOMBINANT OPC

Prince[1], S.M., Feron[2], C., Janssens[2], D., Lobet[2], Y., Achtman[3], M.A. & Derrick[1], J.P.
[1] Dept. Biomolecular Sciences, UMIST, PO Box 88, Manchester, UK.
[2] SmithKline Beecham Biologicals, Rixensart, Belgium.
[3] Max-Planck Institut fur Molekulare Genetik, D-1000 Berlin 33, Germany.

Opc is a member of the class 5 group of *N.meningitidis* outer membrane proteins and has been implicated as an adhesin and invasin in meningoccocal disease. Our aim is to determine the 3-dimensional structure of Opc by X-ray crystallography. Opc can be purified in its native state from *N.meningitidis* cell paste (Achtman *et al.*, *J.Exp.Med.* 1988, **168**, 507-525), and we have been able to identify crystallization conditions for this material. The crystals are orthorhombic and diffract isotropically to 2.0Å resolution using synchrotron radiation. Using recombinant Opc solubilised in guanidine HCl from *E.coli* inclusion bodies, we have also been able to refold the protein by rapid dilution into detergent solution. The recombinant Opc was subsequently purified and was identical to the native material, as judged by molecular weight, circular dichroism and reaction with a human monoclonal antibody against a conformational epitope on the protein (LuNm03). Recombinant Opc crystallizes under the same conditions as the native material, with the same spacegroup and unit cell dimensions. Datasets of reflections from native and recombinant crystals merged together, demonstrating that both structures are essentially identical. These results open up the prospect of studying the structures of the more sparse constituents of the outer membrane of this pathogen using the techniques developed here, along with the possibility of studying mutagenized proteins.

#035

IMMUNOGENICITY OF COMBINATIONS OF *Neisseria meningitidis* SEROGROUP B ANTIGENS

Santini L, Brunelli B, Luzzi E, Pizza M, Grandi G, Rappuoli R, Giuliani MM. IRIS, Chiron S.p.A., Siena, Italy.

The genomic sequence of the virulent serogroup B strain MC58 allowed the identification of proteins that are conserved in sequence across a set of surface exposed strains, which induce bactericidal antibodies. We investigated the properties of each of these antigens administered singly with Freund's adjuvant by the i.p. route to CD1 mice. To further characterize these proteins as vaccine candidates we studied the immune response induced by the immunization with the single antigen and as combinations of two or more antigens given with Freund or alum adjuvant to CD1 mice. In the present work we analyze the sera obtained after immunization with combinations of the following proteins: GNA33, GNA1030, GNA1946, GNA2001, GNA992 and GNA1985. In order to evaluate the immunogenicity in a different animal model, immunizations in guinea pigs were performed. Immune sera were tested in enzyme-linked immunoabsorbent assay (ELISA) using capsulated bacteria to coat the plates, in fluorescence-activated cell sorter (FACS) on strains whose capsule had been permeabilized by treatment with ethanol, and for bactericidal activity. The results showed that the combinations of antigens are highly immunogenic and several have high bactericidal titers. For some of the sera a synergistic effect was observed, suggesting that the use of combinations of several antigens could be a promising approach for a *Neisseria* vaccine development.

#036

GENETIC AND FUNCTIONAL ANALYSIS OF PHOSPHORYLCHOLINE DECORATION OF COMMENSAL NEISSERIAL LPS.

Serino L. and Virji M.
Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.

Phosphorylcholine (ChoP) occurs as a surface component in many mucosal pathogens. In our studies we demonstrated the presence of a ChoP epitope on the pili of pathogenic *Neisseriae* and on the lipopolysaccharide (LPS) of several commensal *Neisseria* (Cn) species. Nuclear magnetic resonance studies on Cn LPS confirmed the presence of the phosphorylcholine moiety. We have shown the presence of *lic*-like genes in Cn that are similar to those involved in choline incorporation in *Haemophilus influenzae* LPS. Similar genes were not identified in the pathogenic *Neisseria* strains tested and they are absent from the *N. meningitidis* strains MC58 and Z2491 genome databases.
ChoP undergoes modulation in the level of expression on Cn LPS. A similar modulation was also observed in the binding of C-reactive protein, CRP, a ChoP-binding reactant that is implicated in bacterial clearance. Recent observations support the notion that CRP contributes to killing of $ChoP^+$ variants through bactericidal activity of complement. The data demonstrate that the ability to switch off ChoP expression is important for immune avoidance. Current studies are investigating the significance of ChoP on the pili of pathogenic *Neisseriae*.

#037

CHARACTERIZATION OF LIPOOLIGOSACCHARIDE STRUCTURES FOUND IN *NEISSERIA GONORRHOEAE* PID2.

Stein, DC*, Tong, Y*, Arking, D*, and Reinhold, V#. *University of Maryland, #University of New Hampshire

N. gonorrhoeae strain PID2 is a clinical isolate from a pelvic inflammatory disease patient that expresses at least 6 different lipooligosaccharide (LOS) components, as analyzed on SDS-PAGE gels. In this study we characterized its LOS by genetic analysis of the genes needed for its synthesis, by exoglycosidase digestion, by sugar composition analysis and by mass spectrometry. Genetic analysis showed that the *lgt* gene cluster of this strain has undergone a rearrangement and that it possesses two copies of *lgtA*, one copy of *lgtB*, and *lgtC* and a hybrid gene containing sequences from *lgtB* and *lgtE*. We determined that the hybrid *lgtB/E* retained the *lgtE* gene function. DNA sequence analysis of the gene organization suggests that an intramolecular recombination between *lgtA* and *lgtD* and *lgtB* and *lgtE* had occurred via homologous recombination between similar sequences. Since this recombination/gene duplication allowed the strain to produce additional LOS components, it indicates that an additional mechanisms occurs that could allow for LOS antigenic variation. Exoglycosidase digestion, in combination with Mass spectrometry analysis and compositional analysis, indicated that all of the LOS components produced by PID2 extend off of the α chain, and that there is no phosphorylation on the second heptose. The longest α chain oligosaccharide (OS) structure is Gal-GlcNAc-Gal-GlcNAc-Gal-Glc-Heptose I, and the six LOSs are built up by sequentially adding sugars onto the first heptose.

#038

A 190KDA CONSERVED SURFACE PROTEIN FROM *NEISSERIA MENINGITIDIS* THAT INDUCES BACTERICIDAL ANTIBODIES.

Fukasawa, L.O.[1]; Gorla, M.C.O [2]; Lemos, A.P[2].; Brandileone, M. C.[2]; Schenkman, R.P.F.[1]; Frasch, C.E.[1]; Raw, I.[1]; Tanizaki, M.M.[1] . [1] Centro de Biotecnologia, Instituto Butantan, São Paulo, Brasil. [2] Serviço de Bacteriologia, Instituto Adolfo Lutz, São Paulo, Brasil. [3]National Institute of Health, Rockville, Maryland, USA

The interest in finding conserved surface exposed proteins in serogroup B meningococcus has increased. Thus far, only NspA and GNA 33 have been described as proteins that satisfy the requirements for broadly distributed surface proteins. We report here the identification of a 190kDa protein present on meningococcal OMV having epitopes that cross react with OMV proteins from different serogroup B and C strains. This protein was detected when sera obtained from mice immunized with OMV serogroup B, strain N44/89 presented bactericidal activity against a IMC 2135, a serogroup C meningococcus but not against N1002/90, another group C meningococcus strain. The cross reactivity among these strains was related to a190kDa protein which was present in N44/89 (B:4,7:P1.15) and IMC 2135 (C:2a:P1.5,2) but not in strain 1002/90 (C:2b:P1.3). Using dot blot analysis of 100 group B and 100 group C strains isolated from Brazilian patients in 1999 an anti-190kDa monoclonal antibody recognized about 60% of the sample from both serogroups. The monoclonal antibody showed complement mediated bacteriolysis against homologous (N3006, B:2b:P1.2) and heterologous strains (N44/89, B:4:P1.15 and IMC2135, C:2a:P1.5.2) with high bactericidal titers (around 4000 to 8000), comparable to that induced by an OMV vaccine. Besides inducing antibodies with high bactericidal activity, this protein was present in 80% of Brazilian strains serotype B4,7:P1.15 the most prevalent in Brazil which corresponded to 58% of the all meningococci serogroup B serotyped in 1999.

#039

VARIABILITY IN THE FRPB PROTEIN AMONGST INVASIVE AND CARRIED MENINGOCOCCI AND COMMENSALS *NEISSERIA* SPECIES

Thompson E A L[1,2], Feavers I M[2] and Maiden M C J[1].
[1]Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, Oxford, UK
[2]Division of Bacteriology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, UK

Objective: To identify and classify the antigenic variation within FrpB (FetA), a potential vaccine candidate, from collections of invasive and carried meningococci and commensal *Neisseria* species.
Design: The nucleotide sequence of *frpB* (*fetA*) was determined from several strain collections, including 107 isolates previously characterised by MLST and MLEE; a collection of carried isolates from the Czech Republic and representatives from a collection of commensals *Neisseria* species.
Results: Each unique *frpB* nucleotide sequence from the invasive and commensal data sets was assigned to a different allele. The mean p-distance of the alleles was 5.4%, ranging from 0.05% to 10.1%. A region of hypervariable sequence was defined as the variable region and classified into groups using all data sets. Split decomposition analysis (SPLITSTREE) of these data did not generate a tree like phylogeny and the splits graphs illustrated networks of multiple possible evolutionary paths. SNAP analysis which calculates d_S/d_N values suggested evidence for positive selection within the variable region.
Conclusions: Sequence analysis of FrpB shows high diversity which has implications for vaccine development.

#040

STRUCTURAL AND FUNCTIONAL ANALYSES OF COMMENSAL OPA PROTEINS.

Mark Toleman[1], Debbie Evans[1], Ellen Aho[2] and Mumtaz Virji[1]

[1]Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.
[2]Department of Biology, Concordia College, Moorhead, Minnesota 56562, USA

Besides *Neisseria meningitidis* (Nm), several species of commensal *Neisseriae* (Cn) frequently colonise the human nasopharynx. However, little is known about the adhesion mechanisms of commensal *Neisseria*. In this study, we have demonstrated the expression of Opa-like proteins in 9 strains of Cn and analysed the structures of the Cn *opa* genes. Phylogenetic analysis segregated the majority of the Cn Opa in a cluster separated from the pathogenic cluster with three exceptions. One of these Opa that located within the pathogenic cluster exhibited significantly greater similarity to an Opa of *N. gonorrhoeae* (Ng) strain VP1 (74%) and of Nm strain C1938 (76%) than to the other commensal Opa proteins (49-60%). In addition, like Ng, it did not have the extra tyrosine residue at position 11 or the DKF triplet insert (position: 136-8) conserved among most Nm Opa proteins. Most importantly, many of the Cn Opa proteins were able to interact with human CEACAM1 (CD66a) molecules, previously identified as receptors for pathogenic Opa proteins and target the same region of the receptor. Furthermore, some typical Cn Opa bound to the receptor with affinities that were comparable to the pathogens. This is the first study to demonstrate the expression of adhesins in Cn that are structurally and functionally related to pathogenic adhesins.

#041

SIALYLATION OF *NEISSERIA MENINGITIDIS* LIPOOLIGOSACCHARIDES

Tsai C-M, Kao G, Zhu P. Div. of Bacterial products, Center for Biologics Evaluation and Research, FDA, Rockville, MD 20852 USA

Neisseria meningitidis lipooligosaccharides (LOSs) are divided into 12 immunotypes, L1 to L12. Sialylation of LOS contributes to serum resistance of *N. gonorrhoeae* and *N. meningitidis*. The sialic acid is present as N-acetylneuraminic acid (NeuNAc). LOS sialylation requires an LOS-specific sialyltransferase (STase) and the substrate, CMP-NeuNAc, which is produced in Group B and C organisms of *N. meningitidis* for their capsular biosyntheses. The LOSs of L9 to L12 from Group A organisms are nonsialylated since they do not synthesize CMP-NeuNAc. For the other 8 LOS immunotypes (L1 to L8) from Group B and C organisms, NeuNAc was shown to be α2,3-linked to Gal in all LOSs at the nonreducing end except for L1 and L6. L6 LOS is nonsialylated because it does not have a terminal Gal acceptor (GlcNAc instead) for the STase. In contrast to other sialylated LOSs, NeuNAc in the L1 LOS was reported to be α2,6-linked. Therefore, we prepared the STases from both 126E (L1) and M986 (L3,7) strains to investigate whether the STases from the two strains have different specificities or the acceptors in their LOSs influence the sialic acid linkage formed. Both STases from 126E (L1) and M986 (L3,7) stains formed an α2,3 linkage when the lacto-*N*-neotetraose containing L3,7 LOS or outer-membrane vesicles (OMV) was used as the acceptor. We are currently characterizing the sialic acid linkage synthesized from nonsialylated L1 LOS and OMV. In conclusion, the specificity of 126E (L1) and M986 (L3,7) STases was similar. Either the acceptor or another mechanism present in the L1 strain causes the formation of α2,6-linked NeuNAc in its LOS.

#042

DETECTION OF THE ALPHA 2,3-SIALYLTRANSFERASE GENE IN DIFFERENT SEROGROUPS AND LOS IMMUNOTYPES OF *NEISSERIA MENINGITIDIS*.

Tsang RSW, Bryden L, Tsai CM*, and Ng LK.
Bureau of Microbiology, Laboratory Center for Disease Control, Health Canada, Manitoba, Canada, and *Center for Biologics Evaluation and Research, FDA, Maryland, U.S.A.

Objective: Pathogenic *Neisseria* species produce a lipooligosaccharide (LOS) which can be terminally sialylated. Sialylation of LOS enables the microorganism to resist complement-mediated serum bactericidal activity. In this study we examine the distribution of the alpha 2,3-sialyltransferase gene, responsible for sialylation of LOS in different serogroups and LOS immunotypes of *Neisseria meningitidis*.

Method: Five sets of PCR primers that target different parts of the alpha 2,3-sialyltransferase gene were used to amplify DNA templates prepared from different prototype strains and clinical isolates of *N. meningitidis*.

Results: The alpha 2,3-sialyltransferase gene is present in prototype strains representing the 12 different LOS immunotypes regardless of the oligosaccharide structure of their LOS, and in the five most common serogroups of A, B, C. W135, and Y responsible for invasive meningococcal diseases. Serogroup A strains which do not synthesis CMP-NANA is also found to have the sialyltransferase gene.

Conclusions: Alpha 2,3-sialyltransferase gene is present in *N. meningitidis* strains regardless of their capsular serogroup and LOS structures. Its presence in different clinical isolates of groupable and non-groupable *N. meningitidis* strains suggests that it may be a potential target for rapid detection of the organism in sterile clinical specimens by PCR.

#043

Characterisation of Autotransporter B (AutB) – a pseudogene with homology to Autotransporter A (AutA) in *Neisseria meningitidis*

Turner DPJ, Wooldridge KG, Ala'Aldeen DAA.
Division of Microbiology and Infectious Diseases, University Hospital, Nottingham, UK.

Introduction: We have recently identified AutA (Accession No AJ277536), a meningococcal autotransporter protein that is expressed in-vivo and antigenic to B-cells and T-cells [1]. This protein showed homology with the predicted amino acid sequence from another neisserial ORF, Autotransporter B (AutB).

Objective: To determine whether AutB is expressed in *Neisseria meningitidis* and whether it is antigenically cross-reactive with AutA.

Design: AutB was cloned and expressed in *Escherichia coli* from two possible start codons. Rabbit polyclonal antibodies were raised and used in immunoblot studies.

Results: Recombinant peptides with apparent molecular weights corresponding to the predicted 73 kDa (AutB1) and 35kDa (AutB2) were obtained. Rabbit anti-AutB2 reacted strongly with both expressed proteins, but failed to react with any protein when probed against whole cell protein extracts from a panel of nine stains of *N. meningitidis*. Rabbit anti-AutB2 failed to cross-react with AutA and, conversely, rabbit anti-AutA failed to react with either of the AutB fragments.

Conclusion: AutB is not expressed in *N. meningitidis* and not cross reactive with AutA.

Reference: Ait-Tahar et al. Mol Micro (in press).

#044

IDENTIFICATION AND CHARACTERIZATION OF A PUTATIVE TONB-DEPENDENT RECEPTOR IN PATHOGENIC NEISSERIAE

Turner PC[1,2], Stojiljkovic IR[3], Thomas CE[2], Ala'Aldeen DAA[1], Sparling PF[2]
Meningococcal Research Group, University of Nottingham, UK[1], The University of North Carolina, Chapel Hill, USA[2] and Emory University, Atlanta, Georgia, USA[3].

Objective: A putative gene with homology to the TonB-dependent family (Tdf) of receptors was identified and labelled *tdfH*. This study was designed to help elucidate a role for this protein (NMA1700/NMB1497) in pathogenesis.

Result: Based on DNA sequence analysis, TdfH is probably a TonB-dependent outer membrane protein that is highly conserved amongst pathogenic neisseriae. Orthologs to this protein appear to be present in other mucosal pathogens including *Haemophilus influenzae*, *Campylobacter jejuni* and *Actinobacillus actinomycetocomitans*. TdfH was expressed in 28/28 meningococcal strains and 47/58 gonococcal strains but only 1/17 strains of commensal neisseriae. Analysis of TdfH in outer membrane preparations showed no evidence of iron regulation. Surface exposure of TdfH was confirmed by protease susceptibility in live bacteria. Although TdfH had principal homology (31% similarity, 16% identity) with the haemophore receptor HasR of *Serratia marcescens*, gonoccocal and meningococcal isogenic *tdfH* mutants were unaffected in their ability to use haem. An *E. coli hemA* mutant expressing TdfH and meningococcal TonB, ExbB and ExbD failed to show a haem utilizing phenotype. TdfH was immunologically unrelated to the 97 kDa haemin-binding protein (HmBP) as observed by B.C. Lee.

Conclusion: TdfH is a conserved, surface exposed outer membrane protein. It is probably a TonB-dependent receptor that serves a novel function, which may not be related to iron acquisition. Lack of expression of TdfH by most commensal neisseriae and the presence of orthologs in other mucosal pathogens suggest that this protein may play an important role in pathogenesis.

#045

MIMITOPES OF *N.MENINGITIDIS* SEROGROUP B CAPSULAR POLYSACCHARIDE.

Tzitzilonis[1], C., Brett[3], P., Feavers[3], I., Lian[1], L.Y., Derrick[1], J.P., & Charalambous[2], B.
[1]Dept. Biomolecular Sciences, UMIST, Manchester, UK.
[2] Royal Free & University College, Dept Biochem. & Molec. Biology, London, U.K.
[3]National Institute for Biological Standards and Control, South Mimms, Herts, U.K.

A phage library displaying random cyclic peptides was screened against the anti-LOS MAb 9-2-L379. This is a bactericidal murine monoclonal with high affinity to the meningococcal outer membrane lipo-oligosaccharide immunotype L3,7,9 most frequently associated with invasive disease. Two peptides were identified that were unrelated in sequence and which cross-reacted with the antibody with different affinities: 10.9±0.9 nM (C10) and 23.7±2.1 nM (C22). The sequences were adapted by addition of a polylysine tail (to render them more soluble): the peptides that were studied were, ACNTIGGYECGGGSKKK(C22) and ACSWLHQPYCGGGSKKK (C10). The structures of both peptides were analysed by solution state NMR at 500MHz. Spin systems from the residues between the disulphide bonds were readily identified from 2D $^1H/^1H$ COSY and TOCSY spectra. Several residues appeared to be in more than one conformation, as evidenced from multiple chemical shifts for some sidechain protons. Analysis of 2D $^1H/^1H$ NOESY spectra demonstrated the presence of long range NOEs across the beta hairpin structure. Subsequent determination of the solution structures of both peptides could help to show the molecular basis for the cross-reactivity of both peptides for a single antibody and may shed some light on the molecular basis for recognition of *N.meningitidis* LOS.

#046

EVOLUTION OF ANTIGEN GENES AMONG CLONAL LINEAGES OF *NEISSERIA MENINGITIDIS*

Urwin R[1], Thompson EAL[1], Russell JE[1,2], Feavers IM[2], M.C.J. Maiden[1]
[1]Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, UK. [2]National Institute for Biological Standards and Control, South Mimms, Herts, UK.

Objective: To establish the evolutionary processes that shape variation in a number of vaccine candidate antigens.
Design: We defined antigen gene variation in a collection of 107 meningococcal strains by nucleotide sequence determination. The strain collection included isolates from the main hyper-invasive lineages; antigen genes examined were *porA* and *porB* which encode outer membrane porin proteins; and *frpB* (*fetA*) which encodes an iron regulated protein.
Results: Diverse antigen genes and combinations of antigen genes were observed within clonal groups of genetically related meningococci. For example, among 10 ET-37/ST11 complex meningococci, 4 *porA*, 4 *porB* and 6 *frpB* alleles were identified in 9 antigen combinations. The reconstructed phylogenetic trees from these data were not well supported by bootstrap analysis. None of the loci examined gave a tree-like structure when analysed using the split decomposition method, but generated networks of interlinked evolutionary pathways. Calculation of synonymous and nonsynonymous substitution rates at each locus showed elevated rates of nonsynonymous change in areas of the genes encoding surface exposed regions of the proteins, suggesting the action of positive selection.
Conclusions: Meningococcal antigens and combinations of antigens are not always well conserved among genetically related strains. Therefore, while sequencing of multiple antigen genes may provide some epidemiological discrimination, one antigen is not a reliable epidemiological marker. Evolutionary analyses of these data show that variation in the three antigens examined is evolving as a result of both horizontal genetic exchange and positive selection from the host immune response.

#047

COMPARISON OF SEQUENCING AND SEROLOGICAL SUBTYPING OF *NEISSERIA MENINGITIDIS*

van der Ende A.[1,2], Spanjaard L.[1,2], Keijzer W.[2], Godfried V.[2], Arends A.[2], van Alphen L.[3] and Dankert J.[1,2]
Academic Medical Center, [1]Department of Medical Microbiology and [2]the Netherlands Reference Laboratory for Bacterial Meningitis, AMC/RIVM, Amsterdam, [3]National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands.

Knowledge of the subtype distribution among meningococcal isolates is essential to determine whether a six valent PorA based vaccine against meningococcal disease will be efficacious. During the first quarter of 2000, the Netherlands Reference Laboratory for Bacterial Meningitis received 198 meningococcal isolates from patients with meningococcal disease. Sequencing of *porA* VR1 and VR2, encoding the PorA subtype epitopes revealed 10 new subtypes. Among each of the predominant VR1 subtypes P1.5 and P1.7 five variants were observed. Of the 109 isolates with P1.7 variant subtypes only 17 (16%) were recognized by the monoclonal antibody against P1.7. Of the 48 isolates with P1.5 variant subtypes 31 (35%) were recognized by the monoclonal antibody against P1.5. The predominant VR2 subtype was P1.4 and its derivative subtypes: 62 were P1.4, 1 was P1.4a and of 3 isolates the VR2 loop was partially deleted. One of the P1.4 isolates and the 4 derivatives were not recognized by the monoclonal antibody against P1.4 (5/66: 8%). In conclusion, assuming that antibodies raised against PorA epitopes will not provide protection against derivatives of these epitopes, vaccination with a vaccine based on 6 PorAs containing 12 subtypes (P1.2c, P1.4, P1.5a, P1.5c, P1.7, P1.7b, P1.10, P1.12a, P1.13, P1.15a, P1.16, P1.19) can prevent 79% (128/162) of group B meningococcal disease. The coverage decreases to 71% (142/198) if disease by meningococci with other serogroups is included.

#048

LIPOOLIGOSACCHARIDE (LOS) PRODUCED BY NEISSERIA GONORRHOEAE

Ryohei Yamasaki, Testu Maruyama, Yumiko Nishinaka, and Uichirou Yabe. Department of Biochemistry and Biotechnology, Tottori University, Japan.

Lipooligosaccharides (LOSs) are one of the major antigenic and immunogenic components produced by *Neisseria gonorrhoeae*. The oligosaccharide of gonococcal LOS consists of the variable and core region, and short oligosaccharide chains are linked to either or both of the Hep residues of the conserved GlcNAc-Hep(II)-Hep(I) core. Several investigators have reported that normal human sera contain bactericidal antibodies against gonococcal LOS. However, up to date, human antibodies specific for gonococcal LOSs have not been identified. In order to confirm suggestions made by earlier investigators, we investigated the presence of normal human antibodies against gonococcal LOS.
To isolate specific antibodies specific for LOS, we used LOS from a serum sensitive strain, JW31R as a ligand for affinity chromatography. We isolated IgG that recognizes specific oligosaccharide structure of the LOS. ELISA and PAGE/blot analyses showed 1) this antibody showed no binding to LOSs which have oligosaccharide linked to Hep(I), 2) but binds to 15253 and JW31R LOS which have oligosaccharides linked to both Hep(I) and Hep(II) 3) its epitope is a conserved region of the above LOS, and 4) it competes with a bactericidal MAb 2C7 that is specific for gonococcal LOS.
For the first time, we isolated human IgG that binds to the oligosaccharide moiety of the gonococcal LOS. The epitope is in a conserved region of the LOS expressed on the surface of the gonococci *in vivo*

#049

LPS DEFICIENT MUTANT OF *NEISSERIA MENINGITIDIS* ELICITS ATTENUATED PROINFLAMMATORY CYTOKINE RELEASE BY HUMAN MACROPHAGES AND SIGNALS INDEPENDENTLY OF THE TLR4/MD2 PATHWAY.

Pridmore AC[1], Wyllie DH[1], Abdillahi F[1], Steeghs L[2], van der Ley P[2], Dower SK[1], Read RC[1]. [1]Molecular and Genetic Medicine, University of Sheffield Medical School, UK. [2]Institute of Public Health and the Environment, The Netherlands.

Severity of meningococcal disease correlates with circulating concentrations of lipopolysaccharide (LPS) and proinflammatory cytokines, but LPS is only one of several components of Gram negative bacteria capable of stimulating host responses. The role of LPS in stimulation of proinflammatory responses was examined using a *Neisseria meningitidis* mutant with a disruption of the *lpxA* gene. This mutation generated a viable organism that is absolutely deficient of detectable LPS. The bioactivity of live wild type *N. meningitidis* to stimulate TNFα and IL-8 secretion by human monocyte-derived macrophages was approximately 10 fold greater than the *lpxA* mutant. The recently discovered Toll-like receptors (TLR) are a family of mammalian receptors involved in responses to microbial products. Two have been well characterised and the recognition specificity of these receptors differs. TLR4 (and its cofactor MD2) being required for responses to LPS, while TLR2 responds to lipoproteins and peptidoglycan. Killed wild-type *N. meningitidis* and its soluble products induced IL-8 promoter activity and IL-8 secretion by transfected HeLa cells expressing Toll-like receptor 4 and its cofactor MD2. However, disruption of *lpxA* abolished signalling by *N. meningitidis* via this pathway. Taken together, these data provide evidence that *N. meningitidis* contains components other than LPS which are capable of eliciting potent biological responses via pathways independent of the TLR4/MD2 signalling system. This has implications for future therapeutic strategies against meningococcal disease based on blockade of Toll-like receptors and modulation of LPS activity.

#050

NF-κB PLAYS AN EARLY ROLE IN THE ACTIVATION OF B CELLS PRIOR TO TYROSINE PHOSPHORYLATION IN RESPONSE TO *NEISSERIA MENINGITIDIS* PorB.

Bhasin N and Wetzler LM. Section of Infectious Diseases, Boston Medical Center and Boston University School of Medicine, Boston, Massachusettes.

We have recently initiated studies to characterize the events governing signal transduction in B lymphocytes, in response to PorB from serogroup B *Neisseria meningitidis*. Neisserial PorB has been shown to have adjuvant like properties in that it affects the B cells to inducibly express higher levels of the costimulatory B7-2 molecules, and is currently being investigated for use as an adjuvant in various vaccine compositions. In order to dissect the signal transduction pathway involved in PorB activation of B cells, we investigated (1) The ability of PorB to induce NF-κB nuclear translocation (2) The ability of PorB to induce tyrosine phosphorylation (3) Whether inhibition of tyrosine protein kinases affected PorB's ability to induce NF-κB translocation and (4) Whether other kinases, specifically MAPK kinase, p38 MAP kinase and PI 3-kinase, are involved in PorB activation of B cells. The basic model for this study involved the incubation of naïve murine B cells (isolated from the LPS hypo-responsive strain C3H/HeJ mice) with 10μg/ml of purified PorB isolated from serogroup B *N. meningitidis* strain H44/76, with or without pretreatment with various kinase inhibitors. The cells were then monitored for their state of activation by (1) FACS analysis, to check for the expression of B7-2 molecules on their cell surface, (2) Western Blot analysis to monitor the inducible phosphorylation of various proteins and (3) EMSA analysis for the detection of NF-κB nuclear translocation. We found that PorB induces NF-κB nuclear translocation in an LPS independent manner within 1-2h, peaking at about 3-5h and returning to baseline at 9h. Also, this translocation of NF-κB was not due to tyrosine protein kinase activity, which PorB induces. However, PorB induction of B7-2 expression was affected by the tyrosine protein kinase inhibitors. Finally, there was no evidence that MAPK kinase, p38 MAP kinase and PI 3 kinase were involved in PorB activation of B cells. In conclusion, PorB is a potent B cell mitogen and similar in its mode of activation to CD40 ligation or BCR cross-linking, where nuclear translocation of NF-κB is an early event, followed by tyrosine phosphorylation of various cellular proteins.

#051

THE DEFENCE AND INFLAMMATORY RESPONSES OF HUMAN MENINGEAL CELLS TO *NEISSERIA MENINGITIDIS*.

Christodoulides, M., Partridge, K., Fowler, M.I., Makepeace, B.L., Kaur, D., Weller, R.O., Heckels, J.E.

The interaction of *Neisseria meningitidis* with the meninges that surround and protect the brain is a pivotal event in the progression of bacterial meningitis. Recently, we have established a robust and reproducible *in vitro* cell culture model of the leptomeninges based on cells derived from human meningiomas[1]. We have extended the use of this model to study the role of meningeal cells in host cellular defence and their contribution to the acute, compartmentalised inflammatory response that is characteristic of meningococcal meningitis. In response to meningococcal challenge, meningeal cells secrete high levels of the pro-inflammatory cytokine mediator Il-6, the chemokine Il-8, which signals the influx of neutrophils, and the chemokine MCP-1, which attracts macrophages. However, meningeal cells secrete little or no Il-1α, -β, TNF-α, Il-10, Il-12, GM-CSF, RANTES, TGF-β or MIP-1α, -β. These results demonstrate that meningococcal interactions with meningeal cells induce signalling events that recruit immune effector cells to the subarachnoid space (SAS), resulting in the release of a cascade of pro-inflammatory cytokines. In further studies, we have also investigated the role of innate cellular defence mechanisms in response to infection with pathogenic *Neisseria*, in particular the regulation of expression of human β-defensins-1 and -2 in epithelial, endothelial and meningeal cells.

The use of this model has demonstrated that meningeal cells contribute significantly to the inflammatory and cellular defence responses during meningitis and play a major role in signalling to immune effector cells resident within and without the SAS.

[1]Hardy, S.J., Christodoulides, M., Weller, R.O., Heckels, J.E. *Molecular Microbiology* 36 (4), 817-829 (2000).

#052

HOST INFLAMMATORY RESPONSE TO NEISSERIA GONORRHOEAE INFECTION IN IMMORTALIZED HUMAN CERVICAL AND VAGINAL EPITHELIAL CELLS.

Desai, P.[1], Fichorova, R.[2], Gibson, F.[1] and C. Genco[1].
[1]Department of Medicine, Boston University School of Medicine Boston, MA 02118; [2]Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston MA 02115. USA.

Although signal transduction pathways and cytokine responses *to N.gonorrhoeae* in the upper genital tract have been the focus of in vivo and in vitro studies, little is known about immunobiological consequences of gonococcal interactions with cervical and vaginal epithelial cells. Recently, three cell lines from normal human vaginal,ectocervical, and endocervical epithelia immortalized by expression of human papillomavirus E6E7 oncogenes have been developed. The morphological and immunocytochemical characteristics of these cell lines closely resemble those of their tissues of origin and primary cultures. We have utilized these cell lines as models to study the interactions of gonococci with mucosal epithelium and to characterize the host response to infection. Pilated *N. gonorrhoeae* actively invaded these epithelial cell lines as demonstrated by an antibiotic protection assay and confocal microcopy using GFP-gonococci. Invasion of GFP-gonococci was characterized by co-localization of F-actin. In all three cell lines, elevated levels of the proinflammatory cytokines IL-6 and IL-8 were detected by 4h while IL-1α a and sICAM-1 showed a delayed pattern of release, and were significantly increased at 24h. These results suggest that early cytokine responses are not mediated through the IL-1α a signaling pathway and that the enhanced ICAM-1 shedding and IL-1α a release may be associated with internalization of live bacteria or with increased cell damage which occurred 24h post infection. Although pili were required for adherence and invasion of gonococci, we did not observe differences in IL-6, IL-8 and sICAM-1 expression in response to P+ or P- gonococci. Stimulation of all three cell lines with gonococcal cell lysates resulted in a similar stimulation of IL-6 and IL-8. The ability of gonococci to stimulate proinflammatory cytokines from these morphologically and functionally different compartments of the female genital tract may contribute directly to the inflammatory response which is characteristic of disease caused by *N. gonorrhoeae*.

#053

STUDIES OF THE INVASION OF PRIMARY HUMAN CERVICAL EPITHELIAL CELLS BY NEISSERIA GONORRHOEAE

Edwards, J. L., Shao, J. Q., Ault, K. A., Apicella, M. A.
Department of Microbiology, University of Iowa

Neisseria gonorrhoeae remains one of the leading causes of sexually transmitted disease. Female infection with gonococci can result in an ascending infection of the uterus and fallopian tubes causing an acute pelvic inflammatory disease. Early studies suggested a role for the cervical epithelium in female gonococcal infection; however, gonococcal pathogenesis of the cervical epithelium remains unclear. To elucidate the mechanism(s) associated with gonococcal infection of the cervical epithelium we have developed primary human ecto- and endocervical epithelial cell culture systems. Confocal microscopy has demonstrated that the integrity of each cell system has been maintained with respect to their parental tissues. Confocal and electron microscopy have demonstrated that gonococci adhere to induce cytoskeletal changes within, and invade both epithelial cell systems. Ruffling of the cervical epithelial cells, similar to that observed with *Salmonella* and *Shigella* invasion of the intestinal epithelium, occurs with gonococcal infection. The onset of ruffling can be expedited by the use of primed infection medium in infection studies. Invasion studies have demonstrated that gonococcal internalization is an actin-dependent process, but microtubules may play a secondary role. *De novo* protein synthesis does not appear to be required either by the bacterium or the host cell. Confocal microscopy has suggested a role for the asialoglycoprotein receptor, CD66, and CD46 in gonococcal invasion. These studies demonstrate the ability of *N. gonorrhoeae* to invade the cervical epithelium using a variety of mechanisms. The induction of membrane ruffling by the gonococcus has not been previously described and may be unique to cervical epithelial cells. These studies indicate that the cervical epithelium may serve as the primary site of gonococcal infection in females.

#054

THE CLASS A MACROPHAGE SCAVENGER RECEPTOR IS A MAJOR RECEPTOR FOR NEISSERIA MENINGITIDIS.

Peiser, L*, de Winther M*, Makepeace K†, Hollinshead MS*, Tang C†, Coull P†, Plested JS†, Moxon ER†, Gordon S*. *Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK. †Department of Paediatrics, John Radcliffe Hospital, Headington, UK

The class-A macrophage (Mϕ) scavenger receptor (SR-A), a trimeric cell surface glycoprotein with broad ligand binding specificity, is implicated in the *in vivo* clearance of bacteria and prevention against endotoxic shock. To clarify the role of SR-A in bacterial ingestion, we compared the ability of bone marrow-derived Mϕ, from SR-A$^{-/-}$- and WT mice to phagocytose *Neisseria meningitidis (NM) in vitro*. SR-A$^{-/-}$ Mϕ ingested 90% fewer *NM* (from serogroups A,B,C) than WT Mϕ in the absence of serum. Phagocytosis of *NM* by WT Mϕ was blocked by SR-A inhibitors and Cytochalasin D. SR-A-mediated recognition of *NM* was unaffected by the presence or absence of capsule, pili or terminal lipopolysaccharide structures on the bacteria. Microscopy showed intracellular *NM* in phagosomes with evidence of replicating organisms. Analysis of the Mϕ response to *NM* infection revealed both SR-A$^{-/-}$ and WT Mϕ could kill internalised bacteria and release nitric oxide (NO) and proinflammatory cytokines, including TNFα. SR-A$^{-/-}$ Mϕ produced only slightly less NO and TNFα than WT cells, and NO production was not inhibited by anti-SR-A antibodies. We have also shown primary human Mϕ can phagocytose *NM* via scavenger receptors in the absence of serum. The data above indicate that SR-A is the major non-opsonic receptor for *NM* phagocytosis by Mϕ, although the cell responses to *NM* infection are less dependent on SR-A.

#055

STRATEGIES FOR THE IDENTIFICATION OF NOVEL CD4+ T-CELL STIMULATING VACCINE CANDIDATES IN *NEISSERIA MENINGITIDIS*

Ala'Aldeen DAA and I Todd
MRG, Microbiology & Immunology, University of Nottingham, UK

Protective immunity to meningococcal infection correlates with long-lasting bactericidal IgG, which requires help by CD4$^+$ T-cells. T-cell stimulating meningococcal proteins may enhance the effectiveness of A and C capsular polysaccharide-based vaccines (acting as more appropriate carriers), or as protective antigens (Ags) in their own right. Taking advantage of the known specificity of TCR-MHC-Ag interactions, we developed two main immunological and genetic strategies for the identification of meningococcal CD4+ T-cell Ags.

Results: The first method involved screening fractionated meningococcal proteins of *N. meningitidis* using peripheral blood lymphocytes (PBLs) obtained from normal individuals and patients convalescing from *N. meningitidis* infection. This led to the identification of several proteins which potently stimulated T-cell (1), including TspA, TspB and App. The genes encoding these Ags were detected from a gene library, using polyclonal antibodies, and later cloned and characterised.

In the second approach, PBLs were used to screen a meningococcal genomic library, directly. A representative population of *E. coli* transformants containing recombinant meningococcal DNA in pBluescript plasmids was divided into oligoclones and screened for T-cell stimulation. Several promising positive oligoclones were identified, one of which was selected for re-screening and led to the identification of AutA (68 kDa), a member of the auto-transporter family of proteins (2).

Conclusion: The strategies adopted here proved highly rewarding. All the identified Ags were confirmed to be potent Ags capable of recalling strong secondary T-cell responses in PBLs of patients and induce strong primary T-cell responses in healthy donors. They all are conserved, expressed *in-vivo* and antigenic to human B-cells, generating cross-reactive antibodies in patients. The identified Ags deserve detailed investigations as vaccine candidates.

1. Infect Immun, 1999, 67, 3533-41. 2) Molec Microbiol, 2000, In press.

#056

ATTACHMENT OF NEISSERIA GONORRHOEAE TO THE CELLULAR PILUS RECEPTOR CD46: IDENTIFICATION OF DOMAINS IMPORTANT FOR ADHERENCE

Helena Källström[1], Barbara Albiger, Darcy Blackmer Gill[2], M. Kathryn Liszewski[2], John P. Atkinson[2] and Ann-Beth Jonsson[1]

[1] Microbiology and Tumorbiology Center, Karolinska Institutet, S-171 77 Stockholm, SWEDEN [2] Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

Pili of *Neisseria gonorrhoeae* mediate binding of the bacteria to human host cells. Membrane cofactor protein (MCP or CD46), a human cell surface protein involved in regulation of complement activation, acts as a cellular pilus receptor. In this work, we examined which domains of CD46 mediate bacterial adherence. The CD46 expression was quantified and characterized in human epithelial cell lines. *N. gonorrhoeae* showed the highest adherence to ME180 cells which have BC1 as the dominant phenotype. The BC isoforms of CD46 were expressed in all cell lines tested. The adherence was not enhanced by high expression of other isoforms, showing that the BC domain of CD46 is important in adherence of *N. gonorrhoeae* to human cells. To characterize the pilus-binding site within the CD46 molecule, a set of CD46-BC1 deletion constructs were transfected into COS-7 cells. Piliated *N. gonorrhoeae* attached well to CD46-BC1 expressing COS-7 cells. We show that the complement control protein repeat 3 (CCP-3) and the serine/threonine/proline (STP) rich domain of CD46 are important for efficient adherence to host cells. Further, partial deletion of the cytoplasmic tail of CD46 results in low bacterial binding, indicating that the cytoplasmic tail takes part in the process of establishing a stable interaction between *N. gonorrhoeae* and host cells.

#057

IMMUNE RESPONSES TO MENINGOCOCCAL OUTER MEMBRANE VESICLES AFTER INTRANASAL IMMUNISATION

Andersen SR, Liang B, Guthrie T, Wong SYC, Hou S, Hyland L. EJIVR, Compton, Newbury, Berkshire. RG20 7NN. U.K.

Objectives: To compare the antibody response in different immunological sites upon intranasal immunisation (i.n.) with natural outer membrane vesicles (NOMV) and detergent extracted outer membrane vesicles (DOMV) from the serogroup B strain 44/76.

Design: Mice (C57BL/6, n=3 per group) were immunised i.n. once per week for 4 weeks with NOMV or DOMV from Mu-4 of *N. meningitidis* strain 44/76. Each dose contained 15 μg of protein and ca. 4 or 1.2 μg of LPS for NOMV and DOMV, respectively, in 30 μl. ELISPOT assay was used to determine the frequency and Ig isotype of NOMV-specific antibody-forming cells (AFC) in the organised and diffused nasal associated lymphoid tissue (O-NALT and D-NALT), mediastinal lymph node (MLN), spleen and bone marrow on day 12 and day 33 after the last dose.

Results: NOMV induced a stronger antibody response than DOMV in all tissues except for the D-NALT. The NOMV also induced a wider range of Ig isotypes/IgG subclasses. The D-NALT showed the strongest response of all the tissues with massive IgA response with a ca. 600 IgA producing AFC/5 x 10^5 cells on day 12 with a ca 33 % decline on day 30. The response in the O-NALT was weak and short-lived with no detectable AFC on day 30. The frequency of AFC in spleen, MLN and bone marrow was ca. 10 times lower than that in the D-NALT. In these tissues the proportion of IgG isotypes (IgG2a and IgG2b in particular) and IgM was higher relative to IgA than in the D-NALT.

Conclusion: NOMV induced a stronger and more long-lasting antibody response than DOMV. IgA was the dominating isotype and the D-NALT was the main site for the IgA production. The second most abundant isotype was IgG2b, which was produced in all tissues examined.

#058

THE EFFECT OF VIRAL INFECTION ON SURVIVAL OF MORAXELLA CATARRHALIS IN A HUMAN MONOCYTE CELL LINE (THP-1)

Raza, Muhammad W., Weir Donald, M, . Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Edinburgh, Scotland

Background: *M. catarrhalis* (MC) is being investigated by our research group for its role in development of immunity to meningococci and for induction of inflammatory responses involved in the pathogenesis of diseases due to both species. The objective was to compared binding, ingestion and intracellular survival MC1 and MC2 THP-1 cells, both uninfected infected with respiratory syncytial virus (RSV).

Methods: Bacterial binding and ingestion were determined by flow cytometry using ethidium bromide (EB) labelled bacteria. Extracellular binding was differentiated from intracellular bacteria by quenching the extracellular EB stain with crystal violet. TNF activity was assessed by bioassay with L929 cells.

Results: Both MC1 and MC2 bound in lower numbers to RSV-infected cells compared with uninfected cells (MC1, $P < 0.01$; MC2, $P < 0.002$). RSV infection reduced ingestion of both strains but was significant only for MC2 ($P < 0.05$). Neither survived within infected or uninfected THP-1 cells. Compared with uninfected cells, RSV infection significantly decreased TNF responses to live MC1 ($P < 0.002$).

Conclusions: Virus infection could contribute to susceptibility to bacterial disease in the non-immune host by reducing the efficacy of phagocytic cells for and inflammatory responses. The model system is being adapted to assess viral infection on intracellular survival and inflammatory responses to meningococci.

#059

Effect of bacterial growth conditions on specificity in the mouse serum bactericidal assay.

Bouliane, S., Bonapace, S., Masi, D., Clay, M., Nault, A. and Wunderli, P., BioChem Pharma, Inc. 30 Bearfoot Road, Northborough, MA 01532

Assays for serum bactericidal antibody (SBA) are accepted as the correlate of vaccine-induced protection for *Neisseria meningitidis* (*N.m.*). This assay shows high variability, particularly resulting from the growth characteristics and isolate variability associated with the organism. The conditions for the assay, including that of preparing cell banks, have been optimized for use in evaluating formulation potency for a BPI research vaccine candidate (NspA). The method uses a 96 well format and consists of adding diluted serum samples, *N.m.* 608B strain cells, and baby rabbit sera (BRS) as complement source. Post-incubation, aliquots are spread on chocolate-agar plates and the results are expressed in colony forming units (CFU) and/or in % lysis.

Optimization of the SBA reproducibility included use of frozen cells to control CFU input across experiments. Working cell banks (WCB) are prepared and frozen, CFU are estimated from repetitive plating, and identical dilutions are made for each test point. Acceptance of a WCB is performance-based, with the new bank compared to previous WCBs using a set of standards and several lots of complement. However, growth conditions were observed to play an important role in the sensitivity and specificity in the assay. Plate culture cell density and broth culture supplements were shown to affect assay performance. Higher cell density plate culture resulted in higher assay non-specific lysis, while broth cultures demonstrated good specificity but increased the variability of responses within and across experiments.

The current SBA method uses reduced density cell plating for preparation of *N.m.* 608B WCB, but experiments are underway to investigate alternatives to broth supplement that would retain SBA specificity while reducing variability. This is being extended to studies within and across *N.m.* serogroups and strains, for the purpose of extending SBA evaluations across multiple strains and serogroups.

#060

MODULATION OF EPITHELIAL RECEPTORS BY INFLAMMATORY CYTOKINES AND MENINGOCOCCAL ADHESION.

Bradley, C.J., Heyderman, R.S. and Virji, M.
Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.

During the early phases of colonisation a variety of cytokines may be released by epithelial cells and macrophages in response to meningococcal adhesion. Additionally increased cytokine release may occur due to viral or other bacterial infections. The aim of present studies is to explore the modulatory effects of such cytokines on the expression of multiple receptors on host cells that are targeted by meningococci and the resulting effect on bacterial adhesion and invasion.

Current studies in our laboratory have used FACS analysis to determine the degree of modulation of CD66, CD46 and integrins implicated in interactions of distinct meningococcal adhesins. We have used IFN-γ, TNF-α, IL-1β and IL-4 both individually and in conjunction. IFN-γ and TNF-α were chosen because these are released at high levels during meningococcal infection. Additionally TNF-α, IL-1β and IFN-γ are all secreted from influenza A virus infected cells. IL-4 was chosen as a control. We observed altered expressions of receptors when using these cytokines alone or in conjunction. For example, we found that IFN-γ was the most stimulatory cytokine in epithelial cells with consistent up-regulation of CD66 expression. Co-stimulation with other cytokines such as TNF-α resulted in synergistic upregulation.

Current studies are evaluating how differential modulation of multiple receptors to meningococcal adhesins/invasins affects meningococcal interactions with target cells.

#061

ABSORPTION OF BACTERICIDAL ACTIVITY AGAINST MENINGOCOCCI BY NEISSERIA LACTAMICA.

Braun, Jan M., Weir Donald M, Poxton, Ian R., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Scotland

Background: Carriage of *Neisseria lactamica* (NL) is associated with development of antibodies protective against meningococcal disease. The objective was to assess the ability of one isolate of NL to absorb bactericidal activity against meningococcal strains of different phenotypes and NL from different regions of Europe.
Methods: A heat inactivated serum pool from was absorbed with NL L01 and absorbed and unabsorbed pools tested for bactericidal activity against NL from: Scotland (3), Iceland (1), the Czech Republic (1) and Greece (1); 24 NM isolates including the 12 immunotype strains from Dr. W.D. Zollinger, Washington, D.C.
Results: The unabsorbed pool killed all 24 NM strains (>80% killing) compared with controls. Bactericidal activity was absorbed by strain L01 for: B:2a:P1.5,2:L(3,7,9), B:819:P1.7:L8, B:4:P1.NT:L(3,7,9) B:9:P1.1:L(3,7,9), C:11:P1.16:L4; B:15:P1.7,16; B:NT:NT; a second Scottish NL isolate and one from Iceland. NL strains from the Czech Republic and Greece were killed by the absorbed sera.
Conclusions: There are epitopes on NL strain L01 cross reactive with some outbreak and group B reference strains of immunotypes L(3,7,9), L4 and L8. We suggest LOS epitopes contribute to induction of the cross reactive antibodies. Carriage of NL might contribute to development of natural immunity against meningococci, but there appear to be significant differences in the antigenic structures of NL in different countries.

#062

ABSORPTION OF BACTERICIDAL ACTIVITY AGAINST MENINGOCOCCI BY MORAXELLA (NEISSERIA) CATARRHALIS (MC)

Braun, Jan M., Weir, Donald M., Poxton, Ian R., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Scotland

Background: While 5-14% of young children in European populations carry *N. lactamica* (NL), 66% carry MC during the first year and 77% by the age of two. The objective was to determine if MC expressed antigens cross reactive those on meningococci by determining if two MC isolates of MC absorbed bactericidal activity against meningococcal and NL isolates from different regions of Europe.
Methods: A heat inactivated serum pool was absorbed with NL L01, and absorbed and unabsorbed pools tested for bactericidal activity against: NL from: Scotland (3), Iceland (1), the Czech Republic (1) and Greece (1); 24 NM isolates including the 12 immunotype strains from Dr. W.D. Zollinger, Washington, D.C.
Results: The serum pool killed all 24 *N. meningitidis* strains (>80% killing). Activity against the following strains was absorbed by MC1: A:21:P1.10:L3,7,9, B:4:P1.NT:L3,7,9, C:11:P1.16:L4; B:4:P1.15; B:15:P1.7,16; B:2a:P1.2; MC2; NL from Iceland and Greece. Absorption with MC2 significantly reduced bactericidal activity against: meningococcal strains B:4:P1.15, B:2a:P1.2; both strains of MC; the Greek isolate of NL and one Scottish NL.
Conclusions: There are epitopes on MC cross reactive with some outbreak and reference strains of meningococci of immunotypes L4, L3,7,9 and some NL strains. Antigens responsible of the induction of this cross reactivity are under investigation.

#063

DOES L(3,7,9) LIPOOLIGOSACCHARIDE INDUCE HIGHER INFLAMMATORY RESPONSES THAN OTHER IMMUNOTYPES ?

Braun, Jan M., Weir, Donald, M., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Edinburgh, Scotland

Background: The LOS immunotype most commonly isolated from patients is L (3,7,9) and other types are primarily obtained from carriers. The objective was: 1) to develop an *in vitro* model to screen inflammatory responses induced by LOS obtained from meningococcal immunotypes; 2) to test the hypothesis that the L (3,7,9) LOS induced higher levels of inflammatory responses than other immunotypes.
Methods: The human monocyte cell line THP-1 was cultured with 10^{-7} M 1.25-dihydroxyvitamin D3 for 72 hr then incubated with 100 ng of LOS obtained from the meningococcal and commensal neisseria strains by hot phenol water extraction. TNFα and IL-6 were tested at intervals over a period of 18 hours. The responses induced by LOS from meningococci, *Neisseria lactamica* or *Moraxella catarrhalis* C were compared with those elicited by 100 ng of endotoxin from *Escherichia coli*.
Results: In 18 experiments, LOS from the MC immunotypes induced higher levels of TNF and IL-6 than the endotoxin of *E. coli*. Immunotype L (3,7,9) LOS induced significantly higher TNF and IL-6 responses than LOS from non- L (3,7,9) strain.
Conclusions: The model was useful for screening induction of pro-inflammatory cytokines by LOS of different strains. The L(3,7,9) LOS induced significantly higher levels of TNF and IL-6 which explain the observation that this immunotype is isolated most frequently from patients.

#064

COMPARISON OF INFLAMMATORY RESPONSES TO LIPOOLIGOSACCHARIDE OF MENINGOCOCCI, NEISSERIA LACTAMICA AND MORAXELLA CATARRHALIS

Braun, Jan M., Weir, Donald, M, Blackwell, C. Caroline
Dept. of Medical Microbiology, University of Edinburgh, Scotland

Background: Concurrent studies on serum bactericidal activity against meningococci indicate LOS present on some strains of *Neisseria lactamica* (NL) and *Moraxella catarrhalis* (MC) induce antibodies to NM. If LOS derived from commensal species is to be considered for vaccines, the toxicity must be assessed. The objective was: 1) to compare *in vitro* inflammatory responses induced by LOS obtained from meningococci of different immunotypes, NL and MC.
Methods: THP-1 cells were cultured with 10^{-7} M 1.25-dihydroxyvitamin D3 for 72 hr then incubated with 100 ng of LOS from the test strains by hot phenol water extraction. Production of tumour necrosis factor α (TNF) and interleukin 6 (IL-6) were tested at intervals over a period of 18 hours. The responses induced by endotoxin (100 ng) from NM of different immunotypes, NL, MC and *Escherichia coli* were compared.
Results: In 18 experiments, LOS from each of the neisseriae or MC strains induced higher levels of TNF and IL-6 than the endotoxin of *E. coli*. Immunotype L (3,7,9) LOS induced significantly higher TNF and IL-6 responses compared equivalent amounts of LOS from non- L (3,7,9) strains, NL, MC or *E. coli*. The LOS of MC1 was the next most potent inducer of the cytokines. MC2 induced the lowest levels of TNF or IL-6.
Conclusions: The model was useful for screening the ability of LOS from different sources to induce pro-inflammatory cytokines. The LOS from other strains of NL and the other NM immunotypes are being assessed in on going studies.

#065

TERTIARY STRUCTURE OF PORIN IS CRITICAL IN DETERMINING BINDING OF COMPLEMENT REGULATORS

Cullinane M*, Ram S*, Elkins C‡ and Rice PA*. *Boston Medical Center, Boston, MA and UNC, ‡Chapel Hill, NC.

We have previously shown that factor H (fH; alternative complement pathway regulator) binds specifically to the Vth loop of Por1A, which contributes to serum resistance (SR). Ongoing work in our laboratory has shown that the Ist Por1A loop I is required for C4b-binding protein (C4bp; classical pathway regulator) binding, while loops V and VII of certain SR Por1B strains form a C4bp binding domain. C4bp binding is critical in determining stable gonococcal SR; of 29 strains screened 0/8 serum sensitive (SS) and 16/21 SR strains bound C4bp. Binding of fH and C4bp to 4 gonococcal strains and hybrids constructed using these strains is shown below:

Strain / Por molecule	fH	C4bp
FA19 / Por1A	+	+
UU1 / Por1A	+	–
MS11 / Por1B	–	+
F62 / Por1B	–	–
Hybrid / F62 **(I)** FA19**(II-VIII)***	–	–
Hybrid / F62 **(I-IV)** FA19**(V-VIII)***	+	–
Hybrid / F62 **(I)** UU1 **(II-VIII)***	–	–
Hybrid / FA19 **(I)** MS11**(II-VIII)****	–	–
Hybrid / FA19 **(I-IV)** MS11 **(V-VIII)****	–	–

*Contains fH-binding loop **Contain 2 potential C4bp-binding regions

All hybrid structures listed above, none of which bind C4bp, are fully SS. Collectively, these data suggest that the Por loops distant from actual fH and C4bp binding domains could significantly impact binding of these regulators. These observations may explain the paucity of occurrence of such hybrids in nature.

#066

NEISSERIA MENINGITIDIS INDUCES A DISTINCT PATTERN OF ACTIVATION OF TRANSCRIPTION FACTORS NF-KB, C-JUN/AP-1 AND ATF2 IN ENDOTHELIUM COMPARED TO LIPOPOLYSACCHARIDE.

Dixon GLJ[1], Heyderman RS[2], Vogel U[3], Frosch M[3], and Klein NJ[1]
[1]Immunobiology Unit, Institute of Child Health, London, [2]Department of Pathology and Microbiology, School of Medical Sciences, Bristol, [3]Institut für Hygiene und Mikrobiologie, Universität Würzburg, 97080 Würzburg, Germany

Binding of activated host inflammatory cells to endothelium is a critical contributor to the vascular damage that is characteristic of severe meningococcal disease. The expression of vascular cell adhesion molecules on activated endothelium regulates leukocyte-endothelial interactions. We have previously shown that both parent and an unencapsulated isogenic mutant of the group B *N. meningitidis* strain B1940 are potent inducers of the vascular cell adhesion molecules CD62E, VCAM-1 and ICAM-1 on HUVEC. Furthermore, the pattern of expression differed to that observed in response to purified lipopolysaccharide (LPS). Meningococci, particularly unencapsulated strains, induced higher levels of CD62E expression than LPS alone, whereas LPS is at least as potent as meningococci at inducing both VCAM-1 and ICAM-1 expression. We hypothesised that meningococci may activate endothelial cells via a number of signalling pathways resulting in differential effects on CD62E expression. The nuclear transcription factors Nf-κB, c-jun/AP-1 and ATF2 are critical to the control of CD62E transcription in endothelial cells. In this study, we examined the binding patterns of these transcription factors to consensus oligonucleotide sequences from the CD62E promoter region by gel shift analysis in HUVEC stimulated with parent strains of B1940, the unencapsulated isogenic mutant *siaD-* and purified LPS. We show that both LPS and meningococci are potent activators of Nf-κB in endothelium. However, the binding pattern of c-jun/AP-1 and ATF2 proteins was different in HUVEC stimulated with meningococci compared to LPS. Taken together, these data provide evidence that intact meningococci induce signals in endothelium which are distinct from that seen with LPS alone. This may help to explain why this organism has such a potent effect on CD62E expression on vascular endothelium.

#067

SENSITIVITY OF A CATALASE, L-LDH MUTANT OF *N. GONORRHOEAE* FA1090 TO TNFα-PRIMED, PMA-STIMULATED HUMAN NEUTROPHILS IN VITRO.

Edupuganti S[1], Cruickshank RD[1], Baragona S[1], Cohen MS[1,2], and Hobbs MM[1,2]. Departments of [1]Medicine and [2]Microbiology & Immunology, University of North Carolina, Chapel Hill, NC

Neisseria gonorrhoeae (GC) demonstrate enhanced cellular respiration in response to L-lactate in human serum and genital secretions. The resulting increase in gonococcal oxygen consumption inhibits neutrophil (PMN) reactive oxygen intermediate (ROI) production as measured by chemiluminescence in vitro. GC possess 3 lactate dehydrogenase (LDH) enzymes, 2 specific for L-lactate and 1 for D-lactate. GC catalase also contributes to protection from damage by ROIs. Mutants of strain FA1090 lacking one L-LDH and the D-LDH, alone or in combination, were unable to inhibit PMN chemiluminescence. In our hands, wild type FA1090 is relatively resistant to PMN killing in vitro. We predicted that LDH mutations would render GC more susceptible to these host cells. However, the mutants remained resistant to phagocyte killing by unstimulated PMNs, as was an L-LDH, catalase double mutant. By contrast, the L-LDH, catalase double mutant was more susceptible than the parent strain when PMNs were primed with tumor necrosis factor alpha and stimulated with phorbol myristate acetate. Nevertheless, substantial numbers of wild type and mutant FA1090 survived PMN attack under all experimental conditions tested. Thus, although defenses from oxygen-dependent PMN bactericidal acitivities may contribute to the survival of GC in an inflammatory exudate, resistance to non-oxidative mechanisms is undoubtedly also important. The requirement for LDH and catalase for virulence of FA1090 remains to be tested in the human challenge model of infection.

#068

ANTIBIOTIC SENSITIVITY AND BINDING OF MORAXELLA (NEISSERIA) CATARRHALIS TO RSV INFECTED CELLS

El Ahmer, Omar R., Amyes, Sebastian G.B., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Scotland

Background: *M. catarrhalis* (MC1) which grew on antibiotics present in Modified New York City (MNYC) medium bound in higher numbers to HEp-2 cells infected with respiratory syncytial virus (RSV). MC2 did not grown on MNYC plates and bound in significantly lower numbers to RSV infected cells. The objectives were: 1) to screen more strains of these two types to determine if this was a consistent pattern; 2) to compare outer membrane proteins (OMP) for the two groups
Methods: Binding of fluorescein-labelled MC (2 antibiotic resistant and 48 antibiotic sensitive strains) to HEp-2 cells and HEp-2 cells infected with RSV subgroup A (RSVA) or subgroup B (RSVB) was examined by flow cytometry. OMP profiles were assessed by PAGE gel electrophoresis. Haemagglutination was assessed with group O cells
Results: Compared with binding to uninfected cells, the 2 antibiotic resistant strains bound in significantly greater numbers to cells infected with RSVA ($P < 0.01$) or RSVB ($P < 0.01$). The 48 antibiotic sensitive strains bound in significantly lower numbers to cells infected with RSVA ($P < 0.01$) or RSVB ($P < 0.05$). The antibiotic resistant strains lacked 49 kDa and 81 kDa proteins but had a 29 kDa protein absent in antibiotic sensitive strains. The antibiotic resistant strains did not agglutinate group O cells but the sensitive ones did.
Conclusions: The majority of clinical isolates were sensitive to antibiotics found in MNYC medium and bound in lower numbers to RSV infected cells. Increased binding to RSV-infected cells is not related to serum resistance or haemagglutination. The effect of antibiotic selection on the OMP profiles is under investigation.

#069

SURFACE ANTIGENS OF MORAXELLA (NEISSERIA) CATARRHALIS IN RELATION TO ANTIBIOTIC RESISTANCE AND SERUM SENSITIVITY

El Ahmer, Omar R., Amyes, Sebastian, G.B., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Edinburgh, Scotland

Background: Two groups of *M. catarrhalis* (MC) have been demonstrated: group 1 grows on selective antibiotics present in Modified New York City (MNYC) medium; group 2 does not. A group 1 strain (MC1) was serum resistant and a group 2 strain (MC2) serum sensitive. Both absorbed antibodies cross-reactive with meningococci. The objectives were: 1) to determine what proportion of clinical MC isolates belonged to the two groups; 2) to assess haemagglutination properties of the isolates in relation to sensitivity to normal human serum.
Methods: MC isolates from adult sputum samples (N = 100) were assessed for growth on MNYC medium with selective antibiotics. Haemagglutination was assessed with blood group O cells. Serum killing was determined using a pool from healthy blood donors and a human complement source absorbed with MC1 and MC2.
Results: Two of the patient isolates grew on MNYC, 98 did not. Haemagglutination was observed for all 98 group 2 strains but not for the two group 1 strains. The two group 1 strains were resistant to serum killing as were 93/98 (95%) group 2 strains; 5/98 (5%) of the group 2 strains were serum sensitive.
Conclusions: Among isolates from adult patients, group 2 was predominant. The majority were resistant to serum bactericidal activity. The ability to absorb bactericidal antibodies cross reactive with NM was not associated with haemagglutinating antigens, and haemagglutination was not always associated with serum resistance.

#070

THE MOLECULAR BASIS OF GROUP A MENINGOCOCCAL SERUM RESISTANCE

Getzlaff S*, Ram S‡, Vogel U*, Quynh-Tram Ngo-Viet* and Rice PA‡. *Universitaet Wuerzburg, Wuerzburg, Germany and ‡Boston Medical Center, Boston, MA.

Group A meningococci cause significant morbidity and mortality in sub-Saharan Africa. The mechanism of complement evasion by serogroup A strains, whose capsule is devoid of sialic acid (cf., group B and C meningococci), has never been studied. Using wild-type (wt) strain 2594 (L3,7,9 LOS; no endogenous sialylation), we constructed a capsule deficient mutant (mynB-) and an lgtA mutant, the latter with a truncated LOS structure (Hep1-Glc-Gal). Bactericidal assays with 20% NHS demonstrated almost complete killing (4 $\log_{10}$ cfu reduction from a baseline of 5.2 $\log_{10}$ cfu) of mynB-, while wt and lgtA- showed decreases of 1.5 and 0.6 $\log_{10}$ cfu respectively. No significant differences in amount of IgG and IgM binding among strains was seen. Despite being serum sensitive, mynB- bound 5-fold more factor H (fH; alternative pathway regulator) than capsulated strains; no differences in fH binding were noted between wt and lgtA-, mynB- bound 2-fold more C3b than wt, while lgtA- bound ~20% less C3b than wt. Differences in C5b-9 binding were pronounced, with the ratio of C5b-9 binding to wt:mynB-:lgtA- = 2:4:1. Collectively, these data suggest that capsule is essential for serum resistance, probably by decreasing binding of complement-fixing Ab to surface structures. The relatively shorter LOS of lgtA- could pose less impediment to fH mediated decay of C3- and C5-convertases on the bacterial surfaces, thus rendering it more serum resistant than wt. Saccharide length can influence fH activity; a decrease in factor H-C3b interactions occurs when C3b is bound to saccharides containing 4 or more sugars (Pangburn MK. J Immunol. 1989:142;2766-70).

#071

FREQUENT TH1 RESPONSE TO MENINGOCOCCAL IGA1 PROTEASE ASSOCIATED α-PROTEIN AMONGST ATOPIC PATIENTS

Goedert S, Buttner C, Kunkel G, Kuehl F, Waltz H, Fleckenstein B, Bumann D and Meyer TF Max-Planck-Institute of Infection Biology, Dept. Molecular Biology, Berlin, Germany

Objective: Pathogenic *Neisseria* secrete IgA1 protease that is secreted as pro-protein and extracellularly cleaved into three parts: the enzyme, the α-Protein and the γ-Peptide. Interestingly, α-Proteins of different *Neisseria* strains contains a potential DR1 binding motif that shares homology with a T cell epitope described for pollen allergens. Basing on these findings we investigated the response of peripheral blood T cells of normal and atopic donors to a recombinant histidin tagged α-Protein fusion protein.

Design: 17 normal donors and 26 atopic patients were tested. (22 of the atopic patients had typical symptoms of hay fever, 3 showed no clinical symptoms but positive skin test or pollen specific serum IgE and one suffered from atopic dermatitis.) PBMC were stimulated with α-Protein to measure proliferation by incorporation of tritium labeled thymidin and cytokine production by ELISA and ELISPOT (IFNγ, IL-4). Responding cells were characterized by FACS analysis.

Results: In contrast to 6 % of normal donors, 50 % of atopic donors, responded to neisserial α-Protein as measured by T cell proliferation and IFNγ production, but not IL-4 production. The α-Protein induced stimulation was dose depend and mediated by CD4+ T helper cells, as shown by FACS analysis and blocking studies with anti-MHC II antibodies.

Conclusions: The frequent Th1 response to neisserial α-Protein observed amongst atopic patients could suggests an association between atopic disease and a proceeded contact with meningococci, Alternatively, the phenomenon of antigenic mimicry can account for the increased responsiveness of atopic patients to α-Protein.

#072

WHY IS CIGARETTE SMOKE A RISK FACTOR FOR MENINGOCOCCAL DISEASE?

Gordon, Ann E., El Ahmer, Omar, R. Weir, Donald M., Raza, Muhhamad W., Blackwell, C. Caroline
Department of Medical Microbiology, University of Edinburgh, Edinburgh, Scotland

Background: We examined effects of cigarette smoke on bacterial binding to epithelial cells and inflammatory responses important in dealing with invasive bacteria.
Methods: Flow cytometry was used to assess bacterial binding and expression of epithelial cell antigens. Blood samples from meningitis parents (2 smokers, 8 non-smokers) and controls (5 smokers, 17 non-smokers) were assessed for pro- and anti-inflammatory responses to LPS from *Escherichia coli*. IL-6, IL-10 and IFN-γ were assessed by ELISA and TNF-α by bioassay.
Results. All strains tested bound in significantly higher numbers to buccal epithelial cells of smokers. Increased binding was not due to changes in cell surface antigens but to coating of the cells with water-soluble material(s) present in cigarette smoke. Preliminary results indicate meningitis families had higher IFN-γ responses but levels of TNF-α, IL-6 and IL-10 similar to controls. Smoking affected pro- and anti-inflammatory cytokine production. The highest responses were observed for TNF, IL-6 and IL10 for smokers from meningitis families.
Conclusion: Exposure to cigarette smoke enhances binding of meningococci to epithelial cells. In general, smoking appeared to reduce inflammatory responses examined; however, smokers from meningitis families had higher levels of all the cytokines compared with smokers from control families. The studies need to be expanded to examine larger numbers of smokers.

#073

A CRITICAL RATIO OF NATURAL ANTIBODIES TO GONOCOCAL ANTIGENS INFLUENCES THE RISK OF GONORRHEA TRANSMISSION

Gulati S*, Hook EW III‡, Blake MS¶, Ram S*, Kohl PK§ and Rice PA*,¶,*Boston Medical Center, Boston, MA, ‡University of Alabama, Birmingham, AL, ¶North American Vaccine, Inc., Beltsville, MD, and §Neukoelln Hospital, Berlin, Germany.

Serum antibodies (Ab) against gonococal porin (Por) and lipooligosaccharide (LOS) are bactericidal, while Ab against Rmp may block serum killing. Therefore a lower (Por Ab + LOS Ab)/Rmp Ab ratio (bactericidal activity ratio; BAR) may be predictive in defining susceptibility to gonorrhea. In a vaccine trial performed in 1986, we observed that of 28 male placebo recipients who were subsequently challenged with a Por1B-3 strain, 10 did not get infected (mean BAR=4.97), while the remaining 18 with infection had a mean BAR of 2.75 (p=0.007). Currently, Por1B strains represent over 90% of all strains isolated in the U.S. We proceeded to validate the predictive value of a BAR $\leq$2.75 in facilitating transmission of Por1B gonococcal strains to women exposed to infected sex-partners. Sera were obtained from 45 female partners of men infected with Por1B gonococci 2, 4 and 6 weeks after exposure and were analyzed for antibody levels against Por, LOS and Rmp of the homologous strain. Assuming a linear increase in Ab levels to the individual antigens, using the three time points we deduced Ab levels at the time of infection by extrapolation. Infection was transmitted to 34 of 45 women. 22 of these 34 women had BAR$\leq$2.75, while only 3 of 8 women who were not infected had a BAR$\leq$2.75 (p=0.03). The positive predictive value of a BAR$\leq$2.75 in Por1B gonococcal transmission was 88%. The BAR may prove useful in identifying individuals who are particularly susceptible to gonococcal infection.

#074

The Effects of *Neisseria meningitidis* on Macrophage Function

D. C. T. Hall, S. Omani, M. Koekemoer, and L. Waldbeser
Texas A&M University; Corpus Christi, TX

Neisseria meningitidis (meningococcus) is the bacterial pathogen responsible for meningococcemia and meningitis. Normally, meningococci colonize the nasopharynx in 5%- 30% of the population. However, meningococci are capable of penetrating the mucosal surfaces of the nasopharynx and entering the bloodstream to cause meningococcemia. If the immune system or antimicrobial therapy does not stop their progression, they can cross the blood-brain barrier and cause meningitis. During the infection process, the meningococci will encounter professional phagocytes, such as, granulocytes, monocytes, and macrophages. Several bacterial pathogens have been shown to induce apoptosis in macrophages. Some of these organisms include *Salmonella typhimurium, Mycobacterium avium, Shigella flexneri*, and recently *Neisseria gonorrhoeae*. The effects of *N. meningitidis* on macrophage death have yet to be elucidated. Our studies focused on the interaction between two macrophage-like cell lines and *N. meningitidis*. The two cell lines studied were MonoMac and THP-1. The MonoMac cell line is more macrophage-like and therefore is further differentiated than the THP-1 cell line, which is monocyte-like. We found that *N. meningitidis* causes premature death of both types of macrophages. Following infection, we determined that the most significant macrophage cell death occurred at a MOI of 10:1. Our kinetics study revealed that macrophage cell death was most significant at 90 minutes. However, there was substantial cell death as early as 30-minutes postinfection. Preliminary studies employing the caspase-3 assay revealed elevated levels of caspase-3 activity following infection with *N. meningitidis*. Additionally, annexin V-FITC studies revealed an early increase in cell death by apoptosis as well as necrosis.

#075

SERUM COMPLEMENT ACTIVITY DURING MENSES AS A RISK FACTOR FOR GONOCOCCAL PID

Hart-Van Tassell A,[1] Nowicki B, [1,2] Nguyen K,[2] Urvil P,[1] and Nowicki S,[1,2] Department of Obstetrics and Gynecology,[1]and Department of Microbiology & Immunology,[2]The University of Texas Medical Branch at Galveston, Texas.

Objective: Symptoms of gonococcal PID usually occur at the onset of menses. C1q enhances the virulence of *Neisseria gonorrhoeae*. Our recent study showed the bactericidal activity of normal human serum (NHS) decreased during menses. Since complement C1q plays a crucial role in both bactericidal activity and gonococcal virulence, we evaluated the serum complement component C1q level, C1q binding to *N. gonorrhoeae* and compared it to complement activity pre-, during and post-menses.

Design: NHS from female volunteers of reproductive age without prior history of gonococcal infection was used. NHS was from blood drawn on day pre-, during and post-menses. Serum C1q level and complement activity was measured throughout the menstrual cycle. The interaction of C1q with *N. gonorrhoeae* cell was analyzed in immunoblots.

Results: The results indicated complement activity decreased during menses, corresponding to reduced bactericidal activity of NHS. Complement and bactericidal activity was inversely related to serum level of C1q and C1q interaction with *N. gonorrhoeae*.

Conclusion: Decreased complement activity may account for reduced bactericidal activity of NHS, while increased C1q interaction with *N. gonorrhoeae* may enhance gonococcal virulence during the onset of menses. These results also suggest that complement activity and complement components may be regulated by sex hormones. Decreased complement activity and increased C1q binding combined may predispose women to higher risk of developing gonococcal PID during menses.

#076

DETECTION OF COMPLEMENT-MEDIATED ANTIBODY-DEPENDENT BACTERICIDAL ACTIVITY IN A FLUORESCENCE-BASED SERUM BACTERICIDAL ASSAY FOR GROUP B *NEISSERIA MENINGITIDIS*

Howell A, Mountzouros K, Wyeth-Lederle Vaccines, Rochester, NY.

Traditional complement-mediated antibody-dependent serum bactericidal assays (SBA) tend to vary in protocol among different laboratories and can be labor-intensive. Here we introduce a new fluorescence-based complement-mediated antibody-dependent serum bactericidal assay (fSBA) and compare the results obtained with the fSBA to those obtained with the traditional SBA. The assay conditions for both assays are identical, but differences lie in how the surviving bacteria are quantified at the conclusion of the assay. The fSBA utilizes the fluorescent/colorimetric dye alamarBlue™. Fluorescent signals generated in the fSBA correlate to oxidative respiration of surviving bacteria, and these signals can be measured between 6 and 8 hours directly from the 96-well assay plate. On the other hand, surviving cells in the SBA need to be counted on semi-solid media after 24 hours. The bactericidal titers obtained from both the fSBA and the SBA were comparable (r=0.901). Not only is the fSBA more sensitive and easier to perform, it is extremely useful in screening large amounts of sera as possible complement sources or detecting functional antibodies against Group B *Neisseria meningitidis* in both human and mouse antiserum. The clear benefits of the fSBA make it an excellent alternative to the traditional SBA.

#077

DETECTION OF A CROSS-REACTIVE AGE DEPENDENT IMMUNE RESPONSE TO A PANEL OF SEROGROUP B MENINGOCOCCAL STRAINS.

Ison CA, Anwar N, Harrison OB, Cole M, Levin M*, Meningococcal Study Group, Dept Med Micro/*Academic Pediatrics, Imperial College, London

Objective: To use the whole blood assay to detect the bactericidal response by naturally infected and uninfected children, toward a panel of serogroup B strains of *Neisseria meningitidis*.

Design: Fifty eight children that had been infected by either serogroup B strain (36) or a serogroup C strain (22), and 68 uninfected controls were screened against a panel of five meningococcal strains, MC58(B:15:P1.7,16), NCTC C(C:NT:P1.5), CubanB(B:4:P1.15), NM48(B:4:P1.4), and NM76 (B:2b:P1.10). The activity was calculated as the mean percentage killing of an inoculum of 10^7 CFU/ml at 90mins. Children were separated into age groups and the proportion in each category with >50% killing at 90mins was determined for individual strains. Spearman's Rank Correlation was used to assess the association between age and % killing.

Results: Uninfected children >2 years of age had significant activity against the Cuban B, NM48 and NM76. The percentage with cross reactive activity increased with age, however the proportions of individuals with >50 % killing was less when compared to infected children. Activity in uninfected children <1 year of age was absent. There was a significant correlation between age and killing for NCTC C(P=<0.001), Cuban B (P=<0.001), M48(P=<0.001) and NM76(P=0.004) in serogroup B infected children. Those >12 years of age had strong activity against the entire panel, 75% of 6-12 year olds had activity against all strains. In the 2-6 year old age group, 50-85% had activity against 3/5 strains and between 14-29% of <1 year olds had activity against a heterologous strain. Serogroup C infected children were older hence there were no strong associations between age and killing ability as the majority of children had activity.

Conclusion: As the age of the child increases so does the ability to kill more than one strain. We have shown the whole blood assay can detect specific and cross reactive responses against meningococci in children of all ages.

#078

MANNOSE-BINDING LECTIN REGULATES THE INFLAMMATORY RESPONSE TO *NEISSERIA MENINGITIDIS* SEROGROUP B

DL Jack[1], AJ Tenner[2], M Frosch[3], MW Turner[1], NJ Klein[1]
[1]Institute of Child Health, London, United Kingdom; [2]Univ. of California, Irwine, CA, USA; [3]Univ. of Würzburg, Germany.

Mannose-binding lectin (MBL) is a serum collectin which binds to the repeating sugar residues commonly found on micro-organisms. Genetic MBL deficiency has been linked to an increased susceptibility to and altered severity of meningococcal disease. We examined how MBL altered the interaction of human neutrophils and monocytes with isogenic mutants of serogroup B meningococci which differed in the expression of capsule and the LPS sialic acid acceptor site and MBL binding. Bacteria preincubated with 2μg/ml purified MBL were mixed with dextran separated leukocytes in serum-free media. MBL increased the phagocytosis of most mutants by neutrophils at 60 min but this was not simply related to the amount of MBL bound. In the same assay, MBL delayed the loss of CD62L and the increase of CD11b at 30 min by neutrophils indicating a delay in cellular activation. This effect was also observed in MBL-deficient anti-coagulated blood supplemented with MBL, but it required supraphysiological levels of MBL (8 μg/ml) and was observed at 15 min. We investigated the effect of MBL on monocyte cytokine responses using an intracellular staining technique and flow cytometry. Brefeldin A and differing concentrations of MBL were added to whole blood from MBL deficient individuals. At 3h, we observed a slight increase in TNFα at low MBL concentrations and a decrease at high concentrations. The effect was similar but more pronounced with IL6, but IL1β production was enhanced over a wider range of MBL concentrations. These results indicate that MBL modulates inflammatory responses but with kinetics and magnitudes that differ for IL1β, IL6, TNFα and adhesion molecules. This inflammatory modulation may influence phagocytosis and provides further evidence to explain differences in the severity of meningococcal disease between individuals with different MBL genotypes.

#079

HUMAN MANNOSE-BINDING LECTIN ENHANCES SERUM KILLING OF *NEISSERIA MENINGITIDIS*

DL Jack[1,3], GA Jarvis[2], CL Booth[1], J Li[2], NJ Klein[1] and MW Turner[1]
[1]Institute of Child Health, London, United Kingdom; [2]Univ. of CA and VA Med. Center, San Francisco, CA, USA; [3]Division of Molecular and Genetic Medicine, University of Sheffield, United Kingdom

Mannose-binding lectin (MBL) is a serum collectin which binds to the repeating sugar residues commonly found on micro-organisms and activates the complement system in an antibody-independent manner. Genetic MBL deficiency has been linked to an increased susceptibility to meningococcal disease. We examined the interaction of MBL with three clinical isolates of serogroup C meningococci which differed in endogenous LPS sialylation and serum sensitivity.

MBL and monoclonal antibody 3F11 (which recognises non-sialylated LPS) were shown to bind simultaneously to strain 8026 (4% endogenous sialylation) by dual colour flow cytometry. Binding of both reagents was inhibited to background levels when the organisms were grown with exogenous CMP-NANA to increase LPS sialylation. The binding of MBL or 3F11 to strains 7954 and 7973 (48% and 86% endogenous sialylation, respectively) was below detection limits. Preincubation of non-sialylated 8026 with MBL increased killing by 49% using 0.5% MBL-deficient serum; killing of the sialylated form was increased by 21% in 1% serum. No increased killing was observed in heat-inactivated serum. In 10% serum, MBL increased the proportion of the non-sialylated form staining double positive for C4 and C5b-9 from 30% to 70% at 3 min (flow cytometry). For sialylated forms, similar increases in C4 and C5b-9 double positivity were observed, but were delayed to 20 min. Using transmission electron microscopy with immunogold detection to increase sensitivity, we could still detect MBL binding to sialylated 8026 although this was greatly reduced compared to the non-sialylated form and appeared to be highly localised.

In conclusion, MBL can bind to encapsulated serogroup C meningococci and increases complement-dependent killing.

#080

HOST FACTORS THAT ENHANCE OR RESTRICT EXPERIMENTAL GONOCOCCAL GENITAL TRACT INFECTION OF FEMALE MICE

Jerse AE, Soler-Garcia AA, Valentin-Bon IE, Bordner A, Rahman I, Sharma N, Crow E. Dept. Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.

We recently described a model of long-term gonococcal genital tract infection using 17-β estradiol-treated BALB/c mice. In light of physiological similarities in the female murine and human genital tracts, we tested the effect of selected host factors including estrogen, progesterone (PG), and commensal flora on experimental murine infection. Untreated mice are susceptible to *N. gonorrhoeae* (GC) during the proliferative stage, but not the luteal stage of the estrous cycle. We found that 3/3 GC strains capable of infecting estradiol-treated mice but not untreated mice, were resistant to PG in vitro suggesting that PG does not directly inhibit GC during the luteal phase. Interestingly, ovariectomized (Ov^-) mice were resistant to infection with GC strain FA1090 unless treated with estradiol. An intense inflammatory response occurred in Ov^- mice inoculated with GC, suggesting that anti-inflammatory effects of estradiol increases susceptibility. We also found a positive association between the degree of recovery of GC from intact estradiol-treated mice and the presence of H_2O_2-negative *Lactobacillus*-like rods. Consistent with this observation was the demonstration that these *Lactobacillus*-like organisms enhanced growth of FA1090 on solid agar. In contrast, H_2O_2-producing *L. crispatus*, a species that is frequently isolated from women, inhibited FA1090 *in vitro*. This inhibition was neutralized with exogenous catalase and a genetically defined catalase mutant of FA1090 was more sensitive to *L. crispatus* in vitro than the wild-type parent. Co-infection of mice with *L. crispatus* and FA1090 did not lead to reduced duration of recovery of GC, however, compared to mice infected with GC alone.

Conclusions: The insensitivity of strain FA1090 to PG in vitro suggests that PG does not directly inhibit GC during infection. Estradiol is required for murine infection even in the absence of endogenous PG. Estradiol may promote susceptibility through anti-inflammatory effects, although other effects may also play a role. Vaginal H_2O_2-negative lactobacilli may stimulate growth of GC in vivo, and although H_2O_2-producing lactobacilli inhibit GC in vitro, mechanisms to avoid this inhibition may be induced in GC during infection. We are currently pursuing the hypothesis that up-regulation of GC catalase in vivo may successfully neutralize *Lactobacillus*-generated H_2O_2 during infection.

#081

CONSTRUCTION AND FUNCTIONAL ACTIVITIES OF CHIMERIC ANTIBODIES AGAINST THE PORA P1.16 EPITOPE ON *NEISSERIA MENINGITIDIS.*

Michaelsen T E[1], Ihle Ø[1], Beckstrøm K J[1], Herstad T[1], Aase A[1], Kolberg J[1] and Høiby A[2]

[1]Department of Vaccinology, National Institute of Public Health, Oslo, Norway, [2]Department of Bacteriology, National Institute of Public Health, Oslo, Norway.

Objectives: To construct and study the differences in functional activities of chimeric mouse/human antibodies of IgG1, IgG3 and IgM isotypes with identical binding regions specific for the P1.16 epitope on PorA

Design: We identified the V_H- and V_L-genes of two P1.16 mouse monoclonal antibodies (mAbs) 151,F-9 and 184,F-12 by isolating m-RNA from the hybridoma cells and RT-PCR-amplifying the V-genes using primer sets annealing to the framework and J-regions of the V-genes, repectively. The cloned V_H- and V_L-genes were sequenced and sub-cloned into expression vectors containing the C-genes of human γ1, γ3 or μ and κ, respectively. The constructed heavy- and light-chain vectors were then co-transfected to the hybridoma fusion-partner (NS0-cells).

Results and conclusions: Transfectomas secreting intact chimeric mouse/human IgG1, IgG3 and IgM were cloned and it was verified that the binding specificity was conserved. The antibodies were finally tested for serum bactericidal activity (SBA) and opsonophagocytic activity (OP) against live meningococci. The results showed a superior SBA and OP activities of IgG3 compared to IgG1. On the other hand, IgM to our surprise, showed not higher SBA than IgG3. Even more, IgM was the isotype with highest OP activity. This indicate an efficient activation at the C3-level and involvement of complement-receptors (CR1 and CR3) on the human effector cells (peripheral blood polymorphonuclear leukocytes) during the OP of IgM.

#082

Measurement of Functional Antibody in Human Serum Against Group C *Neisseria meningitidis*, in a New Fluorescence-Based Serum Bactericidal Assay and in an Infant-Rat Model for Bacteremia

Kenneth Mountzouros, Alan Howell, Keith Friedman, Daniel Sikkema, Kelly Belanger, Kimberly Miller, Garvin Bixler, and Stephen Hildreth

Complement-mediated antibody-dependent serum bactericidal assays (SBA) are considered assays of choice when evaluating functional antibody responses to Group C *Neisseria meningitidis*. Results obtained from ELISAs and SBAs are considered indicators of protection against Group C meningococcal disease and are utilized to evaluate clinical trials for capsular polysaccharide and conjugate vaccines. Here we describe results obtained from the serum of 8 adult humans immunized with a tetravalent (A, C, Y and W135) polysaccharide vaccine. Vaccinated individuals were bled and serum was pooled and blended to make 17 different serum samples. The antibody concentration of the 17 blended serum samples ranged from 0.11 to 45.5 μg/ml IgG in an ELISA specific for Group C capsular polysaccharide. Serum samples were tested in a SBA and also in a newly developed fluorescence-based serum bactericidal assay (fSBA). Bactericidal titers obtained from the SBA and fSBA using rabbit (rab) or human agammaglobulinemic (hag) serum as a complement source were compared to ELISA titers. Serum bactericidal titers generated from both the SBA-rab and fSBA-rab were similar (r = 0.932). By linear regression analysis titers obtained in the fSBA-hag were more correlative (r = 0.891) to ELISA then to fSBA-rab (r =0.804) or SBA-rab (r =0.690). The functional activities of the immune serum samples were also evaluated in an infant-rat passive model of protection against meningococcal bacteremia. Levels of bacteremia were determined in rats passively immunized with immune human serum and compared to bactericidal titers obtained with the SBA-rab, the fSBA-rab and the fSBA-hag. Results from the in vivo model for bacteremia demonstrated an inverse relationship between bactericidal titers obtained in each of the bactericidal assays, to the level of bacteremia in challenged rats. With this limited panal of sera the fSBA-rab appears to be more correlated to the functional activity of immune serum in infant rats (r =0.811) than titers obtained from a fSBA-hag (r =0.748), SBA-rab (r = 0.624) or by ELISA (r = 0.580). Data obtained in this study indicate that the fSBA could be considered a substitute for conventional SBA. Bactericidal titers obtained in this study employing the fSBA-hag or fSBA-rab are correlative to ELISA titers, and appear to be more correlated with functional activity than ELISA titers in the infant-rat model for bacteremia with Group C *N. meningitidis*.

#083

SEROGROUP Y MENINGOCOCCI (Y:14:P1.2,5) ARE RAPIDELY KILLED IN AN EX VIVO WHOLE BLOOD MODEL OF INFECTION WHEREAS SEROGROUP B MENINGOCOCCI (B:4:P1.7,16) SURVIVE AND GROW TO LOGARITHMIC PHASE.

Nolte O, Rickert A, Ehrhard I, Sonntag H-G. Hygiene Institute, Dept. of. Hygiene & Med. Microbiology, University of Heidelberg, Germany

OBJECTIVE: To compare the survival and pathogenicity of an invasive and a carrier strain of *Neisseria meningitidis* in an *ex vivo* whole blood model of infection.

DESIGN: Two strains of *N. meningitidis* (Y:14:P1.2,5 —carrier strain— and B:4:P1.7,16 —invasive strain—) were inoculated at low *cfu* (9009 +/-658 and 9863 +/- 1580 per mL, respectively) in heparinized whole blood samples of healthy vaccinated (tetravalent polysaccharide vaccine) and non vaccinated donors. Remaining viable meningococci were determined at intervals up to 24 h after onset of experiments. In addition, leukocyte cell populations were studied using flow cytometry. Cytokine release was measured using ELISA.

RESULTS: Serogroup Y meningococci were killed within 20 to 30 min after inoculation in whole blood independently of the status of the blood donor (e.g. vaccinated or not). Also independently of the donor, serogroup B meningococci reached logarithmic growth phase in whole blood samples usually within 4 hours after setup of the experiment. Accordingly, high amounts of cytokines (namely IL-1β, IL-6, IL-10 and TNFα, but usually not INFγ) were found to be released after incubation with serogroup B meningococci whereas only comparatively low amounts of IL-6 were released after incubation with serogroup Y meningococci. After incubation with serogroup B meningococci a considerable loss of neutrophils was observed. Neutrophils remained unchanged after incubation with serogroup Y meningococci.

CONCLUSIONS: The *ex vivo* whole blood model with low *cfu* seems to be highly useful to reflect the host-pathogen interactions as taking place *in vivo*. Results obtained using B meningococci indicate a pathogen mediated killing of neutrophils. However, host response to meningococci seems to be strain specific.

#084

COMPARISON OF DIFFERENT LIQUID CELL CULTURE MEDIA FOR CULTURING *NEISSERIA MENINGITIDIS.*

Nolte O, Ledig S, Rickert A, Ehrhard I, Sonntag H-G. Hygiene Institute, Dept. of. Hygiene & Med. Microbiology, University of Heidelberg, Germany

OBJECTIVE: To compare and evaluate different liquid media for culturing *Neisseria meningitidis* (meningococci).

DESIGN: Strains of *N. meningitidis* were grown in different cell culture media under different conditions (e.g. with or without iron source; with or without serum source). As serum source fetal calf serum (FCS) was used to avoid the presence of anti meningococcal antibodies. Growth rate was determined in 30 min intervals for the first six hours and than in longer intervals until 48 h of culture were completed. Optical density (OD) of the culture media were measured (λ=600 nm). Following 24 h of incubation 10^4 viable meningococci were used in a whole blood model of infection. The bacterial survival and cytokine release from leukocytes were compared to results obtained by using meningococci grown on GC agar.

RESULTS: Growth rate was different in various cell culture media. The OD_{600} for 10^6 meningococci/mL was higher for those meningococci grown in liquid media than for those grown on solid media. Most interestingly, when grown in standard cell culture medium (RPMI 1640; Gibco BRL, Paisley, UK) without iron in the medium but supplemented with 10% FCS meningococci grew to a logarithmic growth phase within the first six to eight hours. Between 8 and 24 h of culture we observed an equilibrium phase or a decrease in *cfu*. The behavior of those meningococci was significantly different in a whole blood model when compared to meningococci grown on GC agar. If, for instance, serogroup B meningococci were grown under iron starvation, they were rapidly killed in human whole blood whereas those grown on GC showed logarithmic growth.

CONCLUSIONS: There are considerable differences concerning the growth rate of meningococci and subsequent behavior in a whole blood model of infection depending on the culture medium and the culture conditions used. This should be taken into account when meningococci are used for the study of pathogenicity or host—pathogen interactions.

#085

POSSIBLE VIRULENCE MECHANISM OF GONOCOCCAL PID

Nowicki S.[1,2] Nguyen K,[2] Hart-Van Tassell A,[1] Urvil P,[1] Nowicki B,[1,2]
Department of Obstetrics and Gynecology [1]and Department of Microbiology and Immunology,[2] The University of Texas Medical Branch at Galveston, Texas

This study was designed to test hypothesis that strains of *N. gonorrhoeae* causing PID are more virulent than strains causing local urethritis and the underlying mechanism is mediated by an unknown virulence gene(s). We have demonstrated that the majority of PID isolates, but not isolates from local infection (LI) were ser^R and could developed C1q mediated infection in a rat pup model. The ser^R and C1q responsive PID isolates contain a unique 344-bp DNA sequence. We completed cloning, sequencing and characterization of a 2.6 kb DNA fragment that contained the 344-bp region. This 2.6-kb fragment contains one coding sequence of 1476 bp. The protein deduced from the *pid* gene sequence consists of 492 amino acids with a molecular size of 52.3 kDa. The transmembrane region in the N-terminal half of the hypothetical Pid protein suggests that Pid may be an outer membrane peptide, which could hypothetically directly interact with C1q. *N. gonorrhoeae* PID 2005 (donor of the *pid* gene) express a 52 kDa protein that cross-reacts with antibodies raised against synthetic peptide (P1) corresponding to the most hydrophilic region of the deduced Pid protein. *N. gonorrhoeae* F62 serum sensitive avirulent recipient strain upon transformation of *pid* gene became ser^R and displayed C1q mediated virulence to rat pups. The *pid* gene was found predominately among PID strains (80%) and occurred with low frequency in LI isolates (14%). Contribution of Pid to C1q interaction the virulence mechanism of *N. gonorrhoeae in vitro* and *in vivo* is further investigated

#086

STABLE SERUM RESISTANCE OF *NEISSERIA GONORRHOEAE* IS MEDIATED BY BINDING OF C4B-BINDING PROTEIN TO GONOCOCCAL PORIN

Ram S*, Cullinane M*, Gulati S*, Blom A[¶], Dahlback B[¶], Elkins C[‡], Pangburn MK[§], McQuillen DP*, Monks BG* and Rice PA*. *Boston Medical Center, Boston, MA, [¶]Lund University, Malmo, Sweden, [‡]UNC, Chapel Hill, NC and [§]UTHSC, Tyler, TX.

We have previously shown that stable serum resistance (not mediated by sialic acid) of porin (Por) 1A bearing gonococci is mediated by C4bp binding to Por1A. Cofactor (C4b-degrading) function of C4bp bound to bacteria has also been demonstrated. Using allelic exchange to construct strains with hybrid Por1A/B molecules, we have shown that the N-terminal loop (loop I) of Por1A is required for C4bp binding. We have observed that certain serum resistant Por1B gonococcal strains also bind C4bp via their Por molecule. Using allelic exchange and site-directed mutagenesis we have demonstrated that loops V, VI and VII of Por1B are required for C4bp binding. The three loops together participate in the formation of a negative patch that mediates C4bp binding. C4bp-Por1B interactions are ionic in nature (inhibited by high-salt as well as by heparin), while the C4bp-Por1A bond is hydrophobic. mAbs directed against SCR1 of the α-chain of C4bp inhibit C4bp binding to both Por1A and Por1B. Furthermore, only recombinant C4bp mutant molecules that contain α-chain SCR1 bind Por1A and Por1B gonococci, confirming that SCR1 contains Por binding sites. C4bp α-chain monomers do not bind strains with either Por molecule, suggesting that the polymeric form of C4bp is required for binding to gonococci. Inhibition of C4bp binding to serum resistant Por1A and Por1B strains in a serum bactericidal assay using Fab fragments against C4bp SCR1 resulted in complete killing at 30 min of otherwise fully serum resistant strains in only 10% normal serum, underscoring the role of C4bp in mediating gonococcal serum resistance.

#087

COMPLEMENT INTERACTIONS WITH GROUP B AND GROUP C MENINGOCOCCI: A STUDY USING ISOGENIC MUTANT STRAINS

Ram S[‡], Vogel U*, Getzlaff S*, Gulati S[‡], Van der Ley P[§] and Rice PA[‡], [‡]Boston Medical Center, Boston, MA, *Universität Würzburg, Germany and [§]RIVM, The Netherlands.

Lipooligosaccharide (LOS) sialylation and polysialic acid capsule (Cap) play key roles in mediating group B and group C meningococcal serum resistance. The interactions of complement (C) with isogenic mutant strains that lacked either Cap (siaD-), LOS sialic acid (lst-), or both (siaD-/lst-) were studied to define serum resistance (SR) mechanisms. All Cap+ strains were SR; LOS sialylation conferred low-level SR only to the Cap- group C derivative. Although siaD- and siaD-/lst- bound similar amounts of factor H (fH), C3 and C4 at 30 min, the former bound 50% less C5b-9 and generated 40% less C5a, suggesting that LOS sialic acid probably enhanced fH-induced convertase decay. Cap+ strains bound 2-fold less C3 and C4 compared to Cap- mutants. Only ~50% of C3b on all strains was processed to iC3b. Using western blots, PorA was found to be the main acceptor molecule for C3b. Cap+ strains bound 33% less fH than Cap- strains, suggesting that polysialic acid inhibited fH binding to surface structure(s). The presence of LOS sialic acid did not enhance fH binding to either Cap+ or Cap- strains. Deleting PorB3 from the wt (Cap+) group B strain decreased fH binding 5-fold, while deleting PorA decreased fH binding 2 to 3-fold, suggesting that PorB3 was the main fH acceptor on Cap+ strains. Deleting either Por molecule from Cap- mutants did not influence fH binding, suggesting that either Por could bind fH in the absence of Cap. Thus, Cap and LOS sialic acid (the latter in certain strains) act in concert at different stages of the C cascade to mediate meningococcal SR.

#088

THE MOLECULAR BASIS OF GROUP B MENINGOCOCCAL SERUM RESISTANCE; A STUDY USING CLINICAL STRAINS

Ram S*, Mackinnon FG[‡], Gulati S*, Vogel U[§], Getzlaff S[§], Gorringe A[¶], and Rice PA*. *Boston Medical Center, Boston, MA, [‡]Oxford University, Oxford, U.K, [§]Universität Würzburg, Würzburg, Germany and [¶]CAMR, Salisbury, U.K.

We studied the interaction of complement with 14 epidemiologically related group B meningococcal strains that differed only with respect to capsule (Cap) expression or lipooligosaccharide (LOS) immunotype. LOS sialylation of Cap- strains resulted in resistance to 10%, but not 50% normal serum. LOS sialylation did not enhance the amount of factor H (fH) binding nor decrease total C3 binding at 30 min, but did consistently reduce the rate of C3 accumulation on bacteria. This resulted in 50% less C5b-9 binding and 40% less C5a generation by sialylated strains. Cap+ strains with sialylated LOS were highly resistant to even 50% serum, while those with nonsialylated (and truncated) LOS were significantly less resistant. Cap+ strains bound 1.5-fold less C3 and 2-fold less C4 compared to Cap- strains. Cap+ strains bound 30% less fH than Cap- strains, suggesting that Cap (2,8-linked polysialic acid; colominic acid) interfered with factor H binding to other surface receptor(s). Cap+ strains bound 2-fold less IgG, but 3 to 5-fold more IgM than Cap- strains. IgM bound exclusively to Cap on Cap+ strains, and binding was completely inhibited by pure colominic acid. Decreased C3 and C4 binding to Cap+ strains was not due to inaccessibility of C3 or C4 to surface target(s), because Cap specific mAb 2-2-B fixed complement very efficiently. Thus, LOS sialylation and Cap regulate complement at different stages in the complement cascade and mediate group B meningococcal serum resistance.

#089

MOLECULAR BASIS OF LOW-LEVEL SERUM RESISTANCE CONFERRED BY L1,8,10 LIPOOLIGOSACCHARIDE

Ram S*, Getzlaff S[§], Mackinnon F[‡], Gulati S*, Vogel U[§], and Rice PA*. Boston Medical Center, Boston, MA, [§]Universitaet Wuerzburg, Wuerzburg, Germany and [‡]Oxford University, Oxford, U.K.

Two principal lipooligosaccharide (LOS) immunotypes of *N. meningitidis* include L3,7,9 (lacto-N-neotetraose off the Hep1 chain) and L1,8,10 (lactose off Hep1). Unlike the former, L1,8,10 LOS is not associated with the ability to sialylate. Carrier noncapsulated *N. meningitidis* isolates frequently bear the L1,8,10 LOS structure. Evasion of complement-mediated killing in the absence of LOS sialylation is critical for survival of these isolates in vivo. Thus, we studied the interaction of complement with 5 noncapsulated carrier isolates (3 L3,7,9 and 2 L1,8,10; all identical by PFGE) that were isolated from asymptomatic individuals during a meningitis outbreak in the U.K. Despite binding equal amounts of IgG, IgM and factor H (an alternative pathway regulator), L1,8,10 strains had ~25% less C3 and 50%-60% less C5b-9 than L3,7,9 strains, the latter in the unsialylated state. Less than 50% C3b was processed to iC3b, and no significant difference among strains was noted. 2 strains, S/H1497 (L3,7,9) and S/H155 (L1,8,10) were studied further. Both were fully killed by 10% NHS at 30 min, but S/H155 showed 0.5 and 1.5 $\log_{10}$ greater survival at 5 and 10 min respectively, indicating slower killing. Collectively these data suggest that the shorter L1,8,10 LOS renders C3- and C5-convertases on the bacterial surface more amenable to factor H-mediated decay. A decrease in factor H-C3b interactions occurs when C3b is bound to saccharides containing 4 or more sugars, but 3 or less sugars do not influence this interaction (Pangburn MK. J Immunol. 1989:142;2766-70).

#090

SERUM ANTIBODY RESPONSE TO MENINGOCOCCAL CARRIAGE: EVIDENCE OF HERD IMMUNITY.

Robinson, K.[1], Neal, K.R.[1], Atkinson, K.[1], Stockton, J.[1], Scarth, E.[1], Kaczmarski, E.[2], Gray, S.[2], Slack, R.[1], Nguyen-Van-Tam[1], & Ala'Aldeen, D.A.A.[1]
[1]Meningococcal Research Group, Divisions of Microbiology and Public Health, University Hospital, Nottingham, UK.
[2]Meningococcal Reference Unit, PHLS, Manchester, UK.

The humoral response elicited by asymptomatic carriage of *Neisseria meningitidis* was studied in a cohort of 228 first year undergraduates. On the first day of term the carriage rate was 4.4%, rising to 21.7% one week later, and 20.8% after a further 6 weeks. The very dramatic increase in carriage over the first week of the academic year confirms previously published data from an epidemiological study of carriage in university students[1].

The levels of serum IgG specific for the heterologous strain H44/76 (B:15:P1.7,16) increased significantly from a median of 17.7μg/ml on day 1 to 81.25μg/ml 20 weeks later ($p<0.001$). The IgG responses of acquirers to their homologous meningococcal isolates were analysed and, as shown previously by Jones *et al.*[2], colonisation elicited a rise in specific serum IgG that was significantly higher than the response to H44/76 ($p<0.01$).

The serum anti-H44/76 IgG responses of acquirers of meningococcal carriage were not significantly different from those of non-acquirers during the study. All but three of the individuals had rising serum anti-H44/76 IgG levels. This indicates that the vast majority of the students in the study, whether demonstrated to be acquirers or not, must have been exposed to meningococcal antigens through colonisation at some stage and their humoral immunity boosted. The definition of individuals as non-acquirers based merely upon swabbing is therefore misleading.

1 Neal *et al.* (2000) *BMJ*, **320:** 846-849.
2 Jones *et al.* (1998) *J. Infect. Dis.*, **178:** 451-459.

#091

CARRIAGE OF *NEISSERIA MENINGITIDIS* ELICITS AN ANTIGEN-SPECIFIC MUCOSAL IgG RESPONSE.

Robinson, K., Neal, K.R. & Ala'Aldeen, D.A.A.
Meningococcal Research Group, Divisions of Microbiology and Public Health, University Hospital, Nottingham, UK.

The *N. meningitidis*-specific serum and salivary antibody responses of a group of first year undergraduate students were studied. Posterior pharyngeal swabs were collected together with samples of blood and saliva, from 8 meningococcal carriers and 29 non-carriers. The samples were tested for IgG and IgA specific for the H44/76 strain (B:15:P1.7,16) by ELISA. No significant differences were found in the levels of total or specific salivary IgA of carriers and non-carriers. There were no significant differences in the concentration of total IgG in saliva samples, however, a significantly elevated ($p<0.005$) anti-H44/76 IgG was detected in saliva from those individuals whose swabs tested positive for *N. meningitidis* at that time. The mean specific IgG concentration in saliva samples from these carriers was 103.45ng/ml (±95%CI 77.20), compared with a mean of 21.69ng/ml found in samples from non-carriers (±95%CI 10.91). Twelve weeks later, another set of samples was taken and the elevated salivary IgG response of current carriers was again detected although not as pronounced as before.

No significant difference in the serum anti-H44/76 IgG of carriers and non-carriers was detected and there appeared to be no correlation between specific serum and salivary IgG responses (correlation coefficient 0.087). It appears possible therefore that the high-level salivary IgG was not derived from serum but was elicited at the mucosal surface in response to meningococcal colonisation.

#092

CELLULAR IMMUNE RESPONSES OF UNIVERSITY STUDENTS ELICITED BY CARRIAGE OF *NEISSERIA MENINGITIDIS*.

Robinson, K., Howard, C., Neal, K.R., Robins, A., Todd, I. & Ala'Aldeen, D.A.A.
Meningococcal Research Group, Division of Microbiology, University Hospital, Nottingham, UK.

Blood samples were collected from first year university students and peripheral blood mononuclear cells (PBMCs) were isolated and stored. On the first day of term there were no significant differences in the T-cell proliferative responses to meningococcal strain H44/76 between future acquirers and non-acquirers. 8 weeks later, after the carriage rate of the study population had increased, PBMCs from acquirers responded significantly better ($p=0.033$) to H44/76 than non-acquirers.

In order to determine the T-helper (Th) cell subset response elicited by carriage, H44/76-stimulated PBMCs were stained to detect intracellular cytokines, and examined by flow cytometry. There were no differences in $IFN\gamma^+$ or $IL\text{-}5^+$ events in PBMCs collected on day 1 from future acquirers and non-acquirers. PBMCs collected 8 weeks later from acquirers, however, produced significantly lower $CD4^+$ $IFN\gamma^+$ ($p=0.038$) and $CD4^+$ $IL\text{-}5^+$ ($p=0.016$) events than those of non-acquirers. There was no apparent bias of $IFN\gamma^+$ or $IL\text{-}5^+$ events, and preliminary data have shown the presence of TGFβ in H44/76-stimulated cell supernatants, leading to the possibility that the Th3 cell subset may be elicited by meningococcal carriage. Th3 cells have suppressive properties that have been associated with immunological tolerance to mucosal commensal bacteria.

#093

NEW *NEISSERIA MENINGITIDIS* SEROGROUP B COLORIMETRIC SERUM BACTERICIDAL ASSAY

Rodríguez T, Lastre M, Cedré B, del Campo J, Bracho G, Zayas C, Taboada C, Díaz M, Sierra G and Pérez O. Basic and Clinical Immunology Department, Finlay Institute, Cuba.

Objective: To standardize a new *N. meningitidis* serogroup B colorimetric serum bactericidal assay (CSBA).

Design: The CSBA was developed based on the capability of *N. meningitidis* serogroup B to consume glucose leading to acid production. Glucose and Bromocresol Purple pH indicator were added to the medium in order to estimate growth of CSBA target cell survivors through color change. The assay parameters growth of target cells, target cell number and complement source were optimized. The standardization was conducted on 13 immunized sera.

Results: Mueller-Hinton agar plates, 100 CFU per well, and 67% human complement source were selected. The titers were measured as the major serum dilution that totally inhibited the bacterial growth marked by the color invariability of the pH indicator. This was detected visually and by spectrophotometric lecture. A high coincidence (86%) between both ways of titration was found. The color change was closely related to a significant difference in the growth of target cell survivors determined using Student's t-test. Intralaboratory reproducibility was ± 1 dilution. The correlation between median titers and ELISA anti-OMV IgG serum concentration was high (r= 0.83, β=0.91, P<0.01).

Conclusions: We optimized the assay to obtain intralaboratory reproducible titers with a variety of sera from adults. This standardized CSBA allows an easy, fast, and efficient evaluation of a high number of samples.

#094

THE PAUCITY OF MUCOSAL IMMUNE RESPONSES IN HUMAN GONORRHEA FAVORS VACCINE DEVELOPMENT

Russell MW, Hedges SJ, Sibley DA, Mayo MS, Mestecky J, Hook EW. Depts. of Microbiology and Medicine/Infectious Diseases, and Biostatistics Unit, University of Alabama at Birmingham, Birmingham, AL.

Objective: The fact that gonorrhea can be repeatedly acquired with little or no increased resistance due to previous exposure implies that effective immunity is not induced by natural infection. Conventionally, this is attributed to the extraordinary antigenic variability of the gonococcus which may allow it to evade the host's specific responses. We postulate that such variability also enables the organism to avoid inducing responses in the first place, and we have therefore evaluated mucosal immune responses in subjects having uncomplicated genital gonococcal infections.

Design: Patients were recruited from the Jefferson County STD Clinic with informed consent, and diagnosed by standard clinical procedures for STDs; gonorrhea was confirmed by culture. Samples of serum, saliva, vaginal wash, cervical mucus, male urethral swabs were tested for IgM, IgG, IgA1, and IgA2 antibodies against gonococci (strain MS11 and homologous isolates), total Ig isotype concentrations, and cytokines by ELISA. Data were statistically analysed by non-parametric procedures.

Results: Antibody responses of all isotypes against gonococci in all fluids were minimal in subjects currently infected with uncomplicated gonorrhea, and no correlation was found with previous history of infection, or with rectal coinfection. No evidence was seen for loss of IgA1 in secretions due to cleavage by IgA1 protease. Inflammatory cytokine responses were also weak to non-existent, except in the serum of a subset of women who were coinfected with *C. trachomatis* or *T. vaginalis*, and in men who had high seminal levels of IL-8 regardless of infection.

Conclusions: Uncomplicated gonococcal infection induces little or no specific immune response in the infected subjects. Alternative strategies of immunization aimed at inducing antibodies against conserved or cross-reactive gonococcal antigens and delivering them to the genital tract may therefore be capable of eliciting protective immunity.

#095

GONOCOCCAL CATALASE IS NOT REQUIRED FOR INFECTION AND PERSISTENCE IN THE GENITAL TRACT OF ESTRADIOL-TREATED MICE.

Soler-Garcia AA, Jerse AE. Dept. Microbiol. & Immunol. USUHS, Bethesda, MD 20814.

Objective: To determine if the *N. gonorrhoeae* (GC) catalase gene enhances infection of GC in a murine model of gonococcal genital tract infection.

Methodology: A mutant of GC strain FA1090 carrying an in-frame deletion in the *kat* gene was constructed. The mutation was complemented by supplying a wild type copy of the *kat* gene *in trans* using the shuttle vector pLEE20. WT, *kat* and complemented mutant strains were characterized by Southern blot, catalase activity gel and enzymatic assay; susceptibility to H_2O_2, paraquat and H_2O_2-producing lactobacilli was measured by disc diffusion and zone inhibition assays. Estradiol-treated BALB/c mice were inoculated intravaginally with equivalent numbers of the wt GC or *kat* mutant. Infection was monitored by quantitative culture of vaginal suspension at selected time points following inoculation. The degree of inflammation was measured as the percent of PMNs in vaginal smears.

Results: The GC *kat* mutant was more sensitive to increasing concentrations of H_2O_2, paraquat and H_2O_2-producing lactobacilli compared to the parental strain. Complementation of the mutation *in trans* restored wild type levels of catalase, and resistance to paraquat and H_2O_2-producing lactobacilli. Although both wt and *kat* mutant GC were seen within vaginal PMNs, no difference in the duration of recovery was observed (average = 8 days and 10 days, respectively).

Conclusions: GC catalase confers resistance to H_2O_2, paraquat and H_2O_2-producing lactobacilli *in vitro*. However, GC can withstand the inflammatory response mounted by estradiol-treated mice without a functional catalase gene. These results suggest that *N. gonorrhoeae* may utilize mechanisms other than catalase to survive the host inflammatory response.

#096

ACTIVITY OF HUMAN IgG AND IgA SUBCLASSES IN IMMUNE DEFENSE AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP B

Vidarsson G, van der Pol WL, van den Elsen JMH, Vilé H, Jansen M, Duijs J, Morton HC, Boel E, Daha MR, Corthésy B, and van de Winkel JGJ

Objective: To study the relative contributions of human immunoglobulin subclasses in immunity against meningococci serogroup B.

Design: We generated a unique panel of V-gene matched human chimeric antibodies of all human IgG and IgA subclasses against PorA, a major subcapsular protein antigen of *Neisseria meningitidis* and an important vaccine candidate. Chimeric antibodies were produced in BHK cells, and IgA producing clones were co-transfected with human J chain and/or human secretory component (SC).

Results: While IgG (isotypes IgG1 to 3) mediated efficient complement-dependent lysis, IgA was unable to do so and in fact blocked IgG-mediated lysis. IgG1 and IgG3 also proved most efficient in stimulating phagocytosis of meningococci, while mIgA and IgG2 had lower activity. However, IgA proved more active in stimulating PMN respiratory burst than IgG, and both antibody classes together triggered respiratory bursts in an additive manner. We also show that anti-meningococcal IgA effector functions are subclass dependent, as IgA1, but not IgA2 is cleaved off the surface of live meningococci. Remarkably, in contrast to monomeric IgA, secretory IgA blocked the phagocytosis of meningococci by PMN.

Conclusions: Both IgA and IgG effectively stimulate anti-meningococcal effector functions, with noticeable qualitative and quantitative differences between isotypes and their subclasses. These studies reveal unique roles for IgG and IgA antibodies in defense against meningococcal infections.

#097

THE IMMUNE STATUS OF UNIVERSITY STUDENTS BEFORE AND DURING AN OUTBREAK OF SEROGROUP C MENINGOCOCCAL INFECTION

Williams JN,[1] Jones GR,[1,2] Christodoulides M,[1] Heckels JE[1]
[1]Molecular Microbiology Group, University of Southampton Medical School, and [2]Public Health Laboratory, Southampton, UK

Background: An outbreak of serogroup C meningococcal disease occurred at an English university in October 1997, which consisted of 6 cases with 3 fatalities. Routine sera had been taken 1 month prior to the outbreak in a subgroup of 81 students living in the same halls of residence as 5 of the cases.
Aim: To study the levels of immunity to meningococcal infection in uninfected and infected students prior to, and during, the outbreak.
Design: The immune response to the outbreak strain (C:NT:P1.5,10 was examined in detail. Antibodies to whole-cells, outer membranes and serogroup C capsule were determined by specific ELISA assays. The immune response to outer membrane protein antigens was further investigated by SDS PAGE and immunoblotting. The sera were also examined for bactericidal activity.
Results: Ninety percent of the at-risk students exhibited no bactericidal activity against the outbreak strain. A significant correlation was found between anti-C capsule antibodies and serum bactericidal activity against serogroup C meningococci. Western blots of serogroup C organisms discerned antibodies to outer membrane proteins, including the class 1 and 2 porins, in most sera, which did not correlate with bactericidal activity.
Conclusions: The student population had low levels of immunity to serogroup C organisms prior to the outbreak. The bactericidal activity present in the sera of around 10% of the students appeared to correlate with the presence of antibodies to serogroup C capsule.

#098

CONJUGATES AND REVERSE VACCINOLOGY TO ELIMINATE BACTERIAL MENINGITIS

Pizza MG, Giuliani MM, Masignani V, Scarlato V, Grandi G, Tettelin H*, Moxon ER#, Rappuoli R. IRIS, Chiron SpA Siena, Italy, *TIGR, Bethesda, USA and #Dept. of Pediatrics, Oxford, UK

Bacterial meningitis is caused by *H. influenzae*, *P. pneumoniae* and *N. meningitidis*. Hib has been extremely successfully eliminated from those countries where the vaccine has been introduced. The recent licensure of the conjugated pneumococcal vaccine suggests that also this pathogen will be soon under control. To provide means to control *N. meningitidis*, a decade ago we started the development of a conjugate vaccine against meningococcus C, which induced bactericidal antibodies and memory at all ages. The licensure of this conjugate vaccine in the UK and the immunization of the whole population below 18 years of age is already preventing hundreds of cases. Conjugated vaccines against serogroups A, Y and W, are presently being developed. While we can be confident that the conjugate vaccines will take care of meningococcus A, C, Y and W, we cannot rely on this technology for a vaccine against meningococcus B. Using an approach that we can name Reverse Vaccinology, we started from the genome of serogoup B meningococcus to identify novel. Computer analysis predicted 600 novel antigens. 350 of these were expressed in *E. coli*, purified and used to immunize mice. Sera were tested for bactericidal antibodies. We found 85 novel surface-exposed antigens, 25 of which induced bactericidal antibodies. Most of the new antigens are conserved in sequence. We are confident that some of these antigens will be used for the development of an effective vaccine against serogroup B meningococcus.

#099

RECOMBINANT MENINGOCOCCAL TbpA IS A PROTECTIVE ANTIGEN

West D., Reddin K., Matheson M., Heath R., Hudson M., Robinson A. and Gorringe A. Centre for Applied Microbiology and Research, Salisbury, SP4 0JG, UK

Introduction: *Neisseria meningitidis* transferrin binding proteins (TbpA and TbpB) are a focus for vaccine development, particularly against serogroup B disease. The TbpA+B complex and TbpB have been shown to be protective and elicit bactericidal antibodies in laboratory animals. We have expressed both TbpA and TbpB in *Escherichia coli* and assessed their vaccine potential.
Methods: *tbpA* and *tbpB* genes were cloned by PCR from *N.meningitidis* strain K454 (B15:P1.7,16) and subcloned into *E.coli* expression vectors. Expressed protein was solubilised by deteregent extraction and purified using a human transferrin affinity matrix.
Results: Purified rTbpA and rTbpB were functional and recognised by antisera to native protein. In protection studies, each recombinant protein protected against challenge with homologous and heterologous meningococcal strains. rTbpA and rTbpB were both recognised by human patient convalescent sera, and antisera raised against them cross reacted with a range of serogroup B and C *N.meningitidis* strains. Whilst some SBA activity was detected in rabbits immunised with TbpA or TbpB, SBA activity was not detected in sera from protected mice, indicating that this may not be an appropriate assay for these antigens.
Conclusions: TbpA alone has been shown to be a protective antigen. TbpA appears to be better conserved than TbpB, and this is borne out by the stronger cross reactivity in ELISA studies. These results argue strongly in favour of the inclusion of TbpA in a Tbp-based vaccine.

#100

EFFECTS OF DE-O-ACETYLATION ON THE IMMUNOGENICITY OF *NEISSERIA MENINGITIDIS* GROUP A POLYSACCHARIDE

Lynn, F.[1], Berry, D.[1,2], Bash, M.[1], Lee, R.[1], Frasch, C.[1]
[1]Laboratory of Bacterial Polysaccharides, CBER, Bethesda, MD, [2]Department of Pediatric Infectious Diseases, USUHS, Bethesda MD

The importance of O-acetyls (OA) in the immune response to Group A *N. meninigitidis* polysaccharide (PS) has not been fully elucidated. Gambian infants who received a primary series of an A/C conjugate vaccine failed to demonstrate immunologic memory to Group A when boosted 1 year later with either conjugate or PS vaccine. These results may have been due to alteration of a critical epitope of the Group A PS during conjugation. For *S. typhi* Vi PS and *E. coli* K1 PS, the presence of OA has been shown to be critical to immunogenicity. In our preliminary studies using inhibition ELISA, De-OA PS inhibited antibody binding to native PS in only one of 18 human post vaccination sera. This finding was independent of the effects of polymer size reduction. To examine whether the OA groups of the Group A PS are essential to an immunodominant epitope, we conducted immunization studies in mice using native and De-OA PS and PS-protein conjugates. Results demonstrated a marked loss in immunogenicity as measured by a native PS ELISA when the OA groups were removed from the PS. Bactericidal titers following immunization with DeOA conjugate vaccine were 10-fold lower than those obtained following immunization with native PS-conjugate vaccine and 5-fold lower than those following immunization with native PS. Inhibition studies confirmed the specificity of the antibody to the native PS. The dramatic reduction in immunogenicity of Group A meningococcal PS when the OA groups are removed indicate that O-acetylation is essential to important and immunodominant epitopes of Group A PS vaccines. Studies to determine the bactericidal epitope present in the DeOA PS are ongoing.
Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

#101

IMMUNOGENICITY OF PORA, SUBTYPE P1.4

Pettersson A, Kuipers B, van Eijndhoven L, van den Dobbelsteen G, van Loon M, van Alphen L, van der Ley P.

National Institute of Public Health and the Environment, Bilthoven, NL.

Immunization studies in mice with a hexavalent PorA outer membrane vesicle (OMV) vaccine showed, that the immune response against the P1.4 subtype was low compared with some of the other PorA subtypes. Studies with human volunteers showed essentially the same result. Since P1.4 is the most prevalent subtype in many countries, it is desirable to improve the immune response against it.
To investigate whether the immune response against PorA is T cell-dependent, a comparison was made between the response in wildtype versus nude (thymus deficient) mice. When immunized with the hexavalent vaccine, nude mice showed a much lower antibody titer against most PorA subtypes, as compared to the wildtype mice, indicating that the immune response is T cell-dependent. However, there was no difference in the titer against P1.4 between the two groups of mice. However, when immunized with a monovalent P1.4 vaccine, the nude mice did show a lower titer than the normal mice did. The results suggest that there is a competition at the level of T cells among the various PorA subtypes when mice are immunized with the hexavalent vaccine. Genetic modification of PorA P1.4 was used to improve its immunogenicity. First, known T cell epitopes from tetanus toxin or from an Opa protein were inserted into loop 5. Second, to improve the B cell response amino acid substitutions were introduced into loop 4 to make it more hydrophilic than the normal P1.4 epitope. Surprisingly, one of the constructs induced an increased bactericidal response against subtypes other than P1.4, suggesting that more conserved regions of PorA became more immunogenic. Third, the P1.4-containing loop 4 was lengthened in various ways to make it more accessible at the cell surface. Results from these different approaches will be presented.

#102

RIVM PorA BASED OMV VACCINES: UPDATE OF CLINICAL EXPERIENCE

Rümke HC, Van Alphen L. RIVM, Vaccine division, Bilthoven, Netherlands

Objective: RIVM developed a hexavalent vaccine against group B meningococci with PorA (from 6 different sero-subtypes) as protective antigen expressed in outer membrane vesicles (OMV). A prototype vaccine was immunogenic and safe in humans. Recently, we produced monovalent P1.h7,4 OMV vaccine (MonoMen) and upgraded the hexavalent vaccine (HexaMen). MonoMen-P and MonoMen-OH were adjuvated with alum-phosphate or -hydroxide respectively; HexaMen with alum-phosphate.
Design: Serum bactericidal activity (SBA) was measured using isogenic meningococcal strains, expressing the same PorAs as in the vaccines. Adverse events were studied by questionnaires and direct observations. Three studies will be reported.

	vaccines	age (y)	schedule (mo)	objective
1	MonoMen P vs MonoMen-OH	2-3	0-2-8 vs 0-1-2-8	selection of adjuvant schedule
2	MonoMen-P, >2 yrs after 6-valent vaccine	6-8 and 10-11	1 booster	memory and persistence of SBA
3	HexaMen (7.5-15-30 mcg per PorA)	adult	0-2-8	safety, dose finding

Results: Adverse events after MonoMen and HexaMen were acceptable. Only mild reactions were seen. Results of the safety trial with HexaMen favoured continuation of studies in infants also with the highest dose.
MonoMen was immunogenic. Alum-phosphate was the better adjuvant, and therefore used in HexaMen. After the primary vaccinations with 2 doses MonoMen-P induced 4+-fold rise of anti P1.h7,4 SBA in 81.2%, after 3 doses in 66.7% of children. Conversely, post booster the 3 dose priming gave better results: GMT: 70.4, and 100% 4+-fold, vs GMT 25.6 and 66.4% after 2 doses. 2-3 years after the prototype hexavalent vaccine SBA was negligible, but after one dose of MonoMen-P a 4+-fold rise of anti-P1.h7,4 SBA was seen in 41-75% of prior recipients of hexavalent vaccine, compared to 0-19% of control children that received hepatitis B vaccine. SBA to non-P1.h7,4 increased also, but in a lower percentage of children. There was no SBA rise to a PorA-minus strain.
Conclusions: Both vaccines are well tolerated. MonoMen is immunogenic. Most children vaccinated >2 years earlier with hexavalent vaccines had a P1.h7,4-specific memory response.

#103

IMPACT OF MENINGOCOCCAL C CONJUGATE (MCC) VACCINATION PROGRAMME IN THE UK

Miller E, Ramsay M, Rush M, Gungabissoon U (PHLS CDSC, London, UK), Borrow R, Mallard RH, Kaczmarski EB (Meningococcal Reference Unit, Manchester PHL, UK)

Objective: To devise a national surveillance strategy to measure the impact of the new MCC vaccination programme on age-specific disease incidence of C disease, to obtain formal estimates of age-specific vaccine efficacy, to document the risk factors for vaccine failure, to develop an active system for monitoring vaccine safety, and to monitor any changes in the genotypic characteristics of invasive and carriage strains of meningococci.
Design: Comprehensive national surveillance based on linked epidemiologic and microbiologic data with active follow up of all meningococcal C cases (for details see http://www.phls.co.uk/advice/mensurvw.pdf)
Results: In November 1999, the MCC vaccination programme and accompanying surveillance strategy was launched. The target population comprises all children under 18 years of age with the aim of completing immunisation by end 2000. A single dose is given from 12 months upwards. The first targeted age groups were infants and 15-17 year olds, the two groups at most risk of C disease. Coverage in the adolescents was around 80% with early data indicating coverage of around 90% in infants. By April 2000, a reduction in disease incidence in the targeted age groups was already evident even in infants under a year many of whom would not have had time to complete their primary immunisation course. No change in the epidemiology of serogroup B disease has been seen and adverse event reports are consistent with the pre-licensure safety profile of the vaccine.
Conclusions: The MCC vaccines are proving safe and effective under routine use. The reduction in incidence in infants is the first direct evidence of efficacy in an age group in which the plain meningococcal C polysaccharide vaccines are not effective. This validates the extrapolation of the serum bactericidal antibody correlate of protection derived from adults with natural immunity to infants and should facilitate the licensure of MCC vaccines in other countries.

#104

PHASE I-II STUDY WITH HEXAVALENT RIVM MENINGOCOCCAL B OMP VESICLE VACCINE (HEXAMEN)

Berbers GAM, Lafeber AB, van Limpt CJP, Rümke HC, van Alphen AJW.
National Institute of Public Health and the Environment, The Netherlands

Background: In the Netherlands Men B is the major causative of bacterial meningitis and meningococcal septic shock. Therefore the RIVM has developed a hexavalent MenB OMP vesicle vaccine (HexaMen) in which six PorA-proteins (subtypes P1.7,16; P1.19,15; P1.5,2; P1.5^{c},10; P1.12,13 & P1.7^{h},4) are embedded in outer membrane vesicles. Three PorA's are expressed simultaneously on the surface of a single vesicle, and two sets of vesicles are included in the vaccine. The six PorA antigens cover 70-80% of all clinical MenB (and MenC) isolates in the Netherlands.
Objectives: To study the safety and immunogenicity of HexaMen in adults in three different concentrations. Four different treatment groups (0-7.5-15 and 30 µg of each PorA) administered in a 2+1 schedule (at 0, 1 and 7 months).
Main parameters:
Safety: Directly after each vaccination the investigators observed participants with respect to local and systemic reactions. Vital functions as well as routine blood and urine parameters were monitored before and after each vaccination. Adverse events data were further collected during one month after vaccination.
Immunogenicity: Responses to the RIVM HexaMen administered in different concentrations were measured in the serum samples of the participants by:

1. Serum Bactericidal Activity (SBA 90%) against six isogenic variants of strain H44/76 in which each PorA of HexaMen is expressed individually. The PorA negative mutant strain H1.5 was used as control for PorA specificity.
2. Serum antibody levels by OMV ELISA's and Whole Cell ELISA's.
3. T-cell reactivity.

Results: The vaccine was well tolerated. The frequency and nature of adverse reactions after vaccination were acceptable. Immunogenicity data will be presented and a comparison between the different assays will be discussed.

#105

SEROLOGICAL BASIS FOR USEAGE OF MENINGOCOCCAL C CONJUGATE VACCINES IN THE UNITED KINGDOM

Borrow R[1], Andrews N[2], Goldblatt D[3], Miller E[2] ([1] PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK. [2] PHLS CDSC, London, UK. [3] Immunobiology Unit, Institute of Child Health, London, UK.)

Objective: Meningococcal C conjugate (MCC) vaccines have been successfully licensed in the UK without efficacy trials. Serological correlates have instead been relied upon. This paper proposes an approach for utilization of baby rabbit serum as an exogenous C' source in the menC SBA that was used as a marker for protection against menC to support the licensure of MCC vaccines in the UK.

Results: From the UK experience, we propose the following definitions using SBA titers generated with rabbit C': ≤ 4 susceptible, ≥ 128 protected and 8 to 64 protected if any of the following demonstrated. Re-testing with human C' SBA giving a titer ≥ 4, a 4-fold or greater rise in titer pre to post primary vaccination or a response ≥ 128 after boosting with a 10 μg dose of C polysaccharide. These definitions are based on: i) for sera where both assays were performed 15% of those with rabbit < 8 were protected according to the human SBA titer compared to 40% for rabbit SBA titer of 8 to 64 and 99% for a rabbit SBA titer of ≥ 128; ii) the high specificity of rabbit C' demonstrated by the lack of false positives in pre-vaccination sera; iii) 4-fold or greater rises in titer occurred in 94% of UK toddlers after a single dose of candidate MCC vaccines; iv) of toddlers with titers < 128 post primary MCC vaccination, 90% had post booster titers of ≥ 128; v) regardless of post primary MCC vaccination titers, the avidity indices of these toddlers were consistent with the formation of immunological memory.

Conclusions: On the basis of these definitions of protection, data from various UK MCC trials were interrogated and MCC vaccines introduced into the UK immunization schedule. Direct evidence of the effectiveness of a MCC vaccination programme in which these immunological criteria have been will shortly become available from the UK and will be crucial for planning of other international mass immunisation programmes.

#106

PREVALENCE OF DE-O-ACETYLATED SEROGROUP C MENINGOCOCCI BEFORE AND AFTER THE INTRODUCTION OF MENINGOCOCCAL SEROGROUP C CONJUGATE VACCINES IN THE UNITED KINGDOM.

Borrow R, Longworth E, Gray SJ, Kaczmarski EB (PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK.)

Objectives: Meningococcal serogroup C conjugate (MCC) vaccines have been introduced in the UK to combat the rise in serogroup C meningococcal disease. Serogroup C meningococci may occur naturally expressing either O-acetylated (Oac+) or de-O-acetylated (Oac-) polysaccharide capsules. In a study in the United States in the 1970s 15% of serogroup C meningococcal case isolates were reported to be Oac- though the prevalence of these Oac- isolates has not been recorded in the UK. This is of interest as the first MCC vaccines to be introduced are Oac+ and the potential impact of this on Oac- serogroup C isolates is unclear.

Methods: Serogroup C isolates from cases of disease were submitted to the Public Health Laboratory Service Meningococcal Reference Unit in January 1998 (n = 113), January 1999 (n = 162), January 2000 (n = 181), February 2000 (n = 57), March 2000 (n = 64), April (n = 43) were investigated by dot blotting using monoclonals specific for Oac+ and Oac- serogroup C polysaccharides. A group of 21 serogroup C carrier isolates (from Oct 1999) were also included.

Results: This revealed 12% Oac- isolates for both January 1998 and January 1999 and 18% for January 2000. For February, March and April 2000 there were 18%, 16% and 14% respectively. The proportion of fatal cases was found to similar for both Oac- and Oac+, 14% and 9% for 1998 and 5% and 3% for 1999. Only one carrier isolate (5%) was found to Oac-.

Conclusion: No significant changes have been noted in serogroup C acetylation status though continued monitoring is necessary throughout the introduction of MCC vaccines. The similar percentages of fatalities for Oac- and Oac+ isolates indicates that the pathogenic potential of these isolates is similar.

#107

REDUCED ANTIBODY RESPONSE TO REVACCINATION WITH MENINGOCOCCAL A POLYSACCHARIDE VACCINE IN ADULTS

Borrow R[1], Joseph H[1], Andrews N[2], Acuna M[1], Longworth E[1], Martin S[1], Peake N[1], Rahim R[1], Richmond P[2], Kaczmarski E[1], Miller E[2]. ([1] PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK. [2] PHLS CDSC, London, UK.)

Objectives: Widespread use of meningococcal A and C polysaccharide (MACP) vaccines has raised concerns about induction of hyporesponsiveness to these polysaccharides. Immunological hyporesponsiveness to C polysaccharide has been clearly documented in infants, children and adults but only limited data from Gambian children are available for A polysaccharide. We investigated whether a second dose of MACP, given 6 months after an initial dose affected the immunological response to serogroup A polysaccharide.

Methods: Serogroup A antibody responses were measured by serum bactericidal assay (SBA) and enzyme-linked immunosorbent assay (ELISA) to serogroup A meningococci in young adults (university students, n = 36).

Results: Serogroup A SBA responses one month following the second dose of MACP (geometric mean titre (GMT) 103.6, 95% CI 45.6 – 235.1) were about half (geometric change 0.49, P = 0.017) that of one month post the first dose (GMT 281.87, 95% CI 134.8 – 581.4). The serogroup A-specific IgG levels were significantly lower (geometric change 0.58, P = 0.010) post second dose (GMC 16.8, 95% CI 5.29 – 47.4) as compared to post the first dose (GMC 28.7, 95% CI 20.79 – 39.7).

Conclusions: This confirms that revaccination with MACP vaccine results in reduced antibody responses to A polysaccharide in adults. Repeated vaccination with MACP vaccine may be ineffective and development and use of meningococcal serogroup A conjugate vaccines should be encouraged.

#108

COMPARISON OF ANTIBODY KINETICS FOLLOWING MENINGOCOCCAL SEROGROUP C CONJUGATE VACCINE BETWEEN HEALTHY ADULTS PREVIOUSLY VACCINATED WITH MENINGOCOCCAL A/C POLYSACCHARIDE VACCINE AND VACCINE-NAÏVE CONTROLS.

Borrow R[1], Miller E[2], Peake N[1], Rahim R[1], Acuna M[1], Martin S[1], Andrews N[2], Kaczmarski EB[1]. ([1] PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK. [2] PHLS CDSC, London, UK.)

Objectives: Few data are available on the kinetics of meningococcal serogroup C-specific antibody production following MCC vaccination in particularly in those who have received prior meningococcal A/C polysaccharide (MACP) vaccination(s).

Methods: Laboratory staff who had previously received either one dose (n = 33), two doses (n = 18) of MACP vaccine or naïve to previous meningococcal vaccination (n = 44) were vaccinated with MCC and bled pre-vaccination, on the subsequent four days and on days 10 and 28. Serogroup C serum bactericidal antibody (SBA), and anti-serogroup C-specific IgG, IgA and IgM were measured.

Results: No decrease in serogroup C SBA, IgG, IgM or IgA was observed immediately following vaccination. Significant fold rises were seen in serogroup C SBA, IgG, IgA and IgM between pre-vaccination and day 10 levels of 27.3, 5.3, 6.3, 2.7, respectively. The cohorts who had received either 2 or 1 prior dose(s) of MACP had SBA GMTs 3.2 (GMT 877.8, 95% CI 309.9 - 2486.5) and 1.8 (GMT 1571.2, 95% CI 776.7 - 3178.3) fold lower than the MACP naïve cohort (GMT 2848.9, 95% CI 1431.2 - 5671.0).

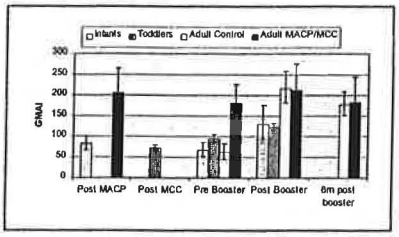
Conclusions: No decrease in serogroup C-specific antibody occurs immediately following MCC vaccination but significant increases are observed by day 10. Prior MACP effects the functional antibody response to MCC vaccination and appears to be dose dependent.

109

ANTIBODY AVIDITY FOLLOWING MENINGOCOCCAL C VACCINES IN ADULTS AND CHILDREN

Goldblatt D[1], Borrow R[2], Richmond P[1,3], Miller E[3]. [1]Inst of Child Health, Univ. College London, London, UK; [2]Publ. Health Lab. Service (PHLS) Meningococcal Reference Unit, Manchester, and [3]Immunisation Div. Communicable Disease Surveillance Centre, PHLS, London UK.

Background: One of the major advantages of the meningococcal C conjugate vaccines (MCC) over pure polysaccharide vaccines (MACP) is the ability of the former to induce immunological memory. Antibody avidity increases over time following primary immunisation and may thus be used as a surrogate marker for the generation of memory. We have studied the kinetics of Men C specific IgG avidity following MCC or MACP in young children and adults. **Methods:** Antibody avidity was measured by a modified ELISA. Infants and adults were assayed one month after MACP (Av Pasteur), 6m later prior to a MCC (Wyeth) boost and 1m and 6m (adults only) after the boost. Toddlers were assayed 1m after MCC, and before and 1m after a booster dose of MACP given 6m later while naïve adults (no previous MACP) were assessed before and 1m and 6m after MCC. **Results**: The figure shows comparative data for the different groups. Neither infants nor adults showed avidity maturation following MACP and avidity was significantly higher in the adults. Toddlers showed an increase in avidity between 1m and 6m following MCC and a further increase after a booster dose. Infants and naive adults showed a significant increase in avidity following MCC. Adults who had previously been immunised with MACP however showed no increase in avidity 1m or 6m following MCC and naïve adults failed to demonstrate further avidity maturation 6m after an MCC vaccine. **Conclusions**: Antibody avidity does not increase after plain polysaccharide vaccine but does increase after conjugate vaccination. Adults have a higher overall IgG avidity which may be a consequence of previous encounter with Men C or cross reactive antigen. Avidity measurements appear to be a sensitive marker of the generation of memory in naïve individuals.



#110

HAS THE A+C POLYSACCHARIDE VACCINE ANY EFFECT OVER THE ISOLATION CAPABILITY ?

MALVAR A., FERNÁNDEZ S., SANTIAGO I. , HERVADA X., MOREMO MJ.
Dirección Xeral de Saúde Pública. Xunta de Galicia. Santiago de Compostela. Spain.

During the following year of the vaccination campaign with meningococcal A+C polysaccharide vaccine took place in Galicia (Spain) *N. meningitidis* was isolated from 41 vaccinated people, 32 of then belonged to serogroup B and 9 to serogroup C, and 79 unvaccinated people, 40 of the serogroup B and 39 of the serogroup C.

At the same time, we studied with PCR samples from vaccinated and unvaccinated people (38 and 27 respectively) all of them with well-founded suspicion of meningococcal disease without isolement. The PCR was positive in 27 vaccinated, 14 from serogroup B and 13 from serogroup C, and in 13 unvaccinated people, 8 and 5 from serogroup B and C respectively.

Suppose to be true that, like *N. meningitidis*, the proportion of serogroup C isolations must be equal to the proportion serogroup C ascertained by PCR our results show, during the time of this study, with regard to vaccinated people the isolation of serogroup C was statistically lower than we expected (X^2, $p<0.025$). This results wasn't observed with regard to unvaccinated people.

We didn't find any other factors that could explain this fact. Due to this we think that, in a short period of time, the vaccination with meningococcal A+C polysaccharide vaccine makes the serogroup C isolation difficult.

#111

CHANGE IN *N. MENIGITIDIS* C CARRIERS AFTER A VACCINATION CAMPAING

FERNÁNDEZ S.[1] ARREAZA L.[2] SANTIAGO I.[1] MALVAR A.[1] BERRON S.[2] VAZQUEZ JA.[2] HERVADA X.[1]
1 Dirección Xeral de Saúde Pública. Xunta de Galicia. Santiago de Compostela. Spain
2 Laboratorio de *Neisserias*. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Majadahonda. Madrid. Spain

In order to learn about *N. meningitidis* carriers, specially of serogroup C, and its relation with meningococcal A+C polysaccharide vaccine two studies was performed. The first before vaccination campaign and the second a year later. The sample was selected from people among 5-19 years old, in two different areas related with high or low group C meningococcal disease rates before vaccination campaign, through a complex design sample. In both studies the samples were weighted to account for unequal sampling probabilities due to different factors. The serogroup C carriers prevalence was 1.51% (IC95% 0.92 – 2.11) in the high incidence area before vaccination and 0.79% (IC95% 0.79 – 1.13) after. In the low incidence area the prevalence was 0.94% (IC95% 0.14 – 1.74) and 0.32 % (IC95% 0.04 – 0.60) respectively. The percentage of decrease was 47% in the high incidence area and 65 in the low incidence area. By age group a decrease was seen in 10-14 and 15-19 groups of age.

This work support a possible effect of meningococcal A+C polysaccharide vaccine over carriage state even thinking on the influence of the natural evolution of the meningococcal infection over carriage state.

#112

SAFETY AND REACTOGENICITY OF AN INTRANASAL *NEISSERIA MENINGITIDIS* SEROGROUP B OUTER MEMBRANE VESICLE VACCINE IN ADULTS

Fischer M[1], Holst J[2], Aaberge IS[2], Haugen IL[2], Burns JL[3], Perkins BA[1], Haneberg B[2]. [1]CDC, Atlanta, GA; [2]Natl Institute of Public Health, Oslo, Norway, [3]Univ of Washington, Seattle, WA

Objective: To evaluate the reactogenicity of an intranasal *N. meningitidis* serogroup B outer membrane vesicle [MenB(OMV)] vaccine developed at NIPH (Norway).

Methods: Eighty participants were randomized to receive 3 intranasal doses of either investigational vaccine (n=40) or saline (n=40) at 0, 6 and 12 weeks. Local and systemic adverse reactions were monitored for 72 hours following each vaccination.

Results: One participant in each group did not complete the 3 dose regimen. In the 72 hours following any dose, vaccinees were more likely than controls to report burning/irritation in the nose (58% vs. 35%; p=0.04) and mild epistaxis (15% vs. 3%; p<0.05). Headache occurred in 20 (50%) of 40 vaccinees and 12 (38%) of 40 controls (p=0.07). Aftertaste was commonly reported just after vaccination but occurred with similar frequency between groups (60% vs 58%; p=0.8). Most reactions were mild and did not interfere with normal activities; none were severe. Side effects peaked within 24 hours postvaccination, and all resolved within several days.

Conclusions: The NIPH intranasal MenB(OMV) vaccine was well tolerated, with local irritation being the most common side effect. Mucosal administration may provide a simpler and less reactogenic route of delivery for MenB(OMV) vaccines.

#113

IMMUNOGENICITY OF AN INTRANASAL *NEISSERIA MENINGITIDIS* SEROGROUP B OUTER MEMBRANE VESICLE VACCINE IN ADULTS

Fischer M[1], Holst J[2], Aaberge IS[2], Høiby EA[2], Haugen IL[2], Burns JL[3], Perkins BA[1], Haneberg B[2]. [1]CDC, Atlanta, GA; [2]Natl Institute of Public Health, Oslo, Norway, [3]Univ of Washington, Seattle, WA

Objective: To evaluate serum bactericidal activity (SBA) elicited by an intranasal *N. meningitidis* serogroup B outer membrane vesicle [MenB(OMV)] vaccine developed at NIPH (Norway).
Methods: Eighty participants were randomized to receive 3 intranasal doses of either investigational vaccine (n=40) or placebo (n=40) at 0, 6 and 12 weeks. SBA titers were measured against the vaccine type-strain (B:15:P1.7,16) at 0, 6, 12 and 14 weeks.
Results: The mean age among enrollees was 30.1 years (range 19-47). At baseline, 23 (59%) vaccinees and 20 (51%) controls had an SBA titer ≥1:8 (p=0.5). Following 3 doses, 10 (26%) vaccine recipients showed a 4-fold rise in SBA compared with 2 (5%) controls (p=0.01); geometric mean SBA titers were also higher among vaccinees (16.6; 95% CI 10.6, 25.8) versus controls (7.5; 95% CI 5.4, 10.4) (p<0.01). Post-vaccination, 30 (77%) vaccinees and 23 (59%) controls had an SBA titer ≥1:8 (p=0.09).
Conclusions: The NIPH intranasal MenB(OMV) vaccine elicits systemic bactericidal antibodies without the use of additional adjuvants. Further work is underway to define the mucosal immune response and measure SBA against heterologous strains. Some refinement of the vaccine formulation or immunization schedule may be needed to optimize the bactericidal response.

#114

T-CELL EPITOPE MAPPING OF THE P64k MENINGOCOCCAL PROTEIN IN BALB/C MICE

González S, Nazábal C, Rao KVS*, Reyes O, Garay HE, Sardiñas G, Caballero E, Silva R.
Center for Genetic Engineering and Biotechnology, Havana, Cuba and *International Center for Genetic Engineering and Biotechnology, New Delhi, India

Our group has previously characterized the P64k protein of *Neisseria meningitidis*. Cloning and expression in *Escherichia coli* of the *lpdA* gene, which encodes for this protein, yielded a soluble antigen accounting for more than 20% of the total host protein. Due to its relatively high molecular weight, demonstrated immunogenicity and availability, P64k was employed as a carrier protein for poorly immunogenic peptides and *Neisseria meningitidis* serogroup C polysaccharide with good results. In this study, 59 overlapping synthetic peptides that encompassed the full-length 596 amino acids of the protein were tested for proliferation in P64k-sensitized mice. The highest proliferative responses were induced against peptides P1 (amino acids 1-20) and P48 (amino acids 470-490). However, only lymph node cells obtained from either P48- or P64k-sensitized mice, produced a statistically significant proliferative response when challenged with the homologous peptide or the recombinant protein, respectively. Moreover, P48 was able of priming BALB/c mice (n=7) *in vivo* for a secondary antibody response, after a booster dose with the recombinant protein, as ascertained by ELISA of individual sera. Finally, three overlapping peptides spanning the P48 sequence were tested for proliferation in homologous peptide- and P48-sensitized mice. The highest proliferative response was obtained against a peptide that includes amino acids 470-485. We can conclude that this peptide (IPGVAYTSPEVAWVG) contains an immunodominant T-cell epitope of P64k in BALB/c mice.

#115

CHARACTERIZATION OF THE ANTIBODY RESPONSE ELICITED AFTER IMMUNIZATION OF HUMAN HEALTHY VOLUNTEERS WITH RECOMBINANT P64k MENINGOCOCCAL PROTEIN

González S, Sardiñas G, Caballero E, Musacchio A, Nazábal C, Reyes O, Rodríguez E, Cinza Z, Silva RC. Center for Genetic Engineering and Biotechnology, Havana, Cuba.

Recombinant P64k, expressed in *Escherichia coli*, acts as an efficient carrier protein for poorly immunogenic peptides and meningococcal serogroup C polysaccharide. We have recently shown that the recombinant protein is safe and immunogenic in human healthy volunteers in a Phase I clinical trial. The volunteers received three doses of recombinant P64k and were boosted 9 months after the third dose. In the present study, we further characterized the human antibody response against this protein, by using a panel of 15 volunteer sera collected during the trial. The subclasses of specific IgG directed against P64k were determined. As expected, IgG1 was the main subclass of anti-P64k antibodies all over the study. However, after the booster dose, a statistically significant amount of anti-P64k IgG4 was detected in the sera. A maturation of the antibody affinity was found in most of the sera. The presence of antinuclear and antimitochondrial antibodies in paired volunteer sera was examined by immunofluorescence on rat kidney sections and HEp-2 cells. None of the sera contained such autoantobodies. Additionally, 84 overlapping peptides spanning the entire sequence of P64k were synthesized on a cellulose membrane and probed by SPOTscan. As an outcome of the mapping, the assayed sera recognized more frequently 12 peptides. We can conclude that IgG1 and IgG4 are the main subclasses of anti-P64k antibodies developed after immunization, the antibodies do not react with mammalian tissue and the continuous B-cell epitopes they recognized are mainly exposed in the protein.

#116

INFLUENCE OF THE CONJUGATION METHOD ON THE ANTIBODY RESPONSE AGAINST POLYSACHARIDE CONJUGATES.

Canaán L, Carmenate T, Alvarez A, Delgado M, Menéndez T, Rodés A, Guillen G.
Centro de Ingeniería Genética y Biotecnología, Habana, POBox 6162, Habana 10600, Cuba.

Objective: To compare the antibody response generated in mice against meningococcal group C polysaccharide (C-Ps) conjugated to the P64k protein using different conjugation procedures.
Design: The polysaccharide conjugates were obtained either by the selective periodate oxidation method or using carbodiimide as coupling reagent. Balb/c mice were immunized twice with both conjugates (2.5 µg of polysaccharide) using aluminum hydroxide as adjuvant. As controls mice were immunized to with free C-Ps and with C-Ps mixed with outer membrane vesicles from meningococcal strain CU385. The antibody response was studied in each case. The antibody titers, bactericidal activity and avidity indexes were determined.
Results: Mice immunized with the C-Ps conjugates obtained by both methods generated high antibody responses significantly superiors than control groups. The second immunization generated an increment of the antibody level and the avidity indexes as it can be expected for a T cell dependent antigen. The best antibody response was generated against the polysaccharide conjugate obtained by the selective oxidation procedure and the mean bactericidal titer of this group was of 1:1024.
Conclusions: The selective oxidation procedure rendered the conjugate with better immunogenic properties for vaccine purposes.

#117

HUMORAL IMMUNE RESPONSE TO MENINGOCOCCAL CLASS 1 PROTEIN IN VOLUNTEERS IMMUNISED WITH VA-MENGOC-BC®.

Menéndez T, Domenech M, Niebla O, Cinza Z, Delgado M and Guillén G.
Centro de Ingeniería Genética y Biotecnología, POBox 6162, Havana 10600, Cuba

Objective: To characterize humoral immune response against Class 1 protein in individuals vaccinated with Cuban vaccine VA-Mengoc-BC®.

Design: Preimmune and third dose sera from 53 individuals vaccinated with VA-Mengoc-BC® (schedule 0-2-5 months) were evaluated by ELISA. Plates were coated with either *E. coli*-expressed recombinant Class 1 protein from meningococcal strain B385 (B:4. P1.19,15) or linear and cyclic synthetic peptides corresponding to VR1 and VR2. Immunoreactivity against Class 1 protein was also evaluated by Western-blotting. Sera with the highest anti- Class 1 protein titres were used for: 1) Mapping of immune response using synthetic peptides that cover Class 1 protein sequence, 2) Immunoblotting of strains 19/92 (B:4. P1.19,2c) and 305/95 (B:4. P1.7,15) in the presence of Empigen BB, 3) Determination of IgG subclasses profile and 4) Serum bactericidal activity assay.

Results: Antibodies specific to Class 1 protein were detected in the sera of 43% of volunteers. Linear and cyclic VR1 and linear and cyclic VR2 peptides were recognised by 31.9%, 56.5%, 82.6% and 86.9% of Class 1 protein-positive sera, respectively. Epitope mapping of the immune response revealed the major immunogenicity to be confined to VR2 and partially to VR1. The adition of Empigen BB enhanced the immunoblotting signals for strain 19/92 in 47% of sera, but had no effect for strain 305/95. IgG_1 was the predominant immunoglobulin subclass. Bactericidal titers (ranging from 1/16 to 1/512) were detected in 76% of tested sera.

Conclusions: VA-Mengoc-BC® induced an immune response against Class 1 protein in 43% of vaccinees, which was mainly directed against VR2. Conformational epitopes of VR1 and linear epitopes of VR2 were the targets of the response. IgG_1 was the predominant immunoglobulin subclass. Killing of strain B385 in the presence of human complement was induced by 76% of sera with the highest anti-Class-1 antibody levels.

#118

COMPARISON OF THE IMMUNE RESPONSE IN MICE AGAINST MENINGOCOCCAL GROUP C POLYSACCHARIDES CONJUGATE TO THREE CARRIER PROTEINS.

Alvarez A, Canaan L, Carmenate T, Menendez T, Delgado D, Rodes L, Guillén G.
Centro de Ingeniería Genética y Biotecnología. PO Box 6162, Habana 10600 Cuba.

Objective: To study the nature and kinetics of the serum antibody response in mice to meningococcal group C polysaccharide (C-Ps) conjugates to three carriers: Bovine serum albumin (BSA), Tetanus toxoids (TT) and recombinant P64k.

Design: Meningococcal group C polysaccharide was conjugated using carbodiimide as coupling reagent and adipic acid (ADH) as spacer between the C-Ps and the carrier. Carrier proteins, conjugates and free C-Ps were employed to immunize Balb/C mice. Aluminum hydroxide was used as adjuvant. The murine humoral immune responses were evaluated by ELISA after the third dose.

Result: The polysaccharide-protein ratios of the three conjugates were: TT-1.26, BSA-1.13 and P64k-0.4 mg/mL.

The immune response against the three C-Ps conjugates was higher than against free C-Ps.

The levels of antibodies detected in the sera of mice immunized with C-Ps-P64k conjugate were higher than those detected against C-Ps-TT and C-Ps-BSA conjugates.

Conclusions: The carrier protein present in the C-Ps conjugates influenced the levels of the IgG antibodies elicited against to the Ps. In this regard P64k was superior to TT and BSA.

#119

***NEISSERIA LACTAMICA* PROVIDES A CROSS-REACTIVE VACCINE AGAINST MENINGOCOCCAL DISEASE**

Gorringe AR, Oliver K[1], Bracegirdle P[1], Reddin K[1], Cartwright K[2], Feavers I[3], Borrow R[4], Hudson M[1], Robinson A[1]. [1]Centre for Applied Microbiology and Research, Salisbury UK. [2]Public Health Laboratory Gloucester, UK. [3]National Institute of Biological Standardisation and Control, UK. [4]Public Health Laboratory, Manchester, UK.

Background: Development of natural immunity to meningococcal disease in childhood is thought to result from nasopharyngeal colonisation by non-pathogenic *Neisseria* spp. *N. lactamica* may be the most important of these non-pathogenic species. We have therefore explored the potential of *N. lactamica* to form the basis of a broadly cross-reactive vaccine against meningococcal disease.

Methods: Adult mice were immunised with *N. lactamica* whole cells or outer membrane proteins (OMP). In addition, *N. lactamica* OMPs separated by semi-native preparative electrophoresis and pooled into <43kDa, 43-67kDa and >67kDa groups were also used to immunise mice. Mice were then given an intraperitoneal challenge with *N. meningitidis*.

Results: *N. lactamica* whole cells, OMP and the <43kDa pool protected mice from lethal challenge doses of *N. meningitidis*. In addition, the OMV and <43kDa pool protected against challenge with 5 meningococcal strains representing serogroups B and C and differing in serotype and serosubtype. Sera from mice immunized with *N. lactamica* contained antibodies that recognised a range of meningococcal strains and showed bactericidal activity.

Conclusion: The commensal bacterium *N. lactamica* has the potential to provide novel, serogroup independent, vaccine components that protect against meningococcal disease and may mimic and enhance the natural immunity provided by carriage of the organism.

#120

CROSS-PROTECTIVE POTENTIAL OF THE OMP85 PROTEIN IN OUTER MEMBRANE VESICLE VACCINES AGAINST MENINGOCOCCAL GROUP B DISEASE

Holst J[1], Wedege E[1], Rosenqvist E[1], Næss LM[1], Griffiths E[2], Rappuoli R[3].
[1]Department of Vaccinology, NIPH, Oslo, Norway; [2]WHO, Geneva, Switzerland; [3]Chiron Vaccines, Siena, Italy.

The efficacy of the current outer membrane vesicle (OMV) vaccines against homologous strains has mainly been attributed to the PorA porin. However, regarding the serum bactericidal activity (SBA) against heterologous strains, reported for these types of vaccines, other antigens may play an important role. The Omp85 protein possesses features that might contribute to the cross-reactive functional immunity. This antigen is present in all *Neisseriacea*, including the commensals, with a high degree of homology. Following vaccination, induction of antibodies against Omp85 is one of the significant features. A good correlation is observed between the degree of antibody binding to Omp85 on immunoblots and SBA. Here we report further evaluation of the protective potential of this antigen.

Results: The Omp85 has been found in several OMV vaccines and is likely to be present in all formulations from different manufacturers. Quantitative analysis of 20 batches produced at NIPH from 1987 to 2000 showed the amount to be 1-3% of the total protein. In spite of the low amount of Omp85, vaccinees show significant immune response against it. Initial work on expression in *E. coli* resulted in an insoluble protein. Immunization of mice with the recombinant protein gave specific antibodies, but no SBA. With various *N. menigitidis* strains in ELISA and a rabbit serum specific for Omp85, significantly higher binding was observed to systemic case isolates than with strains from healthy carriers.

Conclusion: Omp85 may be an important component of OMV vaccines, which should be thoroughly characterized and determined in Quality Control tests. Work is in progress for establishing mutants able to overexpress Omp85 and thus increase the amount in vaccine formulations. Such OMV vaccines might have an improved performance against heterologous strains.

#121

A COMPARATIVE PROTECTION STUDY OF N. meningitidis SEROGROUP B ANTIGENS USING A MOUSE MODEL OF LETHAL N meningitidis INFECTION. Jessouroun E[1], Silveira IAFB[1], Larangeira AP[2], Frash CE[3], Castro-Faria-Neto HC[2], Bozza PT[2] [1]BioManguinhos and [2]Lab. Imunofarmacologia/IOC, FIOCRUZ, RJ Brazil. [3]National Institute of Health, Bethesda, MD, USA.

N.meningitidis is a serious bacterial pathogen that infects only humans. It is a cause of lifethreatening invasive bacterial infections especially in youngs below 2 years old, teenagers and young adults. Bacterial septicemia and meningitis are characterized by a great deal of vascular and tissue damage. When accompanied by widespread purpuric rash and shock is associate with death in up to 40% of cases. LPS liberation during the disease process results in inflammatory cell activation and the elaboration of a wide range of inflammatory mediators. Due to the non-immunogenicity and lack of lytic activity with human complement of serogroup B polysaccharide, it remains a public heath problem all over the world. Most efforts to develop an effective vaccine to **N.menigitidis** serogroup B have focused on outer membrane proteins (OMV) and LPS as alternative immunogens. In the present study we compared the immunogenic ability of OMV and LPS obtained from **N.meningitidis** group B Brazilian prevalent strains (B:4,7P1,19,15 and B:4:P1.7,1:P5.5,7) to a reference vaccine (VAMENGOC) in a lethal model of **N.menigitidis** infection in Swiss mice. Mice were challenged with a lethal dose of **N.menigitidis** ($2,1x10^6$ cfu/ml, ip) plus iron-dextran (9mg/mouse) eight weeks after immunization (2 boosters were performed with 14 days intervals). The model of N.menigitidis-induced sepsis were characterized by a significant increase in serum levels of TNF and IL-6 maximum within 3 - 6 hs of infection, that returned to basal levels within 48 Hs. A significant thrombocytopenia and an increase in neutrophil numbers at the peritoneum cavity accompanied the cytokine alterations. The antigen preparation showed a survival rate of 100% compared to 87% in the reference vaccine and 25% in the non-immunized control group. The antigen preparation also inhibited by 91% the serum IL-6 and by 90% the thrombocytopenia induced by **N.meningitidis**, whereas the reference vaccine reduced IL-6 levels by 65% and failed to inhibit the thrombocytopenia. In conclusion, our results demonstrated that the OMV and LPS formulation obtained from Brazilian prevalent strains of **N.meningitidis** group B showed a better performence in protecting mice from the cytokine response, thrombocytopenia and lethal effects of **N.meningitidis** when compared to the reference vaccine.

#122

MICE ANTIBODY RESPONSE TO MENINGOCOCCAL ANTIGENS DETERMINED BY IMMUNOBLOTTING ASSAY.

Jessouroun E[1], Silveira I.A.F.B.[1], Zanatta A.[1] , Pereira S.[1] and Frash CE[2], [1]BioManguinhos , FIOCRUZ, RJ Brazil. [2]National Institute of Health, Bethesda, MD, USA.

Meningococcal B disease still represents a global health problem. In Brazil it has been responsible for most meningococcal diseases since the beginning of eighties. The low avidity of anti-group B polysaccharide, their lack of lytic activity and the poor immunogenicity, have result in new approaches to develop alternative vaccines. This investigation was carried out to evaluate the immune response of Swiss mice to antigens obtained from *N. meningitidis* group Brazilian prevalent strains, by immunoblotting assay. We wanted to investigate the immunogenicity of different components of outer membrane vesicles (OMV) and, if possible, to correlate the results with bactericidal activities of the serum. Swiss female mice were immunized intraperitoneally with OMV antigens, C polysaccharide with and without detoxified LPS. They received one dose and two boosters with one-month interval. The concentrations used for OMV and C polysaccharide were the same (1–2,5 µg/dose) and was half for the detoxified LPS. We observed that the response to OMV components was increased with the boosters in agreement to the bactericidal titers when compared to the control group. The formulation with detoxified LOS showed, in preliminary studies, the best response. The sera analyzed one moth after the last booster , for all formulations tested, showed good responses to the class 1, 3, 4, 5 and iron regulated proteins.

#123

AN ENHANCED LABORATORY SURVEILLANCE PROGRAM FOR MONITORING THE IMPACT OF THE RECENTLY INTRODUCED MENINGOCOCCAL CONJUGATE VACCINE ON SEROGROUP C DISEASE INCIDENCE IN ENGLAND & WALES

Kaczmarski EB, Gray SJ, Borrow R, Ward C, Fox AJ, Mallard RH (PHLS Meningococcal Reference Unit, Withington Hospital, Manchester M20 2LR, UK) Miller E, Gungabissoon U and Ramsay M (PHLS Communicable Disease Surveillance Centre, London NW9 5EQ,UK)

Objectives The introduction of meningococcal C conjugate (MCC) vaccine into the UK occurred as a phased rollout starting in November 1999. To evaluate the clinical and epidemiological impact of MCC it is essential to accurately ascertain the disease burden by obtaining microbiological confirmation on any affected individuals. A comprehensive laboratory-based surveillance program was developed.

Methods Surveillance has been improved by making meningococcal PCR assays widely available at the national reference unit. Patients aged under 20 years with confirmed serogroup C infection or where the sole laboratory evidence is a positive 'screening' PCR (*ctrA*) result are followed up by a telephone/fax contact which facilitates collection of acute serological samples (3-5 days post onset). These can show a developing functional antibody response when compared with convalescent serum. Assays of serosubtype antibody and avidity are also being assessed as tests to differentiate vaccine-induced antibodies from concurrent or subsequent acute serogroup C infection. Acetylation status and MLST designation are being determined on strains from clinical cases and large scale carriage studies.

Results Over 40% of laboratory confirmations are by PCR and serogroup is identified in about 90%. Serological tests have proved valuable, serving to confirm or refute the diagnosis of serogroup C meningococcal disease. Assessment of functional antibody response in individual patients has been used to guide advice on vaccination with MCC following infection.

Conclusion Availability of a range of assays is necessary to enable maximal laboratory confirmation so as to accurately ascertain the biological impact and clinical effectiveness of vaccine introduction.

#124

Immunogenicity and safety of a booster dose Monovalent RIVM meningococcal B OMP vesicle F91 vaccine in children that received Hexavalent RIVM meningococcal B OMP vesicle vaccine 2.5 years ago

Lafeber AB, van Limpt CJP, Berbers GAM, Rümke HC, van Alphen AJW.
National Institute of Public Health and the Environment, The Netherlands

Background: In the Netherlands as well as in other Western European countries, P1.4 is the most prevalent one among the meningococcal subtypes. However, compared to the other PorA's present in the hexavalent vaccine the anti-P1.4 response induced after vaccination was weaker. For this reason, a monovalent model vaccine (MonoMen), using a vaccine production strain expressing $P1.7^h$,4 PorA (designated F91), was developed. This vaccine was used to revaccinate children who were vaccinated with the hexavalent vaccine (or hepatitis B vaccine as a control) in a previous trial. The aim of this follow-up study was to investigate whether the hexavalent vaccine had stimulated the induction of P1.4 specific memory. Therefore, the immunogenicity of MonoMen was assessed in healthy children previously vaccinated with the hexavalent vaccine. In addition, the reactogenicity of MonoMen was investigated.

Methods: Local and systemic adverse reactions were assessed during the week after vaccination. Before and 4-6 weeks after vaccination the serum bactericidal antibody (SBA) response was measured against six isogenic variants of strain H44/76 in which each PorA of the HexaMen (P1.7,16; P1.19,15; P1.5,2; $P1.5^c$,10; P1.12,13 & $P1.7^h$,4) was expressed individually.

Results: The rise of the GMT against $P1.7^h$,4 in children primed with the hexavalent vaccine was much higher as compared to the HepB group. Of the children with an immune response against $P1.7^h$,4 after the primary series in the earlier study, 80% showed also an immune response after the booster vaccination with MonoMen. Unexpected immune responses against strains not present in MonoMen were observed, this was most pronounced for the strains $P1.5^c$,10 and P1.5,2, possibly caused by cross-reacting antibodies.

Discussion: The frequency and nature of adverse reactions after vaccination with MonoMen are acceptable. This study showed that priming with the RIVM hexavalent vaccine leaded to the development of an immunological memory. In spite of a weaker response against $P1.7^h$,4 after vaccination with the hexavalent vaccine, an adequate response against this strain was found after boosting with MonoMen.

#125

BOOSTER RESPONSES AFTER PRIMING WITH MENINGOCOCCAL C CONJUGATE VACCINE

MacLennan JM[1], Decks JJ[1], English ME[1], Bowen-Morris J[1], Lockhart S[2], Clarke J[2], Moxon ER[1] [1]Oxford Vaccine Group, Oxford, [2]Wyeth Lederle Vaccines, UK

Aim: To assess persistence of antibody, induction of immunologic memory, and the effect of concurrent administration of measles/mumps/rubella (MMR) vaccine following priming with meningococcal group C conjugate vaccine (MenC).
Design: A randomised controlled trial in 248 British toddlers.
Methods: Children randomised to receive MenC or Hepatitis B vaccine (HepB) at 2, 3 & 4 months with their routine immunisations (DTP, HibTITER, OPV) were boosted at 12.5 months with either meningococcal A/C polysaccharide (MPS) or MenC. MMR was given either concurrently or separately (see table). Parents completed a 3 day observation diary following vaccination. Blood was taken before and 14 days (Gp1 & Gp4) or six weeks (Gps 2, 3, 5, & 6) after vaccination.
Results: 231/248 children completed the booster phase. Geometric mean ELISA antibodies (& 95%CI) to meningococcal C polysaccharide are shown in the table.

Vaccination Schedule	Group 1 n=24	Group 2 n=47	Group 3 n=44	Group 4 N=23	Group 5 n=47	Group 6 n=46
2,3,4 months	MenC	MenC	MenC	HepB	HepB	HepB
12.5 months	MPS	MenC & MMR	MenC	MPS	MenC & MMR	MenC
13 or 14 months	MMR	-	MMR	MMR	-	MMR
MenC ELISA Ab	2.48	1.97	1.73	0.54	0.08	0.06
pre booster	1.66, 3.68	1.54, 2.52	1.33, 2.24	0.48, 0.60	0.05,0.11	0.05, 0.08
% ≥ 2µg/ml	59%	52%	51%	0%	3%	0%
MenC ELISA Ab	7.12	36.1	24.7	3.42	13.46	12.11
post booster	5.21, 9.73	29.5, 44.1	19.8, 30.8	2.32, 5.05	10.1, 18.0	10.0, 14.6
% ≥ 2µg/ml	95%	100%	100%	81%	97%	100%

Priming with MenC resulted in significantly higher pre and post boost antibody titres regardless of the booster vaccination (Post boost: Gp1 vs Gp4 p=0.004: Gp2 vs Gp5, p<0.0001: Gp3 vs Gp 6, p<0.0001) showing that immunologic memory had been induced. Concurrent administration of MMR did not affect the primary response to MenC (Gp5 vs Gp6, p=0.53) and enhanced the booster response (Gp2 vs Gp3, p=0.01).
Conclusions: MenC is well tolerated (data not shown) when administered to toddlers and induces immunologic memory. Concurrent administration of MMR did not affect the primary response to MenC and enhanced the booster response to MenC

#126

IMMUNOGENICITY OF A SEROGROUP B MENINGOCOCCAL VACCINE AGAINST MULTIPLE MENINGOCOCCAL STRAINS IN ROUTINELY VACCINATED CUBAN INFANTS.

Morley SL, Cole MJ, Ison CA, Sotolongo F, Anwar N, Cuevas I, Fernández M, Camaraza MA and Levin M. Dept of Paediatrics, Imperial College, London, UK and Instituto Finlay, La Habana, Cuba.

Objective: To assess the immunogenicity in infants of VA-MENGOC-BC® (which contains an outer membrane protein (OMP) complex from the serogroup B (GpB) *N.meningitidis* strain (B:4:P1,15) and serogroup C (GpC) capsular polysaccharide) against multiple *N.meningitidis* strains using both a novel whole blood bactericidal assay (WBA) and the established serum bactericidal assay (SBA).
Study design: Prospective immunogenicity study of vaccination with 2 doses of VA-MENGOC-BC® given at 3.5 and 5.5 months of age to 104 healthy Cuban children (50% also received a 3rd dose at 7.5 months). Immune response immediately pre- and 2 months post- vaccination determined by SBA and WBA.
Results: Mean percentage inoculum survival in the WBA was significantly reduced post-vaccination for both the GpB vaccine strain and a GpC strain (C:NT:P1,1) in contrast to the heterologous GpB control strain (B:15:P1.7,16):

Strain	Mean Percentage Survival of Inoculum in Whole Blood		
	Pre-vaccination	Post- 2 doses	
B:4:P1,15	97.3 (80.3-117.8)	23.4 (18.98-28.86)	p<0.001
C:NT:P1,1	106.3 (87.8-128.8)	26.99 (21.87-33.26)	p<0.001
B:15:P1.7,16	126.5 (103.6-154.4)	169.7 (138.6-207.7)	p<0.001

51.65 and 45.56% of infants respectively demonstrated greater than 50% killing of the GpB vaccine strain and the GpC strain in the WBA after 2 doses of the vaccine. In contrast, a four- fold increase in SBA titre was induced in only 26.7% of infants against the GpB vaccine strain and in 13.8% against the GpC strain. Potential cross-reactive immunogenicity was noted for only 1 (B:2b:P1,10) of the 3 further heterologous GpB strains assessed by WBA. The administration of a third dose of vaccine to half of the children at 7.5 months of age did not significantly influence residual immunogenicity at 16-18 months.
Conclusion: VA-MENGOC-BC® induces an immune response in infants against both the GpB vaccine strain and against a reference GpC strain. Despite the increased sensitivity of the WBA little cross-reactive immunogenicity against heterologous GpB strains is apparent in this age-group.

#127

Immunogenicity and Potency of rNspA Vaccines in Mice, an Indication of Stability

Nault, A.; Bonapace, S.; Masi, D.; Bouliane, S. and Wunderli, P. BioChem Pharma Inc., Northborough, MA

Determination of potency is essential for assessing efficacy of a candidate vaccine product. While a protein may be immunogenic and elicit high levels of antibodies, these antibodies must be capable of conferring protection. A novel and conserved *N. meningitidis* outer membrane protein, NspA, has been described. Formulations of this recombinant protein induce specific antibodies, as measured by ELISA, that also exhibit bactericidal activity. While the rNspA ELISA can measure an amount of specific IgG, the serum bacteri-cidal assay (SBA) evaluates the level of functional (protective) antibody. As bactericidal assays are technically cumbersome, a correlation of the results from these two assays is highly desirable.

Reproducible assays to determine both anti-rNspA specific antibody and bactericidal activity were used to evaluate the immunogenicity and potency of rNspA vaccine formulations. Evaluation of sera from rNspA-immunized mice using these two methods show dose-dependent responses. Although no correlation was observed for individual mice, higher ELISA responses were indicative of stronger SBA responses within groups of rNspA-immunized mice.

The application of these assays has also allowed us to evaluate formulation stability. Immunizations of mice using rNspA formulations were performed over a period of 6 months after preparation and have shown consistent responses, as measured by both assays. Whether storage was at 4 or 37° C, the formulations retained comparable immunogenicity and SBA potency.

#128

PEPTIDE FRAGMENTS OF *NEISSERIA MENINGITIDIS* SEROGROUP B OUTER MEMBRANE PROTEINS AS INDUCERS OF ANTI-MENINGITIS IMMUNITY

Nesmeyanov V.A., Kotelnikova O.V., Volpina O.V., Arkhipova V.S., Zmak M.N., Koroev D.O., Kupriyanova M.A., Ivanov V.T. Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia.

Objectives: To synthesize peptide fragments of *N.meningitidis* serogroup B PorA and OpaB proteins and to select peptides for development of peptide vaccine.

Design: Selection of peptides for synthesis was based on prediction of T-epitopes deduced from known MHC class II peptide-binding motifs and literary data on PorA and OpaB epitope structures. The ability of free peptides was evaluated to induce antibody response and resistance to *N.meningitidis* serogroup B strain 44/76 challenge infection in mice. The binding of peptide-induced antibodies to heterologous strains of *N.meningitidis* was evaluated as well.

Results: Twenty six 12-26-mer peptides were synthesized. A number of these peptides induced antibody response in three genetically different strains of mice without conjugation to carrier protein and possessed considerable protective activity. Eight peptides, predominantly from conservative regions of PorA and OpaB proteins were selected for further studies, namely, 178-199, 273-292, 306-332, 346-363 PorA peptides, and 30-51, 64-83, 74-93, 109-130, 131-150 OpaB peptides. Among them peptides inducing bactericidal antibodies and antibodies capable of binding to heterologous strains of *N.meningitidis* serogroup B were identified.

Conclusions: Certain free peptides representing conservative fragments of *N.meningitidis* serogroup B outer membrane proteins are immunogenic in mice and have a potential as components anti-meningitis B synthetic vaccine

#129

CHARACTERIZATION OF A PEPTIDE MIMIC OF A CARBOHYDRATE EPITOPE ON *NEISSERIA GONORRHOEAE*

Jutamas Ngampasutadol, Sunita Gulati, and Peter A. Rice. Boston University School of Medicine, Boston, MA

Monoclonal antibody (mAb) 2C7 recognizes a widely expressed gonococcal epitope expressed on the oligosaccharide portion of gonococcal lipooligosaccharide (LOS). In humans, the 2C7 epitope elicits a significant antibody response that mediates both killing and opsonophagocytosis after either natural infection or vaccination. Because oligosaccharides are poor immunogens that usually result in a T-cell independent response, we approached this problem by developing peptide(s) that mimic the 2C7 epitope, and which might elicit a T-cell dependent response when used as immunogens. Using a random peptide library expressed on *E. coli* flagella, we identified a consensus sequence that bound mAb 2C7. A peptide containing this consensus sequence has been shown to inhibit binding of mAb 2C7 to LOS in a dose-responsive manner. A multiple antigen form of this peptide (MAP) has also been shown to inhibit mAb 2C7 binding to LOS. To investigate the immunogenicity of this peptide, we immunized mice with four doses of MAP. 2 out of 8 mice showed both IgG and IgM antibody responses to LOS measured by ELISA. Bactericidal antibody activities, directed against "serum-resistant" strain 15253 (2C7 epitope-positive), of these two mouse sera that contained 2.5 and 1.1 μg of anti-LOS IgG antibodies demonstrated 74.1 and 30.8 ± 3.0 % killing respectively. No killing of the 2C7 epitope-negative isogenic mutant strain 15253 *lgtG* was observed. These results indicate that a peptide can mimic the 2C7 oligosaccharide epitope and can also induce a specific active immune response in mice. Strategies to improve the antibody response to this peptide are currently underway.

#130

IMMUNOGENICITY OF RECOMBINANT CLASS 1 PROTEIN FROM *N. meningitidis* REFOLDED INTO PHOSPHOLIPIDS VESICLES AND DETERGENT

Niebla O, Alvarez A, Martín A, Delgado M, Falcón V and Guillén G. Center for Genetic Engineering and Biotechnology, Havana, Cuba

The purpose of this study was to examine the possibility of eliciting bactericidal antibodies in mice against recombinant class 1 protein (PM82) obtained from inclusion bodies in *E. coli*, using detergent and phospholipid vesicles for its refolding.
The recombinant polypeptide was refolded by dialysis against 0.1% SDS (PM82-SDS) and into phospholipid vesicles (PM82-PC). When Balb/c mice were immunized, high titers of subtype-specific antibodies against P1 were obtained in both cases. However the frequency of bactericidal response of the individual animals was different, PM82-SDS generated highest levels of antibodies, but the frequency of responders was slightly lower than PM82-PC. These results were in correspondence with the phagocytosis assay.
A moderate protein refolding in solution with a low SDS content or complete exposition of the immunodominant regions, allowing eliciting functional antibodies to immunodominant epitopes.
Our results support the alternative of the vaccine candidate based on the class1 protein, owing to the advantages of expression of a meningococcal OMP in nonpathogenic *E. coli*.

#131

NEISSERIA LACTAMICA PROTEINS PROTECT MICE AGAINST MENINGOCOCCAL CHALLENGE.

Oliver K[1], Bracegirdle P[1], Reddin K[1], Gorringe AR[1], Borrow R[2], Cartwright K[3], Feavers I[4], Robinson A[1], Hudson M[1], [1]Centre for Applied Microbiology and Research, Salisbury, UK; [2]Manchester Public Health Laboratory, Manchester, UK; [3]Public Health Laboratory, Gloucester, UK; [4]National Institute for Biological Standardisation and Control, South Mimms, UK.

Background: Immunological and epidemiological evidence suggests that carriage of the commensal organism *Neisseria lactamica* may be involved in the development of natural immunity against meningococcal disease. *N. lactamica* has many surface structures in common with *N. meningitidis* and we have shown that *N. lactamica* antigens provide protection in an animal infection model of meningococcal disease. Previous work has shown that *N. lactamica* outer membrane vesicles (OMV) and antigens less that 43kDa are important for this protection
Results: Outer membrane proteins (OMP) of *N. lactamica*, extracted from whole cells using 0.3% (v/v) Elugent, were separated by semi-native preparative electrophoresis. The proteins were pooled into <25kDa, 25-35kDa and >35-67kDa molecular weight groups and used to immunise mice. The mice were then challenged with serogroup B *N. meningitidis* at two challenge doses. The 25-35kDa and 35-43kDa proteins provided the best protection and sera raised against these proteins had higher bactericidal titres against *N. meningitidis* than sera raised against the <25kDa proteins.
Proteins of <43kDa, as well as eliciting antibodies that cross-react with a range of *N. meningitidis* strains, have been shown to protect mice against challenge by a range of *N. meningitidis* strains. Protection was comparable to that provided by *N. lactamica* OMVs.
Conclusion: *N. lactamica* antigens have potential as vaccine candidates to provide protection against a wide range of meningococcal strains.

#132

DIVERSITY AND PREVALENCE OF PorA TYPES IN *NEISSERIA MENINGITIDIS* SEROGROUP B IN THE UNITED STATES, 1992-1998 - IMPLICATIONS FOR DEVELOPMENT OF OMP-BASED VACCINES

Popovic T[1], Sacchi CT[1,2], Whitney AW [1], Beall DS [1], Reeves MW [1], Plikaytis BD[1], Rosenstein NE[1], Perkins BA[1], Tondella MLC [1], and Mayer LW[1],
[1]Centers for Disease Control and Prevention, Atlanta, USA; and
[2]Adolfo Lutz Institute, São Paulo, Brazil.

Objective: Two-hundred-eighty-one *N. meningitidis* serogroup B isolates (NMSB) collected in the US through active laboratory-based surveillance from 1992-1998 were analyzed by porA variable region (VR) typing to determine the prevalence of PorA types in the US.
Results: Thirty-one different VR1 types belonging to 10 VR1 families were identified with only 6, observed in 44% of the strains, identical to those seen in the family prototype strains; 8 of the 25 variants have not been previously reported. Forty-one different VR2 types belonging to 13 VR2 families were identified. Sequences of 13 were identical to those of the family prototypes while 28 were variants, 16 of which are reported for the first time. A total of 73 different VR1 and VR2 combinations (PorA types) were identified and 77% of them comprise non-prototype sequences in VR1, VR2, or both. The most prevalent PorA types identified in 54% of our isolates were P1.7,16-20; P1.22,14; P1.22-1,14; P1.7,16; P1.7-1,1; P1.19,15; and P1.17,16-3. The remaining 66 different PorA types were identified in 1 to 9 isolates each. No correlation was observed between the PorA types and geographic origin of the isolates.
Conclusion: This diversity of the PorA types, along with the present lack of information on the extent of cross-protection in humans among the variants and prototypes in each PorA VR family, create a unique challenge for development of an efficacious outer-membrane protein-based vaccine.

#133

NOVEL CARRIER PROTEINS FOR MENINGOCOCCAL SEROGROUP C CONJUGATE VACCINES : *NEISSERIA MENINGITIDIS* TRANSFERRIN BINDING PROTEIN B (TbpB) AND *BORDETELLA PERTUSSIS* FIMBRIAE

Reddin K M, Crowley-Luke A, Paxton, D, Gorringe A, Hudson M, Robinson A. Centre for Applied Microbiology and Research, Salisbury, UK.

Introduction: Current serogroup C conjugate vaccines use diphtheria or tetanus toxoids as the protein carrier. This does not broaden protection and where mice have been primed with carrier, reduced antibody responses to the polysaccharide are observed. The use of multiple carrier proteins may allow multivalent conjugate vaccines to be formulated into a single injection. Therefore, meningococcal TbpB and *B. pertussis* fimbriae were assessed as novel carrier proteins for *N. meningitidis* serogroup C polysaccharide.

Results: *B. pertussis* fimbriae or recombinant TbpB were conjugated to meningococcal serogroup C polysaccharide and characterised by size exclusion chromatography. Co-elution of protein and sugar confirmed conjugation. The conjugates elicited boostable IgG responses in mice and IgG:IgM ratios indicated that the responses were thymus-dependent. High bactericidal antibody titres were also observed. In animal models, the conjugate vaccines were protective against lethal infection with *N. meningitidis* and the fimbriae conjugate also protected against *B. pertussis* infection.

Conclusions: Meningococcal TbpB and *B. pertussis* fimbriae are effective carrier proteins for meningococcal serogroup C polysaccharide. Using TbpB as a carrier protein provides a vaccine that would broaden protection to other meningococcal serogroups. Use of *B. pertussis* fimbriae as carrier would produce a vaccine to protect against meningococcal disease and also to augment protection against whooping cough.

#134

DE-*O*-ACETYLATED MENINGOCOCCAL C -TETANUS TOXOID CONJUGATE VACCINE IN INFANCY : IMMUNOGENICITY AND BACTERICIDAL ACTIVITY AGAINST *O*-ACETYLATED AND DE-*O*-ACETYLATED SEROGROUP C STRAINS.

Peter Richmond [1], Ray Borrow[2], Jamie Findlow[2], Sarah Martin[2], Keith Cartwright[3], Elizabeth Miller[1]. PHLS Communicable Disease Surveillance Centre, London[1]; PHLS Meningococcal Reference Unit, Manchester[2] and Public Health Laboratory, Gloucester, UK.[3]

Background: Licensed meningococcal C (MenC) polysaccharide vaccines contain the *O*-acetylated(OAc+) form of polysaccharide. Some MenC strains have de-*O*-acetylated (OAc-) polysaccharide that may affect antibody specificity and functional activity if used in a vaccine.

Methods OAc- MenC-tetanus toxoid conjugate (MCC-TT) vaccine was given with DTP/Hib immunisation in 83 infants at 2-3-4 months of age. Serum bactericidal activity (SBA) against OAc+ and OAc- MenC strains and OAc+ and OAc- -specific IgG were measured.

Results: MCC-TT vaccine was well tolerated & highly immunogenic after a single dose. SBA GMT for OAc+ C11 strain increased from 2.7 (95%CI 2.2-3.2) to 320 (95%CI 237-432) , 773 (95%CI 609-982) and 1063 (95%CI 856-1319) after 1, 2 and 3 doses of MCC-TT. OAc- IgG levels were twice as high as OAc+ IgG after the primary series of MCC-TT vaccine. SBA GMT for OAc- C2a strain after 3 doses (2114 95%CI 1504-2969) was significantly higher than C11 SBA GMT ($P<0.001$). Antibody responses to boosting with either OAc+ MenC polysaccharide vaccine (MACP) or a fourth dose of MCC-TT at 14 months of age demonstrated immunologic memory. The acetylation status of the booster vaccine influenced the specificity of the response with significantly higher OAc- IgG and SBA after MCC-TT vaccine compared to OAc+ MACP vaccine but similar OAc+ IgG levels.

Conclusion: MCC-TT vaccine is highly immunogenic and primes for immunologic memory against OAc+ & OAc- MenC strains in infancy.

#135

REDUCED IMMUNE RESPONSE AFTER REPEATED IMMUNISATIONS WITH SEROGROUP A+C POLYSACCHARIDE VACCINE IN NORWEGIAN TEEN-AGERS

Rosenqvist E, Høiby EA, Fuglesang JE, Feiring B, Aaaberge I, Jenum P, Konsmo K, Forsdahl B, Nøkleby H. Departments of Vaccinology and Bacteriology, National Institute of Public Health, N-0403 Oslo, Norway

Design: In a clinical trial with the Norwegian group B outer membrane vesicle vaccine (phase II-10), a control group of 45 secondary school students (average age 13 years old) were immunised with a A+C polysaccharide vaccine (Meningovax A+C "Pasteur Mérieux"). The students received 2 doses of 50 µg of each polysaccharide at weeks 0 and 6, and a third dose 10 months later. Sera were collected at vaccination, six weeks after each immunisation and one year after the third dose. Serum bactericidal antibodies (SBA) against a group C meningococcal strain and IgG levels against C-ps were determined.

Results: Before the first immunisation the GM IgG level against C-ps. was 0.9 µg/mL, and only 4/45 vaccinees (9%) had detectable bactericidal antibodies against the group C strain. Six weeks after the first dose the GM IgG level was 49.3 µg/mL, and the vaccine had induced bactericidal activity in all vaccinees. The second dose induced no further increase in IgG or SBA titers, and 9 vaccinees showed a decrease in SBA titers, compared to the peak value after the first dose. Ten months later the GM IgG anti C-ps level had decreased to 27.8 µg/mL. A "booster dose" at month 10 induced ≥4-fold increase in SBA in 12 vaccinees, a fall in 3 vaccinees and no change in the remaining. Six weeks after the third dose the GM SBA titer was significantly lower than six weeks after the first.

Conclusions: The results demonstrate that repeated immunisation with a plain A+C polysaccharide vaccine do not induce increased SBA response to group C meningococci, and actually it may reduce the titres in some individuals.

#136

IS A PorA BASED VACCINE APPROPRIATE FOR BRAZIL AT THIS TIME?

Sacchi CT[1,2], Mayer LW[1], Lemos APS[2], Brondi LMG[3], Melles CEA[2], Monteiro LMC[3], Popovic T[1]

[1]Centers for Disease Control and Prevention, Atlanta, USA: [2]Adolfo Lutz Institute, São Paulo, Brazil; and [3]National Foundation of Health. Brasilia, Brazil

Objective: To determine the number, prevalence and distribution of PorA variants in serogroup B *N. meningitidis* isolates (MenB) in Brazil in order to evaluate the possible use of an outer-membrane vesicle/PorA based vaccine to prevent meningococcal disease.

Results: During 1997 and 1998 a total of 11,493 cases of meningococcal disease were reported in Brazil (3.5/ 100,000 per year). Only 33% of reported cases had an isolate that was serogrouped, but 66% of these were serogroup B. A set 1,532 B MenB (62% of serogroup B) collected through a passive surveillance system in 1997 and 1998 were serosubtyped (SST) and 124 from greater São Paulo had PorA VR typing. These reported cases and isolates were submitted by health departments in 10 states along the southeast coast and the Federal District totaling 73% of the population of Brazil. By SST 1,018 (66%) were P1.19,15 with P1.7,1 being the next most prevalent SST with 145 (10%) of isolates. Only 74 (5%) were non-serosubtypable. By PorA VR typing of 77 SST P1.19,15 from São Paulo, 70 (91%) were prototype VR1-19 and VR2-15 and 76 (99%) had at least 1 prototype VR. Extrapolating from this data, with a number of assumptions, an estimated 6300 cases of meningococcal disease was caused by B MenB P1.19,15 prototype during this 2 year period in Brazil.

Conclusions: Although improvement are needed in the reporting of cases and collection of strains our data suggest that the use of an effective MenB P1.19,15 PorA based vaccine could prevent significant morbidity and mortality from meningococcal disease in Brazil. Development of a production facility to produce a PorA P1.19,15 vaccine is underway at the Butantan Institute, São Paulo, FIOCRUZ Institute, RJ and Adolfo Lutz Institute.

#137

PLANT OILBODIES: A NOVEL AND VERSATILE VACCINE DELIVERY SYSTEM

Alcantara J[1,2], Hutchins W [1], Yu R-H [1], Moloney MM.[2], and Schryvers AB. [1]

[1]Department of Microbiology and Infectious Diseases, and [2]Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N4N1

Suitable and effective delivery systems are required for the development of effective vaccines. We are developing a novel delivery system based on transgenic plant oilbodies. Oilbodies are 0.5-2.0 uM subcellular organelles in seeds that are composed of triacylglycerol surrounded by a half unit phospholipid membrane and a protein coat containing the seed-specific protein, oleosin. Translational fusions to oleosin target foreign proteins to the oil body surface and can be readily purified from other seed material by gravitational separation. This can potentially provide a simple and inexpensive means of preparing complex vaccine formulations. To circumvent the lengthy and labor-intensive process for generating transgenic plants, we developed a model oil body system in which enzymatically biotinylated antigen is coupled to the oilbody surface via a biotin-streptavidin interaction. Transgenic and model oilbodies using several test antigens (meningococcal TbpB, beta-glucuronidase, tetanus toxin) were tested in parenteral, mucosal (intranasal and intragastric) and transdermal immunizations. The results demonstrate that oilbodies significantly enhance the immune response against antigen in all three routes of immunization and that inclusion of targeting or modulating molecules on the oilbody can further improve the response. The ability to use a single oilbody preparation for different routes of administration provides a considerable advantage in developing an efficient and convenient vaccination regimen.

#138

PREPARATION, IMMUNOGENICITY AND SAFETY OF OUTER MEMBRANE PROTEIN COMPLEX EXTRACTED FROM THE STRAIN 3407 OF *NM* SEROGROUP B

Sun Yinyan, Hu Xujing, Wang Jun and Xu Li. Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing 102206.

Objective: In order to prepare meningococcal serogroup B vaccine a high immunogenicity and broadly cross reactive outer membrane protein complex (BOMPC) was extracted from the strain 3407 of *Nm* serogroup B and its immunogenicity and safety was determined.

Design: A few methods extracted BOMPC from the case strain 3407 of *Nm* serogroup B were compared, then the immunogenicity, safety was determined by the animal experiment, ELISA and the bactericidal test.

Result: The method that the cultural supernatant of the bacteria was primarily precipitated with 70% saturated ammonium sulfate and was further purified on Sephacryl S-300 HR column was chosen. The 42, 39, 30, 26, 19kDa and minor 92, 72kDa proteins in the BOMPC were determined by SDS-PAGE and Western-blotting. The reactive titers of the BOMPC with the sera of rabit anti-the strains 3407 and with the sera of the mice anti-BOMPC were 1:32000 and 1:25600, respectively. In addition, the reactive titers of the antibody against BOMPC with some different serotype and subserotype strains of *Nm* serogroup B were 1:640 to 1:3200. The bactericidal titer of anti-BOMPC sera to the strain 3407 was 1:256. BOMPC induced high antibody titers in rabbit and maintained more than five months. The safety of the BOMPC was defined safe in the toxicity test of mice and guinea pig as well as its pyrogen was normal in pyrogenic rabbit test. The BOMPC was also used as a nice carrier coupling with the pure OS and CPS from the strain of *Nm* by carbodiimide mediated condensation.

Conclusion: The above BOMPC of *Nm* serogroup B possessed high immunogenicity, good solubility and broadly cross reactivity. It seems to be hopeful that BOMPC is chosen as a vaccine candidate of *Nm* serogroup B and as a carrier coupling with polysaccharide.

#139

IMMUNOGENICITY, SAFETY AND STABILITY OF THE ACPS-3407BOMPC CONJUGATE

Sun Yinyan, Hu Xujing, Wang Jun and Xu Li. Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing 102206.

Objective: To get a kind of conjugate which can prevent the infections both of *Neisseria meningitidis* serogroup A and serogroup B (*Nm*) and can enhance the immunogenicity of the capsular polysaccharide of *Nm* serogroup A (ACPS), the immunogenicity, safety, stability of the conjugate were determined.

Design: Outer membrane protein complex (BOMPC) from the strain 3407 of the *Nm* serogroup B was purified on sephacryl S-300 HR after the cultural supernatant was precipitated by 70% ammonium sulfate. ACPS was conjugated to BOMPC by carbodiimmide mediated condensation. The immunogenicity of the ACPS-BOMPC conjugate was determined by immunological experiment in mice, and rabbit, ELISA, Bactericidal test and Western-blotting analysis. The safety of the above conjugate was confirmed in the toxic test of mice and guinea pig.

Results: The antibody titlers against the above conjugate in 87.5% (14/16) mice reached up to 1:7240-1:14481 and the antibody titers against ACPS in 81.25% (13/16) mice reach up to over 1:320. The immunogenicity of the conjugates was enhanced 8 to 128 times as large as unconjugated ACPS or simple mixture of ACPS and BOMPC. Antiserum evoked by the conjugate not only possessed a stronger bactericidal activity to the serogroup A strains (29019) and the serogroup B strains (3407,542852,29021) but also showed broadly cross-reactions to other eight serogroup B strains of different bacterial type. It was primarily found by western-blotting analysis that the sera elicited by the above conjugates obviously reacted with 42, 39 and 26 kDa proteins in BOMPC. Among the reactive bands, the 42kDa protein was class 1 OMP. The high antibody titers (over 1:25600 against the conjugate and 1:3200-6400 against ACPS) was induced by the conjugate and maintain over five months in rabbit. Seven mice were immunized by use of the above conjugate, the mice which had been immunized by the conjugate were attacked with the strain 3407(5×10^8CFU/ml 3407). In the immunologic group five mice survived, but only one mouse survived in the control group in which seven mice were injected normal saline instead of the conjugate. The immunological experiments primarily showed that the conjugate possessed some protective effect for the infection of *Nm* serogroup B. The safety of the conjugate was defined to be safe in the toxic test of mice and guinea pig.

Conclusion: The above conjugate not only possessed strong immunogenicity for *Nm* serogroup A and serogroup B but also enhanced the immuogencity of ACPS in the immunologic experiments of mice and rabbit. It is a hopeful that the conjugate is used for preparing a vaccine to prevent both of the infecions of *Nm* serogroup A and B.

#140

COMPARISON OF IMMUNOGENICITY OF INDIVIDUAL POR A IN TRIVALENT AND MONOVALENT OMV'S

Germie van den Dobbelsteen, Betsy Kuipers, Harry van Dijken and Loek van Alphen

Lab. for Vaccine Research, RIVM, Bilthoven, The Netherlands

A recombinant hexavalent PorA outer membrane vesicle (OMV) vaccine, developed at the RIVM, has given promising results in phase I in adults and II trials in infants, toddlers and schoolchildren. The vaccine was shown to be immunogenic although multiple doses of vaccine were required to induce a significant SBA response and the magnitudes of SBA responses to differed by PorA. The strongest booster responses were found against PorAs P1.5a,2c and P1.5c,10. The bactericidal antibodies against sero-subtypes P1.7,16 and P1.12a,13 were generally high as well. The responses against P1.7b,4 and P1.19,15a were lower, suggesting that some PorA are less immunogenic compared to other PorAs or that three PorAs presented on one vesicle might be a suboptimal formulation.

Objective To: compare to the immunogenicity of individual PorA's in mice after vaccination with trivalent andmonovalent OMVs.

Design: Balb/c mice were subcutanously immunised (day 0, 14 and 28) with a mixture of 6 monovalent OMVs or 2 trivalent OMVs at three concentration namely 4.5 µg, 1.5 µg or 0.5 µg per PorA protein. Sera of day 42 were analysed in whole cell ELISA and serum bactericidal assay.

Results: For each of the 6 PorA the response was similar after vaccination with 2x trivalent and 6x monovalent OMVs. However, the magnitude of the response varied by PorA. Only at very low concentrations 6x monovalent preparation showed a higher antibody response for P1.7b,4 than 2x trivalent OMVs, possibly due to differences in LPS content.

Conclusion: Studies in mice indicate that the difference in magnitude of response to various PorAs is not due to the presentation of three PorAs on one vesicle but due to the characteristics of the PorA itself. This result suggets that PorA subtypes differ in immunogenicity.

#141

MONOVALENT RIVM MENINGOCOCCAL OMP VESCICLE F91 VACCINES IN TODDLERS (Phase II study)

van Limpt CJP, Lafeber AB, Berbers GAM, Rümke HC, van Alphen AJW.
National Institute of Public Health and the Environment, The Netherlands

Objectives:

1. To study the safety and immunogenicity of monovalent RIVM Meningococcal OMP (F91 B: P1.7^h,4) vesicle vaccine in toddlers
2. To compare two different adjuvants ($AlPO_4$ vs $Al(OH)_3$) in this RIVM meningoccal OMP vesicle vaccine.
3. To compare two different vaccination schedules (2+1 vs 3+1, resp. 0-2-8 vs 0-1-2-8 months)

Main parameters:

- Safety: local and systemic symptoms or complaints, e.g. pain, redness, swelling, temperature and headache.
- Immunogenicity: serum antibody titers induced by the vaccine vesicles, measured by:
  1. SBA 90%: An isogenic strain expressing the serosubtype class 1 protein P1.7^h,4 was used to determine the bactericidal activity of the serum antibodies. The PorA negative mutant strain H1.5 was used as control for PorA specificity.
  2. P1.7^h,4 OMV-ELISA.

Results and discussion:

The monovalent OMP vesicle vaccines were well tolerated, only mild local and systematic reactions were reported during the observation period.
None of the children showed bactericidal activity against the PorA negative mutant strain H1.5, illustrating the PorA specificity of the antibody response. In general, the SBA response was highest in the $AlPO_4$ groups, which means that adsorption of the RIVM meningococcal vaccines to $AlPO_4$ seems preferable to $Al(OH)_3$.
After the primary series slightly higher titres were found in children who received two vaccinations instead of three, which is probably due to the longer intervals between the vaccinations in the 2+1-schedule. After the booster vaccination significantly higher GMT's were found in children vaccinated according to the 3+1-schedule. However, the percentages of children with a fourfold rise in SBA titres showed only minor differences between the two schedules.

#142

PORA ANTIBODY SPECIFICITIES OF VACCINEES RECEIVING THE NORWEGIAN B:15:P1.7,16 OMV VACCINE

Wedege E[1], Bolstad K[1], van Dijken H[2], van den Dobbelsteen G[2], Kuipers B[2], van Alphen L[2]. [1]NIPH, PO Box 4404 Torshov, N-0403 Oslo, Norway and [2]RIVM, PO Box 1, 3720 BA Bilthoven, The Netherlands.

Serum bactericidal assay (SBA) and immunoblotting have been used to compare the VR specificity of the PorA antibody responses of volunteers who received 3 doses of the Norwegian OMV vaccine (Perkins et al. 1998). In SBA, recombinant mutants of the vaccine strain 44/76 (B:15:P1.7,16) lacking VR1 and/or VR2 or PorA were employed as well as a mutant in which the P1.7,16 PorA was exchanged with a P1.19,15 PorA. For blotting, the antigens were 44/76, Cu385/83 (B:4:P1.19,15), S3032 (B:NT:P1.12,16) and M1080 (B:1:P1.7,1), the latter two antigens each shared one of the VR of P1.7,16 PorA.

From densitometric analysis of blots, 71% and 19% of the 42 vaccinees showed more than 4-fold increases in IgG binding to the P1.7,16 and P1.19,15 PorA, respectively, six weeks after the third dose compared to prevaccination levels. Antibody levels to P1.19,15 were only 27% of those to P1.7,16. In SBA, 63% and 21% of the vaccinees showed > 4-fold increases against the P1.7,16 and P1.19,15 strains, respectively. No significant difference in GMT between the latter strain and the PorA- mutant was observed after vaccination, indicating little contribution of antibodies to common PorA epitopes.

Both the VR1 and the VR2 mutants revealed significantly higher GMT with the postvaccination sera compared to the P1.7,16 strain, suggesting exposure of otherwise masked epitopes. However, ten of the 11 sera that showed a decrease in titre with the mutant lacking both VR1 and VR2 demonstrated distinct VR2 specificity on blots. This P1.16 antibody activity thus seems to contribute to the SBA activity.

#143

CARRIAGE OF A SEROGROUP C MENINGOCOCCAL STRAIN AFTER A-C MENINGOCOCCAL VACCINATION.

Williams JN,[1] Jordens JZ,[1,2] Jones GR,[1,2] Christodoulides M,[1] Heckels JE[1]
[1]Molecular Microbiology Group, University of Southampton Medical School, and [2]Public Health Laboratory, Southampton, UK

Aim: To determine the effects prior immunisation with the non-conjugated serogroup A/C capsular polysaccharide vaccine (MACPS) on the dynamics of meningococcal carriage and the immune response to colonisation.

Design: Mouth washings, throat swabs, and blood samples were collected at 4 time points during the academic year from a cohort of 42 post-vaccination first year university students. Meningococcal carriage was determined and the humoral immune response to serogroup C meningococcal colonisation analysed in detail.

Results: In this cohort, one student was found to be carrying a serogroup C meningococcal strain, despite recent vaccination. The strain was isolated at weeks 3 and 6, but was undetectable by week 21 of the academic year. High levels of serum bactericidal antibody against the homologous group C strain and against purified group capsule were detected in this student on entry and throughout the study.

Conclusions: This study shows that meningococcal C carriage can persist after MACPS vaccination, despite high levels of anti-C capsular antibodies and bactericidal antibodies, creating a potential reservoir of infection for vulnerable persons. Following the introduction of the new conjugated group C capsular polysaccharide vaccine in the UK, the induction of mucosal immunity and its effect on the persistence of short-term carriage will need to be addressed.

#144

EPITOPE SPECIFICITY OF BACTERICIDAL ANTIBODIES TO MENINGOCOCCAL L3,7 LIPOOLIGOSACCHARIDE (LOS)

Zollinger, W. D., Moran, E. E., and Brandt, B. L.
Walter Reed Army Institute of Research, Silver Spring, Maryland

We have previously reported the presence of L3,7 specific bactericidal antibodies in normal and post-vaccination human sera. The murine monoclonal antibody (MAb) 9-2-L3,7,9 has also been shown to have strong bactericidal activity. In this study, we investigated the epitope specificity of 9-2-L3,7,9 and human bactericidal antibodies specific for L3,7 LOS to attempt to identify the nature of the immunogenic epitope(s) eliciting the bactericidal antibodies. The binding of the MAb 9-2-L3,7,9 in ELISA to different LOS immunotypes with and without sialylation was compared to that of MAb 1B2-1B7, which is known to bind to the terminal two sugars of lacto-N-neotetraose. 1B2-1B7 bound to all immunotypes containing unsialylated lacto-N-neotetraose, but 9-2-L3,7,9 required the presence of both lacto-N-neotetraose and phosphoethanolamine at position 3 of heptose II. Sialylation reduced binding of 9-2-L3,7,9 by about 50%. Human bactericidal antibodies with L3,7 specificity were analyzed by selectively removing antibodies from diluted serum by adsorption to defined antigens bound to the wells of a 96-well microplate. The fine specificity of human bactericidal antibodies varied from person to person, but was generally similar to that of MAb 9-2-L3,7,9. Human erythrocytes or monocytes could not bind the bactericidal antibodies. Compared to unsialylated L3,7 LOS maximum absorption of antibody by sialylated L3,7 LOS or L4 LOS was 15%-85% less. Bactericidal antibodies to L3,7 LOS appear to bind to uniquely bacterial epitopes not present on human cells.

#336

IN VIVO EVALUATION OF LIVE ATTENUATED *NEISSERIA MENINGITIDIS* SEROGROUP B CONSTRUCTS.

McLean, C.S.[2], Barber, L.[1], Turner, J.[1], McDowell, P. Martin, G.[2], Holmes, L.[2], Goringe, A[3], Reddin, K.[3], Dennis, M.[3] and Baldwin, T.[1]
[1]Institute of Infections and Immunity, University of Nottingham, UK, [2]Cantab Pharmaceuticals, 310 Science Park, Cambridge, UK and [3]Centre for Applied Microbiology and Research, Salisbury, UK.

Aim: To determine attenuation and protective efficacy of prototype live attenuated vaccine constructs based on *N. meningitidis* serogroup B.
Rationale: A live attenuated meningococcal vaccine would have a number of important advantages over subunit or conjugate vaccines. Infection with a live organism should lead to expression of all potential immunogens, including those only seen during replication in the human host. As seen after meningococcal disease, this is likely to induce long-lasting and cross-protective immunity to many strains of *N. meningitidis.* Intranasal administration should lead to local mucosal immunity. We have constructed a number of single-deletion mutants of *N. meningitidis* and made a preliminary assessment of their vaccine potential.
Results: The intraperitoneal mouse infection model was used to investigate attenuation of *N. meningitidis* constructs deleted in *aroA*, *aroB*, *galE* or *recA*. *aroA* and *aroB* deleted mutants were attenuated compared to the wild-type. Survivors infected with the higher doses showed protection against subsequent challenge with the parental strain. Antibody responses were investigated by serum bactericidal assay and ELISA.
Conclusion: Early results support the concept that attenuated live vaccine strains of *N. meningitidis* serogroup B can be constructed and lead to protection against challenge. These results will be used in the design of a vaccine strain with multiple deletions.

#145

FUNCTIONAL GENOMICS OF *NEISSERIA MENINGITIDIS* PATHOGENESIS.

Sun Y-H[1], Bakshi S[1], Chalmers R[2], Tang CM[1]. University Department of Paediatrics[1] and Department of Biochemistry[2], University of Oxford University, Oxford, OX3 9DU, UK.

The recent publication of the whole genome sequence of two isolates of Neisseria meningitidis provides a complete picture of the genetic complement of this important human pathogen. What is now needed are methods to perform high-throughput analyses of gene function. Signature tagged mutagenesis (STM) is a powerful method for identifying genes required for pathogenesis. However a prerequisite for STM is the ability to generate diverse insertional mutants in the organism of interest. We have developed in vitro transposition for N. meningitidis mutagenesis and performed STM to identify genes required for meningococcal bacteraemia, a critical step in pathogenesis.
A library of 2,850 mutants containing signature tags was constructed and screened for strains that are defective for bacteraemia in the infant rat model. 234 putatively attenuated mutants were identified in the initial screen. To confirm the link between the transposon insertion and attenuation, all mutations were backcrossed into the original genetic background, and the strains re-tested in animals. A total of 85 insertions that result in attenuation were identified.
The transposon insertion sites in attenuated mutants are widely distributed throughout the serogroup B N. meningitidis genome, and include disruptions in known virulence genes, validating the approach. Furthermore, genes encoding putative transporters, regulators, and hypothetical proteins, none of which have been previously implicated in pathogenesis, were also isolated. The majority of genes are also present in serogroup A N. meningitidis and in N. gonorrhoeae genomes.

#146

MANY, BUT NOT ALL, INVASIVE ISOLATES OF *NEISSERIA MENINGITIDIS* (*NM*) HAVE INSERTIONAL INACTIVATION OF *DAM* BY *DRG*

Sun L, Jolley K, Makepeace K, Hood D, Maiden M, Moxon ER. Dept. of Paediatrics, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK.

The gene *drg* encodes a restriction enzyme which recognises and cleaves the DNA sequence 5'-GATC-3' when it is methylated at the adenine site. Its significance in *Nm* has been underlined by its association with a mutation in *dam*, a DNA adenine methylase. Bucci *et al* have recently reported that a *drg* insertional inactivation of *dam* is characteristic of invasive *Nm* strains. They suggest that Dam deficiency results in a mutator genotype that increases the rate of phase variation of capsule, a property associated with heightened virulence. To further investigate this question, we surveyed the status of *drg/dam* genes in a phylogenetically well-characterised population of *Nm* strains. We observed a strict correlation between the presence of *drg* and the inactivation of *dam*. Of the 100 isolates studied, we found that 75% are *dam* (-), caused without exception by an insertional replacement of *drg* at the same sites within the *dam* locus. The *drg* genes were sequenced, a phylogenetic gene tree was constructed and the degree of polymorphism was compared with that of neutral genes of the same group of isolates. The data indicate that sequence variation in *drg* is comparable to that of *Nm* neutral genes. But, importantly, 25% of strains associated with invasive disease are *dam+*, *drg-*, including hypervirulent clones belonging to the lineages of the A4 cluster and the ET 35 complex.

#147

AcfP, AN ESSENTIAL COFACTOR FOR *NEISSERIA GONORRHOEAE* PILUS-MEDIATED ADHERENCE TO HUMAN EPITHELIAL CELLS.

Winther-Larsen HC[1], Hegge FT[1], Wolfgang M[2], van Putten JPM[3], Hayes SF[4], and Koomey M[1]. [1]Biotechnology Center, University of Oslo, Oslo, Norway. [2]Department of Microbiology, University of Washington, Seattle, Washington [3]Department of Bacteriology, University of Utrecht, Nederland. [4]Microscopy Branch, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana.

Objective; Type IV pili (Tfp) of neisseria species play important roles in host -microbe biology and together with the associated PilC proteins, promote adherence to human tissue. This study's objective was to assess the potential roles of subunit prepilin-like molecules in Tfp-mediated adherence to human epithelial cells.
Results; Using the nearly complete *N. gonorrhoeae* and *N. meningitidis* genomes, we searched for genes that encode proteins related to PilE, the Tfp subunit protein. Seven open reading frames that shared identity with PilE have been found, characterised and shown to correspond to functional genes products. Studies defining one such locus (*comP*) have previously been published. Gene disruption mutants in five of the remaining loci are largely deficient in Tfp expression and thus, these molecules function in pilus biogenesis. Mutants in the last remaining locus are indistinguishable from the wildtype parental strain in Tfp-associated phenotypes except that they are grossly defective in adherence to human epithelial cells. We designated this molecule AcfP for adherence cofactor pilin and demonstrated that AcfP co-purifies with Tfp fibers. Furthermore, we have found that PilC, the putative pilus-associated adhesin for human tissue remains associated with Tfp fibers in *acfP* mutants. Therefore, an adherence defect cannot be ascribed to the absence of PilC.
Conclusion; We have identified the novel AcfP protein as an essential factor in Tfp-mediated adherence of *N. gonorrhoeae* to human epithelial cells. Tfp-mediated adherence therefore requires the expression of at least three distinct components: Tfp fibers, PilC and AcfP.

#148

INTERACTIONS BETWEEN PATHOGENIC NEISSERIA AND HOST CELLS

Albiger, B., Hansson-Palo, P., Källström, H., and Jonsson A-B.
MTC, Karolinska Institutet, S-171 77 Stockholm, SWEDEN.

Pili of pathogenic *Neisseria* mediate binding of the bacteria to human host cells. CD46, also called membrane cofactor protein (MCP), a human cell surface protein involved in regulation of complement activation, acts as a cellular pilus receptor. We have examined which domains of CD46 that mediate bacterial adherence, and identified bacterial pilus components that interact with CD46. We show that the complement control protein 3 (CCP-3) repeat and the serine/threonine/proline (STP) rich domain of CD46 are important for efficient adherence to host cells of piliated *Neisseria*. Also, partial deletion of the cytoplasmic tail of CD46 results in low bacterial binding.

We have also characterized the clear but low interaction between nonpiliated (P^-) *Neisseria gonorrhoeae* and human epithelial cells. Although the binding may not lead to heavy inflammatory responses, the interaction between P^- *Neisseria* and host cells most likely play a role in colonization and asymptomatic carriage of the pathogen. Adherence of P^- *N. gonorrhoeae* is blocked by G-protein inhibitors, and induced by G-protein activators such as cholera toxin and CNF-1. Further, increase of the extracellular free [Ca^{2+}] dramatically enhanced adherence of nonpiliated *Neisseria*. The pharynx and the urogenital tract are natural entry sites of the pathogenic *Neisseria* species, and at both sites the epithelial cells can be exposed to wide variations in Ca^{2+} concentration.

To further understand the process of adherence we used 2-D gel electrophoresis to identify novel proteins that are up- or down-regulated upon pilus-mediated binding to host cells. The role of these proteins in the bacteria-host cell interactions is currently under investigation.

Conclusions: Taken together, we have used a broad approach to map the molecular events during *Neisseria* adherence, by mapping the pilus-receptor interaction and by identifying novel proteins that are up- or down-regulated during the initial adherence to host cells.

#149

RETRACTION OF NEISSERIA TYPE IV PILI POWERS BACTERIAL MOTILITY

So, M.[1], Merz, A[1,2]. and Sheetz, M.P.[3]
[1]Dept. of Molecular Microbiology and Immunology, L220 Oregon Health Sciences University, Portland OR, 97201-3098; [2]Dept. of Biochemistry, Dartmouth Medical School, 7200 Vail Bldg, Hanover, N.H. 03755-3844; [3]Dept. of Biological Sciences, Columbia University, NY, NY 10027.

Type IV pili (Tfp) of pathogenic *Neisseria* are required for cell adhesion, DNA transformation and twitching motility. Using laser tweezers technology, we have obtained physical evidence that the gonococcal Tfp forcefully retract. Tfp-producing diplococci actively crawled on a glass surface and adhered to form microcolonies. When laser tweezers were used to place and hold an isolated wildtype piliated (P^+) diplococcus near a microcolony, retractile forces pulled the bacterium toward the microcolony. Such retractile forces were not observed with a P^+ *pilT* null mutant. In quantitative experiments, Tfp of immobilized bacteria were bound to latex beads and the force of Tfp retraction was measured. Retraction pulled beads from the laser trap at forces that can exceed 80 pN. Episodes of retraction terminated with release or breakage of the Tfp tether. Both Tfp-mediated motility and Tfp retraction occured at ~1 μm/sec and required protein synthesis and PilT function. Our experiments establish that Tfp filaments retract, generating substantial force and directly mediating cell movement. The significance of Tfp retraction to cell adhesion will be discussed.

#150

THE CGM1a (CEACAM3/CD66d) MEDIATED PHAGOCYTIC PATHWAY OF *NEISSERIA GONORRHOEAE* EXPRESSING OPACITY (OPA) PROTEINS IS ALSO THE PATHWAY TO CELL DEATH

Tie Chen*, Silvia Bolland†, Ines Chen¶, James Parker¶, Milica Pantelic*, Fritz Grunert§ and Wolfgang Zimmermann§
*Department of Microbiology, Immunology and Medicine, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202, USA
¶Laboratory of Bacterial Pathogenesis and Immunology, †Laboratory of Molecular Genetics and Immunology, The Rockefeller University, 1230 York Ave, New York, NY 10021, §Institute of Molecular Medicine and Cell Reasearch, University of Freiburg, D-79104 Freiburg, Germany

Abstract
Phagocytosis of Opa^+ *Neisseria gonorrhoeae* by neutrophils may be dependent on the interaction of Opa proteins with CGM1/CD66d (CEACAM3), a neutrophil specific receptor. However, the signalling pathways leading to phagocytosis have not been characterized. Here we show that interaction of Opa1 bacteria with neutrophils or CGM1a-transfected DT40 cells induces calcium flux, which correlates with phagocytosis of bacteria. We identified an immunoreceptor tyrosine-based activation motif (ITAM) in CGM1a, and showed that the ability of CGM1a to transduce signals and mediate phagocytosis was abolished by mutation of the ITAM tyrosines. We also demonstrated that CGM1a-ITAM-mediated bacterial phagocytosis is dependent on Syk, BTK and PLC-r activity in DT40 cells. Unexpectedly, the activation of the CGM1a-ITAM phagocytic pathway by Opa^+ GC results in induction of cell death.

#151

PRO-APOPTOTIC VDAC-LIKE PORIN (PORB) OF *NEISSERIA GONORRHOEAE* IS TARGETED TO THE MITOCHONDRIA OF INFECTED CELLS

Meyer TF, Müller A and Rudel T. Max Planck Institute for Infection Biology, Department of Molecular Biology, Monbijoustr.2, 10117 Berlin, Germany

The porin (PorB) of *Neisseria gonorrhoeae*, the causative agent of gonorrhea, is an intriguing bacterial factor because of its ability to translocate into host cells and mediate the cytotoxic properties of this gram negative bacterial pathogen. We have reported earlier that a transient Ca^{2+} influx as well as the subsequent activation of the Ca^{2+} dependent protease calpain and proteases of the caspase family are key events in the induction of apoptosis by purified porin. Meanwhile a central role for the mitochondria as the orchestrators of apoptosis has been firmly established and several regulatory factors located to the mitochondria have been shown to have bacterial homologues. The typical apoptotic features seen at the mitochondrial level, such as cytochrome C release, loss of membrane potential and the inhibitory capacity of members of the Bcl-2 family can also be observed in porin induced apoptosis. Surprisingly, the same effects can be triggered by addition of porin to isolated mitochondria. Moreover, porin is specifically targeted to the mitochondria of porin treated as well as *Neisseria* infected cells. This targeting also occurs if porin is transiently expressed in HeLa cells. We speculate that the neisserial porin constitutes a deregulated component of the permeability transition pore, a protein complex that plays a major role in the regulation of apoptosis.

#152

NEISSERIAL PORIN INTERACTS WITH MITOCHONDRIA AND PROTECTS CELLS FROM APOPTOSIS

Paola Massari , Yu Ho and Lee M. Wetzler
Evans Biomedical Research Center, Boston Medical Center, Boston University School of Medicine, Boston, MA

The major outer membrane proteins of the pathogenic Neisseria, termed porins or Por, act as B cell mitogens and immune adjuvants. To explore the possible mechanisms of their immunostimulatory activity related to apoptosis and the specific interactions of Por with eukaryotic cells, meningococcal PorB interaction with lymphocytes was examined. Cells were treated with purified PorB and the porin was detected in association with membrane fraction components, specifically with isolated mitochondria. Utilizing co-immunoprecipitation experiments, an interaction of PorB with mitochondrial porin, VDAC, was detected. Apoptosis was induced by treatment with staurosporine and the effect of PorB on parameters affected by apoptosis was examined. Preincubation of cells with PorB reduced the loss of mitochondrial volume and membrane potential ($\Delta\Psi_m$). Loss of $\Delta\Psi_m$ leads to release of cytochrome *c* followed by activation of caspases involved with apoptosis and DNA degradation. PorB pre-incubation of cells reduced the release of cytochrome *c* into the cytosol, reduced the activation of caspases 9 and 3 and decreased the extent of DNA degradation induced by staurosporine. The interaction between PorB and mitochondrial VDAC and the anti-apoptotic effect of PorB are similar to what occurs between VDAC and anti-apoptotic members of the Bcl-2 family and could explain these findings. Moreover, the anti-apoptotic effect of Neisserial porins could potentially play a role in the adjuvanticity of this protein.

This work was supported by NIH/NIAID grant AI40944.

#153

CLASS A SCAVENGER RECEPTOR MEDIATES NON-OPSONIC INTERNALISATION OF *NEISSERIA MENINGITIDIS* BY HUMAN MACROPHAGES

Abdillahi F, Jack D, Klein N, Steeghs L, Dower SK, Read RC.
Sheffield University Medical School, UK

Objective: To measure binding and internalisation of *N. meningitidis* by human macrophages in the absence of opsonins, and the role of the scavenger receptor in this interaction.

Methods: Human monocyte-derived macrophages (MDM) were isolated by density centrifugation and cultured for 12 days prior to inoculation with strains of *N. meningitidis*. At intervals over 3h incubation, cells were fixed and the binding and internalisation of *N. meningitidis* was measured using fluorescence microscopy.

Results: Binding of opsonised *N. meningitidis* was 2-fold greater than non-opsonised organisms; when binding was equalised by modifying the multiplicity of infection, non-opsonised *N. meningitidis* were internalised less efficiently ($n = 6$, $P<0.05$). Non-opsonic internalisation of the wild-type *N .meningitidis* was 4-fold more efficient than of an LPS-deficient isogenic mutant ($n = 8$, $P <0.01$). Therefore, function of the Class A scavenger receptor of MDM (a receptor responsible for LPS clearance) was inhibited using poly(I); this did not inhibit non-opsonic-binding of wild-type *N. meningitidis* to human macrophages, but subsequent internalisation was reduced to 34% of control ($n = 5$, $P <0.01$). Poly(I) did not influence opsonic internalisation, or internalisation of the LPS-deficient mutant.

Interpretation: Both binding, and internalisation, of *N. meningitidis* by human macrophages is less efficient in the absence of opsonins. The Class A Scavenger Receptor contributes to non-opsonic internalisation of *N. meningitidis* by human macrophages.

#154

ISOLATION OF A SEROGROUP 29E MENINGOCOCCAL STRAIN CARRYING GROUP-C *siaD* CAPSULAR GENE.

Ala'Aldeen DAA[1], English A[2], Neal K[1], Gascoyne-Binzi D[2], Hawkey P[2].
[1]Meningococcal Research Group, Microbiology, University Hospital, Nottingham, and [2]Department of Microbiology, Leeds University, UK

Capsular expression in *Neisseria meningitidis* is phase variable. In a major cohort study on meningococcal carriage, we demonstrated that the organism can switch its capsular expression (off and on) *in-vivo* (1). Recent studies have also suggested that strains can swap capsules as a result of allelic exchange of the polysialyltransferase gene. Here we demonstrate that additional siaD genes can naturally be acquired, horizontally, without the loss of the host allele.

In 1997, we isolated a serologically non-groupable (NG) meningococcal strain, from a persistent carrier. All NG strains were screened by PCR for Group B and C *siaD* capsular genes (100% specific). The PCR test genogrouped the isolate as Group C. However, subsequent isolation of the same strain (PFGE-confirmed) from the same carrier showed that the isolate expresses serogroup 29E capsule (29E:NT:P1.5), while continues to carry Group C *siaD*. The PCR-amplified *siaD* fragment from this strain was sequenced and was identical to the published Group C *siaD* gene.

The capsule-biosynthesis genes of serogroup 29E have not been sequenced and no PCR based genogrouping test is available for this Group. Therefore, explanation for these results will remain as a matter of speculation. However, it is most likely that this isolate is a 29E strain, and has horizontally acquired the *siaD* gene of a Group C strain which may or may not be functional. The reverse could also be true although unlikely.

Conclusion: Meningococci are capable of acquiring/possessing more than one capsular genes. These will have important implications on the long term efficacy of capsular polysaccharide-based vaccines.

1. Ala'Aldeen *et al* (2000), *J Clin Microbiol.* 38, 2311-6

#155

THE VIABILITY OF AN *lpxA* NEISSERIA MUTANT CORRELATES WITH AN OPACITY PHENOTYPE EXPRESSION

Albiger B and Jonsson AB. Microbiology and Tumorbiology Centre (MTC). Karolinska Institutet, 171 77 Stockholm, Sweden.

The *lpxA* gene encodes for a protein responsible of the first step of lipid A biosynthesis pathway, by adding the O-linked 3-OH fatty acid to UDP-N-acetylglucosamine. The endotoxic shock is mediated via the lipid A of LOS, which interacts with various serum components that act to neutralize or enhance its endotoxic properties. This endotoxic shock is characterized by an activation of macrophages and by the production of a diverse array of cytokines. Meningococcal septic shock is a direct result of the overstimulation of this response and is characterized by hypotension, organ failure, and death.

We have constructed a viable *lpxA* deficient mutant of *Neisseria mengitidis* FAM20 (serogroup C), by knocking out the *lpxA* allele in FAM20 wild type with a kanamycin resistance cassette. The KnR mutants isolated were shown to be deficient in the biosynthesis of any LOS and they showed unexpected characteristics.

The inactivation of the *lpxA* gene was always correlated with a strong opacity phenotype. By comparison of the outer-membrane proteins composition of both wild type and *lpxA* mutant, preliminary data show that the opacity phenotype observed is not associated to an overexpression of Opa proteins but to an overexpression of a 28 kDa protein, identified as peroxiredoxin 2 familly protein. The correlation between the opacity phenotype of the *lpxA* mutant and the overexpression of this protein and the role of this protein in the viability of the *lpxA* mutants are under investigation.

Futhermore, these mutants showed no abilities to adhere or invade different epithelial cell lines and these results raise the possibility to use this endotoxin free strain as a vaccine producer.

#156

GONOCOCCAL PILC1 IDENTIFIED AS THE ADHESIN INVOLVED IN THE INITIAL BINDING TO HUMAN CD46

Albiger B and Jonsson AB. Microbiology and Tumorbiology Center (MTC). Karolinska Institutet, 171 77 Stockholm, Sweden.

A key factor in *Neisseria* pathogenesis is the ability of the bacteria to attach to human host cell receptors. It has been shown that expression of the type IV pili of pathogenic *Neisseria* is essential for adherence. CD46 (also called membrane cofactor protein, MCP) has identified as the pilus receptor. We are currently investigating the pilus domain that interacts with the pilus receptor. We found that purified recombinant CD46, produced from both *E. coli* and CHO cells, bound to the 110 kDa PilC protein on SDS-PAGE of separated pili preparation. It has been postulated that PilC, a minor 110 kDa pilus-associated protein acts as an adhesin for initial attachment of the bacteria to the human cells. To demonstrate that PilC interacts with CD46 in the pilus-mediated adherence, we have generated a full-length gonococcal PilC1 Histag fusion protein and three different PilC1 fusion proteins covering the different regions of the complete PilC1 protein. We have used these purified recombinant proteins to demonstrate the direct interaction between purified PilC1 and CD46. We have shown that a direct interaction between purified recombinant PilC1 and recombinant CD46 and that both amino terminal and carboxyl terminal region of PilC1 are involved in the binding to CD46. We can postulate that the PilC1 binding-domain to CD46 forms a putative three-dimensional structure. Taken together, our data show that the gonococcal PilC1 is the adhesin involved in the initial binding of pathogenic *Neisseria* to target cells.

#157

Characterisation of a putative type III restriction-modification system in *Neisseria meningitidis*

Al-Bulushi I, Turner DPJ, Kizil G, Ala'Aldeen DAA.
Division of Microbiology and Infectious Diseases, University Hospital, Nottingham, UK.

Introduction: A 2.8kb ORF was identified in *Neisseria meningitidis* by screening of a genomic library with rabbit antiserum raised against meningococcal insoluble proteins [1]. The predicted peptide sequence showed features consistent with a periplasmic protein.

Objective: To determine in vivo expression and functionality.

Results: Significant homology was found between the ORF and several Res protein subunits of type III restriction-modification (R-M) systems including HinfIII in *Haemophilus influenzae*. The ORF was hence designated R-M system restriction enzyme (RmsR, Accession No AJ010114). The predicted peptide sequence derived from an ORF upstream of and overlapping RmsR was found to have strong homology to the corresponding Mod protein subunits of the same R-M systems. This ORF has been designated R-M system modification enzyme (RmsM). In MC58, RmsM contains 20 tetranucleotide repeats, similar to the modification enzyme genes in other organisms. RmsR was cloned from MC58 and work is in progress to produce a deletion mutant for function studies.

Conclusion: Based on sequence homology RmsR and RmsM are the respective restriction and modification components of a novel type III R-M system in *N. meningitidis*. The whole operon is likely to be phase-variable.

Reference: [1] Kizil et al. Infect Immun **67**: 3533-3541.

#158

THE PILUS-INDUCED CA2+ FLUX INCREASES THE AMOUNT OF LAMP1 ACCESSIBLE TO THE IgA1 PROTEASE

Ayala, B.P., Vasquez, B., Clary, S., So, M.
Dept. of Molecular Microbiology and Immunology, L220 Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098

The IgA1 protease secreted by the pathogenic *Neisseriae* cleaves Lamp1, a major integral membrane glycoprotein of lysosomes, and significantly reduces its steady state levels in an infected cell. IgA1 protease hydrolysis of Lamp1 is inefficient at the low pH of lysosomes, strongly suggesting that the enzyme is unlikely to reduce Lamp1 levels within lysosomes to any appreciable extent. We therefore explored the possibility that the protease may reach Lamp1 through an alternate route. We demonstrate that Neisserial pili induce a transient increase in the levels of cytosolic free Ca2+ in A431 human epithelial cells, as was demonstrated previously for ME180 cells (Kllstrom et al. 1998. J Biol Chem. 273:21777). This Ca2+ flux triggers lysosome exocytosis, quickly altering the cellular distribution of Lamp1 and increasing surface Lamp1 levels. Finally, we demonstrate that surface Lamp1 is cleaved by IgA1 protease secreted by adherent bacteria. We conclude that the pilus-induced Ca2+ flux serves to increase the amount of Lamp1 accessible to the IgA1 protease.

#159

PHASE VARIATION IN MENINGOCOCCAL LIPOPOLYSACCHARIDE BIOSYNTHETIC GENES

Berrington AW, Tan Y-C, Srikhanta Y, Kuipers B, van der Ley P, Jennings MP
Dept of Microbiology and Parasitology, University of Queensland, St Lucia, Qld, Australia

Neisseria meningitidis expresses a diverse range of LPS structures, comprising 12 immunotypes (ITs). A feature of meningococcal LPS is the reversible, high-frequency switching of expression (phase variation) of terminal LPS structures. This allows switching between ITs, which is proposed to have functional significance in disease. The objective of this study was to investigate the role of phase variable expression of LPS biosynthetic genes in the pathogenesis of meningococcal disease.

Molecular investigation of the LPS biosynthesis genes in a large collection of *N. meningitidis* strains was performed, using Southern blotting to detect the presence of the known LPS biosynthetic genes. If the phase-variable genes *lgtA*, *lgtC* or *lgtG* were present, they were amplified by PCR and the homopolymeric tracts responsible for their phase variable expression sequenced. The association of the phase variation repertoire with virulence was studied in strains that had been assigned to lineages of greater or lesser virulence by other workers. Further, the importance of phase variation in the pathogenesis of invasive disease was studied in a clinical collection comprising sets of isolates from different body compartments in individual patients with invasive disease.

The results of the phase variation repertoire study revealed the potential for variation between alternate LPS structures in a large set of isolates. The structures predicted to be expressed were confirmed by conventional immunotyping. These data extend our previous studies, performed on a limited collection of LPS immunotype typing strains. No strong correlation was observed between the structural repertoire and virulence. In the clinical collection, one patient demonstrated a phase variation event that altered LPS expression between compartments. In all other cases the colonising organisms isolated from different compartments expressed the same LPS structure. Therefore there was little evidence that phase variation of terminal structures is a prerequisite for the translocation of meningococci between compartments.

#160

OPA PROTEIN BINDING TO CEACAM (CD66) RECEPTORS REQUIRES SPECIFIC SETS OF HYPERVARIABLE REGIONS

Bos MP, [1,2] Kao D[1], Hogan D[1], Belland RJ [1]
[1]LMSF, Rocky Mountain Labs, NIAID, Hamilton, MT
[2]Dept. of Molecular Microbiology, University of Utrecht, 3584 CH Utrecht, The Netherlands

Opa proteins are adhesins that bind to heparan sulfate proteoglycan (HSPG) or CEACAM receptors on human host cells. Development of compounds that interfere with this binding as a strategy to prevent disease requires a detailed understanding of Opa-receptor interactions. Towards that goal we studied the Opa protein domains involved in CEACAM binding. Opa proteins contain 4 extracellular loops of which one is semi-variable (SV), 2 are hypervariable (HV-1, HV-2) and one is highly conserved. We focussed on three Opa proteins of gonococcal strain MS11: OpaB, OpaC and OpaI that contain very different SV and HV regions. but all bind a broad spectrum of CEACAM receptors. We constructed mutant Opa genes with extracellular loop deletions, and after expression in MS11 tested these in infection assays with CEACAM transfected HeLa cell lines. Deletion of the SV region did not influence CEACAM receptor binding. In contrast, deletions of either the HV-1 or HV-2 region almost completely abolished receptor recognition. We were unable to test deletion of the conserved 4th loop, since this deletion resulted in a significant decline in Opa expression level. To understand the role of individual HV regions in receptor binding, we constructed chimeric Opa variants containing combinations of HV regions of OpaB, C and OpaI. Surprisingly, none of the chimeric variants recognized CEACAM receptors to the extent the parent Opa's did. We conclude that both HV loops have to act in a very coordinate manner to form a binding domain for CEACAM. In addition, some chimeric Opa proteins showed HSPG binding, while the parent Opa's did not. Thus, Opa proteins may contain latent HSPG binding activity.

#161

MUTAGENESIS OF GENES FOR PEPTIDOGLYCAN-DERIVED CYTOTOXIN PRODUCTION AND RELEASE

Cloud KA Dillard JP Dept. Medical Microbiology and Immunology University of Wisconsin, Madison, WI 53706 USA

Peptidoglycan (PG) is an important *Neisseria gonorrhoeae* virulence factor. Gonococci release PG fragments during growth, the major fragment being the1,6 anhydro-disaccharide tetrapeptide monomer (PG-cytotoxin). PG-cytotoxin kills ciliated fallopian tube cells and induces IL-1 and IL-6 production, arthritis, and slow wave sleep. We are characterizing the roles of two putative PG transglycosylases (AtlA and Slt70) and a predicted PG fragment transporter (AmpG) in PG-cytotoxin production and release. AtlA is significantly similar to bacteriophage PG transglycosylases. An AtlA truncation mutant is reduced in PG-cytotoxin production by 60% indicating a reduction in PG-transglycosylase activity and suggesting that other enzymes are also involved in PG-cytotoxin production. Slt70 is a soluble lytic transglycosylase in *E. coli*; sequence analysis of the gonococcal genome revealed a sequence with significant similarity to *E. coli slt70*. We created gonococcal mutants containing a 900-bp internal deletion in this gene using a *rpsL*[5]*ermC* cassette following the methods of Johnston and Cannon. In *E. coli*, AmpG internalizes PG monomers. In *E. coli ampG* mutants, PG fragments are released. The gonococcal AmpG homologue is 70 amino acids shorter than the *E. coli* AmpG. Thus, the *N. gonorrhoeae* AmpG may be inactive in PG transport. We are characterizing gonococcal strains containing *E. coli ampG* and a gonococcal *ampG* knockout in order to compare PG turnover and release. Using gel filtration chromotography, we are characterizing and quantitating PG fragments released by *atlA*, *slt70* and *ampG* mutants.

#162

EFFECT OF REPRODUCTIVE HORMONES ON NEISSERIA GONORRHEA AND HUMAN ENDOMETRIAL CELLS

Deal, CD, Miller, T, and Hu, L. Div of Bacterial, Parasitic, and Allergenic Products, CBER, FDA, Bethesda, MD.

Objective: To determine the effect of reproductive hormones, such as estradiol, on human endometrial cells and their use in the study of gonococci pathogenesis.
Design: Hec1B cells were cultured with various concentrations of estradiol to demonstrate estrogen responsiveness and to evaluate adherence of gonococci to cells.
Results: Phenol red has a weak estrogen effect on estrogen-responsive tissue culture cells. To eliminate this variable, Hec1B cells were grown in phenol red free MEM media. Supplementation of media with phenol red or 17-beta-estradiol water soluble (Sigma) stimulated Hec1B cell growth, with 10^{-8} and 10^{-9}M estradiol appearing optimal; physiological hormone levels compared to estrogen variation during the menstrual cycle. As observed for other cells derived from reproductive tract tissue, estradiol grown cells reacted with monoclonal antibody to the 67kDa human estrogen receptor. Addition of estradiol to standard GC media did not alter gonococcal growth rates. Growth of Hec1B cells in estradiol containing media increased observed gonococcal adherence and outer membranes from these gonococci displayed additional protein bands in the 60kD range on SDS-PAGE. Studies on the mechanism of these changes are in progress.
Conclusions: Estradiol containing media altered Hec1B cell growth and varied the adherence of gonococci, suggesting tissue culture systems for the study of pathogenesis may want to incorporate a hormone parameter.

#163

POPULATION DYNAMICS OF GONOCOCCI DURING EXPERIMENTAL HUMAN INFECTION: ANALYSIS OF PILIN VARIATION REVEALS SELECTIVE BOTTLENECKS

Dempsey JAF, Hoffman N, Cohen MS, Cannon JG. University of North Carolina, Chapel Hill, NC, USA

For most mucosal pathogens of humans, virtually nothing is known about the population biology of the bacteria as they interact with mucosal surfaces and host defenses during the period before signs or symptoms of infection appear. The human challenge model of gonococcal (GC) urethritis provides a unique opportunity to study this period in infection. We infected 3 subjects each with strains FA1090 and MS11. After inoculation, urine specimens were cultured daily until the subjects developed urethral discharge; they were then treated. To track the appearance or disappearance of different clonal lineages of GC, we characterized the pilin variants expressed in individual colonies growing on primary isolation plates. The number of pilin sequences a strain can express is so large that two colonies with identical pilins most probably grew from GC that were clonally derived from a single ancestor. We analyzed 50 colonies from each day for each subject, using a pilin gene fingerprinting technique supplemented by DNA sequencing.

Apparent bottlenecks in the GC population, leading to the outgrowth of a population composed predominantly of one new pilin type, occurred in all 6 subjects. However, no single pattern occurred in all of them. In one subject, there were 3 selective bottlenecks within the first 4 days (he had a urethral discharge on day 4); in others, there appear to have been only 1 or 2 such bottlenecks before the subject developed urethritis. In general, the GC population became more complex, consisting of a mixture of multiple different pilin variants, at the time a subject developed a urethral discharge.

Based on these results, we hypothesize that one GC with the right set of adhesins can be sufficient to cause infection in a human host, and that there are multiple stages at which the GC population is reduced by host defenses to one or a few organisms. Those survivors proliferate, generating a clonal population sharing new surface features. The selective pressures responsible for these population bottlenecks are not known, although the rapid timing makes it unlikely that the pressures are acquired antibody responses.

#164

THE INTERACTION BETWEEN TRANSFERRIN AND TRANSFERRIN BINDING PROTEIN B

Derekh U[1], Krewulak K[1], Yu R-H[2], Tari LW [1] and Schryvers AB[2], Department of Biological Sciences[1] and Department of Microbiology and Infectious Diseases[2], University of Calgary, Calgary, Alberta, Canada T2N 4N1

The transferrin (Tf) receptor in pathogenic *Neisseria* is comprised of an integral outer membrane protein, TbpA, and a largely surface exposed lipoprotein TbpB. To characterize the interaction between TbpB and hTf, recombinant forms of TbpB and its N-terminal and C-terminal subfragments were produced in *E. coli* from a novel expression vector that provides a consensus biotinylation peptide and polyhistidine region fused to the N-terminus. This provides a readily purified form of TbpB that can be used in a variety of different assay formats. Dynamic light scattering analysis demonstrated that the preparations of recombinant TbpB were homogenous and affinity capture experiments demonstrated that the recombinant proteins were functionally homogenous. The recombinant proteins were tested for their ability to form complexes with intact Tf and proteolytically-derived N-lobe and C-lobe subfragments by dynamic light scattering and affinity capture experiments. Dynamic light scattering experiments demonstrated that a homogenous 1:1 complex was formed between hTf and TbpB at moderate protein concentrations and that a homogenous heterotetramer was formed with high protein concentrations. These results indicate that we can readily prepare homogeneous preparations of TbpB and TbpB:hTf complexes that are suitable for crystallization studies. A suite of biochemical and biophysical techniques, including dynamic light scattering, are being used to monitor the lobe-lobe interactions between TbpB and hTf as well as subfragments of both proteins.

#165

REMOVAL OF IRON FROM HUMAN TRANSFERRIN BY BACTERIAL TRANSFERRIN RECEPTOR PROTEINS.

De Winter LM, and Schryvers, AB. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Neisseria meningitidis and *Moraxella catarrhalis* are capable of using iron-loaded transferrin as a sole source of iron for growth. Although it has been demonstrated that this process is mediated by the outer membrane receptor proteins, transferrin binding protein A (TbpA) and transferrin binding protein B (TbpB), little is currently known about the iron removal process. Most growth experiments have been performed with diferric transferrin (iron in both the N-lobe and C-lobe iron binding sites), even though monoferric forms of transferrin are the most prevalent forms available *in vivo*. In an attempt to gain further insights into the iron removal process, the ability of wild-type *N. meningitidis* and *M. catarrhalis* and isogenic *tbpA, tbpB* and *tbpAB* mutants to remove iron from different human transferrin preparations was investigated. In these studies the iron status of transferrin in bacterial cultures was monitored by urea PAGE, which is capable of resolving the four forms of human transferrin (apo, monoferric C-lobe loaded, monoferric N-lobe loaded, and diferric). Urea PAGE analysis demonstrates the TbpA and TbpB-mediated removal of iron from diferric transferrin giving rise to apo transferrin in growing cultures of *N. meningitidis* and *M. catarrhalis*.

#166

Rho GTPases are required for the internalization of piliated-capsulated *Neisseria meningitidis* inside endothelial cells: a process associated with meningeal invasion.

Emmanuel Eugène, Isabelle Hoffman, Céline Pujol, Pierre-Olivier Couraud, Sandrine Bourdoulous, and Xavier Nassif.

Neisseria meningtidis is a human pathogen responsible for septicemia and meningitis. *In vivo* observations suggest that internalization of piliated-capsulated meningococcal strains is essential in meningeal invasion. We observed *in vitro* that following pilus-mediated adhesion, capsulated meningococci induced, within endothelial cells, the localized formation of focal plaques characterized by actin polymerization and recruitment of several transmembrane proteins and ezrin, but not of vinculin or paxillin. These modifications lead to the transient formation of membrane protrusions which can lead to bacterial engulfment inside the cells. Pretreatment of the endothelial cell monolayer by the *Clostridium* toxin ToxB, an inhibitor of GTPases of the Rho family, dramatically reduced actin polymerisation at the site of bacterial interaction without preventing the recruitment of ezrin or transmembrane proteins underneath the bacteria. These data suggest that Rho GTPases are essential for actin polymerization, while they are not involved in ezrin or transmembrane protein recruitment. Using dominant negative mutants of Rho GTPases and selected inhibitors, we showed that actin polymerization process required the activation of both RhoA and Cdc42 but not of Rac. Furthermore bacterial invasion was inhibited by incubation of the endothelial cell monolayer with ToxB or Y27632, a specific inhibitor of the Rho kinase. We propose that following pilus mediated adhesion, *N. meningitidis* recruits ezrin at the site of bacterial attachment and that both RhoA and Cdc42 are critical for subsequent actin polymerization necessary for bacterial internalization.

#167

EXPRESSION OF HUMAN β-DEFENSINS IN EPITHELIAL AND MENINGEAL CELLS FOLLOWING CHALLENGE WITH NEISSERIA MENINGITIDIS.

Fowler MI, Deng Y, Pickett, MA, Heckels JE, Christodoulides M. Molecular Microbiology Group, University of Southampton Medical School, Southampton, UK.

Human β-defensins (HBD) are small, cationic peptides with broad-spectrum antimicrobial activity. They are produced by various tissue epithelial cells where they contribute to local innate host defence. The aims of this study were to investigate and compare the expression of HBD-1 and HBD-2 mRNA in epithelial and meningeal cells, and to determine the effects on expression of challenge with meningococci and other bacterial pathogens that cause meningitis.

Semi-quantitative TaqMan RT-PCR assays were developed to detect the presence and relative abundance of HBD-1 and HBD-2 mRNA transcripts in epithelial (Chang) and meningeal (meningioma) cells. HBD-1 mRNA expression was constitutively expressed to high levels by Chang epithelial cell cultures and to a lesser extent by meningeal cell cultures. In contrast, In HBD-2 mRNA was not constitutively expressed by these cell cultures.

Epithelial and meningeal cell cultures were subsequently challenged *in vitro* with both pathogenic and commensal *Neisseria* spp. and other bacterial pathogens including *Haemophilus* and *Streptococcus* spp. Challenge with meningococci appeared to modulate the expression of HBD-1 mRNA expression from both Chang and meningeal cells, but did not stimulate *de novo* HBD-2 transcription.

These results suggest that meningococci may alter the innate defence mechanisms of host cells with important consequences for disease progression.

#168

INVASION OF HUMAN FALLOPIAN TUBE EPITHELIUM BY ESCHERICHIA COLI EXPRESSING POR AND/OR OPA IS INHIBITED BY THE 3F11 LOS EPITOPE.

Gorby GL[a,b], Ehrhardt AF[b], Apicella MA[c], Elkins C[d]. [a]Omaha VAMC, and [b]Dept. Med. Micro. Creighton University SOM, Omaha, NE; [c]Dept. Micro., Univ. of Iowa COM, Iowa City, IA; [d]Dept. Micro. and Immuno., Univ. of N. Carolina, Chapel Hill, NC.

Objective: To determine the role of Por, Opa, and the 3F11 LOS epitope constitutively expressed in *E. coli* and tested in the human fallopian tube organ culture model (FTOC).

Design: Opa (F62-SFG1), Por (Por-$1_{(FA19,\ A)}$), and the 3F11 LOS epitope were expressed in combination utilizing pGEM2 and pACYC184 vectors in *E. coli* DH5alpha. Computerized microscopic image analysis of human FTOCs infected with these variants was used to measure attached, intracellular, and submucosal bacteria (n=1308 images). Measured bacterial areas in these regions were divided by the corresponding epithelial or submucosal area measured to normalize the data. ANOVA was used for statistics.

Results: Opa-producing variants consistently showed statistically larger areas of attachment ($p<0.01$), and Por-producing variants showed the greatest degree of submucosal invasion ($p<0.01$). The ratios of the attached, intracellular, and submucosal area of each variant compared to the *E. coli* control are seen in the table.

Region	Opa	Por	LOS	Opa/Por	Opa/LOS	Por/LOS	All 3	Cntrl
Attached	44.8	6.01	1.01	21.8	6.41	1.88	20.5	1.00
Inside	37.3	21.8	0.53	17.2	1.27	1.49	24.4	1.00
Subepi	2.95	579	0.26	18.7	0.21	0.25	71.5	1.00

Conclusions: Opa enhances *E. coli* attachment to and invasion of fallopian tube epithelium. Por dramatically enhances invasion. The 3F11 LOS epitope markedly reduces attachment and invasion mediated by Opa and Por, although this effect can be partially overcome when all three factors are expressed.

#169

THE USE OF INSERTION-DUPLICATION MUTAGENESIS TO STUDY A PUTATIVE TYPE IV SECRETION SYSTEM ENCODED IN THE PATHOGENICITY ISLAND OF *NEISSERIA GONORRHOEAE*

Hamilton HL, Schwartz KJ, Dillard JP. Dept. of Med. Micro. & Immunol. University of Wisconsin-Madison, Madison, WI 53706 USA

A system of plasmids was created for use in insertion-duplication mutagenesis (IDM) of *Neisseria gonorrhoeae*. Using this system, we are studying the role of several genes within the gonococcal pathogenicity island (GcPI). IDM has the advantage over other methods of mutagenesis in that it only requires a single cloning step prior to transformation into gonococci. Chromosomal DNA cloned into the IDM plasmids directs insertion into the chromosome at the site of homology by a single crossover recombination event. Using various chromosomal DNA insert sizes, we have determined a correlation between the size of the insert and the frequency of gonococcal transformation. In addition, we have shown that even an insert as small as 290 base pairs can target an insertion in the gonococcal chromosome. We have used IDM to create knockouts in two genes in the GcPI, *traG* and *traH*. Both *traG* and *traH* are homologous to genes of a type IV secretion system, with greatest similarity being to the *E. coli* F-plasmid transfer genes. TraG is a predicted inner membrane protein that may interact with other proteins to form a secretion channel, while TraH is predicted to be a periplasmic protein of unknown function. Type IV secretion systems are characterized by their unique ability to transfer both DNA and protein molecules. Therefore, the gonococcal *traG* and *traH* knockouts were assayed for their ability to secrete DNA into the extracellular medium. Data from these experiments show that both the *traG* and *traH* mutations decrease DNA secretion by gonococci during growth, suggesting that TraG and TraH play a role in a novel type IV secretion system in *N. gonorrhoeae*.

#170

A SPECIFIC INTERACTION BETWEEN THE TERMINAL LACTO-N-NEOTETRAOSE ON GONOCOCCAL LIPOOLIGOSACCHARIDE AND THE HUMAN ASIALOGLYCOPROTEIN RECEPTOR ALLOWS ADHERENCE AND INVASION OF PRIMARY URETHRAL EPITHELIAL CELLS

Harvey HA[1], Jennings MP[2], Williams RD[3], Apicella MA[1], [1]Dept. of Microbiology, Univ. of Iowa, Iowa City, IA, [2]Dept. of Microbiology and Parasitology, Univ. of Queensland, Brisbane, AUS, [3]Dept. of Urology, Univ. of Iowa Hospitals and Clinics, Iowa City, IA.

Objective: One goal of these studies was to determine the importance of lacto-N-neotetraose-terminal lipooligosaccharide (LOS) structure in gonococcal invasion of primary urethral epithelial cells (uec). These studies also explored the possibility that this structure binds the human asialoglycoprotein receptor (ASGP-R) and that this interaction contributes to internalization of gonococci into host cells.

Design: Invasion assays were performed using the previously described primary human urethral epithelial cell tissue culture system. Invasion assays were performed with *Neisseria gonorrhoeae* strain 1291 and four mutants derived from this strain which express truncated LOS. Invasion assays were also performed in the presence of two purified ASGP-R agonists, asialofetuin (ASF) and gonococcal LOS.

Results: Invasion assays showed the parent strain 1291 invades uec at significantly higher levels than the following four strain 1291 mutants expressing truncated LOS: *lgtB* ($P<.0001$), *lgtA* ($P=.0002$), *lgtE* ($P<.0001$), and *pgm* ($P=.0002$). Preincubation of uec with ASF resulted in significantly reduced gonococcal invasion of uec ($P=.0046$). When cells were preincubated in increasing concentrations (1µg/ml-500µg/ml) of purified detoxified strain 1291 LOS, a dose response reduction in gonococcal invasion was observed ($P=.04-<.0001$).

Conclusions: Lacto-N-neotetraose-terminal LOS is important for gonococcal invasion of primary urethral epithelial cells through a specific interaction with ASGP-R.

#171

CEACAM-DEPENDENT EVENTS INDUCED IN TARGET CELLS BY *NEISSERIA MENINGITIDIS* AND *HAEMOPHILUS INFLUENZAE*

Darryl Hill, Jo Griffith, Silvia Villullas Rincón, and Mumtaz Virji
Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK.

Adhesion of mucosal pathogens, *Neisseria meningitidis* (Nm) and *Haemophilus influenzae* (Hi) at the apical surfaces of epithelial and endothelial cells may involve human CEA related cell adhesion molecules (CEACAMs, previously CD66). We have shown that the N-terminal domain of the receptor is sufficient for Nm interactions and is required for Hi interactions. Studies using deletion constructs of receptors have identified the additional domains required by Hi for efficient interactions. However, the binding domains overlap sufficiently to create a competitive situation for targeting the receptors. To study the consequences of these interactions, we have used transfectants expressing either CEACAM1-L or CEACAM1-S with identical extracellular domains but different cytoplasmic domains. Our studies indicate that ligation with either molecule leads to similar early events in target cells and are indistinguishable between Nm and Hi. Both bacteria induce changes within target cells that result in detergent soluble receptors to become insoluble and polymerised actin to become localised underneath bacterially induced receptor caps. A Similar situation is observed in human cell lines expressing CEACAMs. However, using polarised epithelial cells, the final outcome of Hi and Nm targeting CEACAMs appears to be distinct and traversal of polarised monolayers occurs by divergent mechanisms. The precise signalling mechanisms that determine the mode of cellular transmigration are under investigation.

#172

PILUS-MEDIATED ADHESION OF CAPSULATED *NEISSERIA MENINGITIDIS* ONTO ENDOTHELIAL CELLS ACTIVATES *SRC* TYROSINE KINASES, A PATHWAY ESSENTIAL FOR BACTERIAL INTERNALISATION

Hoffmann I, Eugène E,Nassif X, Couraud PO and Bourdoulous S.
CNRS UPR 415, ICGM, 22 rue Méchain, 75014 Paris, France

Interaction of virulent capsulated *Neisseria meningitidis* with endothelial cells occurs in a multi-step process: a pilus-mediated adhesion resulting in an initial localized adhesion is followed by the dispersion of the bacteria at the cell surface. Pilus-mediated adhesion induces RhoA- and Cdc42-dependent actin modifications leading to the localized formation of membrane protrusions and bacterial internalization. We show here that *N. meningitidis* adhesion induces tyrosine phosphorylation of the host cell protein cortactin and the concomitant activation of the protein tyrosine kinase *src*. These effects disappear during the dispersion of *N. meningitidis*. A meningococcal mutant PilT$^-$, deficient for pilus-retraction, does not disperse and induces a persistent activation of *src* and cortactin tyrosine phosphorylation, indicating that *src*/cortactin signaling pathway is specifically activated during the early stage of meningococcal-cell interaction. When phosphorylated, cortactin is recruited at the site of cortical actin polymerization within cellular protrusions underneath the bacteria. Selective inhibition of *src* activity by PP2 results in the inhibition of both cortactin phosphorylation and recruitment to membrane protrusions, while actin polymerization or ezrin recruitment in these structures are not affected. PP2 largely enhances actin stress fiber formation induced by bacterial adhesion, a process which is prevented by the selective inhibitor of Rho-kinase, Y27632. This observation suggests that *src* downregulates the Rho-dependent cytoskeleton modifications induced by bacterial adhesion. Moreover, PP2, as well as Y27632, significantly reduced *N. meningitidis* internalization into endothelial cells, both effects being additive. Taken together, these data demonstrate that activation of *src*- and Rho-dependent pathways contribute to internalization of piliated capsulated bacteria inside endothelial cells.

#173

HUMAN MANNOSE-BINDING LECTIN ENHANCES NON-OPSONIC PHAGOCYTOSIS OF MENINGOCOCCI BY MACROPHAGES

DL Jack[1,2], MW Turner[1], NJ Klein[1], RC Read[2]
[1]Institute of Child Health, University College London, [2] Division of Molecular and Genetic Medicine, University of Sheffield, United Kingdom

Background Mannose-binding lectin (MBL) is a serum collagenous lectin which recognises the sugars and sugar conformations commonly found on micro-organisms. It activates complement and may also interact directly with a number of receptors on phagocytic cells. Genetic deficiency of MBL appears to increase susceptibility to infection by *N. meningitidis*. We measured the influence of MBL upon non-opsonic phagocytosis of *N. meningitidis* by human monocyte-derived macrophages (MDMs).

Method A galE mutant of *N. meningitidis* B1940, known to bind MBL, was incubated with 5 μg/ml of the lectin. Organisms were washed and added to MDMs, cultured for 12-14 days on coverslips, at an MOI of 300:1. After 30 min at 37°C, cells were fixed and meningococcal binding to, and internalisation by, MDMs was measured by an established fluorescent microscopic technique.

Results MBL increased the median proportion of MDMs associated with one or more meningococci from 19% to 33% (p=0.05; Wilcoxon), but the median number of organisms bound per cell was not significantly increased (1.8 to 2.1 organisms per cell). The proportion of bound meningococci internalised was increased from 19% to 34 % by MBL (p=0.05). The number of organisms internalised by a population of 100 macrophages increased from 7 to 21 (p<0.02) in the presence of MBL.

Interpretation We conclude that mannose-binding lectin contributes to non-opsonic phagocytosis by facilitating increased recognition of meningococci by macrophage populations capable of phagocytosis.

#174

CEACAM IS NOT NECESSARY FOR *NEISSERIA GONORRHOEAE* TO ADHERE TO AND INVADE FEMALE GENITAL EPITHELIAL CELLS

Swanson, K. V.[1], Jarvis, G. A.[1,2], Brooks, G. F.[2], Barham, B. J.[3], Cooper, M. D.[3], and Griffiss, J. M.[1,2]
[1]Centre for Immunochemistry, VA Med. Center, and [2]Dept. of Laboratory Medicine, Univ. of California, San Francisco, CA; [3]Dept. of Medical Microbiology and Immunology, Southern Illinois Univ., Springfield, IL

Neisseria gonorrhoeae has a repertoire of up to 11 opacity-associated (Opa) proteins that are adhesins. Most Opa proteins adhere to CEACAM (formerly called CD66) antigens and when CEACAM molecules are present on the surface of transfected epithelial cells their binding by Opa is thought to induce gonococcal invasion. In this study we investigated whether several malignant epithelial cell lines, as well as normal cervical and fallopian tube epithelial cell cultures express any of the CEACAM molecules, and whether gonococci use these molecules for adherence and invasion of these female genital epithelial cells. A primary cervical cell culture and metastatic cervical cell line ME180 both expressed CEACAM as shown by whole cell ELISA and flow cytometry, and increased the surface expression of total CEACAM during incubation with Opa$^+$ gonococci. Opa$^+$ gonococci both adhered to and invaded these cells; CEACAM-specific MAb partially abolished this interaction. A primary fallopian tube cell culture, a primary cervical cell culture, and two malignant cell lines, HEC-1-B and HeLa, did not express CEACAM nor was CEACAM mRNA present. No evidence of either intracellular or secreted extracellular CEACAM was found with HEC-1-B and HeLa cells. Opa$^+$ gonococci both adhered to and invaded CEACAM non-expressing cells; however Opa$^+$ gonococcal association with these non-expressing cell lines could not be inhibited with CEACAM-specific MAb. These data show that CEACAM is not always expressed on female genital epithelial cells and is not essential for gonococcal adherence and invasion. However when CEACAM is expressed, Opa$^+$ gonococci exploit it for the adherence to and invasion of these cells.

#175

PROBING THE FBPA-FBPB INTERACTION BY HETEREOLOGOUS GENETIC EXCHANGE EXPERIMENTS.

Lam S-L, Kirby SD, and Schryvers AB, Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada T2N 4N1

The transport of iron from transferrin (Tf), lactoferrin (Lf) and other ferric iron sources requires a functional periplasm to cytoplasm ABC transport system. This system consists of a periplasmic iron binding protein, FbpA, and two proteins, FbpB and FbpC, that comprise the inner membrane transport complex. To probe the FbpA-FbpB interactions, we inititiated hetereologous genetic exchange experiments by introducing foreign *fbpA* genes into *Haemophilus influenzae* and into a reconstituted pathway expressed in *Escherichia coli*. In *H. influenzae* none of the foreign *fbpA* genes was able to reconstitute a functional pathway. However, hybrid *N. meningitidis/H. influenzae fbpA* genes, including ones that encoded only the signal peptide region from *H. influenzae*, were functional. These results led to the conclusion that, unlike *E. coli*, *H. influenzae* is not capable of properly processing proteins with foreign signal peptides. The *N. meningitidis* but not *Pasteurella haemolytica fbpA* gene was able to function with the *H. influenzae fbpB&C* genes in *E. coli* by supporting growth of an *entA* mutant on 200 uM dipyridyl. Since the intact pathways from all species tested were functional, the deficiency was attributed to the interaction between FbpA and the FbpB/C inner membrane complex. Additional experiments with hybrid and mutant FbpAs and FbpBs are being implemented in an attempt to specifically identify the sites of interaction. The available crystal structures of the *N. meningitidis* and *H. influenzae* FbpAs provide an opportunity to map the sites of interaction on the FbpA protein.

#176

NEISSERIA MENINGITIDIS CAN ACQUIRE IRON FROM CULTURED EPITHELIAL CELLS

JA Larson and M So
Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland, OR, USA

Pathogenic Neisseria have multiple means of acquiring iron from host iron binding proteins both on mucosal surfaces and within the bloodstream. We wished to determine if N. *meningitidis* has the additional ability to acquire iron from within human cells. MC 8013 has the ability to invade and grow within A431 cells. This intracellular growth can be greatly reduced after iron chelation with desferal. An isogenic *hmbR* mutant, due to its inability to utilize bovine hemoglobin, cannot grow in tissue culture media without the addition of iron. After invasion of A431 cells, the *hmbR* mutant, without iron supplementation grows at the same rate as the wildtype strain, indicating that iron is acquired from the host cell. A *tonB* mutant, which also fails to grow in media without iron supplement, did not grow within A431 cells indicating that a Tonb dependant outer membrane receptor may play a role in intracellular iron acquisition.

#177

RELATIVE INVASIVENESS OF GONOCOCCAL OPA PROTEINS IN THE HUMAN FALLOPIAN TUBE ORGAN CULTURE MODEL

Lund SJ, Carlson DJ and Gorby GL. Dept. Med. Micro., Creighton University SOM and Omaha VA Medical Center, Omaha, NE.

Objective: To determine the relative invasion-promoting potential of gonococcal Opa proteins in human fallopian tube tissue.
Design: Human fallopian tube sections were infected with an equal number mix of each Opa-expressing variant of either *N. gonorrhoeae* FA1090 or *E. coli* expressing recombinant β-lactamase/Opa fusion proteins. Intracellular bacteria were recovered following a 40 h incubation using an antibiotic/tissue homogenization assay. The Opa expression of randomly selected colonies was then determined.
Results: The overall extent of invasion by *N. gonorrhoeae* FA1090 varied with the patient from whom the fallopian tube tissue was acquired. Whereas, the degree of invasion by *E. coli* Opa$^+$ was relatively independent of tissue source throughout this study. The frequency of invasive Opa phenotypes differed somewhat with each experiment. However, preliminary analysis of early *E. coli* Opa$^+$ invasion experiments revealed the relative frequency of invasive Opa phenotypes to be approximately 19% (Opa B) and 4% (Opa E). Each of the remaining phenotype frequencies (A, C, D, F, I, J, K) hovered around the expected value of 11% (n=167). Further analysis of the *E. coli* data is in progress as is the analysis of parallel studies with *N. gonorrhoeae* FA1090.
Conclusion: This preliminary data suggests that gonococcal Opa proteins expressed in *E. coli* differ significantly in their invasion-promoting ability for human fallopian tube epithelium. In addition, invasion of this model by *N. gonorrhoeae* FA1090 may be patient specific.

#178

STRUCTURE-FUNCTION ANALYSIS OF THE PILC PROTEINS IN *N. MENINGITIDIS*

MORAND P.C. and NASSIF X.. INSERM U411, Faculté de Médecine Necker, 156 rue de Vaugirard, 75730 Paris Cedex 15, France.

Pilus-mediated adhesion to human cells is essential in the pathogenesis of *Neisseria meningitidis* (Nm). Pili are assembled from a protein subunit called pilin. Two proteins, PilC1 and PilC2, are also key elements in pilus biogenesis since the production of at least one of the PilC proteins is required for pilus assembly. In addition, PilC1 but not PilC2 modulates adhesiveness. The genes have been demonstrated to be controlled by separate promotors : *pilC2* is expressed from a single transcription starting point (TSP) whereas *pilC1* has three TSPs, PC1.1 to PC1.3. PC1.1 of *pilC1* is identical to the unique TSP of *pilC2* and the two others are located in a region upstream of *pilC1*, not found for *pilC2*. We have shown that the expression of *pilC1*, but not that of *pilC2*, is transiently induced by bacteria-cell contact.

The aim of this work was to determine whether the specific regulation of PilC1 was responsible for adhesion of Nm or if, beside regulation, PilC-mediated adhesion was confered by some specific protein pattern not present in the meningococcal PilC2.

We first engineered a PilC1$^-$ / PilC2$^+$ meningococcal strain in which: i) *pilC2* was under control of the *pilC1* promotor by replacing the ORF of *pilC1* by that of *pilC2*, ii) the original wild type *pilC2* locus was deleted. This strain expressing PilC2 at the *pilC1* locus showed a non adhesive wild type PilC2$^+$ phenotype, although expressing PilC2 in similar amounts as the PilC1$^+$ control. This result shows that beside regulation, structural differences between PilC1 and PilC2 lead to different adhesion phenotypes.

In order to locate the specific PilC1 adhesion-promoting regions, we engineered a series of meningococcal mutants expressing different PilC1-PilC2 hybrids at the *pilC1* locus. All hybrids were expressed by Nm in the same amount as in the wild type PilC1$^+$ strain and were functionally active since all strains were piliated and competent. Our results show that the adhesion-promoting regions are located in the aminoterminal region of the molecule. A single specific domain that would be responsible for adhesion could not be identified within this region, suggesting that several domains of the N-terminal part of PilC1 need to interact with each-other to promote adhesion to human cells.

#179

CLONING OF A GENE FROM *NEISSERIA GONORRHOEAE* STRAINS CAUSING PELVIC INFLAMMATORY DISEASE

Nowicki S[1,2], Nguyen K[1], Hart A[2], Urvil P[2], Nowicki BJ[1,2]. [1]Dept. Microbiol. & Immunol. and [2]Dept. Ob/Gyn, Univ. Texas Medical Branch, Galveston, TX.

Objective: To clone the complete coding region of a *sac*-4 gene fragment that was present in the majority of *N. gonorrhoeae* strains isolated from patients with pelvic inflammatory disease (PID).

Design: Gonococcal PID strains could be differentiated from local infection strains by the presence of a 0.3 kb *sac*-4-associated fragment. Multiplex restriction-site PCR (mRS-PCR) was employed to isolate sequences flanking the known sequence, whose transcription was demonstrated by RT-PCR. The acquired sequences were assembled and analyzed for open-reading frames and potential genes using the Bacterial Gene Finder program.

Results: Analysis of the DNA sequence inclusive of the 0.3 kb *sac*-4 fragment identified a putative gene, named *pid*, having a coding potential for a polypeptide of 492 amino acids. The DNA and deduce amino acid sequences showed no significant homology with any sequences in databases of GenBank and Gonococcal Sequencing Project. The protein sequence contained a putative transmembrane region in the N-terminal half, suggesting that product of *pid* gene may reside on the surface of *N. gonorrhoeae* and may play a role in the bacterial virulence.

Conclusions: We report here the isolation of the first gene that can significantly differentiate gonococcal strains associated with local infections and PID. Studies are in progress to determine whether the gene product contributes to the pathogenesis of gonococcal PID. The discovery of the *pid* gene offers a prospect of a specific diagnostic test for identification of pid-positive strains patients with increased risk of developing PID.

#180

THE ROLE OF MENINGEAL CELLS IN THE INFLAMMATORY RESPONSE TOWARDS MENINGOCOCCI AND THE NATURE OF THE CELL SURFACE RECEPTORS RECOGNISED BY MENINGOCOCCAL LIGANDS.

Partridge, K., Kaur, D., Weller, R.O., Christodoulides, M., Heckels, J.E. Molecular Microbiology, Division of Cell and Molecular Medicine, University of Southampton School of Medicine, Southampton, UK.

The interactions of meningococci with the meninges that surround and protect the brain are pivotal events in the progression of bacterial meningitis. An *in vitro* model of the meninges using human meningioma cells has been used to study the nature of the inflammatory response following bacterial challenge and the identity of putative cell surface receptors that may be recognised by meningococcal ligands.

Cell surface receptors present on meningeal cells were identified by immunocytochemistry with specific antibodies. Meningeal cells expressed CD46, the putative pilus-binding receptor, integrin subunits recognised by Opc and heparan sulphate proteoglycan recognised by some Opa proteins: in contrast, CD66 receptors recognised by Opa were expressed weakly or not at all.

The nature of the inflammatory response *in vitro* was determined by challenging meningioma cells with meningococci, and using RT-PCR to measure mRNA expression, and specific ELISA to quantify protein secretion. IL-8, IL-6, MCP-1, RANTES, and GM-CSF mRNAs were strongly up-regulated in response to infection with a corresponding high level of protein secretion. These results indicate that the meninges, in response to infection, produce cytokines that recruit immune effector cells, and therefore play a role in host defence.

#181

IDENTIFICATION AND CHARACTERIZATION OF A KDO-DEPENDENT ACYLTRANSFERASE IN PATHOGENIC NEISSERIA

Post DMB1, Phillips N2, Harvey HA1, Gibson BW2, Apicella MA1. 1Dept. of Microbiology University of Iowa, Iowa City, IA. 2 Dept. of Pharmaceutical Chemistry University of California San Francisco, San Francisco, CA.

The lipid A of pathogenic *Neisseria* plays an important role in pathogenesis. Modifications in *htrB* can alter the toxicity of this LOS component. A PCR amplicon of a region of DNA from the meningococcal genome had high homology to *E. coli htrB* (29% identity and 63% homology). A deletion/insertion mutant was made in this gene using a non-polar kanamycin cassette. *htrB* mutations were made in *N. meningitidis* strain NMB, an acapsular NMB mutant and *N. gonorrhoeae* strain 1291. Southern blot analyses showed the expected mutations in *htrB*. LOS could be isolated from the acapsular NMB*htrB* and 1291*htrB* but not from NMB*htrB*. Mass spectroscopy analysis showed that the lipid A from 1291*htrB* was pentaacyl. The biological effects of the *htrB* mutation of 1291 were studied using invasion and IL-6 release assays. Invasion assays showed that strain 1291*htrB* was significantly less ($p= 0.0368$) able to invade male urethral epithelial cells than strain 1291; however, there was no significant difference ($p=0.242$) in the two strains ability to adhere to urethral epithelial cells. Urethral epithelial cells were stimulated with different concentrations of LOS from strains 1291 and 1291*htrB* to measure IL-6 release. IL-6 production did not rise above background levels when cells were stimulated with 1291*htrB* LOS, while IL-6 production was 10-50 times higher than background levels in the challenges performed with 1291 LOS.

Our studies have shown that the 1291*htrB* LOS elicited no IL-6 response from urethral epithelial cells. It was more surprising that this lipid A mutant had a reduced ability to invade urethral epithelial cells. This difference may reflect configurational effects on the LOS oligosaccharide by lipid A. Further research should enable us to determine more clearly the role that HtrB plays in *Neisseria* pathogenesis.

#182

SURVIVAL WITHIN ADULT HUMAN NASOPHARYNGEAL (NP) MUCOSA IS A FEATURE OF PATHOGENIC *NEISSERIA*, BUT VARIES WIDELY BY HUMAN HOST

Read RC, Goodwin L, Townsend R, Maiden MCJ, Silcocks PB. Sheffield University Medical School, and Oxford University, UK.

Objective: To determine whether survival within human np mucosa is a discriminating feature of pathogenic *Neisseria*, and to measure variability between mucosa derived from different hosts.

Methods: Explants of np mucosa were cultured with epithelial surfaces exposed to humidified 5% CO_2 in air, and inoculated with *Neisseria*. At intervals over 24h incubation, washed explants were homogenised by an automated method, and viable counts recovered. To estimate bacterial penetration of mucosa, explants were exposed to 0.25% sodium taurocholate for 30s prior to homogenisation.

Results: Counts of *N. meningitidis* (ET-5) recovered from mucosa increased gradually over 24h incubation and the organism penetrated the tissue. In contrast, *N. lactamica* could be recovered at lower counts, but did not penetrate; *N. animalis* was not recovered ($n=9$, $P<0.008$). We then compared representatives of different clusters/clones of meningococci. Strains of clones ET-5, ET-37, and Lineage III were recovered from and penetrated tissue. Strains of clones A4, A:subgroup I, A:subgroup III, and A:subgroup IV-1, were recovered but did not penetrate ($n=6$). MBC s of sodium taurocholate for all strains were identical. To measure host variation, survival of *N. meningitidis* (ET-5) within np mucosa of 40 different donors was measured. Intra-class correlation of replicates was 0.97, but the co-efficient of variation of recovered viable counts was 1335% after 4h and 77% after 24h incubation.

Interpretation: In this model of human np mucosa, survival within the tissue varies between *Neisseria*. Meningococcal survival varies widely between mucosae derived from different human hosts.

#183

NEISSERIAL CARRIAGE IN HUMAN TONSILLAR TISSUE AND IDENTIFICATION OF BACTERIAL FACTORS REQUIRED FOR NASOPHARYNGEAL COLONISATION.

Sim RJ, Moxon ER, Read RC*, Tang CM. University Department of Paediatrics, John Radcliffe Hospital, Oxford, and *Division of Molecular and Genetic Medicine, University of Sheffield Medical School, UK.

Objective To define the precise cellular location of *Neisseria meningitidis* (*Nm*) during carriage, and to examine the genetic basis of colonisation using a biologically relevant model.

Design Tonsillar tissue was obtained from 32 patients undergoing tonsillectomy and examined for the presence of *Nm* by immunohistochemistry (IHC) using antibodies against PorA serosubtypes and whole bacteria. A library of 600 mutants was examined to identify those that fail to colonise the human nasopharyngeal (NP) explant model.

Results Preliminary experiments established that anti-PorA monoclonal antibodies recognise *Nm* in a sero-subtype specific fashion and do not cross-react with commensal species. *Nm* was detected by IHC in 14 of the 32 patients, and was present in a sub-epithelial site in four individuals. A library of insertional mutants was constructed in the serogroup B isolate C311, by in vitro mutagenesis. Each mutant contained an identifying signature tag allowing high throughput analysis. Mutants were screened in banks of 96 at an infecting dose of 5×10^7 cfu over an interval of 20 hours. 600 mutants were examined for their ability to colonise human tissue in the NP model. Several colonisation deficient mutants were identified.

Conclusions Colonisation of individuals without invasive meningococcal disease includes residency in a sub-epithelial site. The NP model was successfully employed to identify colonisation deficient mutants

#184

INVASION OF NEISSERIA GONORRHOEAE INTO HUMAN EPITHELIAL CELLS

Song W, Ma L, and Stein D, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742

Lipooligosaccharide and Opa are major outer membrane components of *Neisseria gonorrhoeae* that have been implicated in adhesion to and invasion of host epithelial cells. Using *N. gonorrhoeae* that express defined LOS structures we recently described the role of specific LOS molecules in adhesion and invasion of the human cervical epidermoid carcinoma cell line, ME180 (Song et al., *J. Exp. Med.* 2000, 191:949-959). We have expanded these studies to investigate the ability of these mutants to adhere to and invade polarized human intestine epithelial cells, T84. We have also investigated the adhesive and invasive capacities of strains expressing defined LOS, in combination with the expression of specific Opa proteins. Our data indicate that the requirements for gonococcal adhesion differ from the requirements for invasion. Using electron microscopy, we have been able to visualize differences in cellular associations formed between the gonococci expressing different surface molecules and host epithelial cells. We have also demonstrated that gonococci differing in surface molecules activate the rearrangement of actin cytoskeleton to different degrees. The results from our studies indicate that adherence and invasion of gonococci to epithelial cells can proceed via several different receptor/ligand interactions. These data further support the view that gonococcal invasion into specific cells can utilize redundant entry mechanisms.

#185

THE IDENTIFICATION OF THE GONOCOCCAL 'hCG-LIKE MOLECULE AND ITS ROLES IN LHr-MEDIATED INVASION OF HEC1B CELLS

Spence JM, Tyler R, Domaoal R, Clark VL. Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY

Our model of lutropin receptor (LHr) mediated gonococcal invasion proposes the expression of gonococcal mimetics of hCG, a ligand for LHr. Using a commercially available pooled antisera to hCG, we identified, by Western analysis of whole cell lysates, a GC protein that shares structural similarites to hCG. This protein was purified by both immunoprecipitation and 2D gel analysis and found, by N-terminal sequencing, to be the ribosomal protein L12. We have evidence that L12 is membrane associated and surface exposed on gonococci from western analysis of cell fractionation and antibody adsorption studies. We purified recombinant gonococcal L12 (rL12) and found that exogenous rL12 can significantly influence GC invasion of Hec1B cells. When Inv-GC are pre-treated with μM concentrations of rL12, they become 5- to 8-fold more invasive. This is the range of enhancement observed between Inv+ and Inv-GC. In addition, invasion of Hec1B cells by both Inv+ and Inv-GC is inhibited by nM concentrations of rL12, suggesting a physiological function for this molecule. Preliminary transcytosis studies, using polarized Hec1B cells, found similar results, in that rL12 pre-treatment of Inv-GC led to transcytosis rates identical to Inv+GC. In addition, this enhancement of transcytosis could be completely blocked by the addition of luteinizing hormone (LH), another ligand for LHr. These data supports a role for L12 in gonococci as the invasin that interacts with LHr to achieve internalization and transcytosis.

#186

MODULATION OF THE ADHESION OF NEISSERIA MENINGITIDIS TO HUMAN CELLS THROUGH CROSS-TALK BETWEEN MENINGOCOCCI AND TARGET CELLS

Deghmane[1] A, Petit[1] P, Topilko[2] A, Pereira[1] Y, Giorgini[1] D, Larribe[1] M, and Taha[1] M-K.

Unité des Neisseria[1], and Station Centrale de Microscopie Electronique[2], Institut Pasteur, 25, 28 rue du Dr Roux, 75724 Paris cedex15, France

Adhesion, crucial step in meningococcal pathogenesis, is usually viewed as a two-step process: (i) initial adhesion, which is pilus-mediated, and (ii) intimate adhesion, which involves other bacterial and cellular structures. Pilin, the major subunit of pilus and PilC1 are key elements in initial meningococcal adhesion to target cells. We have shown that these proteins are downregulated upon bacteria-cell interaction (after after the intital adhesion). Moreover, capsule which is another surface structure that may hinder intimate adhesion, is also down regulated. This downregulation seems to necessitate crgA (contact-regulated gene A) which encodes a transcriptional regulator (a LysR-type transcriptional regulators). crgA expression is induced upon cell contact. Inactivation in meningococci provokes a dramatic reduction in bacterial adhesion to epithelial cells. Moreover, this mutant is unable to undergo intimate adhesion to epithelial cells or to provoke effacing of microvilli on infected cells. Purified CrgA is able to bind to pilC1, crgA, pilE and sia promoters and CrgA seems to repress the expression of pilC1, crgA, pilE and probably sia genes . Our results are in favour of a dynamic model of bacteria-cell interaction involving a network of regulators acting in cascade. CrgA could be an intermediate regulator in such a network.

#187

HIGH GENETIC DIVERSITY WITHIN THE TWO MOST COMMON SEROVARS (IB-2 AND IB-3) OF *NEISSERIA GONORRHOEAE* ISOLATES IN SWEDEN

Unemo M[1], Berglund T[2], Olcén P[1], Fredlund H[1].

[1]National Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiology & Immunology, Örebro Medical Centre Hospital, Sweden. [2]Unit for Infectious Disease Epidemiology, Swedish Institute for Infectious Disease Control, Solna, Sweden.

Gonorrhea in Sweden reached an all time low of 2.4/100000 inhabitants in 1996. Since 1997 the incidence has however increased for each year, mainly due to an increase of domestic cases (1). From all reported cases of gonorrhea from February 1998 to January 1999, epidemiological and microbiological data were obtained in 89% (348 patients with isolates). Two core groups of domestic cases have been identified: homosexual men with serovar IB-2 as the most prevalent *N. gonorrhoeae* (Ng) isolate and young heterosexuals of both sexes with IB-3. The aim of the present study was to examine the homogeneity within these serovars. Genomic DNA of all IB-2 (n=44) and IB-3 (n=84) isolates was digested using two restriction endonucleases, *Spe*I and *Bgl*II, and the DNA fragments were separated by PFGE. Among the IB-2 isolates, 19 different restriction enzyme patterns were identified. IB-2 isolates from domestic homosexual cases (n=19) gave indistinguishable patterns in 15 (79%). The IB-3 isolates gave 15 different patterns. 61% (51/84) of these isolates had the same indistinguishable pattern. This major clone mainly (73%) comprised isolates from domestic cases of young (less than 25 years of age) heterosexuals of both sexes. PFGE fingerprinting identified a high genetic diversity within both the serovars. Still the results indicate an introduction and spread of one Ng clone of each serovar within the two domestic core groups.

Reference: 1. Berglund T et al. Sex Transm Dis 1999; **26**:390-391.

#188

SIGLECS: MODULATORS OF SIALIC ACID MEDIATED VIRULENCE OF *NEISSERIA MENINGITIDIS*?

Claire Jones, Debbie Evans, Mumtaz Virji and Paul R. Crocker
Department of Pathology and Microbiology, University of Bristol, UK and The Wellcome Trust Biocentre, University of Dundee, UK

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are transmembrane proteins of the immunoglobulin superfamily. Members of this family share extensive sequence similarity (*c.* 30-85% identity) and are expressed on distinct subsets of haemopoietic cells e.g. macrophages (sialoadhesin / Siglec-1), neutrophils (Siglecs 5,9), monocytes (Siglecs 3,5,7,9) etc. Each Siglec also exhibits distinct carbohydrate binding properties with specificity for both the linkage and the nature of the sialic acid. The differences in the expression and the ligand binding suggest that each Siglec mediates a specific, non-redundant function in haemopoietic cell biology. In order to investigate if any of the Siglecs also function as receptors for sialylated bacteria, we studied the interactions of L3 (sialylated) and L8 (non-sialylated) LPS variants of *N. meningitidis* (Nm) sergroup B strain MC58. Studies using purified chimeric soluble receptors showed that sialylated but not non-sialylated Nm were recognised by Siglec-1 and Siglec-5 whereas other Siglecs were ineffective. In addition, Siglec-1-expressing CHO cell transfectants as well as macrophages bound and phagocytosed sialylated Nm. These interactions could be inhibited by treatment with Vibrio cholerae sialidase and by antibodies against Siglecs. The results were reproduced using serogroup A strain C751 grown in the presence or absence of CMP-NANA. The specificity exhibited by some Siglecs in the recognition of sialylated Nm suggests that certain haemopoietic cells possess the exquisite capacity for targeting the sialic acid structure present on Nm LPS. The studies imply that Siglecs may modulate the virulence properties associated with Nm LPS sialylation.

#189

Title: Isolation of *Neisseria gonorrhoeae* mutants that show enhanced trafficking across polarized T84 epithelial monolayers.

Authors: Hopper S, **Wilbur JS**, , Vasquez BL, Larson J, Clary S, Mehr IJ, Seifert HS, So M

Abstract: Initiation of a gonococcal infection involves attachment of Neisseria gonorrhoeae to the plasma membrane of an epithelial cell in the mucosal epithelium and its internalization, transepithelial trafficking, and exocytosis from the basal membrane. Piliation and expression of certain Opa proteins and the immunoglobulin A1 protease influence the transcytosis process. We are interested in identifying other genetic determinants of N. gonorrhoeae that play a role in transcellular trafficking. Using polarized T84 monolayers as a model epithelial barrier, we have assayed an *N. gonorrhoeae* FA1090 minitransposon (mTn) mutant bank for isolates that traverse the monolayer more quickly than the isogenic wild-type (WT) strain. From an initial screen, we isolated four mutants, defining three genetic loci, that traverse monolayers significantly more quickly than their WT parent strain. These mutants adhere to and invade cells normally and do not affect the integrity of the monolayer barrier. In two mutants, the mTns had inserted 370 bp apart into the same locus, which we have named fit, for fast intracellular trafficker. The *fit* locus contains two overlapping open reading frames, *fit*A and *fit*B, whose deduced amino acid sequences have predicted molecular weights of 8.6 and 15.3, respectively. Replication of the MS11A *fit*A mutant in A431 and T84 cells is significantly accelerated compared to that of the isogenic WT strain. In contrast, growth of this mutant in liquid media is normal. Taken together, these results strongly suggest that traversal of *N. gonorrhoeae* across an epithelial barrier is linked to intracellular bacterial growth.

#190

CONSTRUCTION OF A SHUTTLE VECTOR TO ADAPT THE IN VIVO EXPRESSION TECHNOLOGY (IVET) TO NEISSERIA MENINGITIDIS

Wintermeyer E, Dümig M, Frosch M
Institut für Hygiene und Mikrobiologie, Universität Würzburg, Germany

Objectives: To determine the activity of various promotors in *Neisseria meningitidis* under in vitro and in vivo conditions and to select for the promotors, that are specifically expressed under in vivo conditions.
Design: We used the recently described transformation hotspot *hrtA* to develop a shuttle vector (pEWIVET-I) which enables the integration of the promotor probe reporter gene fusions into the chromosome of *Neisseria meningitidis*. A promotorless transcriptional fusion of the selection markers *lacZ* and *cat* was integrated together with an erythromycin resistence gene into the 4,4 kb *hrtA* locus derived from *Neisseria meningitidis* B1940. We evaluated our system by expressing the reporter genes under the control of known promotors such as the *sia*, and *opa* promotor in *Neisseria meningitidis*.
Results: Plasmids derived from pEWIVET-I can be transformed at high rate of transformation and recombination into different pathogenic *Neisseria meningitidis* strains. Determination of ß-galactosidase expression show, that the activity of the respective promotors (*sia, opa*) is directly correlated with the rate of chloramphenicol resistance, that can be used as positive selection marker under in vivo conditions. Further, the degradation of X-Gal by ß-galactosidase appears to be toxic constitutive promotors.
Conclusions: Our shuttle vector, enables the selection of promotors, which are active under in vivo conditions and can be used for the investigation of any given promotor in different *Neisseria meningitidis* strains.

#191

MAPPING THE BACTERIAL RECEPTORS-BINDING EPITOPE(S) ON HUMAN LACTOFERRIN AND BOVINE TRANSFERRIN

Wong H and Schryvers AB. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Transferrin (Tf) and lactoferrin (Lf) are mammalian iron (Fe) binding proteins that are structurally conserved. Their presence in the extracellular milieu reduces the free Fe concentration to well below that which is required for microbial growth. However, pathogenic bacteria from the families Neisseriaceae and Pasteurellaceae possess outer membrane receptors that function to acquire Fe from Tfs and Lfs. The bacterial Tf and Lf receptors are distinct receptor complexes and they are designated Tf binding protein A/B (TbpA/B) and Lf binding protein A/B (LbpA/B), respectively. These receptor complexes display a strict binding specificity; where they exclusively bind the ligand from their host species. As part of the continuing effort to understand the mechanism behind this highly evolved form of Fe acquisition, we took advantage of the high degree of sequence homology between Tfs and Lfs to construct a series of chimeric human Lf (hLf) / bovine Tf (bTf). This involved splicing together regions in the two genes that encoded defined structural domains. The chimeric hLf/bTfs were then expressed in insect cells and used in a variety of binding and affinity isolation assays to probe their interaction with the bacterial receptors. Results from one particular construct, hLf-N/bTf-C, indicate that LbpA from *Moraxella catarrhalis* is not capable of binding the N-terminus of hLf. This construct also confirms previous reports that TbpB from *Pasteurella haemolytica* binds the C-terminal half of bTf.

#192

THE FATE OF HEME IN *NEISSERIAE*

Stojiljkovic I, Balding-Perkins D, Zhu W, and Richardson AR.
Department of Microbiology, Emory University, Atlanta, GA.

The heme utilization system of neisseriae consists of outer membrane receptors, proteins that energize the transport of heme across bacterial membranes, and intracellular enzymes that participate in heme processing and degradation. All heme/Hb receptors function by binding heme-protein complexes, stripping the heme from them and transporting the heme into the periplasm. Analysis of the binding characteristics of HmbR, a Hb-binding receptor, demonstrated that this receptor possesses several orders of magnitude higher affinity for Hb than the related Hb receptor from *Y. enterocolitica*. Using genetic and biochemical approaches functionally important HmbR receptor domains were discerned. Two HmbR regions, residing on putative surface-exposed loops III and X, form a high affinity binding site for Hb. A highly conserved domain in all heme/Hb receptors that encompasses loop VII of HmbR, is essential for Hb but not heme utilization. The deletion in the putative cork-like domain of HmbR affected Hb but not heme use and Hb binding, suggesting the involvement of the cork domain in utilization steps that follow Hb binding. Neisserial mutants that are deficient in the transport of heme across the cytoplasmic membrane have not been isolated. Very little is known about the fate of heme after entering the cytoplasm. We have identified a neisserial gene, *hemO*, that is essential for heme, hemoglobin (Hb), and haptoglobin-Hb utilization. The product of the *hemO* gene is homologous to enzymes that degrade heme; 21% of its amino acid residues are identical and 44% are similar to those of the human heme oxygenase-1. HemO genetic knockout strains were unable to use any heme source, while the assimilation of transferrin-iron and iron-citrate complexes was unaffected. The expression of HemO also protected *N. meningitidis* cells against heme toxicity. HemO was purified and analysis has shown that it is a heme binding protein that, in the presence of NDPH, oxygen and NADPH reductase, degrades heme to biliverdin, iron and CO. The product of this reaction is α-biliverdin, the same isomer as one produced by the human heme oxygenase.

#193

THE TRANSFERRIN RECEPTOR OF *NEISSERIA GONORRHOEAE*

Cornelissen CN[1], Boulton IC[1], Kenney CD[2], Masri HP[1], Ronpirin C[1], Yost MK[1] Depts. of Microbiology/Immunology[1] and Pathology[2], Virginia Commonwealth University, Richmond, VA, USA

Efficient iron acquisition from human transferrin (Tf) by *N. gonorrhoeae* depends on expression of two iron-regulated proteins, TbpA and TbpB, which together form the functional Tf receptor. TbpA is similar to TonB-dependent, integral, outer membrane receptors, while TbpB is lipid modified and largely surface exposed. We have developed a two-dimensional topology model for gonococcal TbpA, and have begun to test its predictions using genetic and biochemical approaches. We have identified putative, surface-exposed loops of TbpA that are responsible for ligand binding and for Tf-iron internalization functions. Because we are also interested in complex formation between TbpA and TbpB and the stoichiometry of such a complex, we have sought to determine the relative amounts of TbpA and TbpB expressed as a function of iron stress, at both transcriptional and translational levels. At the transcriptional level, steady-state levels of *tbpB*- and *tbpA*-specific mRNAs are distinct with *tbpB*-specific message in excess of *tbpA*-specific message. Iron concentration and the pH of the growth medium influence the levels of both mRNA species. Using immunoprecipitation assays, we have detected only a fraction of the maximally expressed Tbps within a TbpA:TbpB complex. We are currently evaluating growth, solubilization, and assay conditions that might effect the presence and/or our ability to detect a complex between the gonococcal Tbps. Models will be presented that illustrate our current hypotheses about the structure and stoichiometry of the gonococcal Tf receptor.

#194

GNA33: A NOVEL GENE INVOLVED IN PEPTIDOGLYCAN METABOLISM AND CELL SEPARATION

Adu-Bobie J, Jennings GT, Lupetti P#, Savino S, Marchetti E, Aricò B, Baldi L, Holtje J-V*, Dallai R#, Pizza MG, Grandi G, and Rappuoli R. IRIS, Chiron SpA, Siena, Italy, *The Max Planck Institute für Entwicklungsbiologie, Abteilung Biochemie, Tübingen, Germany and #Unit of Electron Microscopy and Cryotechniques, Dipartmento di Biologia Evolutiva, Università di Siena, Siena, Italy

The entire genomic sequence of serogroup B Meningococcus was used to identify novel vaccine candidates. One molecule identified, GNA33, induces antibodies with bactericidal activity and is well conserved among Meningococcus B strains, other serogroups and Gonococcus. The ORF for GNA33 encodes a 48 kDa protein containing a lipopolypeptide signaling sequence and is 33% identical to membrane bound lytic transglycosylase (MltA) from *E. coli*. We expressed GNA33 as a lipoprotein in *E. coli*, demonstrated by incorporation of [^{3}H] palmitate. The protein was purified and murein hydrolase activity confirmed when GNA33 was shown to degrade both insoluble murein sacculi and unsubstituted glycan strands. The formation of 1,6 anhydrodisaccharide tetra and tri peptide reaction products demonstrated the enzyme is indeed a transglycosylase. To study the function of the gene and its role in pathogenesis and virulence an isogenic mutant was generated. Electron microscopy revealed the mutant grows in clusters and exhibits retarded growth *in vitro*. This phenotype suggests a defect in cell separation. In conclusion we have identified a highly conserved protein from meningococcus that plays an important role in peptidoglycan metabolism and cell separation.

#195

FUNCTION AND REGULATION OF GONOCOCCAL GENES REQUIRED FOR ANAEROBIC GROWTH

Clark VL, Cardinale JA, Householder,TC, Silver, LE. Dept. Microbiology and Immunology, School of Medicine and Dentistry, University of Rochester, Rochester, NY

Neisseria gonorrhoeae grows anaerobically by respiration, using nitrite as a terminal electron acceptor and producing nitrous oxide. We have identified three genes that are required for anaerobic growth, *aniA*, *norB*, and *fnr*, which encode, respectively, a copper containing nitrite reductase, nitric oxide (NO) reductase, and an anaerobically active transcriptional regulator. AniA and NorB are induced during anaerobic growth in the presence of nitrite. AniA expression requires FNR and an *fnr* insertion mutation can grow anaerobically if *aniA* is controlled by the *tac* promoter, indicating that *aniA* is the only gene regulated by FNR that is required for anaerobic growth. NorB expression requires both AniA and nitrite. This was demonstrated to be due to a requirement for NO production by AniA, as exogenous NO induces *norB* in an *aniA* null mutant. NO is not toxic to the gonococcus anaerobically, but is toxic aerobically in a *norB* null mutant, probably due to production of peroxynitrite. AniA is present on the surface of gonococci, as evidenced by its ability to protect against killing by normal human serum. Its location suggests that it would not be directly linked to an anaerobic respiratory chain. NorB is most likely an integral cytoplasmic membrane protein, as it contains 14 potential transmembrane domains, and it is probable that NorB functions in anaerobic respiration to maintain an oxidation reduction balance. As NO reductases have not been demonstrated to couple energy production to respiration, it is unlikely that this occurs in the gonococcus, suggesting that anaerobic ATP production occurs only by substrate level phosphorylation. *N. gonorrhoeae* thus as the ability to both produce and degrade NO, an important host defense molecule. The gonococcus may also be able to modulate the inflammatory response by determining NO levels in the host.

#196

TRANSCRIPTION ACTIVATORS AND TARGET GENES INVOLVED IN ANAEROBIC GONOCOCCAL GROWTH REVEALED BY GENOMIC, PROTEOMIC, CLONING AND REPORTER GENE FUSION TECHNIQUES

Reid EG, Overton TW, Mohan SB, Smith H, Cole JA School of Biosciences, University of Birmingham, Birmingham, UK

Genes encoding proteins similar to the *Escherichia coli* transcription factor, FNR, that regulates adaptation to anaerobic growth, and to the nitrate and nitrite sensing proteins, NarX/NarQ and the response regulators, NarP/NarL, were identified in the gonococcal genome database. All three genes were cloned into *E. coli* and inactivated *in vitro*. The mutated genes were recombined into the gonococcal chromosome. The *fnr* mutation totally abolished and the *narP* mutation severely diminished the ability of gonococci to: (1) grow anaerobically; (2) adapt to oxygen-limited growth; (3) initiate transcription from the *aniA* promoter in the presence of nitrite; and (4) reduce nitrite to nitrous oxide during growth in oxygen-limited media. Anaerobic transcription from the *aniA* promoter was activated by nitrite but not by nitrate, so the gonococcal system is designated NarQ-NarP. This is the first experimentally documented example of a two-component regulatory system working with a transcription activator in pathogenic neisseria. An anaerobically induced, 50 kDa, *c*-type cytochrome was identified as a homologue of cytochrome *c* peroxidases (CCP) of other bacteria. Transcription from the *ccp* promoter cloned into the promoter probe vector, pLES94, was totally FNR-dependent but, unlike AniA, insensitive to the presence of nitrite during oxygen-limited growth. Proteomic comparison of wild type and *fnr* mutant gonococci revealed other proteins that are regulated as part of the FNR regulon. No FNR-dependent gonococcal promoter has yet been expressed in *E. coli*.

#197

THE ROLE OF MANGANESE IN THE PROTECTION OF *NEISSERIA GONORRHOEAE* FROM OXIDATIVE STRESS

Jennings, M.P, Tseng, H-J, Srikhanta, Y. McEwan, A.G. Department of Microbiology and Parasitology, University of Queensland, St. Lucia, Queensland 4072, Australia

Objective *Neisseria gonorrhoeae* is a facultative aerobe with a high iron requirement and a highly active aerobic respiratory chain. These factors suggest that this bacterium requires defense systems to respond to toxic oxygen species. In view of the key role of superoxide dismutase (SOD) in the removal of this highly toxic oxygen species it has been postulated that SOD is essential to aerobic life. A major unanswered question concerning the pathogenicity of *N. gonorrhoeae* is how this bacterium defends itself against O_2^- since it is one of only a very few aerotolerant bacteria that does not appear to possess this enzyme activity. Certain lactobacilli can defend against oxygen toxicity in the apparent absence of SOD. In *Lactobacillus plantarum*, it has been established that the Mn(II) ion itself plays the central role in the detoxification of O_2^-. Therefore, the objective of this study was to test the hypothesis that in *N. gonorrhoeae* intracellular oxidation of Mn(II) to Mn(III) may substitute for SOD in protecting the cell from damage caused by O_2^-.
Design. The presence and role of SOD was examined in a range of strains. The role of Mn in protection against oxidative stress was examined by confirming accumulation of Mn provided in growth media and determining whether there was a correlation with increased protection against killing by oxidative stress. Once confirmed specific knockout mutants were made in a gene involved in Mn uptake to confirm its role in this system.
Results. *N. gonorrhoeae* strain 1291 was shown to accumulate Mn when grown on Mn supplemented media and this accumulation of Mn was shown to correlate with increased resistance to killing in a paraquat killing assay (which generates O_2^- in the cytoplasm). Having confirmed that Mn-dependent protection occurs we created a knockout mutant in a putative Mn uptake system Compared to the wild type, the mutant was deficient in Mn uptake and was very sensitive to killing in the paraquat assay. Using Southern blotting we confirmed that 100% of strains tested contained the *sodB* gene. None contained *sodC*. A knockout mutant in *sodB* of strain 1291 confirmed that SODB plays no role in the observedMn-dependent protection.
Conclusion. We believe that our studies have identified a novel mechanism for protection against oxidative stress in *N. gonorrhoeae* that may be key in the pathogenesis of this organism and other bacterial pathogens.

#198

THE ROLE OF THE HEMOGLOBIN RECEPTOR IN GONOCOCCAL INFECTION DURING MENSES

Anderson, J.E.[1], Leone, P.A.[1] and Sparling, P.F.[1,2]

Departments of [1]Medicine and [2]Microbiology & Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 USA

The ability of gonococci to acquire iron (Fe) from transferrin (Tf), lactoferrin (Lf), hemoglobin (Hb), enterobactin (En) and other sources has been established. Most gonococci can utilize more than one source of Fe using specific receptors for each ligand. It remains unclear whether there is a selective advantage to expressing more than one receptor. All gonococci encode a Hb-receptor (Hb-R), but it is phase-variable and most laboratory and clinical isolates are in the Hb-R "off" phase. In this study, we address the question of whether there is a selective advantage to expressing the Hb-R during early phases of the menstrual cycle, when Hb is readily available from menstrual blood. We collected isolates in Raleigh, North Carolina from 67 male and female patients attending the Wake County Health Department Sexually Transmitted Diseases Clinic. Hb utilization by gonococci isolated from women was stratified by early (first 14 days) or later stages of the menstrual cycle. A significant correlation was found between Hb utilization in vitro (i.e., Hb-R "on" phase) and isolation within the first 14 days of the menstrual cycle: 5/12 were "on" in the first 14 days compared to 1/16 "on" in the later stages of the cycle (P = 0.36, Fisher's exact test, 1-tailed). Almost all gonococci from men were in the Hb-R "off" phase. All isolates were able to use Tf, and about half were able to use Lf for growth; no differences in expression of a functional Lf receptor were found between men and women or during different stages of the menstrual cycle. Thus, there appears to be a selective advantage to expression of the Hb-receptor in women during the early phase of the menstrual cycle.

#199

EXPRESSING A LACTOFERRIN RECEPTOR IN THE ABSENCE OF A TRANSFERRIN RECEPTOR IS SUFFICIENT TO ELICIT INFECTION IN HUMAN MALE VOLUNTEERS WITH GONOCOCCAL STRAIN FA1090.

Anderson, J.E.[1], Hobbs, M.M. [1,2], Cohen M.S. [1,2] and Sparling, P.F.[1,2] Departments of [1]Medicine and [2]Microbiology & Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 USA

Neisseria gonorrhoeae have evolved multiple receptor-dependent pathways for competitively acquiring iron (Fe) from host-proteins transferrin (Tf) , lactoferrin (Lf) and hemoglobin (Hb). We previously showed that the Tf receptor is required for infection by a naturally occurring Lf receptor negative strain, (Cornelissen, et al., 1998) in the male urethral human challenge model (Cohen, et al., 1994). In the current study, we repaired the naturally occurring Lf receptor deletion in the Tf deletion mutant, FA6916, and tested this Tf-, Lf+ mutant in the human challenge model. Eight volunteers received inocula between 5.1 and 6.4 Log_{10} colony forming units. Four (50%) became infected, but only two (25%) developed a urethral discharge. Thus, the presence of the Lf receptor enabled infection, suggesting that there is sufficient Lf on the urethral surface to support growth of gonococci. The biological advantages to Lf utilization in vivo are unclear since all gonococcal clinical isolates are Tf+ and only about one half are Tf+. Conceivably, Tf+Lf+ gonococci have a selective advantage in initiation of infection, compared to Tf+Lf- strains. Consistent with this hypothesis, we demonstrated a modest in vitro growth advantage of the Tf+Lf+ strain in a mixed culture of Tf+Lf+ and Tf+Lf- gonococci grown with both Tf and Lf.

#200

HEMOGLOBIN UTILIZATION IN GONOCOCCAL HPUA/HPUB MUTANTS

Chen CJ, McLean DS, Anderson JE, Thomas CE, Sparling PF. Dept. Medicine, University of North Carolina, Chapel Hill, NC, USA.

Objective: To examine the genetic variations that allow mutants of the *hpuA/B* operon to grow on hemoglobin as the sole source of iron.

Design: FA1090 does not make functional HmbR and depends on the HpuA/B receptor for growth on hemoglobin. Both HpuA and HpuB are essential for the utilization of hemoglobin as the iron source (Hgb+). An *hpuA* deletion mutant of FA1090, FA7169, was constructed and was unable to grow on hemoglobin (Hgb-). Hgb+ revertants of FA7169 were selected at a frequency of about $1x10^{-7}$ on hemoglobin/desferal plates. This selection procedure also gave rise to Hgb+ revertants of the intermediary strain FA7167 (*hpuA* deletion and *hpuB* with CAT insertion) used in the construction of FA7169.

Results: Two classes of Hgb+ revertants were detected. The first one showed elevated sensitivity to the detergent Triton X, rifampin and other antibiotics. The Hgb+ phenotype of these revertants was not dependent on TonB or HmbR and the responsible locus mapped outside the *hpuA/B* operon. These revertants apparently accumulated heme from hemoglobin in a receptor independent manner. The second class of Hgb+ revertants, all derived from *hmbR* mutants of FA7169, were not hypersensitive to detergent or antibiotics. Each had one single point mutation in *hpuB*.

Conclusions: Point mutations in *hpuB* can override the dependency of the HpuA/B hemoglobin receptor on HpuA. There also is a newly discovered pathway for obtaining heme from hemoglobin that may involve an efflux pump.

#201

IDENTIFICATION AND CHARACTERIZATION OF A HIGH-AFFINITY ZINC UPTAKE SYSTEM IN *NEISSERIA GONORRHOEAE*

Chen CY, Morse SA. Division of AIDS, STD and TB Laboratory Research, Centers for Disease Control and Prevention, Atlanta, GA.

Objective: Zinc is required for the normal growth of all organisms, however, very little is known about specific mechanisms for zinc transport and accumulation in *Neisseria gonorrhoeae*. The purpose of this study was to identify and characterize a putative gonococcal high-affinity zinc uptake system.

Design: The gonococcal genome database was searched using the known zinc-binding protein (*ZnuA*) sequences from *Escherichia coli* and *Haemophilus influenzae*. The sequence encoding the putative gonococcal *ZnuA* was PCR amplified, cloned in *E. coli* TB1, and the protein purified. A gonococcal *ZnuA* knockout was constructed by insertional inactivation with a spectinomycin-resistant gene (*aad9*) to examine the specificity of the zinc uptake system. A *H. ducreyi ZnuA* mutant was complemented with the gonococcal *ZnuA* to demonstrate the function of the gonococcal *ZnuA in vivo*.

Results: Three adjacent ORFs encoding a putative ZnuC (ATP-binding protein), ZnuB (permease), and ZnuA were located in the gonococcal genome. Gonococcal ZnuA possessed a characteristic 30 amino acid histidine-rich metal binding motif (repetitive HDH sequence) containing 43% H and 37% D and E. *ZnuB* and *ZnuC* were located upstream of *ZnuA* and possessed a consensus permease motif and highly conserved Walker A and B motifs in the ATP-binding domain, respectively. When partially purified from sonicated cell-free supernatants by CTB extraction and CM-sepharose chromatography, the mature gonococcal ZnuA had an estimated MW of 32-kDal. The presence of a 19 amino acid signal peptide and its solubility suggest that ZnuA was located in the periplasm. Using a chemically defined agar medium, the gonococcal ZnuA mutant grew only in the presence of Zn^{2+}; whereas Mg^{2+}, Ca^{2+}, Ni^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, and Cd^{2+} had either no effect or were growth inhibitory.

Conclusions: A putative gonococcal high-affinity zinc ABC tranpsort system was identified with homology to a growing family of prokaryotic zinc transporters. Inactivation of the *N. gonorrhoeae* F62 *ZnuA* gene by insertional mutagenesis resulted in a mutant that grew more slowly than the wild-type parent strain when even $ZnCl_2$ was provided at a final concentration of 400 μM. Other cations did not restore the growth of this mutant.

#202

VARIABILITY AND EXPRESSION OF Omp85 IN *NEISSERIA GONORRHOEAE* AND *NEISSERIA MENINGITIDIS*

Judd, RC[1], Manning, DS[1], Nycz, D, Poolman, J[2] and Tommassen, J [3]. [1]Universtiy of Montana, Missoula, MT; [2] SmithKline Beecham, Rixensart, Belgium; [3] University of Utrecht, The Netherlands

Our laboratory described an 85kDa outer membrane protein (Omp85), found in both *Neisseria gonorrhoeae* (GC) and *Neisseria meningitidis* (MC), which was highly similar to protective antigens found in *Hemophilus influenzae* and *Pasteurella multocida*.

The Omp85s of GC and MC were 95% identical with 40 amino acids varying between the two species, 19 (48%) of which occurred between amino acids 720-750. MC had five extra amino acids in this region. A preliminary topographical model of Omp85 showed this region was highly surface-exposed on a penultimate loop of the protein. Other surface-exposed loops in the C-terminal region showed much less variability (3 amino acid differences or less).

Variability of *opm*85 within GC and MC was evaluated by sequencing this region from geographically and structurally diverse isolates. GC studied included urethral, PID and DGI isolates. No variability was observed in the isolates sequenced to date. Expression of Omp85 from cultured organisms was evaluated by Western blot analysis. Omp85 was observed in all GC and MC isolates tested thus far and showed no variation in molecular mass within each species.

These data suggest that Omp85 is universally and invariantly expressed in GC and MC and therefore maybe a useful prophylactic molecule. Studies are currently underway to evaluate the utility of Omp85 as a vaccine to prevent meningococcal disease and will soon commence using *in vitro* models to evaluate the potential of Omp85 to elicit antibacterial responses to gonococci.

#203

COMPLEX FORMATION BETWEEN GONOCOCCAL TRANSFERRIN-BINDING PROTEINS TBPA AND TBPB

Kenney, CD[1], Cornelissen, CN[2] Department of Pathology[1] and Department of Microbiology and Immunology[2] Virginia Commonwealth University/Medical College of Virginia, Richmond, VA.

The use of human transferrin as an iron source by *Neisseria gonorrhoeae* is attributable to two separate transferrin-binding proteins designated TbpA and TbpB. TbpA, an integral outer membrane protein, and TbpB, a lipidated peripheral outer membrane protein are thought to interact to form the functional transferrin receptor. Previous evidence of complex formation between TbpA and TbpB has been based largely on the co-purification of TbpA and TbpB using transferrin-affinity chromatography. In our studies strain MCV601 (Lbp- derivative of strain FA19) was solubilized with 1% Elugent and the solublized Tbps immunoprecipitated with anti-rTbpA and anti-rTbpB antibodies. The specificity of our immunoprecipitating antibodies was confirmed by dot blot analysis as well as conducting immunoprecipitation assays with isogenic mutants. We can demonstrate a stable complex between TbpA and TbpB when immunoprecipitating with anti-TbpA polyclonal sera. Immunoprecipitation of the complex with anti-TbpB polyclonal sera however has been unsuccessful. We conclude from these studies that there is a direct and stable interaction between gonococcal TbpA and TbpB even in the absence of transferrin. Future experiments will address factors that may effect the nature of this interaction (i.e. the presence of transferrin) as well as the determination of the stoichiometry of the individual components of the TbpA/B complex.

#204

CLONING AND FUNCTIONAL CHARACTERIZATION OF INDIVIDUAL LOOPS OF GONOCOCCAL TRANSFERRIN BINDING PROTEIN A (TBPA)

Masri HP, Cornelissen CN
Department of Microbiology/Immunology, Virginia Commonwealth University, Richmond, USA

Two iron-regulated proteins, TbpA and TbpB, comprise the gonococcal transferrin receptor complex. TbpA is a TonB-dependent, integral outer membrane protein, which is essential for Tf-iron uptake, while TbpB is lipid modified, surface exposed and is thought to increase the efficiency of iron uptake from Tf. To assess the structure and function of one of the components of the Tf receptor, TbpA, we have cloned individual, putatively surface exposed loops of TbpA fused to an amino terminal cellulose-binding domain. We then overexpressed the recombinant proteins in *E. coli*. These proteins were found mainly in inclusion bodies, which were then solubilized, and the fusion proteins renatured by slow dialysis. To test the loops for their ability to bind Tf we employed dot blots. Loop fusions were applied to PVDF and then probed with HRP-Tf. We observed a specific, dose dependent binding phenomenon for a loop 5 –fusion and to a lesser extent, for a fusion to loops 4 + 5. Solid phase quantitative chemiluminescent Tf-binding assays support these results. To investigate the immunogenicity of the fusion proteins, we are in the process of immunizing New Zealand white rabbits. We will characterize any antibodies produced and determine if these antibodies prevent Tf binding, Tf-iron internalization or if they prevent bacterial growth.

#205

CONSTRUCTION AND CHARACTERISATION OF MUTANTS IN THE AROMATIC PATHWAY OF *NEISSERIA MENINGITIDIS* FOR USE AS PART OF A LIVE-ATTENUATED VACCINE

[1]McDowell PW, [1]Zhang-Barber, L, [1]Turner G., [1]Litt, D, [1]Bishop K, [1]Winzer K., [2]McLean L, [2]Martin G., [3]Gorringe A. and [1]Baldwin, T.J. [1]Institute of Infections and Immunity, Queens Medical Centre, The University of Nottingham, Nottingham, UK. [2]Cantab Pharmaceuticals, Cambridge UK. [3]CAMR Porton Down, Salisbury. UK.

Background: Microorganisms, including *S. typhi* and *N. gonorrhoea*, are attenuated in animal models when mutated in one or more genes of the aromatic amino acid pathway. The unavailability of essential nutrients or their intermediates in mammalian tissues limits the growth of *aro* mutants, making them potential live attenuated vaccine candidates.

Results: We have cloned, sequenced and made deletion/insertion mutants in the *aro*A and *aro*B genes from *N. meningitidis* strain B16:B6. Both mutants were auxotrophic for aromatic amino acids, being unable to grow in minimal essential medium (MEM) deficient in tryptophan, tyrosine and phenylalanine. High levels of aromatic amino acid supplements restored growth of the mutants in this medium.

Proteins encoded by both genes were expressed as His-tagged fusions, which, after affinity purification, were used to raise polyclonal and monoclonal antibodies. The absence of AroA and AroB proteins in the mutant strains was confirmed using these antibodies in western blot analysis.

Both mutants were evaluated for attenuation and their ability to protect on subsequent infection with wild-type B16:B6 in a mouse IP model of infection. The results of these studies are shown on another poster.

Conclusions: *aro* mutants of *N. meningitidis* show many of the characteristics of *aro* mutants of other bacterial species and therefore may be promising live attenuated vaccine candidates.

#206

GONOCOCCAL TRANSFERRIN-BINDING PROTEINS ARE ESSENTIAL FOR EXPERIMENTAL BACTEREMIA

Nowicki S,[1,2] Hart-Van Tassell A,[1] Pawelczyk E,[1] Sparling F,[3]
Dept. of Obstetrics and Gynecology, [1] and Dept. of Microbiology & Immunology,[2] The University of Texas Medical Branch at Galveston, Texas. UNC/Infectious Diseases, University of North Carolina, Chapel Hill. North Carolina [3]

Objective: Since transferrin binding proteins Tbp have major importance in an iron utilization and survival of *N. gonorrhoeae* in humans, therefore, in this study, we investigated the possible role of two transferrin-binding proteins Tbp1 and Tbp2 on development of experimental gonococcal infection in rat pup model.

Design: Virulence of three different Tbp mutants of *N. gonorrhoeae* (FA6819, FA6747 and FA6815) were characterized in experimental rat pup model. Quantitative culture of peritoneal fluid and blood was used as an indicator of gonococcal virulence. Binding of rat and human transferring to tested strains was evaluated by immunoblotting.

Results: *N. gonorrhoeae* FA19 and its mutants FA6819 and FA6747 was recovered from blood and peritoneal fluid of infected animals in concentration of 1.6×10^4 CFU/ml, 4×10^2 CFU/ml and 1.3×10^2 CFU/ml respectively. Double mutant FA6815 was not recovered from blood and peritoneal fluid of tested animals.

Different concentrations of human and rat transferrin were incubated with membrane fixed Tbp mutants. A dose dependent binding detected with anti-transferrin antibody was recorded for the rat and control human transferrin.

Conclusion: We conclude that Tbp plays an important role in virulence of wild type strain of *N. gonorrhoeae* FA19 in the rat pup model. We provide the first evidence that *N. gonorrhoeae* FA19 was able to utilize an iron from a source other than human, namely, the rat. Mutation of gonococcal Tbp abolished capacity of *N. gonorrhoeae* to compete binding to both human and rat transferrin abolished also survival of mutated strains in rat pup model. Altogether suggests that the rat pup model may provide opportunities for a variety of experimental approaches that will compliment those on male volunteers.

#207

CHARACTERIZATION OF THE ENZYME DIHYDROPTERIN PYROPHOSPHOKINASE FROM *NEISSERIA MENINGITIDIS* AND INTERACTIONS WITH OTHER ENZYMES IN THE FOLATE SYNTHESIS PATHWAY.

Qvarnstrom Y. and Swedberg G.
Dept. of Pharmaceutical Biosciences, Div. of Microbiology, Biomedical Center, Uppsala University, Uppsala, Sweden.

Resistance to antibiotics is an important issue in today's health care. For example, the drug of choice for treatment of infections caused by pathogenic *Neisseria* has been changed several times because of the occurrence of resistant strains. Since this development is likely to continue, new antibiotic drugs will probably be needed in the future.

One approach is to find new targets for antimicrobial chemotherapy. The folate synthesis pathway is unique for prokaryotes and lower eukaryotes and therefore interesting in that respect. The enzyme DHPS (dihydropteroate synthase) is well recognized as the target for sulphonamides. The enzyme preceding DHPS in the pathway, the PPPK (dihydropterin pyrophosphokinase), is another interesting candidate. We have cloned and sequenced the gene coding for PPPK (*folK*) from different clinical strains of *Neisseria meningitidis*. Comparisons revealed one strain with a mosaic structure in its *folK* gene, suggesting that horizontal transfer of genetic material has occurred. The PPPK enzymes have also been purified and characterized with respect of their kinetic properties.

The metabolic role of PPPK is to provide one of the substrates for DHPS. Earlier studies of DHPS enzymes have suggested that PPPK and DHPS enzymes need to have physical contact with each other for full enzyme activity. Substances that interfere with such interactions could lead to impaired growth and thus be used as growth limiting drugs. Studies of potential interactions between the enzymes from *Neisseria meningitidis*, using the two-hybrid system in yeast, have been initiated. However, no convincing conclusions can be drawn from the preliminary results so far.

#208

REGULATION OF THE GONOCOCCAL TRANSFERRIN RECEPTOR

Ronpirin C, Cornelissen CN
Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA, USA

The gonococcal human transferrin receptor consists of two transferrin-binding proteins, TbpA and TbpB, both of which are iron-regulated. TbpA is an integral outer membrane protein and is similar to TonB-dependent receptors in Gram-negative bacteria. In contrast, TbpB is a surface-exposed, lipidated protein. The *tbpB* gene is located upstream of and separated from the *tbpA* gene by an inverted repeat, which is predicted to form a hairpin-loop structure. Because polar transposon insertions in *tbpB* generate mutants that express neither *tbpB* nor *tbpA*, it is likely that transcription of both genes is regulated by a common upstream promoter. Using RT-PCR, we confirmed that both genes are co-transcribed on a single mRNA species. As determined by *lacZ* transcriptional fusions in both *tbpB* and *tbpA* genes, the *tbpB*-specific transcription was approximately 2- fold higher than that of *tbpA*-specific transcription, and the *tbp* genes were derepressed 25- to 27-fold during iron starvation. Interestingly, we also found that *tbp* expression was influenced by the pH of the growth medium. The low pH condition, which repressed *tbp* expression, might do so by increasing iron solubilization and availability. At present, the steady-state levels of wild-type *tbpA*-and *tbpB*-specific mRNAs are being quantitated using ribonuclease protection assay and competitive RT-PCR.

#209

INTERACTIONS OF THE NEISSERIA MENINGITIDIS RECEPTOR HPUAB WITH HEMOGLOBIN AND HEMOGLOBIN-HAPTOGLOBIN

Rohde, KH, Dyer DW. Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center

Objectives: To characterize the interaction of the TonB-dependent receptor HpuAB with its ligands - hemoglobin (Hb) and hemoglobin-haptoglobin (HbHp) - and to determine the role of TonB in this receptor-ligand interaction.
Design: To assess the role of the HpuA lipoprotein in HpuAB function, a nonpolar hpuA deletion mutant was constructed. The ability of HpuA-B+ DNM69 to bind and use Hb and HbHp as Fe sources was determined using growth assays and dot blots. The structure of HpuAB was probed using a protease accessibility assay. The binding kinetics of 125-I Hb to wild-type (wt) HpuAB or either component alone was determined using a liquid-phase equilibrium binding assay. The ability of heterologous animal Hb to compete with 125-I labeled human Hb was assayed to determine the species specificity of HpuAB. Finally, the role of TonB in HpuAB structure-function was characterized using the methods above to analyze a TonB- mutant as well as wild-type meningococci treated with the proton motive force uncoupler CCCP.
Results: Phenotypic analysis of DNM69 demonstrated that an HpuA- mutant is unable to use Hb or HbHp as sole Fe sources, a defect explained by the inability of DNM69 to bind these ligands. Similar results were obtained upon analysis of HpuA+B- and HpuA-B- strains. Protease accessibility studies suggest that HpuA and HpuB are both surface exposed, HpuB physically interacts with HpuA, both components are protected from cleavage by pre-incubation with specific ligand, and TonB energization affects the conformation of HpuAB. The binding of radiolabeled Hb to HpuAB yielded a saturable binding curve (Kd=90nM) that best fit a one-site model of receptor-ligand kinetics. Competitive binding assays did not reveal any species specificity in ligand recognition. The Hb binding kinetics of HpuAB were dramatically altered in the TonB- mutant and in wt meningococci treated with CCCP.
Conclusions: We hypothesize that the HpuB and HpuA assemble to form a receptor complex in the OM, with both components absolutely required for receptor function. The data suggest that HpuA and HpuB interact to form a single Hb binding site, an interaction that is significantly affected by the TonB-mediated energy state of the receptor.

#210

TOWARDS DEFINING THE FUR REGULON. **Sebastian S, Murphy J.R, and C.A.Genco. Department of Medicine, Section of Infectious Diseases, Boston University School of Medicine, Boston, MA 02118, USA.**

In the majority of gram-negative pathogens, genes involved in iron acquisition and virulence are transcriptionally regulated by iron availability through the ferric uptake regulator protein (FUR). Fur dimerizes in the presence of ferrous iron and binds to a specific DNA sequence (Fur box), which overlaps the promoters of iron-regulated genes, resulting in the inhibition of transcription. A *fur* homolog has been identified in *N.gonorrhoeae*, however its role within the organism is not well defined. This study was undertaken to characterize the interactions of gonococcal Fur with the promoter/operators of gonococcal iron regulated genes and to begin to define the gonococcal Fur regulon. The *fur* gene was PCR amplified from *N.gonorrhoeae* F62 and cloned into the expression vector pET11C and the resulting plasmid pKASH1was transformed into the host expression strain *E.coli* HBMV119, (*fur* null mutant with lambda DE3 phage carrying the T7 RNA polymerase). For expression of gonococcal Fur, *E.coli* HBMV119/pKASH1 was grown in luria broth (LB) medium with ampicillin and isopropyl-β-D-thiogalactoside at 39°C. Following induction, the gonococcal Fur was isolated from inclusion bodies (IB) and was purified by ion-exchange chromatography. The interaction of gonococcal Fur with different promoter regions was analyzed by electromobility shift assays and the metal specificity examined. The gonococcal Fur was found to bind to the promoter/operator regions of the gene encoding the periplasmic binding protein (FbpA) and the transferrin receptor (TbpB). We also observed binding to the gonococcal *fur* promoter supporting autoregulation of the gonococcal *fur*. Furthermore binding of gonococcal Fur to *fbp*A, *tbp*B and *fur* promoters was metal dependent as expected. This is the first report to demonstrate that gonococcal Fur can bind to promoter/operator regions of gonococcal iron-regulated genes and that Fur can regulate its own expression. To further define the gonococcal *fur* regulon we have also constructed a positive selection system to isolate iron-independent mutants of gonococcal *fur*. Characterization of the iron-independent Fur mutants will enable us to define genes which encompass the Fur-regulon in the gonococcus and to study their regulation as well as their role in gonococcal pathogenesis.

#211

IN A MEDIUM CONTAINING GLUCOSE, LACTATE CARBON IS INCORPORATED BY GONOCOCCI PREDOMINANTLY INTO FATTY ACIDS AND GLUCOSE CARBON INCORPORATION IS INCREASED: IMPLICATIONS REGARDING STIMULATION OF METABOLISM

Smith H, Yates EA, Gao L, Woodcock NA, Parsons NJ, Cole JA Medical School, School of Biosciences, University of Birmingham, Birmingham, UK

The reason for stimulation by lactate of metabolism of gonococci growing in a medium containing glucose, which increases LPS synthesis, has been investigated. Tricene SDS-PAGE and TLC on homogenates of gonococci grown in this medium with ^{14}C lactate showed that lactate carbon was preferentially incorporated into lipid and LPS. NMR spectroscopy on lipid from gonococci grown in the glucose containing medium with ^{13}C lactate showed that lactate carbon was incorporated into fatty acid moieties and not into ethanolamine/glycerol moieties. In contrast, NMR on lipid from gonococci grown with ^{13}C glucose indicated glucose carbon in both moieties. When unlabelled lactate was added, lipid synthesis from ^{13}C glucose was stimulated and small amounts of different fatty acids were formed. The NMR data shows that gluconeogenesis from lactate carbon does not occur in the presence of glucose, suggesting that lactate is used solely for rapid production, via pyruvate, of acetyl CoA, the precursor not only for fatty acid synthesis but also for the constituents and products of the citric acid cycle, including ATP. The rapid formation of a high level of acetyl CoA is the probable reason for stimulation of metabolism and oxygen uptake by lactate. ^{14}C label on LPS was in its fatty acids. In contrast to growth with ^{14}C glucose, most proteins that stained with silver in tricene SDS-PAGE were not significantly labelled by ^{14}C lactate in the glucose containing medium. Two of three appreciably labelled proteins were identified by N-terminal sequencing as GroEL and porin 1B, and one of two less labelled proteins was similar to peroxiredoxin proteins. There were no signs of specific induction of these proteins by lactate and labelling was consistent with fatty acids in attached lipid.

#212

GENERATION OF HAEM AUXOTROPHS AND NON-HAEM UTILIZING GONOCOCCAL MUTANTS USING RANDOM CASSETTE MUTAGENESIS

Turner PC[1,2], Thomas CE[2], Ala'Aldeen DAA[1], Sparling PF[2]
Meningococcal Research Group, University of Nottingham, UK[1] and The University of North Carolina, Chapel Hill, USA[2].

Objective: Pathogenic neisseriae internalize haem and use it as an iron or porphyrin source. This process can occur independently of TonB and more than one pathway may be involved in the transport of haem. To help elucidate the steps involved in haem utilization, a library of gonococcal mutants was generated and screened for haem-dependent and non-haem utilizing phenotypes.

Design: A gonococcal *tonB* isogenic mutant was further mutagenized by random insertional mutagenesis using an adaptation of a technique (developed by B.A. Dougherty and H.O. Smith for *H. influenzae*) that involved intramolecular circularization of chromsomal DNA, partial restriction endonuclease digestion and insertion of an antibiotic gene resistance cassette. Transformants were selected by antibiotic resistance and screened for non-haem utilizing or haem-requiring phenotypes on agar plates in the presence or absence of haem as a sole source of iron.

Result: The mutagenesis technique had a transformation efficiency of 1.9 x 10^{-5} in broth using 100 ng of chromosomal DNA (cf. 7.5 x 10^{-5} for control DNA containing a single antibiotic resistance gene cassette). Each mutant contained one copy of the antibiotic resistance gene cassette. Screening of 6,174 mutants revealed that thirty-six (~0.5% of total) had haem-requiring phenotypes and one had a non-haem utilizing (< 0.02% of total) phenotype.

Conclusion: The cassette mutagenesis procedure presented here serves as a simple, rapid and inexpensive tool for the generation of a library of randomly mutagenized gonococcal mutants and aids in the characterization of previously unidentified genes following phenotypic screening.

#213

MUTAGENESIS OF TRANSFERRIN BINDING PROTEIN A (TBPA)

Yost MK*, Boulton IC†, Cornelissen CN*
*Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond VA, USA
†Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario, Canada

The human transferrin receptor of *Neisseria gonorrhoeae* is TonB dependent and consists of two distinct proteins: transferrin binding proteins A and B. TbpA is an integral outer membrane protein that shows homology to other TonB dependant receptors. TbpB is a lipidated surface exposed protein on the outer leaflet of the membrane. Both proteins specifically bind human transferrin (hTf), but the uptake of iron into the gonococcus is dependent on the presence of TbpA. We have used deletion mutagenesis, site specific mutagenesis, and insertion mutagenesis to analyze the topology and function of TbpA. Gonococcal variants were constructed in which putative loops 4, 5, and 8 were deleted individually. In addition to the deletions, a cysteine in putative loop 5 was changed to a serine, and a c-myc epitope was inserted into putative loop 4. While all deletion mutants were structurally sound and surface exposed as evaluated by accessibility to exogenous protease, the deletion of loop 4 or loop 5 resulted in the inability of TbpA to bind hTf and to grow on hTf as a sole iron source. The deletion of loop 8 resulted in a mutant TbpA that was capable of reduced affinity hTf binding but was incapable of supporting growth on hTf as a sole iron source. Therefore, we conclude that putative loops 4 and 5 contain motifs essential for the hTf binding capability of TbpA. Deletion of putative loop 8 resulted in a decreased affinity for transferrin and a concomitant loss of transferrin iron internalization function.

#337

IDENTIFICATION AND CHARACTERISATION OF TWO NEW AUTOTRANSPORTER PROTEINS IN *NEISSERIA MENINGITIDIS*

Turner DPJ, Wooldridge KG, Ala'Aldeen DAA.
Division of Microbiology and Infectious Diseases, University Hospital, Nottingham, UK.

Objective: To identify and characterise novel autotransporter proteins in *Neisseria meningitidis*.

Design: Genes encoding autotransporter proteins from several gram-negative organisms were used to screen the meningococcal predicted coding sequences released by The Sanger Centre for additional putative autotransporter protein genes.

Results: Two putative autotransporter protein genes were identified. The first, *ausP* (autotransported serine protease), with a deduced 112kDa protein, shows homology to autotransported serine proteases in *Serratia marcescens*. The second, *Map* (meningococcal adhesion protein), encodes a putative 62kDa protein and shows homology to autotransported adhesion proteins in *Haemophilus influenzae*. Both genes have been cloned and expressed in *Escherichia coli* and work is in progress to determine the function and immunogenicity of the expressed proteins.

Conclusion: Two new putative autotransporter proteins have been identified in *Neisseria meningitidis*.

#214

MOBILE ACQUIRED ERYTHROMYCIN RESISTANT GENES IN *Neisseria gonorrhoeae*

Roberts MC[1], Luna V[1], Cousin S Jr[1], Whittington WLH[2], Judge K, and Young K[1]. Department of Pathobiology[1] and Medicine[2], University of Washington, Seattle WA. 98195

Objective: To examine gonococci, from weighted randomly sampled males, attending the STD Clinic for the presence of *mtr* mutations, rRNA methylase genes, and *mef*(A) gene, a new Gram-positive macrolide efflux pump.

Design: All isolates (n=24) were serogroup IB, most were prototrophic and isolated in the Seattle during 1996-98. Sequencing the PCR product of the *mtr* promoter identified the *mtr* mutation. DNA probes and PCR assays were used for DNA-DNA hybridization to detect the rRNA methylase genes *erm*(B), *erm*(C), *erm*(F) and the efflux pump *mef*(A). Conjugal experiments were done to examine mobility of the *erm* and *mef*(A) genes.

Results: No isolate carried either β-lactamase or *tet*(M) plasmids. The majority of isolates contained the *mtr* mutation and/or *erm* and/or *mef*(A) genes. Strains, with *erm* and/or *mef*(A) genes, were used as donors to move these genes into 4 different Gram-negative hosts and the Gram-positive *Enterococcus faecalis* recipients, at frequencies ranging from 10^{-6} to 10^{-10} per recipient. The data suggests that both types of genes are on complete conjugative transposons.

Conclusions: Half of these isolates carried multiple determinants conferring resistance to macrolides. Results from the mating experiments suggest that both the *erm* and *mef*(A) are associated with functional conjugative transposons which can be transferred to both related and unrelated genera. Both *erm* and *mef*(A) genes are of Gram-positive origin, as is the previously described *tet*(M) gene, and have been added to *N. gonorrhoeae* antibiotic resistant repertoire.

#215

CHANGING PATTERNS OF GONOCOCCAL RESISTANCE TO THERAPEUTIC ANTIBIOTICS IN LONDON

Ison CA, Martin IMC, Burrowes E, and the London Gonococcal Working Group. Dept. Infectious Diseases & Microbiology, Imperial College School of Medicine, St. Mary's Campus, London, UK

Objective: Patterns of resistance to penicillin, tetracycline and ciprofloxacin among goncoccal isolates collected as part of a London surveillance programme over a three year time period were monitored.

Design: Isolates from consecutive patients with gonorrhoea attending 10 centres in London were collected over the same three month time period between 1997 and 1999. All isolates were tested for plasmid-mediated resistance to penicillin and tetracycline, alone or together (PPNG, TRNG, PP/TRNG) and chromosomal resistance to penicillin (CMRNG) and ciprofloxacin (QRNG) using standard methods. Demographic data was collected from each patient.

Results: A total of 1133 isolates were tested in 1997, 1203 in 1998 and 1559 in 1999. The levels of TRNG (2.4% in 1997; 3.5% in 1998 and 4.2% in 1999) and PP/TRNG (1.3% in 1997; 1.0% in 1998 and 2.7% in 1999) have risen. The majority of infections were heterosexually acquired in the UK (86% and 93% respectively in 1999). In contrast levels of PPNG have remained low, relatively stable (0.5% in 1997; 0.8% in 1998 and 0.6% in 1999) and were more likely to be acquired abroad (46% in 1999). CMRNG have decreased dramatically from 7.6% in 1997, to 3.2% in 1998 to 1.5% in 1999, were isolated more frequently in homosexual men (69% in 1998, 75% in 1999) and were mostly acquired in the UK (92% in 1999). Resistance to ciprofloxacin was low in 1997 and 1998 (0.4%) but increased to 1.0% in 1999, was heterosexually acquired, and predominately from abroad.

Conclusions: Patterns of resistance may reflect changes in therapeutic practice. Surveillance programmes are essential to monitor these trends and to inform the choice of appropriate therapy.

#216

PLASMID MEDIATED RESISTANCE IN *NEISSERIA GONORRHOEAE* STRAINS ISOLATED FROM FEMALE SEX WORKERS IN NORTH SUMATRA, INDONESIA 1996.

XIAOHONG SU[1]*,MD,PhD, NAMYO HUTAPEA[2], MD, JOHN W TAPSALL[3], MB, BS, FRCPA, MD, and INGA LIND[1],MD,DMSc

[1]Neisseria Unit, Department of Respiratory Infections, Meningitis and STIs, Statens Serum Institut, Copenhagen, Denmark

[2]Department of Dermato Venereology, School of Medicine,University of North Sumatra, Medan, Indonesia

[3]Department of Microbiology , Prince of Wales Hospital, Randwick, Sydney, Australia 2031

Background: A high prevalence of plasmid-mediated tetracycline and penicillin resistance among gonococci isolated from female sex workers was observed in North Sumatra, Indonesia in 1996.

Goal: To characterize of *Neisseria gonorrhoeae* strains isolated from a core group of STI patients in North Sumatra, Indonesia in 1996.

Study Design: The strains were characterized by antimicrobial susceptibility testing, auxotype /serotype (A/S) class, plasmid analysis, subtype of the tetM determinant and analysis of genomic DNA by pulsed-field gel electrophoresis (PFGE).

Results: A total 163 *N. gonorrhoeae* strains were isolated from 592 cervical specimens obtained from female sex workers in ten different places in North Sumatra, Indonesia in 1996, corresponding to an average prevalence of gonorrhoea of 28%(range 17% to 56%). All 161 tested strains exhibited plasmid-mediated resistance to tetracycline or penicillin or both:114 strains were PPNG/TRNG (71%), 46 TRNG (29%) and one PPNG. All 161 strains were susceptible to ceftriaxone, ciprofloxacin, kanamycin , and spectinomycin. All PPNG strains tested carried the 7.2 kb (Asian type) plasmid except one which carried the 4.9 kb (Toronto type) plasmid. The tetM gene was identified in all TRNG strains and 99% of tetM positive strains contained Dutch-type tetM gene; the American-type tetM gene was observed only in one strain. Among 161 isolates 55 were selected for auxotyping and serotyping. Overall 20 A/S classes were identified.PFGE analysis of genomic DNA of 156 *N.gonorrhoeae* strains documented that a diversity of strains existed and that a few resistant clones had spread in a certain area or between different areas in North Sumatra.

Conclusions: Monitoring the trends and the changing patterns of antimicrobial resistance in *N. gonorrhoeae* in high-risk populations are important.

#217

gyrA AND *parC* CHANGES IN CIPROFLOXACIN RESISTANT MENINGOCOCCI PARALLEL THOSE SEEN IN GONOCOCCI

Shultz TR, Tapsall J. Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia

Objective: To describe the induction of quinolone resistance in *N. meningitidis* in vitro and the accompanying *gyrA* and *parC* changes.

Design: A non-invasive meningococcal isolate produced colonial variants within the zone of inhibition of quinolone antibiotic discs. These 'first stage' variants had increased MICs to ciprofloxacin and were themselves cultured in the presence of increasing concentrations of ciprofloxacin. 'Second stage' colonial variants were then obtained. The ciprofloxacin MICs of the first and second stage variants were determined and the *gyrA* and *parC* quinolone resistance determining regions (QRDRs) were sequenced using primers described for the equivalent gonococcal QRDRs.

Results: Sequential changes in the *gyrA* and *parC* QRDRs were observed as ciprofloxacin MICs increased. The first stage variants had MICs of 0.25 and 0.5 mg/L and changes in *gyrA* at D95N and T91I respectively. The second stage variants had ciprofloxacin MICs of 1 mg/L and in addition to a *gyrA* change at T91I, there was an EP1K change in the *parC* region.

Conclusions: *gyrA* and *parC* changes were induced in *N. meningitidis* in a manner analagous to those observed in *N. gonorrhoeae*. As in *N. gonorrhoeae*, the changes in the QRDRs of *N. meningitidis* were sequential with *parC* alterations occurring after *gyrA* changes. The *parC* change was the result of a threonine to isoleucine substitution, again a change described in quinolone-resistant gonococci.

#218

MTRA, PILF AND TONB-EXBB-EXBD PROTEINS ARE REQUIRED FOR INDUCTION OF THE MTRCDE EFFLUX PUMP SYSTEM OF *NEISSERIA GONORRHOEAE*

Rouquette CE[1], Stojiljkovic I[1]and Shafer WM[1,2]

[1]Dept of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, 30322, USA and [2]VA medical center, Decatur, GA, 30033, USA

Introduction: The *mtr* (*m*ultiple *t*ransferable *r*esistance) complex in *Neisseria gonorrhoeae* encodes an energy-dependent efflux system, composed of MtrC-MtrD-MtrE cell envelope proteins, that exports antibacterial hydrophobic agents (HAs). The MtrCDE system is inducible by sublethal concentrations of Triton X-100 (TX-100) and a structurally related spermicide, nonoxynol-9. The insertion of a kanamycine cassette into the *mtrA* gene, encoding an AraC-like protein, gave rise to a non-inducible mutant. Several gonococcal clinical isolates were then tested for induction by TX-100, and the *mtrA* genes from 3 inducible strains and 3 non-inducible strains were sequenced. All the non-inducible strains tested had an 11 bp-deletion in the 5'-end of the *mtrA* gene resulting in a truncated MtrA protein.

Results: Using 2D-gel electrophoresis analysis, we were able to identify another protein, PilF, known to be involved in pilus biogenesis, which was regulated by MtrA. We also demonstrated that a *pilF*::Km mutant was no longer inducible. Similarly a deletion of the *tonB-exbB-exbD* genes led to a non-inducible mutant.

Conclusions: The induction of the gonococcal MtrCDE efflux pump by sublethal concentrations of TX-100, required at least 3 other systems: an AraC-like activator termed MtrA, the pilus assembly protein PilF, and the energy-transducer complex TonB-ExbB-ExbD. We hypothesize that induction of elevated resistance to HAs in gonococci requires a secretion process involving PilF and energy provided by the TonB-ExbB-ExbD system.

#219

THE HIGH DEGREE OF POLYMORPHISM OF THE PENA GENE IN NEISSERIA MENINGITIDIS IS DIRECTLY CORRELATED WITH REDUCED SUSCEPTIBILITY TO PENICILLIN

Antignac A[1]., Kriz, P[2], Tzanakaki G[3], Alonso J-M[1], and Taha M-K[1]

1 Unité des Neisseria, and Centre National de Référence des Méningocoques, Institut Pasteur, 28 Rue du Dr Roux, 75724 Paris cedex15, France.
2 National Reference Laboratory for Meningococcal Infections, NIPH, Prague, Czech Republic.
3 National Meningococcal Reference Laboratory and the National School of Public Health (NSPH), 196, Alexandras Avenue, 11521 Athens, Greece.

Meningococcal infections require urgent medical treatment and antibiotics should be administrated immediately. Penicillin is one of the antibiotics of choice and is effectively used in the treatment of meningococcal infections. However, meningococcal strains with reduced susceptibility to penicillin (pen^I) have been reported in several countries. We studied the polymorphism of penA which encodes penicillin-binding protein 2 (PBP2) in 13 strains of Neisseria meningitidis susceptible to penicillin (pen^S) and 12 strains with reduced susceptibility to penicillin (pen^I). These strains differed in geographical origins. Serological and genetic typing showed that these strains were highly diverse and belonged to several genetic lineages. Restriction analysis and DNA sequencing of penA showed that all pen^S strains had the same penA allele regardless of genetic group whereas pen^I strains harbored various penA alleles. Transformation with DNA from a pen^I strain conferred the pen^I phenotype on a pen^S strain. Thus, the reduction in susceptibility to penicillin is directly related to changes in penA and the analysis of penA polymorphism could be used as a reliable tool for characterizing meningococcal strains in terms of their susceptibility to penicillin.

#220

GENES INVOLVED IN TRANSFORMATION OF GONOCOCCI TO HIGH-LEVEL PENICILLIN AND TETRACYCLINE RESISTANCE

Nicholas RA, Ropp PA, Hu M, Olesky M Dept. Pharmacology University of North Carolina at Chapel Hill, Chapel Hill, NC

Rationale: Intermediate-level chromosomally-mediated resistance to penicillin (pen) and tetracycline (tet) in *N. gonorrhoeae* occurs through acquisition of multiple resistance genes (*penA, mtr,* and *penB*). However, the genetic and molecular mechanisms involved in transformation of a *penA mtr penB* strain ($MIC_{pen, tet}$ = 1 μg/ml) to levels of resistance equal to a high-level pen- and tet-resistant strain (FA6140; $MIC_{pen,tet}$ = 4 μg/ml) have not been clearly elucidated.

Results: Alterations in PBP 1 have been correlated with high-level pen resistance. Therefore, the *ponA* gene encoding PBP 1 was sequenced from multiple ß-lactamase-negative, pen-resistant isolates (MICs ≥ 1 μg/ml) separated both geographically and temporally. A single point mutation (Leu-421→Pro, termed *ponA**) 40 amino acids N-terminal to the active site serine (Ser-461) was observed in all resistant isolates. Kinetic analysis showed that PBP 1 harboring the L421P mutation had a 3-4 fold lower rate of acylation (k_2/K') than wild type PBP 1 with a variety of ß-lactam antibiotics. Surprisingly, transformation of a *penA mtr penB* strain with the *ponA** gene did not increase pen (or tet) resistance. However, plating of a *penA mtr penB* strain on pen concentrations slightly above the MIC resulted in a few colonies with increased pen resistance (MIC_{pen} = 2.0 μg/ml). A concomitant 2-fold increase in tet resistance was also observed. Further transformation of these isolates with *ponA** plasmid DNA resulted in colonies with an MIC_{pen} = 4 μg/ml, with no change in tet resistance. Transformation of a *penA mtr penB* strain with FA6140 DNA and selection on tet gave rise to colonies with tet resistance equal to FA6140, but with no increase in pen resistance.

Conclusions: These data indicate that two genetic events (one of which is acquisition of *ponA**) are required to obtain high-level pen resistance in a *penA mtr penB* strain, whereas a single gene is sufficient for mediating donor-level tet resistance.

#227

A VARIATION OF MULTILOCUS SEQUENCE TYPING (MLST), MULTILOCUS RESTRICTION TYPING (MLRT) AS AN ALTERNATIVE FOR *N. MENINGITIDIS* STRAIN DISCRIMINATION

Bennett D, and Cafferkey M.
Meningococcal Reference Laboratory, The Children's Hospital, Dublin, Ireland.

Objective: To assess the potential of MLRT as an alternative multilocus typing method to MLST for *N. meningitidis* strain characterisation.

Design: Irish invasive disease-associated strains of *N. meningitidis* whose serogroup, serotype and serosubtype were already known, were examined by MLRT analysis. This involved restriction fragment length polymorphism (RFLP) analysis of PCR products generated from the seven loci of housekeeping genes used in MLST. As with MLST, the combination of alleles at each of the seven loci gave an allelic profile or restriction type (RT). Restriction patterns obtained with each locus were computer-analysed using the 1D Advanced and Databasing software available from Phoretix International (U.K.).

Results: Restriction analysis of each of the seven loci produced patterns consisting of between one and eight bands, depending on the locus and isolate examined. Banding patterns were easy to analyse because of the small number of bands in each pattern. Several different restriction patterns (alleles) were observed for each of the seven loci examined, potentially producing limitless RTs. Greater allelic variation was observed with the *fumC* and *pgm* loci than with the *abcZ* and *adk* loci, suggesting that the later were more conserved. Optimal strain discrimination was achieved when all seven loci were examined. As with MLST analysis, no strict correlation between RT and serogroup was noted. However, some correlation was observed between serotype and serosubtype and RT as strains with the serotype 4 and serosubtype p1.4 did form a distinct RT as did strains with the serotype 2a and serosubtype p1.2 p1.5.

Conclusions: From this study, MLRT has proved to be an efficient and effective method, as an alternative to MLST, for *N. meningitidis* strain characterisation. MLRT provides a rapid, simple and easy to perform multilocus typing approach without the requirement for high throughput sequencing.

#228

POPULATION STUDY OF *NEISSERIA LACTAMICA* IN INFANTS

Bennett J S[1], Jolley KA[1], Griffiths D[2], Crook DW[2], Maiden MCJ[1].

[1]Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3FY.

[2]Department of Bacteriology, Level 6/7, John Radcliffe Hospital, Headley Way, Headington, Oxford, OX3 9DU.

Objective: To provide the background information necessary for exploring the hypothesis that carriage of the commensal organism *Neisseria lactamica* provides protection against disease caused by *Neisseria meningitidis*.

Design: *Neisseria lactamica* isolates were recovered from a cohort of infants in the first two years of life and then analysed using high throughput automated nucleotide sequence determination to investigate genetic variability and population structure.

Results and conclusions: Preliminary findings indicate that the N. lactamica strains carried by infants represent a genetically diverse population. These results also show that in many infants the same strain is carried for the duration of the study, whereas in others there is a change in isolates recovered.

#229

MULTILOCUS SEQUENCE TYPES OF *NEISSERIA MENINGITIDIS* CAUSING SYSTEMIC DISEASE IN NORWAY, 1999 – THE END OF AN EPIDEMIC.

Caugant DA, Iversen BG, Frøholm LO, Wedege E. Depts. Bacteriology and Vaccines, National Institute of Public Health, Oslo, Norway

Norway was reached in 1975 by an epidemic of meningococcal disease that peaked in 1983 with 367 cases. In 1999, only 80 cases were notified, representing an incidence of 1.79 cases per 100,000. Sixty-one of the 80 cases (76%) were caused by serogroup B strains and 11 (14%) by serogroup C. The peak of incidence in the teen-age group that has characterized the epidemic, has now nearly totally disappeared and >45% of the cases are under 5 years of age. Eleven patients (13.8%) were reported to have died as a result of the disease.

N. meningitidis was isolated and sent to the reference laboratory for further characterization in 71 of the 80 cases (89%). Numerous serotype:serosubtype combinations were represented. P1.7,16 was still the predominant serosubtype (21%), causing 16 of 75 cases (71 strains + 4 cases diagnosed by PCR/sequencing of *porA*). Of 67 strains analyzed by multilocus sequence typing (MLST) for allelic variation at 7 genes, 46 sequence types (STs) were identified. The strains from children under the age of 5 were especially diverse, with 25 different STs differentiated among the 33 strains analyzed by MLST. The ET-5 complex was represented by 38% of the patient strains, followed by lineage III that caused 13% of the cases.

Thus, meningococcal disease in Norway is tending to a normal endemic situation. The disappearance of peak of disease in teen-agers justifies the decision not to offer vaccination with the Men-B outer-membrane vesicle preparation to Norwegian school-children. The diversity of clones in children <5 years, together with their more restricted antibody response, makes it less likely that the monovalent vaccine will offer significant protection in this group.

#230

Current status of the web based Multi Locus Sequence Typing project for *Neisseria meningitidis*

M S Chan & M Maiden. The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, South Parks Road, Oxford OX1 3FY, UK.

Multi locus sequence typing of bacterial pathogens involves the sequencing of seven 400-500 bp fragments of house keeping genes to characterise the isolates. Each unique sequence at a locus is assigned an allele number and a sequence type is defined by a unique combination of alleles at seven loci (Maiden et al, 1998). These data can be unambiguously compared among different laboratories regardless of geographical location. A web-based interface is an ideal medium with which to share this information.

A web site for MLST is available at http://mlst.zoo.ox.ac.uk . This web site contains a database driven query interface, which allows users to enter their own sequences for comparison with existing strains. This includes querying by allelic profile and by sequence as well as by other fields such as country of isolation. As well as *Neisseria meningitidis*, MLST schemes have also been developed for *Streptococcus pneumoniae*, *Campylobacter jejuni* and *Staphylococcus aureus* and are currently being developed for several other species. The site is intended an international forum for MLST projects and includes experimental protocols for the methods used and welcomes submissions of new strains to the databases.

Currently (31st May 2000), the *Neisseria meningitidis* database contains 1079 strains, 579 sequence types and approximately 100 alleles at each locus. The MLST website (all species) has 338 registered users.

#231

SUBTYPING *Neisseria gonorrhoeae*:RANDOM AMPLIFIED POLYMORPHIC DNA AND AMPLIFIED FRAGMENT LENGTH POLYMORPHISM FINGERPRINTING

Chenia HY[1], Pillay B[1], Toth I[2], Birch P[2], Pillay D[1]. [1]Microbial Biotechnology Programme, University of Durban-Westville, KwaZulu-Natal, South Africa; [2]Scottish Crop Research Institute, Invergowrie, Dundee, Scotland.

Objective: To assess the use of two DNA amplification techniques, i.e., random amplified polymorphic DNA (RAPD) analysis and amplified fragment length polymorphism (AFLP) fingerprinting for subtyping gonococcal strains.

Design: The study population consisted of 66 epidemiologically unrelated gonococcal isolates previously subtyped by serotyping, PCR-RFLP of the Por gene and PFGE. RAPD profiles were obtained by amplification with 10-mer primers OPA-03 and OPA-13. AFLP fingerprints were obtained by EcoRI-MseI digestion of gonococcal genomic DNA, ligation to adaptors and amplification with selective primers. The discriminatory ability of RAPD analysis and AFLP fingerprinting was calculated according to Simpson's index of discrimination.

Results: Three types of polymorphisms were seen with both methods and included differences in fragment length, band intensity and presence or absence of a band. With the OPA-03 primer: profiles of 4 -16 DNA fragments, ranging in size from72 bp – 2 808 bp, were obtained. Similarly, OPA-13 profiles consisted of 2-17 fragments, ranging in size from 80 bp – 4 669 bp. Both OPA-03 and OPA-13 profiles had six fragments each, which appeared frequently and showed a larger amount of amplification product. AFLP analysis showed banding patterns with a mean of 35-50 bands. Marker bands were also observed in AFLP profiles. Both RAPD analysis and AFLP showed significant inter- and intra-serotypic discrimination, especially amongst PIB-3-, PIB-1- and PIA-6- expressing strains. Discriminatory indices for OPA-03, OPA-13 and AFLP fingerprinting were 0.9991, 0.9996 and 0.90, respectively.

Conclusions: While both RAPD and AFLP fingerprinting are extremely discriminatory, the latter appears to be a good taxonomic tool for subtyping gonococci. Unlike RAPD subtyping, which is prone to variation, AFLP fingerprinting is reproducible and has high resolution as a result of stringent PCR conditions. It also overcomes the over-discrimination between and among serovars obtained by RAPD analysis.

#232

GENETIC CHARACTERIZATION OF RESISTANCE TO ACTINONIN, AN INHIBITOR OF PEPTIDE DEFORMYLASE IN *Neisseria gonorrhoeae*

Dunham SA, Kuhn M, Holler T, Ortwine DF, Olson E

Pfizer Global Research and Development
2800 Plymouth Rd
Ann Arbor, MI USA

The formylation of methionine t-RNA is usually required to initiate protein synthesis in bacteria and is catalyzed by the gene encoding formyl transferase, *fmt*. This formyl-methionine is subsequently removed from the nascent polypeptide chain by peptide deformylase (PDF), encoded by *def*, to allow for the action of methione amino peptidase. This process is unique to bacteria and homologs of *def* and *fmt* can be identified in all bacterial species sequenced to date. PDF function is essential to viability in *E. coli* and *S. aureus* except in strains carrying deletions of both *def(B)* and *fmt* which have a reduced growth rate. These characteristics make peptide deformylase an attractive target for a novel broad-spectrum antibacterial agent.

Actinonin is a naturally occurring antibacterial agent that has been shown to be an inhibitor of purified *E. coli* PDF. *In vivo* evidence that actinonin's antibacterial activity is due to peptide deformylase inhibition has been demonstrated by an inverse relationship between the expression of *def* and antibacterial activity against *E. coli*. To identify genetic determinants for resistance to actinonin in *N. gonorrhoeae* we subjected FA1090 to chemical mutagenesis and isolated strains with a decreased susceptibility to actinonin. The mutations responsible for resistance were identified by genetic transformation of a susceptible strain followed by phenotypic selection (actinonin resistance) using PCR products of *def* and/or *fmt* from resistant mutants. These experiments identified independent mutations in both *def* and *fmt* associated with actinonin resistance demonstrating that PDF is the primary target of actinonin in *N. gonorrhoeae*. DNA sequencing of 9 independent *fmt* mutants identified 6 different missense mutations all resulting in bacteria with a decreased growth rate on plain GC agar. The failure to identify any *fmt* mutations resulting in the generation of a stop codon suggests that the observed mutations result in bacteria with decreased Fmt activity and that an *fmt*-null mutant is not viable.

#233

ANTIBIOTIC SUSCEPTIBILITY OF *NEISSERIA MENINGITIDIS* ISOLATES OF GERMANY, 1993 - 1999

Ehrhard I, Sonntag H-G. National Reference Centre of Meningococci, Institute of Hygiene, University of Heidelberg, Germany

Objective: Determination of antibiotic susceptibility of *Neisseria meningitidis* isolates of patients and carriers in Germany.

Design: *N. meningitidis* isolates of 1993 - 1999 were examined using agar dilution (in 1993 and 1994) and Etest® (from 1995 to 1999). Antimicrobial agents tested were penicillin G (1993 - 1999), rifampicin (1993 - 1999), ciprofloxacin (1994 - 1999) and cefotaxime (1995 - 1999). Number of isolates examined: 1959 invasive and 1266 carrier strains for penicillin G and rifampicin, respectively, 1749 invasive and 1201 carrier strains for ciprofloxacin and 1640 invasive and 1159 carrier strains for cefotaxime. Medium used for testing was GC Agar Base supplemented with IsovitaleX (1%) and Bovine Hemoglobin (1%).

Results: On an average 3,9% of all *N. meningitidis* isolates were moderately sensitive to penicillin G (MIC 0,25 - 1µg/ml, 4,3% of invasive and 3,2% of carrier strains). In 1999, 7,3% of invasive isolates showed intermediate susceptibility to penicillin G. Among serogroup C isolates from patients and carriers the percentage of strains with decreased susceptibility to penicillin G was 7,2% on an average (in 1999: 15,9%). None of the meningococcal isolates was resistant to penicillin G (MIC >= 2 µg/ml). Resistance to rifampicin (MIC >= 4 µg/ml) was found in 0,4% of invasive and 0,5% of carrier isolates. The high *in vitro* susceptibility to cefotaxime and ciprofloxacin was demonstrated by the low MIC50 and MIC90 values.

Conclusions: In 1999, there was on increase of invasive isolates with intermediate susceptibility to penicillin G. Among group C strains the percentage of isolates moderately susceptible to penicillin G is higher than in non-group C strains.

#234

TYPING AND SUSCEPTIBILITY TO PENICILLIN OF Neisseria meningitidis ISOLATED IN CUBA FROM 1993-1999.

Sosa J[1], Llanes R, Gutiérrez Y[1], Guzmán D[1], Gutiérrez O[1], Franklin S[2]. [1] Trop. Med. Inst. "Pedro Kouri" (IPK) [2] Finlay Institute. Havana, Cuba.

Objetive: know the serogroup, serotypes and subtypes distribution, and susceptibility to penicillin of a group of N. meningitidis strains isolated from years 1993-1999.

Design: One hundred eleven isolates of N. meningitidis were obtained from sporadic cases and small outbreak that occurred in 14 province of Cuba during 1993-1999. The strains were received at National Reference Laboratory for Pathogenic Neisseria. The susceptibility to penicillin was developed by the agar-dilution procedure on Mueller Hinton agar medium supplemented with 7% of calf serum. Serogroups were determined by slide agglutination with policlonal antisera to serogroups A,B,C,W135,X,Y and Z. Serotype and subtype were determined by a whole-cell enzyme-linked immunoassay with the monoclonal antibodies reagents.

Results: Thirty-five (31.5%) isolates showed reduced sensitivity to penicillin (MIC ≥ 0.1 µg/mL and ≤ 0.8 µ/mL) being MIC_{50} and MIC_{90} of 0.3 µg/mL and 0.6 µg/mL respectively. Resistant strains (MIC ≥ 2 µg/mL) were not detected. The 111 isolates belonged to group B and the most common phenotype was B:4:P1.15 (86/77.5 %). The proportion of isolates with phenotypes other than B:4:P1.15 in reduced sensitivity strains to penicillin (28.6%) was greater than in sensitive group (19.7%).

Conclusion: We do not exclude the possibility that strains with MICs higher than 0.8 µg/mL may appear. The increase of nontypeable and nonsubtypeable isolation demand the use of molecular biology techniques for their characterization.

#235

THE USE OF INTACT CELL MASS SPECTROMETRY FOR IDENTIFICATION OF NEISSERIA SPECIES AND POTENTIAL SUB-TYPING OF *N.MENINGITIDIS*

Fox AJ[1], Edwards-Jones V[2], Ralphson K[3], Gray SJ[1], Kaczmarski EB[1].
[1]Public Health Laboratory, Manchester M20 2LR; [2]Biological Sciences, Manchester Metropolitan University, Manchester M1 5GD;
[3]Kratos Analytical Ltd., Manchester M17 1GP, UK

Objective: To assess utility of Intact Cell Mass Spectrometry (ICMS) in providing a spectral fingerprint of the surface of different Neisserial species and isolates of *Neisseria meningitidis*.

Design: Intact cell cultures of Neisserial species and representative isolates of *Neisseria meningitidis* were analysed by ICMS using Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI TOF MS) using the Kratos (Salford,UK) Kompact Alpha instrument and using linear positive ion mode with pulsed extraction set to 100. The matrix used was α-cyano-4-hydroxycinnamic acid.

Results: The spectra obtained for the Neisseria genus were examined over the mass range 500-12000 Da. Between 500-1000 Da, spectra were virtually indistinguishable. Comparison of spectra obtained from different Neisseria species, show a significant number of common mass values, and above 5000 Da, a number of unique masses provide species differentiation. The spectra of *N. gonorrhoeae* and *N.meningitidis* were distinct over the mass ranges 1000-3000 Da allowing the differentiation of the two major pathogenic species, from each other and from other Neisseria species. ICMS analysis of isolates from different serogroups of *N. meningitidis* showed clear differences in the mass range 5000-12000 allowing differentiation within the species.

Conclusions: This preliminary study indicates that it is possible to distinguish between different species of Neisseria and between the different isolates of *N. meningitidis* using ICMS, which may provide a rapid technique for identification and sub-typing of pathogenic micro-organisms such as *N.meningitidis*.

#236

MULTILOCUS SEQUENCE TYPING (MLST) OF *NEISSERIA MENINGITIDIS* C:4:P1.4 ISOLATES IDENTIFIES GENOTYPIC DIVERSITY WITHIN THIS UNUSUAL PHENOTYPE

Gray SJ[1], Urwin R[2], Russell JE[2], Maiden MCJ[2], Kaczmarski EB[1]. ([1]PHLS Meningococcal Reference Unit, Public Health Laboratory, Withington Hospital, Manchester, UK. [2]Wellcome Trust Centre for the Epidemiology of Infectious Disease, University of Oxford, Oxford,UK).

Background: A limited number of *Neisseria meningitidis* phenotypes predominate among organisms associated with clinical disease. The commonest observed in the British Isles are B:4:P1.4 and C:2a:P1.5. Fifteen meningococci with the phenotype C:4:P1.4 were identified from 10,000 isolates during1995-99, 10 of which were from Eire. Investigations were performed to see if they shared common genetic and epidemiological origins and possible relatedness to B:4:P1.4 organisms, which are often assigned to lineage 3 by multilocus enzyme electrophoresis (MEE) and sequence type (ST) 41 by multilocus sequence typing (MLST).

Methods: The isolates were examined by *porA* sequencing, PFGE and MLST and results compared with possibly related organisms.

Results: *PorA* sequencing was identical, specifically P1.7b and P1.4 for all ten isolates. PFGE analysis of nine isolates using *SpeI* showed eight were closely related variants (fewer than 4-band differences). MLST identified three sequence types (ST) from 8 isolates which had previously been assigned to serogroup B lineage 3 meningococci, namely ST 41 (n=6), ST 42 (n=1) and ST 154 (n=1). ST 41 differed from ST 42 by a single base change which defined the abcZ-3 and abcZ-10 alleles. Two isolates from another patient demonstrated abcZ alleles distinct from those typical of lineage 3.

Conclusion: As nine out of ten of the meningococci examined differ from each other at only one or two loci, it is likely that they are all closely related, possibly originating from a common ancestor. A possible mechanism for this is capsular switching which may have occurred serially.

#237

DETERMINATION OF NEISSERIA MENINGITIDIS SUSCEPTIBILITY TO PENICILLIN BY DIFFERENT METHODS

Grzybowska W., Tyski S.
Dept. Antibiotics & Microbiology, Drug Institute, Warsaw, Poland

Several different techniques have been used world wide for determination of N. meningitidis susceptibility to penicillin, drug of choice in meningococcal meningitis treatment. The aim of the study was to compare penicillin MIC of N. meningitidis strains with lower susceptibility to this antibiotic determined by different methods.
All together 89 strains, isolated from meningitis patients (n=79) and carriers (n=10) in Poland, with initially determined penicillin MIC, from 0.06mg/L to 2.0 mg/L, on Mueller Hinton II Agar (MHA) were under investigation. The following techniques and media were used: penicillin E-tests on MHA+5% sheep blood (SB); penicillin agar dilution on: MHA, MHA+SB /NCCLS method/, MHA+5% horse blood (HB), MHA+5% lysed horse blood (LHB), chocolate MHA, Columbia Agar (CA)+SB, CA+HB, CA+LHB, chocolate CA, GC II Agar base + HB; penicillin broth dilution in: cation-adjusted Mueller Hinton Broth (CAMHB) +3% LHB /NCCLS method/. Incubation was performed 24h in 5%CO_2 at 35°C.
It was shown, that penicillin resistant N. meningitidis isolate possessed MIC=2mg/L determined initially on MHA, regardless the medium and method applied. The susceptible strains with MIC=0.06 mg/L where also susceptible by other assays. The strains with MIC between 0.12 and 1.0 mg/L differed sometimes 1-2 dilutions. In general, results obtained on MHA were one dilution lower as compare with CA and obtained on chocolate agars were also 1 dilution lower as compared with blood agars. No significant differences were observed when two kinds of blood applied. Results obtained on CAMHB+LHB were generally 1 dilution lower than MIC performed on agar media.

#238

CHARACTERISTICS OF *NEISSERIA MENINGITIDIS* ISOLATED IN KIELCE REGION OF POLAND

Hryniewicz W.[1], Piotrowska M.[2], Skoczyńska A.[1]
[1]National Reference Centre for Bacterial Meningitis, Sera & Vaccines Central Research Laboratory, Warsaw. [2]Regional Children Hospital, Kielce, Poland

Objectives: To analyse meningococcal isolates from children with meningitis in Kielce region, where the number of laboratory confirmed cases is the highest in Poland.

Material and methods: *N. meningitidis* isolates collected between 1997-99 in Kielce Hospital were studied in the National Reference Centre for Bacterial Meningitis (NRCBM). PCR reactions were run to confirm species and serogroup identifications. Minimal inhibitory concentrations (MICs) were determined by agar dilution method according to the NCCLS guidelines. All isolates were analysed by random amplification of polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) following *Bgl*II restriction.

Results: Between 1997-99, 28 cases of meningococcal meningitis were reported in Kielce Hospital, however the NRCBM received 25 of them. More than 90% of isolates belonged to serogroup B, followed by group C. Twenty percent of isolates had decreased susceptibility to penicillin. By RAPD typing 7 distinct patterns were observed, while PFGE revealed a great heterogeneity with 23 different patterns. Only 3 isolates presented the same pattern. Two of them showed decreased susceptibility to penicillin and were isolated from brother and sister in the interval of 7 months.

Conclusions: Molecular analysis indicates high heterogeneity among isolates from Kielce region, however a reservoir of epidemic strain with decreased susceptibility to penicillin should be taken into consideration.

#239

CHANGING EPIDEMIOLOGY OF ET-37 COMPLEX MENINGOCOCCI IN NEW SOUTH WALES, AUSTRALIA.

Jelfs, J[1,2]., Munro R[1].
[1]Department of Microbiology and Infectious Diseases, SWAPS, Liverpool, NSW. Australia.[2]Faculty of Medicine, University of New South Wales, Kensington, NSW Australia.

The State of New South Wales (NSW), Australia is divided into 17 Health Areas and cases of meningococcal disease (MD) are notified to the Health Department via each Health Area Public Health Unit.
In the late 1990s, ET-37 complex/ET-15 meningococci became the dominant strain in NSW and caused several clusters of MD, both within institutions and in the general community, in several of these Health Areas. Retrospective analysis of MD notification data and the corresponding laboratory data showed that in 1994, the first year the ET-37 complex variant- ET-15 became apparent in NSW, the majority of these early ET-15 cases occurred within the Northern Sydney Health Area, including a MD cluster in a high school. This Health Area bordered onto the Central Coast Health Area, where the first known case of ET-15 MD was identified. ET-15 meningococci then spread into neighbouring Health Areas and was only associated with sporadic disease until 1996, when a large cluster of MD occurred in the Wentworth Health Area. In addition, ET-15 continued to be associated with sporadic MD in the Northern Sydney Health Area. This was in contrast to the other Health areas where ET-15 MD had first occurred in 1994 but where there were no further ET-15 cases until 1996/7. Over the next 18 months, several ET-15 MD clusters occurred in other Health Areas. The Wentworth Health Area experienced hyperendemic ET-15 MD in 1997 and to a lesser degree in 1998. In 1998, ET-15 MD began to decline in NSW. Presently, B:4:P1.4 strains predominate in NSW. The epidemiology of ET-15 in NSW differed from that described in other countries where ET-37 complex/ET-15 caused extended hyper-endemic MD associated with both high mortality and morbidity.

#240

CARRIED MENINGOCOCCI IN THE CZECH REPUBLIC: A DIVERSE RECOMBINING POPULATION

Jolley KA[1], Kalmusova J[2], Feil EJ[1], Gupta S[1], Musilek M[2], Kriz P[2] and Maiden MCJ[1]

[1] Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, Oxford, UK.

[2] National Reference Laboratory for Meningococcal Infections, National Institute of Public Health, Prague, Czech Republic.

Conventional collections of the Gram negative diplococcus *Neisseria meningitidis*, a cause of septicaemia and meningitis world-wide, contain a substantial over-representation of isolates from cases of invasive disease. Here, molecular techniques were used to establish the genetic relationships of 156 meningococci isolated from healthy young adults in the Czech Republic during 1993. None of the individuals sampled had known links to cases of invasive disease.

Results: Multi locus sequence typing (MLST) showed that the bacterial population was highly diverse, comprising 71 different sequence types (STs) which were assigned to 34 distinct complexes or 'lineages'. Three previously identified hyperinvasive lineages were present: 26 isolates (17%) belonged to ST-41 complex (lineage 3); four (2.6%) belonged to the ST-11 (ET-37) complex; and one (0.6%) to the ST-32 (ET-5) complex.

Conclusions: The data were consistent with most nucleotide sequence diversity resulting from the reassortment of alleles by horizontal genetic exchange.

#241

SENSITIVITY AND NEGATIVE PREDICTIVE VALUE OF CULTURE AND PCR OF THROAT SWABS AND GARGLES IN DETERMINING CARRIAGE OF *NEISSERIA MENINGITIDIS*

[1,2]Jordens JZ, [1,2]Jones GR, [2]Williams JN, [2]Heckels JE
[1]Public Health Laboratory, Southampton, UK and [2] Molecular Microbiology Group, University of Southampton, SO16 6YD, UK

Several UK universities have suffered clusters of meningococcal infections and it is now national policy to vaccinate all new university students against group C meningococci. Studies at the University of Southampton indicate that about 25% of students carry meningococci. Traditionally, detection of carriage is by culture of throat swab and this depends heavily on the skill of the operator taking the swab. This study aimed to determine the most sensitive detection method for meningococcal carriage and to eliminate operator variation.

Throat swabs and saline mouth washings obtained from 89 first-year medical students were processed in parallel by conventional culture and PCR. Carriage of meningococci, as detected by the combined methods, was 20%. The sensitivity of throat swab culture, throat swab PCR, gargle culture and gargle PCR was 72%, 56%, 56% and 50% respectively. The probability that a technique would correctly identify the absence of carriage (negative predictive value, NPV) was 93.4%, 89.9%, 89.9% and 88.8% for throat swab culture, throat swab PCR, gargle culture and gargle PCR, respectively. Culturing both throat swab and mouth washings increased the NPV to 98.6%. The further addition of throat swab PCR increased this to 100%. PCR is a useful adjunct to culture for detecting nasopharyngeal carriage, but should not replace it.

#242

GLOBAL SPREAD OF A MENINGOCOCCAL W135 ET-37 COMPLEX CLONE IN TRAVELLERS RETURNING FROM THE ANNUAL HAJJ PILGRIMAGE OF 2000

Kaczmarski EB[(1)], Achtman M[(2)], Taha M-K[(3)]
(1)PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK, (2)Max Planck Institut fuer molekulare Genetik, Berlin, Germany, (3)Institut Pasteur, Paris, France on behalf of multinational W135 Meningococcal Studies Collaborating Group

Background Increased numbers of infections due to *Neisseria meningitidis* serogroup W135 were reported between March and May in UK and France. Initial cases were in persons recently returned from the Hajj pilgrimage while most later patients had had household contact with travellers. Cases of Hajj associated W135 infections were subsequently identified in many countries around the world.

Methods Recent and stored W135 isolates were characterised by a range of phenotyping and genotyping methods. Epidemiological and carriage studies have been initiated.

Results Recently isolated W135 organisms from pilgrims and their household contacts were identical. Furthermore these isolates were closely related to ET-37 complex organisms isolated in Gambia, Mali and Ghana in the 1990s. Similar strains have been identified from sporadic cases occurring between 1994 and 1999 in France.

Conclusion The molecular results show that a W135 clone of the ET-37 complex, which caused disease in West Africa during the 1990s, has been widely transmitted during the Hajj pilgrimage this year. The evolving epidemiology of this outbreak is currently being studied, facilitated by raising awareness of W135 disease through the resources of WHO and several national epidemiological agencies. Comparison with strains from collections in reference centres by applying powerful, reproducible and portable molecular characterisation techniques has enabled rapid and reliable assignment of this unusual disease cluster within a global context. The W135 clusters identified this year indicate that it is important to review the meningococcal vaccination advice for future Hajj pilgrims and other travellers.

#243

RISK FACTORS FOR MENINGOCOCCAL DISEASE IN UNIVERSITY HALLS OF RESIDENCE.

Neal Keith R[1], Nelson Sally J[2], Charlett André[3], Orr Hilary[4], Barker Mike[5], Taylor Clare[6], Monk Phil[7], Evans Meirion[8], Stuart James[4].
[1] Public Health Medicine & Epidemiology, University of Nottingham. [2]Wiltshire HA, [3]CDSC, Colindale, London. [4]CDSC South and West, Gloucester. [5]Southampton and SW Hampshire HA, [6]University of Leicester, [7]Leicestershire HA, [7]Bro Taf HA, Cardiff. UK.

Introduction - UK university students are at greater risk of invasive meningococcal disease (IMD) than other people of the same age. This excess risk was associated with catered accommodation. Some universities had higher rates each year and this was seen in particular halls of residence. It is unclear why some halls of residence should have a greater risk of cases than others.

Methods - Cases of IMD amongst university and higher education college students during the 4 academic years 1994/5 - 7/8 were identified. Data on individual halls were collected using questionnaires on 31 social and environmental variables of halls; design, geographical location, residents, catering arrangements, numbers of bedrooms and kitchens, smoking policy, and features of hall bars. Poisson regression analyses were done using with the cases of disease the dependent variable and the social and environmental factors the explanatory variables.

Results- 90/100 eligible universities and colleges replied giving data for 491 separate halls. 89 cases of IMD occurred for 556,236 resident years. The incidence was 16.0 per 100,000 resident years (95% CI 13.0- 19.7). Factors independently associated with IMD were more first years in hall (>75%, RR=5.4 - 95% CI 1.7 – 17.7), a hall bar opened before 1990 (RR =13.9 – 95% CI 6.6 - 29), more than 14 residents per kitchen and all single rooms in the hall showed weaker associations and smoky bar was associated with a lower risk (RR = 0.1 – 95% CI 0.1 – 0.3).

Conclusion - Social and environmental factors contribute to the increased risk of IMD in students. Most factors have ready epidemiological explanations.

#244

CARRIAGE PATTERNS OF MENINGOCOCCI STRAINS WITHIN UNIVERSITY HALL OF RESIDENCE - IMPLICATIONS FOR DISEASE CONTROL.

Neal Keith R, Ala'Aldeen Dlawer, Slack Richard, Nguyen-Van-Tam Jon, Gray Steve, Kaczmarski Edward.
Department of Public Health Medicine and Epidemiology, University of Nottingham, UK, PHLS Meningococcal Reference Unit, Manchester, UK.

Introduction - University halls of residence (dormitories) with catered facilities have been shown to be linked to higher rates of disease and clusters. To look for patterns of linkage within halls we looked at the distribution of meningococcal strains over 3 years in one hall of residence.

Methods - One hall of residence at the University of Nottingham (UK) with 230 residents was investigated over a period of 3 years (1997-2000). Students underwent serial oro-pharyngeal swabbing for meningococci. Swabs were directly plated onto VCNT media and incubated for 48 hours in 5% CO_2. Characterisation of strains was performed by the PHLS Meningococcal Reference Unit (Manchester, UK). A detailed plan of the hall, by room, was used to look for clusters by floor, sub-blocks and kitchen communal areas.

Results - Carriage rates of over 30% were found after the first week of term. No exclusive clustering of strains by any identifiable structural sub-area was identified. On the 2 occasions (October 1997 and October 1998) when a case of serogroup C disease occurred, phenotypically related organisms were spread widely through the hall.

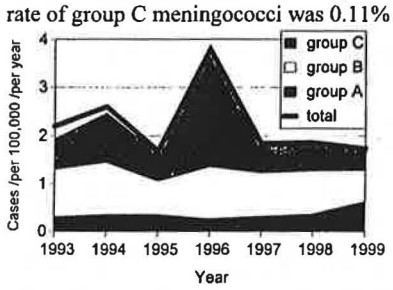
Conclusion - The identification of sub areas is inappropriate in managing IMD cases. The hall should be included as a single unit in terms of further measures.

#245

IS GROUP C MENINGOCOCCAL DISEASE INCREASING IN MOSCOW FOLLOWING EUROPE?

Koroleva IS, Chistyakova GG, Kuijper E, Platonova OV, Platonov AE.
Central Research Institute of Epidemiology, Moscow, Russia;
Moscow State Center for Epidemic Surveillance; Academic Hospital of the University of Amsterdam, the Netherlands.

In 1993-1999 the substantial increase in the general incidence of meningococcal disease (MD) in nine European countries correlated with the increase of the absolute number and proportion of cases caused by serogroup C meningococci. Our surveillance of MD in Moscow 1993-1999 have demonstrated that the incidence of group A and group B disease was stable with the exception of 1996, when the large outbreak of group A disease occurred. In 1999 the incidence of group C disease reached 0.63/100,000 in comparison to 0.34 in 1993-98. Additionally, in four adjacent districts of Moscow, where the average incidence of systemic MD was 2.6/100,000 in 1999, the carriage rate of group C meningococci was 1.7% (22 of 140 carrier strains) in contrast to six other districts with the average incidence 1.3 where the carriage rate of group C meningococci was 0.11% (1 of 48 carrier strains).



This phenomenon may reflect the epidemiological connections between Western and Eastern Europe and serve as the early warning of possible spread of group C disease to the East European and Asian parts of Russia.

#246

LONG-TERM MONITORING OF MENINGOCOCCAL SUSCEPTIBILITY TO ANTIBIOTICS IN THE CZECH REPUBLIC (1981-1999)

Urbaskova P., Kriz P. Center for Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic

Objective: To assess the situation and trends in antibiotic susceptibility of *Neiseria meningitidis* strains isolated between 1981 and 1999 in the Czech Republic.

Materials and methods: Minimal inhibitory concentrations (MIC) of penicillin, cefotaxime, chloramphenicol, rifampicin and sulfonamides were established by the agar dilution method on Mueller Hinton agar with 5% of sheep blood.

Results: In the period from 1981 to 1999, 1367 meningococcal strains were tested for susceptibility, 2.4 % of them were not susceptible to penicillin (MIC 0.125-0.25 mg/l), all of them were susceptible to cefotaxime (MIC_{90}<=0.008 mg/l), chloramphenicol (MIC_{90}<=0.5 mg/l) and rifampicin (MIC_{90}<=0.125 mg/l); 24.9 % were resistant to sulfonamides (MIC_{90}>8 mg/l). Meningococci resistant to penicillin were found with three times higher frequency (3.6 %) among isolates from the nasopharynx of healthy carriers or from patients with non-invasive disease compared to those isolated from patients with invasive meningococcal disease - IMD (1.1 %). Three quarters of strains resistant to penicillin were of serogroup B (21 strains), the others were of serogroup C (7 strains).

Conclusions: Meningococci resistant to penicillin have so far been rare in the Czech Republic. In invasive penicillin resistant isolates, MIC did not exceed 0.125; strains with penicillin MIC 0.25 mg/l were isolated only from the nasopharynx of healthy carriers. Resistance to sulfonamides occurs in about one quarter of strains.

#247

ANTIMICROBIAL SUSCEPTIBILITY AND COMPARISON OF *penA* SEQUENCES IN ISOLATES OF MENINGOCOCCI FROM SOUTH AUSTRALIA.

Lawrence AJ, Hansman DJ, Wati S, Turnidge J
Microbiology and Infectious Diseases Department, Women's and Children's Hospital, Adelaide, South Australia.

Objective : To ascertain the susceptibility (MIC value) to a number of antimicrobial drugs and to compare the *penA* DNA sequence of isolates of *Neisseria meningitidis* from South Australia.

Design: We examined the antimicrobial susceptibility of strains of meningococci from cases of invasive MD in South Australia during the 4 year period, 1996 through to 1999. These strains were predominantly serogroup B and C. Testing was done by agar dilution on Mueller-Hinton (MH) agar with sheep blood, except for tests with sulphonamides and co-trimoxazole when MH agar with lysed horse blood was used. The method was based on National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Drugs tested were azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, co-trimoxazole, minocycline, penicillin G, rifampicin, sulphadiazine, sulphamethoxazole and trimethoprim. Additionally, for 38 of these strains, a 711 base pair portion of the *penA* gene was sequenced. The range of MIC values of the strains sequenced was 0.008 to 0.5µg/mL penicillin.

Results: Relative insusceptibility to penicillin G was detected in 3 of 91 strains (3%), as was insusceptibility to rifampicin, 9 of 108 (8%). Resistance to sulphamethoxazole was common 66 of 124 strains (48%) and resistance to co-trimoxazole was slightly less so, 28 of 85 (32%). 100% of isolates were susceptible to ceftriaxone, chloramphenicol and ciprofloxacin. The DNA sequence identity (for 711 bases, positions 1035 – 1746) of strains with MICs of <=0.03 µg/mL was 98%. However for strains with MIC values greater than 0.03µg/mL there was considerable sequence variation.

Conclusions: All isolates were fully susceptible to ceftriaxone, chloramphenicol and ciprofloxacin. Resistance to sulphamethoxazole was common and resistance to co-trimoxazole less so. Relative insusceptibility to azithromycin, penicillin and rifampicin was uncommon. DNA sequencing of the *penA* gene may be of value for determining reduced susceptibility to penicillin, particularly in culture negative, PCR positive cases.

#248

ANTIBIOTIC SUSCEPTIBILITIES OF *NEISSERIA GONORRHOEAE* IN CUBA (1995-1999).

Llanes R,[1] Sosa J,[1] Guzmán D,[1] Valdés EA,[1] Gutiérrez Y,[1] Gutiérrez O,[1] Llop A,[1] Sotolongo F,[2] [1]Trop. Med. Inst. "Pedro Kouri" (IPK), [2]Finlay Institute. Havana, Cuba.

Objective: To determine antibiotic susceptibilities (AS) of *Neisseria gonorrhoeae* (NG) local strains to guide appropiate therapy.

Design: One hundred twenty NG strains isolated during 1995-1999 from different Cuban provinces were studied at the Natl. Ref. Lab. for Neisseria (IPK). MICs for different antibiotics (see table) were determined by the agar dilution method (NCCLS).

Results: See Table: AS of 120 NG strains in Cuba. 1995-99 (No/%)

Antibiotics	Resistant	Intermediate	Susceptible
Penicillin	72/60	6/5	42/35
Tetracycline	64/53.3	12/10	44/36.7
Ceftriaxone	-	-	120/100
Spectinomycin	-	2/1.7	118/98.3
Ciprofloxacin	1/0.8	3/2.5	116/96.7
Azithromycin	-	-	65/100 *

* For this antibiotic only 65 NG strains were evaluated

Conclusions: A highly proportion of NG strains resistant to both, penicillin and tetracycline, the drugs of choice to treat uncomplicated gonococcal infections in Cuba was detected. The rest of antibiotics showed to be effective. We report here the first NG resistant/moderately resistant to ciprofloxacin in Cuba. It is prudent to continue monitoring antimicrobial susceptibilities of NG strains in our country.

#249

COMPARISON OF FOUR METHODS FOR DETECTION ß-LACTAMASE ACTIVITY IN NEISSERIA GONORRHOEAE

Llanes R,[1] González M,[1] Martínez IF,[2] Sánchez L,[1] Sosa J,[1] Guzmán D,[1] Sotolongo F[2] [1] Trop. Med. Inst. "Pedro Kouri" (IPK) [2] Finlay Institute. Havana, Cuba.

Objective: To compare 4 methods for detection ß-lactamase (BL) activity in *Neisseria gonorrhoeae* (NG) strains in Cuba.

Design: Seventy NG strains isolated in Cuba during 1995-97 were studied. The following methods: chromogenic, iodometric, acidimetric and biologic, to detect BL activity were compared. Statistical analysis was performed using the X^2 test and the index of Kappa (KI), in the latter case the chromogenic method was used as gold standard.

Results: Thirty-one strains (44.3%) were PPNG and 39 (55.7%) were non-PPNG strains. The latter group of strains were BL-negative, by the 4 methods. However, for PPNG strains 29/31, 30/31 and 31/31 were BL positive by the iodometric, acidimetric/biologic, and the chromogenic methods, respectively. There was no statistically differences between the 4 methods studied ($p < 0.01$) existing a high concordance (KI) between the different methods used and the chromogenic one. For iodometric KI= 0,97 and for acidimetric/ biologic KI= 0,98.

Conclusions: A highly percentage of PPNG strains was observed in our Cuban NG strains. No statistically differences existing between the different BL methods employed in our strains, other-otherwise there was a good concordance between them. We recommend to use any of such methods to evaluate BL activity in NG strains, in Cuba.

#250

MENINGOCOCCI WITH REDUCED SUSCEPTIBILITY TO PENICILLIN IN ITALY: GENETIC DIVERSITY OF *pen*A GENE BY FINGERPRINTING OF AMPLIFIED DNA

Stefanelli P.[1], Fazio C.[1], Muscillo M.[2], and Mastrantonio P[1].[1] Dept. of Bacteriology and Medical Mycology and [2] Dept. of Environmental Hygiene, Istituto Superiore di Sanita', Italy

Objective: To characterize meningococcal clinical isolates with reduced susceptibility to penicillin due to the production of altered forms of penicillin-binding protein 2 (PBP2). To this aim the *pen*A gene, coding for PBP2 proteins, was amplified by PCR and analyzed by fingerprinting to identify genetic diversity among strains.

Design: Twenty-eight *Neisseria meningitidis* clinical isolates with decreased susceptibility to penicillin (MIC mean value of 0.11 µg/ml) and 21 susceptible strains (MIC mean value of 0.032 µg/ml) equally distributed among serogroup B and C and representing the most frequent phenotypes isolated in the country from 1994 to 2000 , were studied.

Results: During the 1980s, most strains examined had MIC values for penicillin less than 0.016 µg/ml, whereas in the second half of the 1990s less than 15% of the strains had such a value and 11.7% of isolates were moderately susceptible to penicillin (with MICs ranging between 0.094 and 0.190 µg/ml). To characterize the latter and investigate these strains, the *pen*A gene was amplified by PCR using previously reported primer sets. The resulting amplimers were cut by restriction endonuclease *Hpa*II and electrophoresed with Methaphor agarose. A total of 13 different RFLPs were found. Two major patterns (RFLP I and II) predominated among the strains examined without any correlation with decreased susceptibility. The strains with reduced susceptibility to penicillin exhibited 11 RFLPs , 9 of which exclusive of this group, differing from the most frequent RFLP II for only one band.

Conclusions: The results obtained suggest the presence of two major *pen*A gene populations among the strains analyzed not correlating with decreased susceptibility to penicillin. This genetic homology will be further investigated with DNA sequencing of the gene in order to identify alterations already described as well as to determine a real homology to the sequence identified.

#251

UTILITY OF MULTILOCUS SEQUENCE TYPING (MLST) IN INVESTIGATIONS OF MENINGOCOCCAL DISEASE OUTBREAKS IN THE UNITED STATES

Mayer LW, Reeves MW, and Popovic T

Centers for Disease Control and Prevention, Atlanta, USA

Objective: It has been demonstrated that multilocus sequence typing (MLST) provides results comparable to that of multilocus enzyme electrophoresis (MEE) for population biology and taxonomic purposes. Given that data supporting its utility in outbreak investigations is lacking, we chose for analysis 32 *N. meningitidis* serogroup C isolates collected during 2 epidemiologically well characterized outbreaks (New Mexico, 1994 and California, 1993): 21 were epidemiologically defined as outbreak-associated (OA), while 11 were sporadic. All isolates had been previously subtyped by MEE, pulsed-field gel electrophoresis (PFGE), serotyping, serosubtyping, ribotyping, and arbitrary primer (AP) PCR.

Results: Excellent correlation of MLST and epidemiological data was observed for all New Mexico isolates: all OA isolates were of ET-17, a member of the ET-37 complex, and of 7 gene sequence type (ST7) 11. Even though by MEE, 3 of 5 sporadic isolates were identical to the OA isolates (ET-17), none of 5 sporadic isolates were of ST7-11 The MLST results were not as discriminating for isolates from the California outbreak which occurred in a correctional facility in Los Angeles County All 10 outbreak isolates, from 9 inmates, and all 4 community cases with a reported contact with an inmate were of ET-24, another member of the ET-37 complex, and of ST7-11. Five of 6 sporadic isolates were also ST7-11, compared with 3 of 6 sporadic isolates that were of ET-24.

Conclusion: Although, sensitivity of 100% in identifying an OA isolate in these two outbreaks was achieved by both MEE and MLST, substantial differences in specificity were observed MLST was 100% specific for New Mexico isolates but only 33% specific for those from California, while MEE was 40% specific for New Mexico and 56% for California isolates. Other subtyping methods resulted in similar levels of specificity and sensitivity relative to the epidemiological case definition of an outbreak. Further evaluation of utility of MLST in outbreak investigations is needed. Use of more than a single subtyping method, in conjunction with epidemiologic data should be considered for outbreak investigations.

#252

THE RECENT "HAJJ" ASSOCIATED EPIDEMIC MENINGOCOCCAL W-135 P1.5,2 STRAIN: COMMON IN SWEDEN SINCE THE 1970s

Mölling P, Bäckman A, Olcén P, Fredlund H.
National Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiology & Immunology, Örebro Medical Centre Hospital, SE-701 85 Örebro, Sweden.

The first outbreak of *Neisseria meningitidis* (Nm) serogroup W-135 disease has recently been reported associated with pilgrims returning to Europe and the US from the "Hajj" in Mecca in April 2000 (1). One of these strains in the US has been reported to have genosubtype P1.5,2 by *porA* gene sequencing (1). In Sweden, this genosubtype was found in isolates from two patients and two carriers, who all had been in contact with pilgrims. To investigate the prevalence of this genosubtype over a longer period of time, all preserved invasive and carrier strains of Nm W-135 (n=43), isolated in Sweden during 1978-2000 were genosubtyped in variable regions (VR) 1,2 and also 3 (2). The most frequent genosubtype P1.5,2,36b was found in 10 invasive and 6 carrier strains. Also the second most common genosubtype P1.18a,3,38 was seen in invasive (n=6) and carrier (n=7) strains. Totally 7 different genosubtypes, including a new VR2 variant (26-2), were found. It is not surprising that the recent "Hajj" connected Nm W-135 strain showed genosubtype P1.5,2 since that genosubtype is the most common subtype among invasive Nm W-135 isolates since the 1970s in Sweden.

References:
1. Fine A and Layton M. MMWR April 28, 2000/49(16);345-6
2. Mölling P, Unemo M, Bäckman A and Olcén P. APMIS 2000, in press

#253

EVALUATION OF POLYMERASE CHAIN REACTION (PCR)-BASED DIAGNOSIS OF INVASIVE MENINGOCOCCAL DISEASE IN ENGLAND

Morgan J[1], Kaczmarski E[2], Stuart J[3], Popovic T[1], Guiver M[2], Shutt K[1], Orr H[3], Perkins B[1], Rosenstein N[1], [1] MSPB, CDC, Atlanta; [2] MRU, Manchester, UK; [3] PHLS, Gloucester, UK.

Background: England recently began routinely vaccinating infants against serogroup C meningococcal disease, a leading cause of death among young children. The need to determine accurately the burden of meningococcal disease highlights the importance of improved diagnostic methods. Diagnosis traditionally relied on culture of *Neisseria meningitidis*; newly developed polymerase chain reaction (PCR) testing was introduced and we performed this study to assess its effectiveness.

Methods: We reviewed medical records of 274 patients in South/West England for whom PCR had been performed on blood and/or cerebrospinal fluid (CSF) during June to December 1998. Initial screening PCR assays targeted the capsular transport gene (*ctrA*); reactive specimens were tested by PCR for the sialyltransferase (*siaD*) gene to determine serogroup. A culture-confirmed case was defined as positive blood or CSF culture from a sterile site. A possible meningitis case was defined as negative culture, but with meningismus, altered mental status and fever, or CSF consistent with bacterial meningitis.

Results: All 274 patients had specimens submitted for both culture and PCR. Twenty-nine persons had positive cultures and 45 had a positive PCR. For culture-confirmed cases, PCR sensitivity was 50% (95% confidence interval [CI] 28-72%), and specificity 84% (95% CI 78-89%). For possible meningitis cases, CSF PCR sensitivity was 63% (95% CI 41-81), and specificity 88% (95% CI 75-96). Nine of 14 patients with possible meningitis were laboratory confirmed by PCR.

Conclusions: Despite suboptimal sensitivity, PCR has increased overall laboratory-confirmed cases of meningococcal disease in England by 33% during 1998. Modifications to the PCR technique since this investigation have demonstrated marked improvement in sensitivity and while further advances are needed, PCR could be an important tool for surveillance.

#254

CARRIAGE RATE OF MENINGOCOCCI IN TWO DIFFERENT TEENAGE POPULATIONS.

Muscat I[1], Neal KR[2], Wells D[2], Robinson K[2], Scarth E[2], Gray S[3], Kaczmarski E[3], Slack R[2], Ala'Aldeen DAA[2].
Dept Microbiology, St Helier General Hospital, Jersey; CI
Meningococcal Research Group, University of Nottingham, UK;
PHLS Meningococcal Reference Unit (MRU), Manchester, UK.

Introduction - There is a rising carriage rate of *Neisseria meningitidis* among older teenagers, leading to a peak of invasive meningococcal disease in this age group. We looked at carriage rates in two different populations.

Methods - Pupils in a sixth form college (16y) on the Island of Jersey, Channel Islands (population 80,000) who live at home, and first year students (18y), living in single room dormitories, at Nottingham University (England) underwent serial oro-pharyngeal swabbing for meningococci. Strains were characterised at the MRU.

Results - In Nottingham, we examined rapidity of acquisition and confirmed previous results that carriage increases within days (<1wk). Carriage rate reached around 20% and remained stable.

	September On arrival	After one week	Mid term
University	4.4%	21.7%	20.8%
Jersey	12.4%	Not done	20.0%

In Jersey, carriage reached a similar rate to the University students and remained high at 20% 3 months after mass vaccination with Group C conjugated vaccine. There was no significant difference in strain diversity between the two sites. Group C carriage was documented in one Jersey student 3 months after receiving conjugate vaccine.

Conclusion- Carriage is rapidly acquired, and sustained at around 20% throughout the term. The impact of the new conjugate Group C vaccine on carriage will be presented.

#255

CHARACTERIZATION BY MULTILOCUS SEQUENCE TYPING OF 130 SEROGROUP A *NEISSERIA MENINGITIDIS* FROM AFRICA ISOLATED BETWEEN 1988 AND 2000.

RIGLET V., DECOUSSET L., NICOLAS P.

Unité du méningocoque, Institut de Médecine Tropicale du Service de Santé des Armées, Centre Collaborateur OMS, Parc du Pharo BP 46 13998 Marseille Armées, France.

Objective:

We used Multilocus Sequence Typing (MLST) method developed by Maiden et al. for the characterization of 130 Serogroup A *Neisseria meningitidis* strains isolated in Africa between 1988 and 2000.

Design: 130 strains isolated in 15 African countries between 1988 and 2000 were characterized by grouping, typing, sub-typing and Multilocus Sequence Typing.

Results: The 7 loci of the 130 strains were all unambiguously characterized by their sequences. For most of strains, alleles and sequence type were already on the internet data base (http://mlst.zoo.ox.ac.uk), allowing us to classify them in ST5 or ST7.

Conclusion:

Sequence type 5 (ST5) strains were isolated in most of cases and outbreaks that happened in Africa between 1988 and 1996. As ST5 was still responsible of outbreaks, ST7, a new clone emerged. ST7 strains were isolated in Algeria in 1995, in Chad and in Cameroon 1997, in Zaire in 1998, in Niger and Sudan in 1999.

#256

CIPROFLOXACIN RESISTANT GONOCOCCI IN THE UK – NOW AN ENDEMIC PROBLEM.

Palmer HM, Gough KR, Hemming MN & Turner A
Gonococcus Reference Unit, Genitourinary Infections Reference Laboratory, Public Health Laboratory, Bristol Royal Infirmary, Bristol BS2 8HW UK

Clinically significant resistance to fluoroquinolones in *Neisseria gonorrhoeae* has been reported worldwide. In the UK the first reported cases of intermediate susceptibility to ciprofloxacin (MIC ≥ 0.05mg/l) and resistance (MIC ≥ 1mg/l) were in 1990[1] and 1994[2]. Patient demographics reveals that initially most of the resistant strains were acquired abroad, but recently there has been a trend towards more endemic acquisition. In October 1999 an increase in the number of ciprofloxacin resistant strains belonging to the non-requiring auxotype was reported in the neighbouring towns of Oldham and Rochdale situated in the North West of England. These strains have been investigated in the context of other ciprofloxacin resistant non-requiring *N. gonorrhoeae* isolated elsewhere in England and Wales during the period April 1999-February 2000. Strains were opa-typed[3] (using a modified method) in order to distinguish between related and unrelated strains. Of 75 strains studied, 51 were indistinguishable by opa-typing. This cluster included 33 strains from Oldham and Rochdale, 16 other strains isolated from the north of England and a further 2 strains with known epidemiological links to Oldham but isolated in the south of England. None of these strains were reported to have been acquired abroad. The remaining 24 strains all gave unique opa-types and nine were known to have been acquired abroad. Opa-typing facilitated the identification of an outbreak of antibiotic resistant *N. gonorrhoeae*, which extended beyond the original focus of transmission (two towns in the North West of England), to other areas in the North of England.

1. Gransden WR *et al. Lancet.* 1990;335:51.
2. Birley H *et al. Genitourin Med.* 1994; 70:292-3.
3. O'Rourke M *et al. Mol Microbiol.* 1995; 17: 865-875

#257

EVALUATING THE SURVEILLANCE SYSTEMS FOR MENINGOCOCCAL DISEASE IN FRANCE IN 1996 USING CAPTURE-RECAPTURE METHODS

Perrocheau A[1], Alonso JM[2], Goulet V[1], Levy-Bruhl D[1], Desenclos JCD[1].
[1] Institut de Veille Sanitaire, Paris, France
[2] Centre National de Référence des Méningocoques, Institut Pasteur, Paris, France

Background : The surveillance of Meningococcal Disease in France is based on mandatory notifications (MN). In 1989-90 the sensitivity of the MN system was estimated at 51% using capture-recapture method with 2 sources. To monitor the evolution of the sensitivity of Meningococcal Disease surveillance we conducted a new capture-recapture analysis with 3 sources in 1996.
Methods : A case was defined as a person with meningococcal infection and *Neisseria meningitidis* isolated from blood or CSF admitted in France in 1996. Three sources of cases of meningococcal infection were crossed : 1) MN; 2) patient samples sent to the National Reference Centre for Meningococci (NRC); 3) laboratory reporting from a hospital network "EPIBAC". After identification of matches between sources we estimated the total number of cases that occured in 1996 using log-linear regression modeling. This value was used as a denominator to calculate the sensitivity of MN, NRC and EPIBAC in 1996.
Results : A total of 415 individual cases were identified from the 3 sources (285 from MN, 329 from the NRC and 231 from EPIBAC). 220 cases cases were matched between MN and NRC, 192 between NRC and EPIBAC and 145 between MN and EPIBAC. 127 cases were matched between the 3 sources. Log-linear modeling identified interaction terms between NRC and MN and between NRC and EPIBAC. The best model (smallest AIC) gave an estimate of 470 cases (95%CI; 430-510) to have occured in 1996. From 1989-1990 the sensitivity of the MN increased from 51% to 61% (95%CI; 56-66%) and the sensitivity of the NRC increased from 53% to 70% (95%CI; 65-77%). The sensitivity of EPIBAC was estimated at 49% (95%CI; 45-54%) in 1996.
Conclusions : The sensitivity of MN and NRC improved between 1989-1990 and 1996. A better awareness of the importance of reporting Meningococcal Disease amongst physicians and biologists is the most likely explanation for this improvement. This study demonstrates the value of capture-recapture methods in evaluating surveillance systems and their improvement over time. Evaluation of trends over time should take into account this improvement.

#258

AN AUDIT OF ANTIBIOTIC PRESCRIBING FOR PROPHYLAXIS OF SECONDARY CASES OF MENINGOCOCCAL DISEASE.

Marks Peter, Neal Keith R. Department of Public Health Medicine and Epidemiology, University of Nottingham, UK.

Introduction - If chemoprophylaxis for contacts of cases of invasive meningococcal disease (IMD) is administered to too few contacts, potentially preventable cases of IMD may occur. However, if prophylaxis is given too widely problems may follow as a result of side effects of medication, and clearance of competitive flora, which may increase the risk IMD.
Methods - Details of all 137 cases of IMD and all 952 identified contacts were obtained for two years in one health district of 540,000 people in the UK. Hospital and general practitioner (primary care physician) prescribing data were obtained. Questionnaires were used to obtain further information about prescribing for contacts of IMD.
Results - 66% of prescribing was done by hospital staff and 34% by general practitioners. General practitioners wrote twice as many prescriptions as were recommended, but there was little extra prescribing by hospitals. For every 100 patients recommended to have chemoprophylaxis, 143 prescriptions were written. However, despite this additional prescribing no evidence of a prescription could be found for 32% of the contacts who should have received prophylaxis.
Conclusion - Although almost 50% more prescriptions were written than had been recommended, a significant number of people who should have received prophylaxis did not. This leaves some contacts at increased risk of becoming a secondary case. Over-prescribing may also cause problems of side effects in those people who do not need prophylaxis and even increase their risk of contracting IMD by eliminating carriage of *N lactamica* and other commensal flora.

#259

CRITICAL EVENT AUDIT OF SECONDARY CASES OF MENINGOCOCCAL DISEASE.

Marks Peter, Neal Keith R. Department of Public Health Medicine an Epidemiology, University of Nottingham. UK.

Introduction –Close contacts of patients with invasive meningococcal disease (IMD) are at 1000 fold increased risk of being a developing IMD. Antibiotics are administered to reduce this risk but failure is we documented. These cases occur over 3 months. Research in the effectiveness of prophylaxis is difficult and no ethical randomised tria can be done. We report a critical event audit of second cases of IMD
Methods – The computerised records of 3 health authorities were searched for cases of IMD from 1992-99 Duplicates of surname, addresses and post codes were identified Additional episodes of link were identified from the paper records systems Details of potentially linked cases were examined A time limit of 100 days was used for associated cases Educational establishments and community outbrea were excluded
Results - 0 (A), 1 (B) and 5 (C) linked cases were identified by authority Each authority had 700-800 cases in the study period In authority B, the failure was due to none notification of the first case C, child care contacts were missed (2), co-primary infections (1), mut contact named later (1), and household contacts only (1)
Authorities A and B used ciprofloxacin for adult contacts, included a wider group of contacts and visited nearly cases Authority C used rifampicin to adult contacts, strictly applied the UK guidelines for clo contacts and advised from the office Difference in possibly preventa secondary cases between A & B vs C, p = 0 01 (Fisher exact test)
Conclusion - Missed contacts were generally adults who looked after children. Ciprofloxacin and intervening wider may be more effective controlling second cases.

#260

CHANGING CHARACTERS: FOUR DIFFERENT CLONES PLAYED CONSECUTIVELY THE LEADING PART IN SEROGROUP A MENINGOCOCCAL DISEASE FROM 1969 TO 1997 IN MOSCOW

van der Ende A, Platonov AE, Koroleva IS, Kostyukova NN, Kusecek B, Morelli G, Brieske N, Schuurman IGA, Zurth K, Zhu P, Achtman M. Central Research Institute of Epidemiology, Moscow, Russia; N.F.Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia, Dept. of Medical Microbiology, Academic Medical Center, Amsterdam, The Netherlands; Max-Planck Institut für molekulare Genetik, Berlin, Germany.

Serogroup A *Neisseria meningitidis* have been responsible for a considerable proportion of the bacterial meningitis in Moscow since the 1920's. Bacteria isolated since the 1969 were analyzed by a variety of powerful molecular techniques. The results showed that four independent clonal groupings have successively caused disease in Moscow. Subgroup III bacteria, having the pgm3 allele, were responsible for an epidemic between 1969 and the mid-1970's. A new subgroup, X, and then subgroup VI caused disease through to the early 1990's. A subsequent epidemic in 1996 was caused by the pandemic spread of subgroup III bacteria, having the pgm 19 allele. These data show that subgroup III meningococci continues to spread and may result in group A outbreaks in other countries.

Grouping (Num. of strains)	MLST types	Main sero(sub)types
subgroup III (17)	ST5 (pgm3 allele)	4,21:P1.20,9
subgroup X (23)	ST75, 76, 77, 78	4,21:P1.10 or 21:P1.10
subgroup VI (40)	ST2, ST68-73	4,21:P1.5,10 or 4,21:P1.10
subgroup III (21)	ST7 (pgm19 allele)	4,21:P1.20,9

#261

TOPOLOGY OF MENINGOCOCCAL INFECTION

Platonov AE, Platonova OV, Demina AA, Ermakov SP, Bolshakov KN, Kostyukova NN. Central Research Institute of Epidemiology, Moscow, Russia; N.F.Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia.

Usually surveillance data of of meningococcal infection are presented in tabular form: country by country, region by region in alphabetical order. Nevertheless, the infection does not respect political and administrative borders, so the importance of meningococcal epidemiology is to delineate spatial "epidemiological units" having relatively stable and independent epidemic features. Well-known examples of such units are the "meningitis belt" in Africa and large islands (Cuba, Iceland, Ireland, North Zealand). Mapping meningococcal incidence in Russia 1964-99 in 89 administrative districts we can see a kind of "meningitis belt" in South-Eastern part of Russia adjacent to the borders with China, Mongolia and Kazakhstan. Another region of stable high incidence is the North-Western part of Russia adjacent to the White and Baltic seas. In contrast, Eastern Siberia is the region of low incidence. Other districts tend to cluster also into several large "units" where a cyclical rise and fall of meningococcal incidence was registered. A priori, a "unit" is the complex epidemiological phenomenon summarizing geographical, ecological and socioeconomical effects. The analysis of evolution and re-configuration of "epidemiological units" based on retrospective data should be extended prospectively. In Russia one may expect the introduction of serogroup A disease from the South-Eastern part and group C disease from North-Western part into the central part of country.

#262

INVESTIGATION OF PRE-ADMISSION ANTIBIOTIC TREATMENT IN PATIENTS WITH MENINGOCOCCAL DISEASE

Roznovsky[1] L., Strumcova[2] V., Dostal[3] V., Plisek[3] S., Burget[4] I., Svejda[5] J., Kriz[6] P., Dept. of Infectious Diseases, Ostrava[1], Plzeň[2], Hradec Králové[3], Brno[4], Ústí nad Labem[5], National Institute of Public Health[6], Praha, Czech Republic

Objectives: Pre-admission antibiotic treatment is not compulsory in patients with suspected invasive meningococcal disease in the Czech Republic.

Design: Investigation of pre-admission parenteral and non-parenteral antibiotic treatment was performed in 112 patients (10 deaths) with invasive meningococcal disease, who were treated at 5 university hospitals in Czech Republic from January, 1996 to May, 2000. Statistical analysis was performed using Fisher's exact test.

Results: Pre-admission antibiotic treatment was used in 77 patients and only 5 of them (6.5%) died. Thirty-five patients were not treated before admission and also 5 of them (14.3%) died. Pre-admission treatment was used in 21 of 36 patients with septicaemia (4 treated patients of 7 deaths), in 44 of 54 patients with septicaemia/meningitis (1 treated patient of 3 deaths) and in 12 of 22 patients with meningitis (0 death in this group). The mortality of patients with and without pre-admission antibiotic treatment were 19.4% and 20.0% for septicaemia, 2.3% and 20.0% for septicaemia/meningitis and accordingly 0% for meningitis. The differences are statistically insignificant for all patients (p=0.162) and also for patients with sepsis/meningitis (p=0.085).
Parenteral treatment (especially cefotaxim, ceftriaxon and penicillin G) was administered in 62 patients, combine peroral and then parenteral treatment in 5 patients and only peroral treatment (preferably β-lactam antibiotics) was used in 10 patients. All 5 patient who died received parenteral therapy.
General practitioners and physicians of out-patients departments started antibiotic therapy sporadically (in 13 patients) and preferred peroral treatment except one patient with parenteral treatment. The parenteral therapy was started in local hospital before patients transport to university hospital.

Conclusions: Approximately two third of patient with meningococcal diseases in Czech Republic received pre-admission antibiotic treatment, but the therapy is only rarely started by general practitioners. The mortality of patients with pre-admission antibiotic treatment was more than twice lower then in patients without treatment (6.5% vs. 14.3%), but statistically significant differences were not proved.

Grant support: Grant Agency Czech Republic N. 310/96/K102

#263

Persistence of complexes of disease-causing *Neisseria meningitidis* isolated in England and Wales over twenty years 1975-1995.

J.E. Russell [1&2], I.M. Feavers[2], A.J. Fox[3], S. Gray M.C.J. Maiden[1]

[1]Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom.
[2]Division of Bacteriology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG, United Kingdom.
[3]Meningococcal Reference Unit, Manchester Public Health Laboratory, Withington Hospital, Manchester M20 8LR, United Kingdom.

The meningococci responsible for disease in England and Wales from 1975-1995 were characterised by multilocus sequence typing and nucleotide sequence analysis of their *porA* genes. The sample comprised at least 100 isolates received by the Meningococcal Reference Unit in 1975, 1985 and 1995. Several disease-associated clonal complexes were responsible for the majority of disease over this period, with changes in prevalence of individual complexes. Members of the ET-37 complex (belonging to serogroups C and B) were isolated from all time points, but over twenty years there was a reduction in the number of members of cluster A4. Consistent with previous studies, the levels of disease caused by ET-5 complex rose to a peak in 1985 , the fall in this complex being accompanied by a rise in the proportion of lineages 3 organisms isolated in 1995. A striking observation was the repeated occurrence at all time points of the sequence types associated with the putative founders of most complexes, with variants of these sequence types usually restricted to one or two time points.

#264

SEQUENCE ANALYSIS OF GENETIC DIVERSITY OF MENINGOCOCCAL *PENA* GENE

Sadler F[1], Borrow R[1], Kaczmarski EB[1], Ramsay M[2], Dawson MM [3], Fox AJ[1]. [1]PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK. [2] CDSC, London, UK. [3]Manchester Metropolitan University, Manchester, UK.)

Objective: To analyse the genetic diversity of the meningococcal *pen*A gene of meningococcal isolates from England and Wales and to determine the genetic background of isolates with altered *pen*A genes using multilocus sequence typing (MLST).
Design: The meningococcal *penA* gene was analysed by nucleotide sequencing. Whole gene products obtained by PCR were sequenced using the Beckman CEQ 2000 Analysis System and Red dye chemistry. Isolates representing a range of susceptibilities to penicillin by MIC and E-test were analysed. Isolates were also characterised by MLST to establish the strain background.
Results: *Pen*A sequence variation was evident between isolates showing decreased susceptibility to penicillin whereas sensitive isolates were relatively homogenous. However, while reduced sensitivity to penicillin is most common amongst C:2b:P1.5,2 isolates, the majority of these had identical *penA* sequences.
Conclusions: Sequence analysis of the *pen*A gene has identified the genetic variation underlying altered susceptibility to penicillin amongst recent representative UK isolates . The *penA* sequence types associated with the C:2b:P1.5,2 isolates were homogeneous and associated with a strain of clonal descent as demonstrated by MLST.

#265

MENINGOCOCCAL CARRIER ISOLATES – INVASIVE PATHOGENS OR AVIRULENT COMMENSALS?

Sadler F[1], Borrow R[1], Cartwright KAV[2], Dawson MM [3], Fox AJ ([1] PHLS Meningococcal Reference Unit, Withington Hospital, Manchester, UK. [2] Gloucester Public Health Laboratory, Gloucestershire Royal Hospital,UK. [3] Manchester Metropolitan University, Manchester, UK.)

Objective: The meningococcal polysaccharide capsule is the primary virulence factor necessary for survival in the blood. For invasion, the meningococcus must down-regulate its capsule to pass through the epithelium. During this study, 178 culture isolates of *N. meningitidis* obtained from an outbreak of serogroup C disease in 1995/6 were examined phenotypically. Eighty-nine isolates were found to be non-serogroupable. These 89 isolates were investigated to delineate their mechanism of down-regulation.

Design: To determine the capsular status of the isolates the *siaD* PCR ELISA was used to identify their serogroup (B, C, W135 and Y). Following examination with the *siaD* PCR ELISA three genes: *siaA*, *siaD*, and *ctrA*, were investigated by PCR and sequencing.

Results: Thirty isolates which were phenotypically non-serogroupable were serogroupable using the *siaD* PCR ELISA. Two isolates were positive for the insertion sequence IS*1301* within the *siaA* gene. Previous studies have shown the IS*1301* mediated loss of encapsulation results in both stronger adherence and increased entry of meningococci into epithelial cells. Sixteen isolates which were phenotypically non-serogroupable yet positive on the serogroup B PCR ELISA were investigated for slipped-strand within the *siaD* gene. Of the 16 isolates only 4 exhibited any evidence of slipped-strand mispairing. A *ctrA* PCR was utilised for the detection of this capsular transport gene that contains a conserved region present in all serogroups. Twenty-nine of the isolates investigated did not possess a *ctrA* gene.

Conclusions: Both potentially virulent and avirulent meningococcal carrier isolates exist. Fully avirulent organisms may be of use in a live meningococcal vaccine that could protect against all serogroups.

#266

RAPD AS A RAPID SCREENING TOOL FOR *NEISSERIA MENINGITIDIS* SEROGROUP C ISOLATES OF ET-24

Schmink S, Reeves MW, Plikaytis B, and T. Popovic

Centers for Disease Control and Prevention, Atlanta, GA, 30333.

Objective: Most of the SCMD outbreaks in the U.S. have been caused by *Neisseria meningitidis* serogroup C (NMSC) isolates, identified by multilocus enzyme electrophoresis (MEE) as being of the electrophoretic type (ET) 24, a member of the virulent ET-37 complex, but the same ET is also commonly found in endemic isolates. The objective of this study was to evaluate RAPD as a rapid screening tool to identify NMSC isolates of ET-24.

Results: A total of 199 U.S. NMSC isolates (77 of ET-24 and 122 of 50 other ETs) collected through the active laboratory-based surveillance between 1992 and 1998 were analyzed with two commercially available RAPD primers, P1and P5. Four different RAPD patterns were identified among ET-24 isolates using P1, with P1-II seen in 96% of them. This pattern was also shared by 22 other isolates that were not of ET-24; ETs of 16 of them were only a single enzyme different from ET-24. Using P5, 6 patterns were identified among 77 ET-24 isolates: P5-II in 81%, and P5-VII in 9% of isolates. Twenty-six non ET-24 shared patterns P5-VII and P5-II, and 18 of them had an ET differing from ET-24 by a single enzyme. P1 identified ET-24 isolates with sensitivity of 96% and specificity of 82%, while the use of P5 resulted in sensitivity of 90%, and specificity of 84%. Combining the P1 and P5 patterns a sensitivity of 88% and a specificity of 87% were obtained. The addition of isolates with ETs one enzyme different from ET-24 lowered the sensitivity to 82% but increased the specificity to 96%. RAPD also clearly differentiated ET-24 isolates from those of ET-17 and ET-27, two other major ETs within the ET-37 complex. Moreover, only 1 of 22 isolates outside of the ET-37 complex produced the pattern (P1-II) typical for ET-24 isolates.

Conclusion: RAPD can be very useful for rapid and reliable identification of ET-24 isolates, providing information that could assist public health officials in outbreak investigations and in decision making regarding the extent of an outbreak and the need for vacciantion.

#267

MENINGOCOCCAL MENINGITIS IN POLAND IN 1999

Skoczyńska A., Klarowicz A., Literacka E., Hryniewicz W.

National Reference Centre for Bacterial Meningitis, Sera & Vaccines Central Research Laboratory, Warsaw, Poland

Objectives: To characterise *N. meningitidis* isolated in Poland in 1999.

Material and methods: Meningococci isolated from cerebrospinal fluid (CSF) in 1999 were collected in the National Reference Centre for Bacterial Meningitis (NRCBM). The strains were identified to the species level by standard methods. Serotyping was performed by whole cell ELISA method. Minimal inhibitory concentrations (MIC) of 8 antimicrobial agents were evaluated by agar dilution method according to NCCLS

Results In 1999 113 cases of meningococcal meningitis were reported, however only 57 isolates (50 4%) from CSF were received by the NRCBM The most predominant was serogroup B (n=47, 82 5%), followed by group C (n=6, 10 5%) and W135 (n=4, 7 0%) In two isolates (3 5%) decreased susceptibility to penicillin was identified In general meningococci were susceptible to antimicrobial agents tested with the exception of cotrimoxazole (64 9% nonsusceptible isolates)

Conclusions: In 1999 *N. meningitidis* was still the most common etiologic agent of bacterial meningitis in Poland, causing sporadic cases only. The most predominant was serogroup B, being responsible for more than 80% of cases. After 3 years of activity, the NRCBM received in 1999 20% more of meningococcal isolates from notified cases as compared to previous 2 years.

#268

CLUSTERS OF MENINGOCOCCAL DISEASE IN SCHOOLS IN ENGLAND AND WALES: ASSESSING THE RISK

Stuart JM[1], Rushdy AA[2], White JM[2], Andrews N[3], Ramsay ME[2], Kaczmarski E[4], Monk PM[5]. Regional Services[1], Immunisation Division[2], PHLS Statistics Unit[3] PHLS Communicable Disease Surveillance Centre, London, UK. PHLS Meningococcal Reference Unit[4], Manchester, UK. Leicestershire Health[5], Leicester, UK.

Objective: To estimate the risk of occurrence of clusters of meningococcal disease in educational settings in England and Wales.

Design: Population based retrospective survey of clusters of meningococcal disease in pre-school and school settings in England and Wales from April 1995 to March 1998.

Results: 70 clusters were identified in pre-school and school settings over the 3 year study period. Cases in these clusters accounted for 4% of identified cases in 2-18 year olds. The relative risk of occurrence of a cluster (two or more cases within four weeks) was significantly raised in all settings. The relative risk ranged from 4.4 (95%CI 2.7-6.2) in secondary schools to 27.5 (95%CI 10.1-59.5) in pre-school settings and was highest in the week after the first case. The relative risk of occurrence of a cluster of serogroup C cases was 2-3 times more likely than a cluster of serogroup B cases in all settings.

Conclusions: The relative risk of further cases is low in educational settings compared with households. In conjunction with the uncertain evidence for effectiveness of chemoprophylaxis and the potential harm from widespread antibiotic use, we do not believe that recommending antibiotics for pupils after a single case in educational settings is justified by these data. A significant fall in cluster incidence would be expected to follow the introduction of serogroup C conjugate vaccines in the UK.

#269

RISK OF MENINGOCOCCAL DISEASE IN LABORATORY WORKERS

Stuart JM[1], Boutet R[1], Andrews NJ[2], Kaczmarski EB[3], Jones DM[3]. [1]Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre, Gloucester, UK. [2]PHLS Statistics Unit, London, UK. [3]PHLS Meningococcus Reference Unit, Manchester, UK.

Objective: To estimate the risk of meningococcal disease in laboratory workers from exposure to cultures of *Neisseria meningitidis*.

Design: Cases of meningococcal infection in laboratory workers in England and Wales between 1985 and 1999 were identified by a retrospective survey of NHS microbiology and Public Health Laboratories and by literature review. Absolute and relative risks of secondary meningococcal disease in laboratory workers were calculated from the number of identified secondary cases, estimates of numbers of laboratory workers at risk and incidence of meningococcal disease in the general population. Sensitivity analysis was done to allow for uncertainty around these parameters.

Results: Five secondary cases were identified in laboratory workers. All 5 had been exposed to cultures of *Neisseria meningitidis* in the 10 days before inset, 4 were confirmed by culture from a deep site and one by demonstrating seroconversion. They were all associated with introduction of a procedure involving manipulation of meningococcal suspensions outside a safety cabinet. The absolute risk was estimated as 1 case per 8000 exposed laboratory workers, a relative risk of 318 (95%CI 103 - 744).

Conclusions: The risk of meningococcal disease in laboratory workers who prepare suspensions of meningococci outside safety cabinets are at increased risk. Safety cabinets should always be used for such procedures, and chemoprophylaxis administered in case of inadvertent exposure. Conjugate meningococcal vaccines should be offered to laboratory workers who handle these organisms.

#270

MOLECULAR BASIS OF HIGH-LEVEL CIPROFLOXACIN RESISTANCE IN *NEISSERIA GONORRHOEAE* STRAINS ISOLATED IN DENMARK 1995-1998.

Xiaohong Su† and Inga Lind. Neisseria Unit, Department of Respiratory Infections, Meningitis and STIs, Statens Serum Institut, Copenhagen, Denmark

Background: In Denmark surveillance of the in vitro susceptibility to ciprofloxacin of *Neisseria gonorrhoeae* was established in 1990. The proportion of *Neisseria gonorrhoeae* strains with decreased susceptibility or resistance to ciprofloxacin (MIC ≥ 0.06 μg/ml) was low (0.3~2.3%) up to 1995. Between 1995 and 1998 the rate of less susceptible and resistant strains rose from 6.9% to 13.2%. Among ciprofloxacin resistant strains (MIC ≥1μg/ml), 81% were highly resistant (MIC ≥ 4μg/ml).

Design:Thirty five *Neisseria gonorrhoeae* strains (40 isolates) with ciprofloxacin MICs 4~32 μg/ml were investigated for the frequency and patterns of mutations within the gyrA and parC genes. The quinolone resistance-determining regions of the gyrA and parC genes were amplified by PCR and the amplicons were directly sequenced.The strains were analyzed for genetic relationship by pulsed-field gel electrophoresis (PFGE) .

Results: Alterations at Ser-91 and Asp-95 in the GyrA and a single or double alterations in the ParC were identified in 32 strains (91%). Ser-91 to Phe and Asp-95 to Gly alterations in the GyrA were detected in 28 strains (80%). The most common ParC alteration, Asp-86 to Asn , was found in 19 strains (54%). The analysis of genomic DNA by PFGE showed that nine strains with the same mutation pattern in the gyrA and parC genes, originating from different geographical areas over three years, shared the same PFGE patterns after SpeI as well as NheI digestion (only one strain with one band difference in NheI pattern), suggesting that a resistant clone had spread world-wide.

Conclusions: The results from this study strongly suggest that double gyrA mutations plus parC mutation(s) play an important role in the development of high-level fluoroquinolone resistance in *N. gonorrhoeae*.

†Present address: Xiaohong Su: Department of STD, National Center for STD and Leprosy Control, Institute of Dermatology, Chinese Academy of Medical Sciences, 12 Jiangwangmiao Road, Nanjing 210042, P.R.China

#271

ANTIBOTIC SUSCEPTIBILITY OF *N. meningitidis* FROM INVASIVE INFECTION, AUSTRALIA 1994 – 1999

Tapsall J and the National Neisseria Network c/- Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia

Objective: To describe the susceptibility of *N. meningitidis* from invasive meningococcal disease (IMD) in Australia from 1994 to 1999 to antibiotics used in treatment and prophylaxis.

Design: Isolates from cases of IMD in Australia in the period 1994 – 1999 were examined for trends in susceptibility to penicillin, ceftriaxone, rifampicin and ciprofloxacin by a standardised agar plate dilution technique in Australian reference laboratories. A programme-specific quality assurance system ensured consistency of results.

Results: 1434 strains were examined in the 6 year period. Penicillin MICs ranged between 0.008 and 1 mg/L. The proportion of sensitive strains (MIC <,= 0.03 mg/L) decreased from 45% in 1994 to 34% in 1995 and to 27% in 1996. Subsequently this percentage ranged between 19 and 26% (p =,< 0.0001). Strains less sensitive to penicillin (MIC 0.06 – 0.5 mg/L) comprised all of the remaining isolates except for single strains in 1996 and 1999 with an MIC of 1 mg/L. The geometric mean MIC increased from 0.045 to 0.065 mg/L from 1994 to 1999. No significant difference was noted between serogroup B or C isolates. All isolates were fully sensitive to ceftriaxone (MIC < 0.003 mg/L). Sporadic isolates were resistant to rifampicin. Two isolates in each of the years 1996 –1999 inclusive had MICs of 1 mg/L and a single isolate in 1999 had an MIC of >100 mg/L. All isolates were quinolone susceptible with the exception of a single isolate in 1998 with a ciprofloxacin MIC of 0.25 mg/L.

Conclusions: Penicillins remain suitable treatment for IMD in Australia despite some increased in vitro resistance. Resistance to the prophylactic agents rifampicin and ciprofloxacin is uncommon.

#272

PCR: A USEFUL METHOD FOR THE TYPING OF NON-GROUPABLE MENINGOCOCCAL STRAINS ISOLATED FROM CARRIERS IN GREECE.

G. Tzanakaki[1], E. Themeli[2], S. Levidiotou[2], J. Kremastinou[1] and CC Blackwell[3]

[1] National Meningococcal Ref Laboratory, NSPH, Athens Greece,
[2] Dept of Microbiology, Medical School, University of Ioannina, Greece
[3] Dept of Microbiology, University of Edinburgh, Medical School, U.K

Objective: To assess the Polymerase Chain Reaction (PCR) technique for classification of meningococcal isolates which were non-groupable by conventional anti-capsular antibodies.

Design: A total of 334 meningococcal strains were isolated from 3,100 individuals in 4 regions of Northern Greece (Ioannina, Serres, Evros and Florina). Of these, 198 isolates (60%) were non-groupable and were analysed by PCR. The Isoquick extraction kit (ORGA, USA) was used for the meningococcal DNA extraction. The gene amplified was *dhps* specific for *N. meningitidis* and *siaD* specific for serogroups A,B,C and Y. The PCR products were run on 2% agarose gel and estimated by comparison to a commercial size marker. The amplification products were 650bp for *N meningitidis* and 450 bp, 250 bp, 400 bp, and 120 bp for the serogroups B, C, A, and Y respectively. Controls included strains for which the capsular antigens were detected by grouping sera.

Results: Among the 118 non-groupable stains isolated from the regions of Ioannina, Evros and Florina, 57.6% (68/118) were groupable by PCR. Of these, 49 strains (72%) were serogroup B, 11 (16.1%) and 8 (11.8%) were serogroups C and Y respectively. A lower percentage of the NG strains from Serres, 30% (24/80), could be typed by PCR Twelve (12) strains were serogroup B, 6 serogroup C, and 6 serogroup Y. Most of the 56 strains which were non-groupable did not react with any of the monoclonal serotype or subtype reagents.

Conclusions: PCR is useful and necessary for typing of meningococcal strains when serological methods have failed.

#273

SUBSTITUTION OF THE ET37 COMPLEX BY THE A4 LINEAGE AFTER AN EPIDEMIC WAVE IN SPAIN .

Alcalá B, Vázquez JA.
Laboratorio de Referencia de Meningococos, Centro Nacional de Microbiología (ISCIII). Madrid, Spain.

An increased number of cases of meningococcal disease associated with serogroup C strains was observed during 1996-1997 in Spain. Most of the group C strains isolated before this period showed a 2b:Non subtypable antigenic combination, being associated to the ET37 complex by multilocus enzyme analysis. However the epidemic wave was associated with a C:2b:P1.2(5) strain.

In order to analyze if this phenotypic change was caused by the introduction of a new genetic lineage, we have used two molecular markers: Pulse field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). One thousand thirty four C:2b:P1.2(5) and 76 C:2b:Non subtypable (NST) strains were analyzed by PFGE, and of these, 30 C:2b:P1.2(5) and 14 C:2b:NST meningococci randomly choose were studied by MLST. We identified more than 50 profiles on the C:2b:P1.2(5) and 24 on the C:2b:NST meningococci, both groups sharing 9 different patterns. Most of the C:2b:P1.2(5) isolates (65%) belonged to only two different profiles (with only one band difference). In the analysis of the C:2b:NST strains we found two different situations: before 1994 most of them (60%) belonged to one specific profile, but after that a different pulsotype (PT) became the most frequently found (80%). We were able to correlate some of the PTs with the specific alellic profiles characteristic of both A4 and ET37 lineages. The most important finding was that the A4 complex has almost substitute to the ET37 cluster which is now very rare in serogroup C isolates in Spain.

#274

A NOVEL PROTEIN (MTRF) INVOLVED IN ANTIMICROBIAL RESISTANCE MEDIATED BY THE GONOCOCCAL MTRC-MTRD-MTRE EFFLUX PUMP

[1]Veal WL, [1,2]Shafer WM. [1]Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA and [2]Laboratories of Microbial Pathogenesis, VA Medical Center, Decatur, GA.

Objective: To determine if other genes are involved in resistance to antimicrobial hydrophobic agents (HA) mediated by the MtrCDE efflux system.

Design: Investigation of the function of the *mtrF* gene product through observation of the effects of insertional inactivation, identification of MtrF homologues, and determination of variations in *mtrF* transcriptional regulation.

Results: We recently identified a gene, *mtrF*, located adjacent to the *mtrR* and *mtrCDE* loci, that appears to encode a 56.1 kDa inner membrane protein containing 12 transmembrane domains. MtrF appears to be necessary for high-level resistance to certain hydrophobic agents, notably nonionic detergents. Homologues of MtrF demonstrating significant amino acid identity over the length of the protein were identified in genome sequence databases of both Gram-positive and -negative bacteria, resulting in the identification of previously uncharacterized but similar motifs among these MtrF homologues. Insertional-inactivation of *mtrF* resulted in an altered HA susceptibility profile; however, inactivation of *mtrF* in an HA-sensitive *mtrCDE* mutant strain did not further alter HA susceptibility, indicating that the function of MtrF is dependent upon MtrC-MtrD-MtrE. RT-PCR studies demonstrated increased transcription of *mtrF* in a strain lacking MtrR expression, indicating that *mtrF* expression is directly or indirectly controlled by MtrR.

Conclusions: Based on these observations, we propose that MtrF is involved in *mtr*-mediated HA-resistance. Furthermore, the identification of previously uncharacterized but similar motifs among MtrF homologues possessed by other bacteria suggests that MtrF defines a new protein family, which is of potential importance in microbial resistance to antibacterial agents.

#275

CARRIAGE OF ET-15 MENINGOCOCCI IN BAVARIA

Hessler F, Alber D, Claus H, Frosch M, Vogel U. Institute for Hygiene and Microbiology, University of Wuerzburg, Germany

Objective: ET-15 meningococci have first been reported in Bavaria during an outbreak of the disease in 1998. We determined the carriage rate of the ET-15 clone in Bavaria.

Design: In collaboration with the German military services and the Bavarian government, 830 meningococcal strains were isolated from 8000 Bavarian children and young adults. ET-15 meningococci were identified by molecular methods.

Results: We determined consecutively the *porB* class, the *fumC* sequence (1), the presence of restriction modification systems (2), the *pgm* and *pdhC* alleles, and performed PFGE to finally identify one ET-15 isolate out of 830 strains.

Conclusions: We estimate that the incidence in Bavaria of disease caused by ET-15 meningococci is 1/1.000.000/a. For this epidemiological setting, the carriage rate of the most-affected age groups was shown to be 1/8.000. This suggests a risk of >1/100 for these age-groups to become ill after colonization.

Literature:
1. Vogel, U., Claus, H., Frosch, M., and Caugant, D.A. *J.Clin.Microbiol.* 38(2):941-942, 2000.
2. Claus, H., Friedrich, A., Frosch, M., and Vogel, U. *J. Bacteriol.* 182(5):1296-1303, 2000.

#276

ANTIMICROBIAL SUSCEPTIBILITIES OF *NEISSERIA GONORRHOEAE* IN THE UNITED STATES

Wang SA, Jester-Harvey AB, Levine WC, Knapp JS, and Gonococcal Isolate Surveillance Project (GISP) Investigators, Centers for Disease Control & Prevention, Atlanta, GA.

Objective: To describe recent data on antimicrobial susceptibilities of *N. gonorrhoeae* in the United States.

Methods: Since 1987, STD clinics in approximately 26 U.S. cities have provided the first 25 male urethral gonococcal isolates each month to GISP for antimicrobial susceptibility testing. Clinical information was abstracted from medical records.

Results: Approximately 5,000 isolates are collected through GISP annually. Ciprofloxacin-resistant (MIC≥1.0 μg/ml) isolates in GISP were first identified in 1991 when only one GISP site had a ciprofloxacin-resistant isolate. In 1999, 19 such isolates were reported from ten GISP sites including Honolulu, where 8/49 (16.3%) of isolates were ciprofloxacin-resistant. An isolate with decreased susceptibility to azithromycin (MIC≥1.0 μg/ml) was first identified through GISP in 1993; in 1999, there were 25 such isolates from nine GISP sites. Isolates with decreased susceptibility to cefixime (MIC≥0.5 μg/ml) and ceftriaxone (MIC≥0.5 μg/ml) remain rare; during 1992-1998, there were 41 isolates with decreased susceptibility to cefixime and during 1987-1998, there were four isolates with decreased susceptibility to ceftriaxone. In 1999, there were no isolates with decreased susceptibility to either cefixime or ceftriaxone.

Conclusions: Ciprofloxacin-resistant gonococci remain rare in the continental U. S. but have been identified in an increasing number of locations and have become endemic in Hawaii. Isolates with decreased susceptibility to azithromycin are also rare but are being identified more frequently and in more locations. Only sporadic isolates with decreased susceptibilities to cefixime and ceftriaxone have been identified through GISP. Ongoing nationwide antimicrobial susceptibility monitoring is necessary to ensure appropriateness of gonorrhea treatment recommendations in the U. S.

#277

MOLECULAR EPIDEMIOLOGY OF *NEISSERIA MENINGITIDIS* SEROGROUP Y DISEASE IN THE US AND ISRAEL

Whitney AM, Block C, Sacchi CT, Reeves MW, Tondella MLC, Keller N, and Popovic T

Centers for Disease Control and Prevention, Atlanta, GA, Hadassah-University Hospital, Jerusalem, and National Center for Meningococci, Tel Hashomer, Israel

Design *Neisseria meningitidis* serogroup Y (NMSY), unlike other serogroups, is frequently associated with systemic infections and pneumonia, especially in the elderly. The proportion of meningococcal disease caused by NMSY in the US has increased from 10% in 1992 to 32% in 1998. Likewise, in Israel NMSY now accounts for 15-20% of all identified serogroups, compared to 4% in 1990. We analyzed 167 NMSY isolates collected thought the active laboratory-based surveillance in the US (1992-98) and all isolates collected in Israel from 1990-99 (49 isolates) by serotyping/serosubtyping (ST/SST) and PorA VR typing to investigate if the increase in NMSY disease incidence in these two countries is potentially associated with the appearance and expansion of a single NMSY clone.

Results. Serotypes 14 and 2C were identified in 55% and 41% of the US isolates. Two major serosubtypes were identified: 66% were non-serosubtypeable (NSST), and 34% were serosubtyped as P1.5,2. Five PorA types were identified among the NSST isolates by DNA sequencing with P1.5-2,10-1 predominating (91%), while 100% of the P1.5,2 isolates were sequenced as P1.5-1, 2-2. Similarly, in Israel two PorA VR types predominated: P1.5-1,2-2, one of the two predominant PorA types in the US, was identified in 47% of Israeli isolates. However, P1.5-1,2-13, that accounted for 33% of Israeli isolates, was not detected in the US. Four other PorA VR types were identified in Israel including three not seen among the US isolates. Conversely, P1.5-2,10-1, the most frequently identified PorA VR type in the US (54%), was identified in only 10% of the isolates from Israel.

Conclusions. The increase in incidence of serogroup Y meningococcal disease in the US and Israel is not associated with the appearance of a single serosubtype or PorA type. While PorA type P1.5-1,2-2 was predominant in both the US and Israel, the second most frequent PorA type in Israel is not seen in the US. The most frequent PorA type in the US is the third most frequently isolated type in Israel.

#338

Risk Behaviour Study on Gonnorhoea Infection and other STDs among the CSWs in Jakarta, Indonesia.

Pattiradjawane F.M, Matulessy P F, Lohy J, Simanjuntak M.

PALMA-Foundation; ESG.NH-FKUKI, Christian University of Indonesia, Jakarta, Indonesia.

Jakarta is the biggest and the Capital city of Indonesia, was identified by Department of Health as the most prevalence of HIV/AIDS and STDs among the 26 Provincies in Indonesia.

Objectives: to understand the behaviour of CSWs and the prevalence of Gonnorhoeae infection among the STDs cases; why they are taking risks of their sexual-life.

Methods: 216 CSWs the respondents from several locations in Jakarta, 214 had urine test, LCR, in assessing the prevalence of Gonnorrhea & Chlamydia. Interview & examination about their attitude and sexual life were collected. How they use condom or other prevention were focused in this study. Using EPI-Info 6 to prepare the questioners and dat analysis.

Results: 28.97% had Chlamydia infection, 27.57% had Gonnorrhea and 40.19% had both;19.48% had negative test but suffered from one or more Vaginal Discharge, Dysuria, Genital Lesions and Abdominal Pain.The mean Age is 25.5 Years with average of 15-46 years, which the Age and the amount of the Fee by the last clients is significantly related to the presence of Gonnorrhoea and Chlamydia, and Codom use. About 50% of the married-respondents have permanent sex partners other their husband, and 80% of the CSWs do not care about using or not using Condom for their protection.

Conclusions: Gonnorrhoea and Chlamydia are the most silence infection among the CSWs, and they need protection more than Condom use, because other motivation & their difficult conditions.

#339

MUTATIONS IN PORIN IB PROTEINS THAT MEDIATE GONOCOCCAL RESISTANCE TO PENICILLIN AND TETRACYCLINE.

Olesky M and Nicholas RA Dept of Pharmacology The University of North Carolina at Chapel Hill, Chapel Hill, NC

Rationale: *PenB* is the third determinant in a series of five resistance genes responsible for chromosomally-mediated resistance to penicillin and tetracycline in *Neisseria gonorrhoeae*. It was recently reported that alterations in the outer membrane porin protein PIB are responsible for the *penB* phenotype, but specific mutations were not identified experimentally (Gill et.al., Antimicrob. Agents Chemother. (1998) 42:2799-2803). Therefore, this study focused on determining which amino acid mutation(s) in PIB mediates the *penB* phenotype.

Results: Por_{1B} chimeras were constructed with different portions of FA1090 (PIB) and FA140 (PIB, *penB* phenotype) por_{1B} sequence to determine which of the ten amino acid differences in FA140 porin mediate the increase in penicillin and tetracycline resistance. Studies with these chimeras revealed that two amino acid mutations in PIB (G120→D, A121→D) were sufficient for transformation of FA19/*penA-mtr* (PIB) to the *penB* phenotype. Furthermore, constructs containing a single mutation in PIB of either G120→D or A121→D were also capable of transforming FA19/*penA-mtr* (PIB) to penicillin and tetracycline resistance, although the resistance of the resulting strains for both antibiotics was less than that of a strain harboring mutations at both residues. To determine the importance of specific amino acids at these positions, constructs harboring randomized codons at positions 120 and 121 were used to transform the recipient strain to penicillin and tetracycline resistance. Of twenty-three total resistant isolates, a lysine residue was found at position 120 in twenty-one isolates, whereas arginine and proline were found at that position in two other transformants. Interestingly, numerous charged and uncharged amino acids were found at residue 121.

Conclusion: A charged amino acid at position 120 in PIB is highly preferred for mediating resistance to penicillin and tetracycline in *N. gonorrhoeae*, while the role of residue 121 is less defined.

#278

THE REPEAT ASSOCIATED PUTATIVE PHASE VARIABLE GENE REPERTOIRE OF MC58

Nigel J. Saunders[1], Alex C. Jeffries[1], John F. Peden[1], Derek W. Hood[1], Herve Tettelin[2], Rino Rappuoli[3], and E. Richard Moxon[1]. 1. Univeristy of Oxford, UK. 2. TIGR. 3. Chiron.

Phase variation, mediated through variation in the length of simple sequence repeats, controls the expression of many of the currently recognised virulence determinants of *Neisseria meningitidis*. Based upon the complete genome sequence of the *N. meningitidis* serogroup B strain MC58, we have identified tracts of potentially unstable simple sequence repeats and their potential functional significance determined on the basis of sequence context. Of the 65 potentially phase variable genes identified, only 13 were previously recognized . Comparison with the sequences from the other two pathogenic *Neisseria* sequencing projects show differences in the length of the repeats in 36 of the 65 genes identified, including 25 of those not previously known to be phase variable. Six genes that did not have differences in the length of the repeat instead had polymorphisms such that the gene would not be expected to be phase variable in at least one of the other strains. A further 12 candidates did not have homologues in either of the other two genome sequences. The large proportion of these genes which are associated with frame shifts and with difference in repeat length between the neisserial genome sequences is further corroborative evidence that they are phase variable. The number of potentially phase variable genes is substantially greater than for any other species studied to date, and would allow *N. meningitidis* to generate a very large repertoire of phenotypes through expression of these genes in different combinations. Novel phase variable candidates identified in the strain MC58 genome sequence include a spectrum of genes encoding glycosyltransferases, toxin related products, and metabolic activities as well as several restriction / modification and bacteriocin related genes, and a number of open reading frames for which the function is currently unknown. This suggests that the potential role of phase variation in mediating bacterium – host interactions is much greater than has been appreciated to date.

#279

COMPLEX REPEATS IN THE GENOMES OF *N. GONORRHOEAE* AND *N. MENINGITIDIS*: CORREIA REPEAT-ENCLOSED ELEMENTS (CREE)

Shi V. Liu[1], Nigel J. Saunders[2], Alex C. Jeffries[2], E. Richard Moxon [2,3], and Richard F. Rest[1], [1] Dept of Microbiology & Immunology, MCP Hahnemann Sch of Med, Phila, PA, USA, [2] Inst of Mol Med, Univ of Oxford, UK, [3] Univ Dept of Paediatrics, John Radcliffe Hosp, Oxford, UK

We performed genome sequence analyses of the genomes of *N. meningitidis* (*Nm*) strains Z2491 and MC58, and the almost completed genome of *N. gonorrhoeae* (*Ng*) strain FA1090 to find the numbers, distribution and sequence features of Correia repeats (CR) (Correia et al 1986 J. Bact. 167:1009) and more complex repeats enclosed by CR, that we have termed CR-Enclosed Elements (CREE). We found 533, 510 and 253 CR and 270, 253, and 97 CREE in *Nm* Z2491, *Nm* MC58 and *Ng* FA1090, accounting for 1.74%, 1.60%, and > 0.58% of the respective genomes. CREE lengths range from 28 to 348 bp. Multi-copy CREE appear mainly in two lengths: 154–156 bp and 105–107 bp. The distribution of CREE lengths is similar between the two *Nm* genomes, with 154–156-bp CREE (163 in *Nm* Z2491 and 152 in *Nm* MC58) dominating over the 105–107 bp CREE (72 and 75 copies, respectively). In the *Ng* FA1090 genome there are more 105–107 bp CREE (52 copies) than 154–56 bp CREE (35 copies). All CREE have terminal direct TA dinucleotide repeats and terminal inverted repeats that reflect different combinations of basic sequence blocks.

Because of our interest in regulation of expression of sialyltransferase (stase), we more fully investigated a multi-copy 107 bp CREE. By PCR and sequencing, we observed the 107 bp CREE upstream of the stase gene, *lst*, in 25 of 25 *Nm* strains but in none of 25 *Ng* strains. This 107 bp CREE is repeated 26, 23, and ≥17 times in *Nm* Z2491, *Nm* MC58 and *Ng* FA1090, respectively. Fourteen copies of the element are located in the same loci in the two *Nm* genomes whereas only one unique CREE 107 is located in the same locus in the genomes of both *Nm* and *Ng*. All 107 bp CREE exist in intergenic regions, closely associated with several virulence genes. The abundance of CREE suggests that they may have played a role in genome organization, function, and evolution. Their differential distribution in different pathogenic *Neisseria* may contribute to the distinct behaviors of each neisserial species/strain.

#280

STRUCTURAL VARIABILITY IN GONOCOCCAL LOS DUE TO GENETIC ALTERATIONS WITHIN THE *LGT* GENE REGION.

Stein, DC.*, Tong, Y.*, Braun, D*. and Reinhold, V#. * University of Maryland, #. University of New Hampshire.

We characterized the genetic organization of the *lgt* gene region (Originally described as *lgtA-E* by Gotschlich [J. Exp. Med. 180:2181-2190]) from a variety of gonococcal strains and determined that a significant amount of heterogeneity exists. We determined the transcriptional status of the lgt gene cluster from a variety of strains and found that promoters are embedded within the polyguanine tracts and changes in the number of guanines within the polyguanine tract effect transcription rates of downstream genes. Promoter strength varies with changes in the length of the polyguanine tracts, and these changes are reflected in the ratio of the LOS components expressed. These data explain why the polyguanine tracts never exceed 17 in nature and provide one mechanism for phenotypic modulation of multiple LOS components. Strain PID2 simultaneously expresses 6 different but related LOS molecules. We ascertained the genetic organization of the *lgt* gene region from this strain and determined that it contained two complete copies of *lgtA*, with the second copy positioned where *lgtD* is normally found. The 5'end of *lgtE* was substituted with the 5' end of *lgtB*, suggesting that this genetic organization arose via an intramolecular homologous recombination event. We used sequential enzymatic digestions, mass spectrometry and compositional analysis to determine the structure of each of the LOS components expressed by PID2. A glucosamine or a lactosamine can be added onto the lacto-N-neotetraose structure, due to the overexpression of *lgtA*, demonstrating that the largest LOS structures expressed by this strain differ from those seen in F62. In addition, none of the oligosaccharides were found to be phosphorylated: with the absence of phosphorylation also influencing the heterogeneity of the surface expressed oligosaccharides.

#281

DOES DNA MISMATCH REPAIR AFFECT VIRULENCE OF PATHOGENIC *NEISSERIA* SPECIES?

Cannon JG, Dempsey JAF, Johannsen D, Haskill G, Wilson J, Leone P. University of North Carolina, Chapel Hill, NC, USA

N. gonorrhoeae (GC) and *N. meningitidis* (MC) demonstrate high-frequency antigenic variation of many surface components by processes involving DNA rearrangements, either recombination or slipped-strand mispairing. In general, the GC and MC genomes contain more repeated genes and repetitive DNA than other prokaryotes. We hypothesized that these attributes reflect novel aspects of DNA replication and/or repair that may contribute directly to the success of GC and MC as human pathogens, focusing initially on mismatch repair (MMR).

The GC and MC genomes have *mutS* and *mutL* (but not *mutH*) homologues. We demonstrated that GC has a functional MMR system, in part by constructing a *mutS* strain in which mutation rates were elevated up to 18-fold. The *mutS* strain showed differential effects on antigenic variation of surface components. Rates were elevated for pilin variation and phase variation of *hpuA* (hemoglobin utilization), but not for phase variation of an *opa* gene.

In *E. coli*, the signal for strand targeting in MMR is Dam methylation; *dam* mutants have increased mutation rates. GC and MC strains can be Dam$^+$ or Dam$^-$. Bucci et al. recently concluded that disease-causing strains of MC are hypermutable and hypervariable as a consequence of the Dam$^-$ phenotype (Mol.Cell 3:435,1999). However, our results from several experiments, including comparing mutation rates of a Dam$^+$ GC strain and a *dam* mutant derived from it, indicated that Dam$^-$ GC were not hypermutable relative to Dam$^+$ strains and that Dam methylation was not the signal for strand targeting in MMR in GC. Results of a more limited set of experiments done to date with MC were also consistent with that conclusion. These results argue that the Dam-dependent mechanism proposed by Bucci, et al. does not operate in the pathogenic *Neisseria*. Nonetheless, the link between MMR and rates of surface variation suggests that factors influencing the efficiency of MMR may well contribute to the ability of GC (and MC?) to survive in the ever-changing environment of the infected human host.

#282

TRANSCRIPTIONAL REGULATION OF DIVERGENT CAPSULE OPERON GENE PROMOTERS IN THE SEROGROUP B *NEISSERIA MENINGITIDIS*

Tzeng Y-L, Swartley JS, Miller YK, Nisbet RE, Liu L-J, Ahn JH, and Stephens DS*

Department of Medicine and Microbiology and Immunology, Emory University School of Medicine, and Department of Veterans Affairs Medical Center, Atlanta, GA.

The regulation of extracellular capsular polysaccharide production is a critical element in the pathogenesis of *Neisseria meningitidis*. Clinically important serogroups B, C, Y and W-135 *N. meningitidis* produce capsules that contain sialic acid. In each of these serogroups the capsule transport (*ctrABCD*) and capsule biosynthesis (*synABCD*) operons are separated by a 134-bp intergenic region and are divergently transcribed (Swartley *et al.*, 1996). The transcription of these two operons, identified by primer extension, is initiated from adjacent promoters suggesting possible co-regulation. Insertional mutagenesis or deletion of these promoter sequences in the serogroup B meningococcal strain, NMB, resulted in a reduction of *ctrABCD* and *synABCD* transcription as measured by RNA slot blot, with a concomitant loss of encapsulation as measured by whole cell ELISA. Chromosomal transcriptional *lacZ ermC'* reporter gene fusions of *syn* and *ctr* promoters were constructed through allelic exchange. The biosynthesis operon is constitutively transcribed ~ four-fold higher than the transport operon. Both promoters showed increased activity during stationary growth. Environmental conditions including low pH, low temperature, high osmolarity, iron limitation, altered carbon source and exposure to serum did not affect either *synA* or *ctrA* promoter activity. In addition, truncation of the other surface polysaccharide entity (lipooligosaccharide, LOS) has no significant effect on capsule expression. The 5' un-translated region (UTR) of *synA* contained a direct repeat and a palindromic sequence that overlapped with two putative IHF binding sites and down regulated both *synA* and *ctrA* transcription. This down-regulation by the *synA* UTR was absent in a K1 *E. coli* that produces identical capsular polysaccharide, implicating species-specific regulation. Meningococcal capsule expression is influenced by the 5' UTR of *synA*, is increased during stationary growth, and shows species specific regulation.

Swartley, J.S., Ahn, J.H., Liu, L.J., Kahler, C.M. and Stephens, D.S. (1996) *Journal of Bacteriology*, 178, 4052-9.

#283

MECHANISMS AND RESULTS OF PILIN ANTIGENIC VARIATION

Seifert, HS, Howell-Adams, B, Serkin, CA, Long, CD, Stohl, E, Sechman, EV, Skaar, E, Kline, K. Dept. of Microbiology-Immunology, Northwestern University Medical School, Chicago, IL, USA.

The enormous abundance of gonococcal antigenic variation is one major reason that immunity and vaccines are extremely difficult to develop. We are elucidating the molecular mechanisms used to mediate the high frequency gene conversion reactions that mediate pilin antigenic variation.

Proteins required for antigenic variation include the homologous recombination factors RecA, RecO, and RecQ. The role of a protein that modulates RecA activity and the role of other RecF pathway recombinases in pilin antigenic variation are being investigated. We are also investigating a family of site-specific recombinases that may be involved in antigenic variation. We have developed and tested predictions of unique models that explain how gene conversion can be mediated in a bacterium.

Pilin antigenic variation (and all homologous recombination) is regulated by iron availability. Under iron-limited growth, the entire repertoire of pilin variants changes in a population. All RecA-dependent processes are up regulated when gonococci are starved for iron, but levels of RecA protein do not change. We are testing hypotheses that predict how this iron-dependent regulation is important for gonococcal pathogenesis.

It is well-established that pilin variation affects pilus function. One intriguing observation is that expression of a minute amount of pilin increases transformation competence from 1000 to 10,000-fold without resulting in detectable pili on the cell surface. These data are consistent with a role for the pilus assembly apparatus in transporting DNA for transformation.

#284

STUDIES OF ComP: A PREPILIN LIKE MOLECULE ESSENTIAL FOR DNA UPTAKE IN NATURAL TRANSFORMATION

Aas FE, [1]Wolfgang M, Koomey M. Biotechnology Centre of Oslo, University of Oslo, Gaustadalléen 21, 0316 Oslo, Norway. [1]Department of Microbiology, University of Washington, Seattle, Washington 98195, USA.

Background: ComP is a Type IV prepilin-like protein previously demonstrated to be essential for DNA uptake during natural transformation but dispensable for other Tfp functions. We have carried out further studies to understand how ComP functions in this capacity.
Results: The ComP protein is not detectable in whole cell lysates using immunoblotting. Using northern blotting, we found that levels of *comP* mRNA are also below the level of detection. The absence of ComP antigen therefore reflects corresponding low levels of gene transcription. To facilitate studies of ComP, we utilized strains which expressed high levels by virtue of *pilE::comP* translational fusions. Despite the fact that mature ComP is expressed at levels equivalent to that of PilE in this background, it still functions with levels of transformation being 5-10 fold increased. Overexpression of the protein does not however influence Tfp expression nor does it lead to the presence of significant levels of ComP in purified Tfp fibers. As a further way to examine potential interactions between PilE and ComP, we made point mutations in the prepilin processing site at G^{-1} and in residue E^{+5} which are conserved in PilE and ComP. While each of these classes of mutants in *pilE* abolished Tfp expression and transformability (0.001% of wt), identical mutations in *comP* resulted in only slight defects in transformability (1-2% of wt for G^{-1} and 15-20% of wt for E^{+5}). These findings most likely suggest that ComP need not form a multimer/fiber in order to function.
Conclusion: ComP is essential for DNA uptake, and functions at very low levels. Moreover, it seems to be a limiting factor since overexpression results in increased transformability. Based on biochemical and genetic studies ComP does not seem to be an integral part of the Tfp fiber.

#285

INTERRUPTION OF *siaD* GENE IN A MENINGOCOCCAL CARRIER ISOLATE MEDIATED BY AN INSERTION SEQUENCE.

Arreaza L, Vázquez JA.
Laboratorio de Referencia de Meningococos, Centro Nacional de Microbiología (ISCIII). Madrid, Spain.

A dramatic increase in the incidence of meningococcal disease took place in Galicia (Spain) during 1995-96. In this situation the local health authorities decided to implement an A+C vaccination campaign for the population aged 18 months to 19 years. At the same time a wide meningococcal carrier survey was done.
Meningococcal strains isolated from that study were serogrouping by slide agglutination. An important percentage of strains were defined as non-groupable. In order to determine the capsular genotype, the phenotypically nonserogroupable meningococcal strains were characterized by PCR using two primer pairs to identify serogroup B and C isolates.
In the PCR assay with specific serogroup B primers one strain generated a 1540 bp PCR product while the expected product is of 457 pb. In order to characterized the genetic event that had generated this PCR product we decided to sequence it. We found the *siaD* allele but with an additional DNA fragment (1083 bp) located in an intermediate position, which we identified as the IS*4351N1* Insertion Sequence. So the presence of this IS mediated the loss of encapsulation and for this reason the nongroupable phenotype of this isolate.
Modulation of capsule expression via transposable genetic elements, like insertion sequences, would be the explanation for some of the non-groupable strains, particularly those isolated from asymptomatic carriers.

#286

QUORUM SENSING BY NEISSERIA GONORRHOEAE

Arvidson CG[1,2], Potter LC[1], and So M[1]. [1]Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland, OR and [2]Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Many bacteria communicate with one another via the production of extracellular signalling molecules. These molecules can allow a bacterial population to sense density and/or environmental conditions and to respond by altering the expression of one or more genes. This phenomenon, termed quorum sensing, has been well characterized in the marine bacterium, *Vibrio harveyi*, to control bioluminescence by this organism. *V. harveyi* produces two classes of quorum sensing molecules, autoinducer 1 (AI-1) which is an N-acyl homoserine lactone and AI-2, which is a small soluble heat labile organic molecule whose structure is unknown. AI-2 production is dependent on the *luxS* gene. *LuxS* homologs have been identified in many gram negative organisms, some of which have been shown to regulate the production of virulence factors. We have identified a *luxS* homolog in *N. gonorrhoeae* strain MS11A. The gonococcal *luxS* gene was cloned and when expressed in an *E. coli* strain defective for AI-2 production, resulted in high levels of AI-2 production, as determined by the *V. harveyi* bioluminescence assay. The *luxS* gene of MS11A was mutated by shuttle mutagenesis. Wild-type MS11A, but not isogenic *luxS* mutants, produce an AI-2 molecule that functions in the *V. harveyi* bioluminescence assay. Production of AI-2 varies upon culture conditions. Significant levels of AI-2 are produced when gonococci are grown in the presence of glucose, a phosphotransferase system (PTS) carbohydrate, but not when glycerol and lactate (non-PTS sugars) are provided as carbon sources. This is consistent with what has been observed for *E. coli and S. typhimurium* strains that produce AI-2. We speculate that the gonococcal *luxS* gene may be part of a regulatory system that controls gene expression in response to bacterial cell density and/or environmental conditions.

#287

CLONING EXPRESSION AND MUTATIONAL ANALYSIS OF TWO NEW LYTIC TRANSGLYCOSYLASES FROM MENINGOCOCCUS SEROGROUP B.

Baldi L, Capecchi B, Savino S, Masignani V, Marchetti E, Aricò B, Holtje JV*, Pizza M, Grandi G, Rappuoli R, Jennings GT. IRIS, Chiron S.p.A., Siena, Italy, *The Max Planck Institute fur Entwicklungsbiologie, Abteilung Biochemie, Tubingen, Germany.

Recently, we used the genomic sequence of serogroup B Meningococcus to identify novel vaccine candidates. Two molecules identified during this procedure, GNA33 and GNA1279, were found to be 33% and 37% identical to *E. coli* membrane- bound lytic transglycosylases MltA and MltB, respectively. GNA 33 was expressed in *E. coli* as a lipoprotein and purified. Kinetic analyses confirmed that the protein was indeed a lytic transglycosylase. A prediction of catalytic residues in GNA33 was made by comparing its sequence with that of MltB for which a crystal structure has been resolved. The residues E254, E322 and D361 were mutated to glycine and the resultant mutants proteins purified. We observed a 50% and 70% reduction in specific activity for the E254G and E322G mutants, respectively, and no decrease for D361G. GNA1279 was also expressed in *E.coli* and purified. The protein was demonstrated to be a murein hydrolase by the degradation of insoluble murein sacculi. A comparison of the primary sequence of GNA 1279 with MltB indicated the active site residues are well conserved. The predicted catalytic residues of GNA1279 were mutated and resultant mutant proteins analysed for murein hydrolase activity.

#288

IDENTIFICATION AND ANALYSIS OF A ZINC UPTAKE REGULATORY PROTEIN IN NEISSERIA GONORRHOEAE

Berish, SA, Morse, SA Division of Aids, STD, and Tuberculosis Laboratory Research, Centers for Disease Control and Prevention, Atlanta, Ga

Objective: Zinc plays an essential role as a structural and catalytic cofactor in many metalloproteins. Despite this importance very little is known about the mechanisms and regulation of zinc transport in bacteria. Here, we describe a Neisseria gonorrhoeae Fur paralog, Zur, that regulates an operon implicated in zinc transport.

Design: The genomic sequence of N. gonorrhoeae and N. meningitidis was searched with a consensus Zur sequence derived from the known zur genes characterized in Escherichia coli, Listeria monocytogenes and Bacillus subtilis. A 477bp open reading frame was identified in both N. gonorrhoeae and N. meningitidis that had 52%, 41%, and 40% identity, respectively. A 647bp fragment containing the promoter and the structural gene was amplified by PCR from genomic DNA from N. gonorrhoeae strain F62. This fragment was then cloned into the Hind III site of the plasmid pBAD/HisA to generate clone pBADZur2. A Zur- mutant was constructed in strain F62 by inserting the spectinomyocin resistance gene from aad9 into the Dra III site of pBADZur2.

Results: A putative zur gene from N. gonorrhoeae F62 was identified which had 56% homology with the gonococcal fur gene. SDS/PAGE analysis of whole cell extracts from the Zur- mutant showed that under iron-rich conditions, proteins with molecular weights of 95Kd, 44Kd, and 15Kd were down regulated while proteins of 72 Kd and 17Kd were upregulated. Under iron-limited conditions, expression of the 37Kd ferric binding protein was greatly reduced in the Zur- mutant when compared to the WT strain F62.

Conclusions: A gonococcal gene, which encoded a protein of approximately 18Kd was identified that exhibited homology with the gonococcal fur gene. This gene had a high degree of homology with zur sequences form various other organisms.

#289

ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN INFECTED HUMAN MACROPHAGES

Bowler LD[1], Read RC[2]. [1]Biological Sciences, University of Sussex, Brighton, BN1 9QG, UK., and [2] Division of Molecular Medicine, Sheffield University Medical School, Royal Hallamshire Hospital, Sheffield, S10 2RX, UK.

Objective: To identify changes in gene expression in both host cells and meningococci as they interact.

Design: Representational difference analysis of cDNA (cDNA RDA) offers a powerful approach for the identification of specific differences between two mRNA populations. The technique has great potential for studying changes in gene expression that result from host-pathogen interactions occurring during the course of an infection. To compensate for the abundance of eukaryotic sequences and rRNA species, the cDNA RDA methodology was modified by the inclusion of competitor fragments to increase the stringency of the subtractive hybridisation.

Results: Primary monocyte derived macrophages were infected with *N meningitidis* strain MC58, and subjected to a modified cDNA RDA protocol. The analysis successfully identified a number of differentially expressed transcripts from both host and pathogen. These included a meningococcal homologue of *dnaK* (HSP70), and human monocyte chemotactic protein-3 (MCP-3).

Conclusions: We have further adapted the cDNA RDA approach and have used it to simultaneously identify bacterial and eukaryotic genes whose expression is modified when *Neisseria meningitidis* infects human macrophages. cDNA RDA allows infected host cells to be rapidly processed without the need to first separate bacterial cells from host tissue, greatly increasing the chances of identifying differentially expressed genes whose transcripts are short-lived. The ability to identify both host and pathogen genes in this way should greatly contribute to our understanding of the pathogenesis of meningococcal disease

#290

ComE, A DNA-BINDING PROTEIN INVOLVED IN *NEISSERIA* GENETIC COMPETENCE

Chen I, Gotschlich EC
The Rockefeller University and Public Health Research Institute, New York, NY.

We queried the gonococcal genome sequence against competence proteins from other bacteria which lacked a described ortholog in *Neisseria*, and identified *comE* as the ortholog of *Bacillus subtilis comEA*, whose product is involved in DNA binding and transport during transformation. The gene *comE* is present in four copies in Gc genome, downstream of each of the four rRNA operons (*rrn*); a copy of the Correia element, a neisserial 152bp repetitive element, is found between the *rrn* operon and *comE*. In *N. meningitidis* (Mc), *comE* is also present in four copies, downstream of each *rrn* operon, but the intervening Correia elements are absent. Deletions of *comE* copies in Gc MS11 affected transformability in a cumulative way, leading to $4x10^4$-fold reduction in transformation frequencies when all copies were deleted; piliation and twitching motility were unaltered. The effect of *comE* deletions on competence correlated with impaired ability to take up DNA. The gene *comE* originates a 99aa protein with a predicted signal peptide and two helix-hairpin-helix motifs at the putative mature region. A 8kDa protein was detected in Gc MS11 by immunoblotting, which agrees with the calculated molecular weight of the mature protein; the same band could be observed in different strains of Gc and Mc. Recombinant ComE (rComE) showed DNA-binding properties in a number of assays; this activity lacked any detectable sequence specificity. In conclusion, we identified ComE, a non-specific DNA-binding protein necessary for DNA uptake and transformation in *Neisseria*.

#291

CAPSULAR OPERONS OF SEROGROUP W135 AND Y MENINGOCOCCI

Claus H, Vogel U, Morelli G*, Frosch M, and Achtman M*.
Institute for Hygiene and Microbiology, University of Wuerzburg
* MPI für molekulare Genetik, Berlin, Germany

Objective: To study the genetic variability of capsular operons of serogroup W135 and Y meningococci.

Design: MLST was performed on a collection of serogroup W135 and Y meningococci. Nine genetically unrelated sequence types were selected for further analyses. We sequenced a methyltransferase gene, region E, *ctrD*, *ctrA*, *siaD*, two hypothetical orfs, and *galE*.

Results: Serogroup-specific mutations were found in the *siaD* gene only. There was a tremendous conservation especially of the methyltransferase gene, *ctrA* and two hypothetical orfs downstream of *siaD* (4 nucleotide exchanges in >3, 000 nt).

Conclusions: Further proof was provided that a polymorphic region of the *siaD* gene determines which serogroup is expressed. Parts of the capsular operons were highly conserved suggesting very recent acquisition by serogroup W135 and Y meningococci.

#292

IS*1106* IN *NEISSERIA MENINGITIDIS*

Dolan-Livengood, J.M.[2], Voegele, K.A.[2], Derouine, T.[2], and Stephens, D.S.[1,2]

[1]Department of Veterans Affairs Medical Center, Atlanta, GA, USA
[2]Emory University School of Medicine, Atlanta, GA, USA

IS*1106* was originally described downstream of *porA* in *Neisseria meningitidis* by Knight *et al* (1) and has been associated with recombinational events (2). Southern blots were performed on 131 strains of *N. meningitidis*, three strains of *N. lactamica*, one strain of *N. subflava*, and one strain of *N. sicca* to determine the number of copies of IS*1106* in each genome. IS*1106* was present in every strain examined, and the number of copies per genome ranged from four to fifteen. A survey of the MC58 and Z2491 genome databases revealed that IS*1106* is adjacent to several virulence genes (eg., *lbpA*, adhesin and penetration protein homologs, *opc*). IS*1106* is 1137 bp in length and is flanked by 35- and 36 bp imperfect inverted repeats. Two significant open reading frames, each encoded on a separate strand, were first identified: *orf1* is 863 bp and potentially encodes a protein of 33 kDa; *orf2* is 542 bp and was predicted to encode a protein of 20 kDa. We asked whether IS*1106* *orf1* and/or *orf2* are transcribed. We cloned and sequenced the IS*1106* downstream of *porA* from a B15 strain (F207) into a medium copy-number vector in *E. coli*. The sequence of our clones was 98% identical to the published sequence of IS*1106*, but contained missense mutations in *orf1* but not *orf2*. RT-PCR was performed in both wild-type meningococcal strains and in *E. coli* containing the cloned copies of IS*1106*. RNA transcripts of both *orf1* and *orf2* were detected, indicating that no *Neisseria*-specific host factors are required for IS*1106* RNA synthesis.

References

1. Knight AI, Ni H, Cartwright KAV, and McFadden J. 1992. Identification and characterization of a novel insertion sequence, IS*1106*, downstream of the *porA* gene in B15 *Neisseria meningitidis*. *Mol Micro* **6**: 1565-1573.
2. Dolan HM, Miller YK, Kahler CM, Ajello G and Stephens DS. 1998 IS*1301*-dependent and –independent recombinational events resulting in unencapsulated *Neisseria meningitidis*. In "*Neisseria* genome and genetic regulation", *Abstracts of the Eleventh International Pathogenic Neisseria Conference*. Nassif X, et al. (eds.), Editions E.D.K., Paris, France.

#293

EFFECTS OF PHYSIOLOGIC IRON SOURCES ON TRANSCRIPTION OFGONOCOCCAL IRON TRANSPORT GENES

Garges E, Desai P, Genco CA. Department of Medicine, Section of Infectious Diseases, Boston Medical Center and Boston University School of Medicine, Boston, Massachusetts USA

In response to iron limitation the gonococcus express a set of genes required for iron transport. These include *tbpB* (encoding a transferrin receptor), *hpuB* (encoding a hemoglobin receptor), as well as *fbpA*, the periplasmic binding protein central to iron transport. Few studies have described detailed studies of the transcription of these genes in response to different iron sources. Indeed most *in vitro* experiments have examined the response of the gonococcus to an absolute unavailability of exogenous iron. In this study we have evaluated the expression of *tbpB*, *hpuB*, and *fbpA* in response to growth with several physiologic iron sources by RT-PCR analysis. Gonococcal strains F62 and 340 exhibited unrestricted growth in iron free media supplemented with heme, hemoglobin, or transferrin. cDNA levels of both *tbpB* and *fbpA* were significant in heme, hemoglobin, and transferrin grown cultures. However, transcript levels were substantially lower in cultures grown with inorganic iron. In contrast, transcription of the constitutively expressed gene *rmp* was unaffected by growth condition. These results indicate that heme, hemoglobin, and transferrin bound iron does not repress the transcription of *tbpB* or *fbpA*. The gonococcal hemoglobin receptor *hpuB* demonstrated transcriptional repression when grown in both free inorganic iron and hemoglobin. Taken together, these results suggest that transcription of *tbpB* and *fbpA* is responsive to inorganic iron, but not to heme, transferrin, or hemoglobin. In contrast, *hpuB* appears to be sensitive to both iron and hemoglobin.

#294

NEISSERIA GONORRHOEAE RECJ MUTANTS ARE DEFECTIVE IN RECOMBINATIONAL-REPAIR

Stuart A. Hill Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115 USA

Neisseria gonorrhoeae lacks several common repair pathways found in other organisms. As recent evidence had indicated that gonococci use recombinational repair to repair UV-induced DNA lesions, this study examined whether the gonococcal RecJ homologue contributed in this repair capacity. The *recJ* gene from strain MS11 was cloned and sequenced and showed considerable identity with its *Escherichia coli* homologue. An *N. gonorrhoeae* *Δ recJ* mutant was constructed and was tested for recombinational proficiency as well as for defects in DNA repair. In the absence of the RecJ exonuclease, DNA transformation and pilin switching frequencies occured at wild type levels, indicating that recombination efficiency remained unimpaired. In contrast, *N. gonorrhoeae* *Δ recJ* mutants showed extreme sensitivity to low level UV irradiation as well as to exposure to DNA alkylating reagents (e.g. ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS)). Complementation of the gonococcal *recJ* mutant *in cis* restored low level UV resistance, indicating that in a repair capacity the gonococcal RecJ protein could act independently of other single-strand-specific exonucleases. In addition, transformation competence did not augment recombinational-repair. Overall, the data show that *N. gonorrhoeae recJ* mutants present a unique phenotype when compared to their *E. coli recJ* counterparts, and further support the contention that RecORJ-dependent recombinational repair is a major neisserial DNA repair pathway.

#295

DYNAMICS OF PORA EXPRESSION IN *NEISSERIA MENINGITIDIS* IN CARRIERS

Hopman C.Th.P., Pannekoek Y, Dankert J., and van der Ende A.
Academic Medical Center, Department of Medical Microbiology and the Netherlands Reference Laboratory for Bacterial Meningitis, AMC/RIVM, Amsterdam, the Netherlands.

Stable expression of PorA is a prerequisite for an efficacious PorA based vaccine against meningococcal disease. The dynamics of PorA expression in meningococci present in throats of carriers was studied. From 3 individuals meningococci could be cultured from throat swabs during 10, 12 and more than 30 months, respectively. The PorA expression during the carriership of 10 month did not alter. During the carriership of 12 months the proportion of PorA+ expression variants increased from 21% to 97%. From the carrier with a carriership of 30 months Nm:NG:4:P1.9 was cultured first. After 4 months Nm:C:2a:P1.2,5 was cultured from throat swabs during the following 6 months. During the last 3 months of this period Nm:NG:4:P1.9 was co-cultured and was the only type cultured the next 20 months. The proportion of PorA+ expression variants of C:2a:P1.2,5 in the primary cultures of throat swabs decreased over time. PorA expression of C:2a:P1.2,5 was turned off by deletion of *porA* and by a change in the length of a homopolymeric adenine tract in the *porA* coding region. The sensitivity of the different types of meningococci to bactericidal activity of carrier's own serum depended on the PorA expression. In conclusion, meningococci in the nasopharynx can evade bactericidal activity against PorA by reducing the expression of this protein.

#296

MULTIPLE MECHANISMS OF PHASE VARIATION OF PORA IN *NEISSERIA MENINGITIDIS*.

Hopman C.Th.P., Dankert J., and van der Ende A.
Academic Medical Center, Department of Medical Microbiology and the Netherlands Reference Laboratory for Bacterial Meningitis, AMC/RIVM, Amsterdam, the Netherlands.

Previously, we reported that PorA expression in *Neisseria meningitidis* is modulated by length variation of the homopolymeric tract of guanidine residues between the -35 and -10 regions of the promoter or by deletion of *porA*. To reveal additional mechanisms of PorA expression variation, the meningococcal isolates from 41 patients and 19 carriers were studied. In addition multiple isolates from 11 patients were studied. Sequence analysis of the *porA* promoter showed that the spacer between the -35 and -10 regions varies between 14 and 24 bp, due to the variable polyguanidine tract. PorA promoter sequences did not vary between multiple isolates from single patients. Highest PorA expression was observed in strains with a promoter spacer of 17 or 18 bp. PorA expression was twofold reduced in strains with a *porA* promoter spacer of 16 or 19 bp. Strains, having a 16 bp promoter spacer with substitutions in the polyguanidine tract, displayed increased PorA expression levels as compared to strains with a homopolymeric tract of guanidine residues in the *porA* promoter. All but one strain with a *porA* promoter spacer of 16 to 20 bp and undetectable PorA expression had a homopolymeric tract of 8 or 6 instead of 7 adenine residues in the *porA* coding region. The other PorA negative strain had a nonsense mutation in the coding region. In conclusion, meningococci display multiple mechanisms to vary PorA expression.

#297

DIFFERENCES IN THE SEQUENCES AND GENOME ORGANIZATION OF THE *pilE/pilS* LOCI OF PATHOGENIC *NEISSERIA*.

Alex C. Jeffries, Nigel J. Saunders & E. Richard Moxon
Institute of Molecular Medicine, The University of Oxford

In *Neisseria* species the *pilE* gene product, pilin, is the major subunit of the type 4 pilus and undergoes variation through recombination between an expressed and multiple silent loci. The *pilS* cassettes are transcriptionally silent and encode partial pilin subunits with different amino acid sequences and lack the PilE N-terminal 'leader' region and promoter structure. Non-reciprocal homologous recombination between the expressed *pilE* gene and one of a number of silent *pilS* 'cassettes' (i.e., gene conversion) can occur. This generates diversity that can alter the structure, substitutions, and antigenic properties of pili. This probably has both functional and immune evasive consequences.

We have analysed the sequence and genome organisation of the *pilE/pilS* locus in *N. meningitidis* serogroup B strain MC58 and serogroup A strain Z2491 and compared them to *N. gonorrhoeae* sequences including strain MS11.

The meningococci differ notably from gonococci in having only a single pilin locus containing both the expressed and silent pilin sequences. As with the gonococci, the meningococcal non-coding regions contain RS1, RS2, RS3 and inverted pairs of RS3 (dRS3) repeat sequences together with a single Sma/Cla repeat downstream of the *pilE* gene. No RS4 repeats are present in the meningococci. However, the meningococcal loci contain significantly greater numbers of dRS3 repeats than the gonococcal loci and these repeats are present in all of the non-coding regions where as in the gonococci they are found in only a few.

These observations taken together suggest that the meningococci will have different characteristics in recombination between their silent and expressed pilin loci. This finding illustrates that fundamental differences in molecular organisation between functionally similar systems in closely related organisms can exist in systems important in host interaction.

#298

MISMATCH REPAIR IN *NEISSERIA MENINGITIDIS*: HIGHER MUTATION AND PHASE VARIATION FREQUENCIES DUE TO ABSENCE OF THE DAM METHYLTRANSFERASE ?

Klee S [1], Kusecek B [1], Wintermeyer E [2], Achtman M.[1] Max-Planck-Institut für molekulare Genetik, Berlin, Germany [1] and Institut für Hygiene und Mikrobiologie der Universität Würzburg, Germany [2]

Introduction: Bucci et al. (Mol. Cell 3, 435-4451, 1999) reported that the frequency of capsule phase variation and spontaneous mutation in pathogenic strains of *N. meningitidis* (Nm) isolated from patients is much higher than with commensal isolates. They postulated that this reflects a defect in the mismatch repair system due to the absence of the gene for DNA adenine methyltransferase (*dam*). Dam was absent from all pathogenic isolates and from about 50% of the commensal strains tested. In these strains, the gene is replaced by the *drg* gene that encodes a restriction enzyme which cleaves Dam-methylated DNA.
Objective: We tested the presence and activity of the *dam* gene in a set of 176 strains representing the genetic diversity of Nm according to multilocus sequence typing.
Results and conclusion: In contrast to the published results, we found that *dam* is not restricted to commensal strains, but is also present in two related hypervirulent clonal groupings (A4 cluster and ET-37 complex) and several rare strains from diseased patients. The *dam* gene shows a high, the *drg* gene a lower degree of conservation. Both genes have a low GC-content, and related *drg* genes were found in unrelated clonal groupings. Therefore, it is likely that the genes were acquired by horizontal genetic transfer from other species. We found no correlation of Dam-absence with higher mutation frequencies, and conclude that unlike Enterobacteriaceae, Dam is not essential for effective mismatch repair in Nm. The phase variation frequency in Dam-positive and Dam-negative strains is currently investigated.

#299

COMPUTER ANALYSIS OF A BACTERIAL GENOME

Masignani V, Galeotti CL, Mora M, Scarlato V, Scarselli M, Grandi G, Rappuoli R. IRIS, Chiron SpA, Siena, Italy

Whole-genome sequence of a serogroup B clinical isolate (strain MC58) of *Neisseria meningitidis* has been analyzed by computer-aided methodologies and compared to a number of other available bacterial genomes in order to detect novel factors specific for the virulence of this important human pathogen.
Long before the MC58 complete sequence annotation was complete, we had been able to identify more than 570 novel surface- and membrane-associated ORFs by using a systematic combination of public softwares. Availability of Serogroup A Meningococcus, as well as of *Neisseria gonorrhoeae*, *Haemophilus influenzae* and a still growing number of other bacterial genomes, has allowed a "transversal genome analysis" through which we have been able to identify: 1) a Mu-related prophage which is present in *N. meningitidis* and *H. influenzae* genomes, but absent from *N. gonorrhoeae*, which carries a number of lipoproteins and outer membrane proteins with unknown function and for which a possible role in virulence can be assessed. 2) A new family of adhesins present in multiple copies in *N. meningitidis* and *N. gonorrhoeae* genomes and absent from all the other pathogens which might have a role in the particular phenomenon of adhesion/invasion specific of Neisseria. 3) Six novel surface-exposed antigens which have been sequenced in 34 *N. meningitidis* strains and successfully used for epidemiological studies through a new approach of Multi Locus Sequence Typing technique.
In silico analysis of bacterial genomes and computer-based comparisons among closely related species are extremely useful tools which can help to clarify the mechanisms of pathogenesis and that can also be applied for other purposes such as epidemiological typing.

#300

STRUCTURE AND EVOLUTION OF A NEW FAMILY OF MU-LIKE PROPHAGES

Vega Masignani, Guido Grandi, Rino Rappuoli, Vincenzo Scarlato. IRIS, Chiron S.p.A., Siena, Italy.

Design: Pathogenic bacteria use different routes to exchange genetic material. Among these, lysogenic conversion by bacteriophages is one of the more frequent; a number of major bacterial virulence factors are, in fact, carried on bacteriophages, including diphtheria and cholera toxins and the pore-forming toxin CTX of *Pseudomonas*.
Results: Sequence analysis of serogroup B meningococcus strain MC58 revealed the presence of a 34 kb long Mu-like structure (MuMenB) inserted within a putative ABC transporter. The prophage has two counterparts in serogroup A meningococcus (MuMenA1 and MuMenA2) and one in *Haemophilus influenzae* (FluMu) genomes. Early phase as well as late phase transcription functions are well preserved in the Mu-like prophages and are colinear with respect to Mu gene arrangement. Whereas head, tail sheath and baseplate structures are also maintained in terms of sequence, the tail fiber - which is involved in host recognition and binding – is highly polymorphic in the different prophages, thus suggesting the hypothesis of different host specificities for the ancestral infecting phage. Evolutionary studies performed on conserved functions showed that there are two subfamilies, one comprising Mu and FluMu and the other one represented by the three Neisserial prophages.
Finally, several hot-spots for recombination were mapped by means of atypical nucleotide content studies, and regions potentially acquired by recent events of horizontal transfer were found to be associated in most cases with genes coding for surface-associated proteins.
Conclusions: We suggest that these bacteriophages could have a role in the evolution and transfer of surface-associated structures, probably involved in virulence or immunity.

#301

IDENTIFICATION OF NOVEL VACCINE CANDIDATE ANTIGENS USING PHAGE DISPLAY TECHNOLOGY

Newcombe J and McFadden J. University of Surrey, Guildford, Surrey, UK.

There is a need to develop a new vaccine that is effective for group B meningococcal disease. We are currently using phage display technology to identify new candidate antigens. Our approach is to screen a meningococcal expression library (expressed on the surface of a phage) with convalescent sera taken from meningitis patients. Our assumption is that convalescent sera will contain antibodies that bind to protective antigens. Genomic DNA from *Neisseria meningitidis* H44/76 (B,15 PI 7,16) was DNAse treated to generate random fragments of between 100 and 300bp that were blunt ended and cloned into the M13 phagemid vector, PHEN-1, to generate a genomic library of 350,000 clones. The library was screened by biopanning to identify phage that bind to antibodies present in convalescent sera, but absent in acute sera. Three rounds of biopanning were performed and individual clones from the convalescent sera-enriched library were subjected to DNA sequence analysis. A number of meningococcal proteins were identified including outer membrane proteins, virulence proteins and regulatory proteins. Western blot analysis confirmed the presence of antibodies to these expressed proteins in convalescent sera but not in acute sera. The immunogenicity and protective efficacy of these antigens will be evaluated

#302

GENERATION AND CHARACTERISATION OF A PHOP HOMOLOGUE MUTANT OF NEISSERIA MENINGITIDIS

Johnson, C.R.[1], Newcombe, J.[1], Borde, H.A.[1], Gorringe, A.R.[2], Funnell, S.G.P.[2] and McFadden, J.J.[1]. School of Biological Sciences, University of Surrey, Guildford, Surrey, UK[1] and Centre for Applied Microbiology and Research, Porton Down, Salisbury, UK[2].

Two-component regulatory systems are important regulators of virulence genes in a number of bacteria. Genes encoding a two-component regulator system, with homology to the phoP/phoQ system in salmonella, was identified in the meningococcal genome. Allele replacement was used to generate a meningococcal knock-out mutant of the regulator component of this system and its phenotype was examined. The mutant displayed many differences in protein profiles compared to wild-type, consistent with it being a gene regulatory mutation. Many of the growth characteristics of the mutant were similar to those of phoP mutants of salmonella: it was unable to grow at low concentrations of magnesium and was sensitive to defensins and other environmental stresses. Magnesium-regulated differences in protein expression were abrogated in the mutant, indicating that the meningococcal PhoP/PhoQ system may, as in salmonella, respond to changes in environmental magnesium levels. These results are consistent with the PhoP homologue playing a similar role in the meningococcus as PhoP in salmonella, and suggest it may similarly be involved in the regulation of virulence genes in response to environmental stimuli in the meningococcus. Identification of those genes regulated by the meningococcal PhoP may provide a route towards the identification of virulence genes in the meningococcus.

#303

GENOME-SCALE IDENTIFICATION OF VIRULENCE GENES FROM MENINGOCOCCUS BY SIGNATURE-TAGGED MUTAGENESIS

PELICIC V., NASSIF X.
U411 INSERM, Faculté de Médecine Necker-Enfants Malades, France

The bonanza of new data generated by two complete meningococcal sequences might remain underexploited if the current lack of genome-scale mutagenesis methods persist. However, we demonstrated that meningococcal DNA was efficiently mutated *in vitro*, in the presence of purified *Himar1* transposase, using a mini-transposon consisting of a kanamycin resistance gene and an uptake sequence flanked by *Himar1* inverted repeats. After transformation, we obtained approximately 10 and 10^4 mutants/reaction for PCR amplified DNA and chromosomal DNA, respectively. Sequencing *Himar1* insertion points in more than 60 transposition mutants, confirmed that insertions occurred at random. The high frequency of transposition permits the creation of mutant libraries containing mutants in each and every *N. meningitidis* gene, thus allowing a mutational analysis on a scale that was previously unfeasible. However, screening the mutant libraries in order to identify mutants affected in virulence promises to be a difficult task. In this regard, a major step forward in *N. meningitidis* molecular genetics would undoubtedly be the development of signature-tagged mutagenesis (STM). We therefore engineered our transposon to contain unique DNA tags which allow mutants to be distinguished from each other in STM. An ordered library of 4,608 mutants, composed of 96 pools of 48 mutants, has been constructed in a variant of strain 8013 and is currently being screened in search of attenuated mutants.

#304

GENOME COMPARISON OF PATHOGENIC NEISSERIA USING DNA MICROARRAYS ON HIGH DENSITY MEMBRANES

Perrin A. Bonacorsi S, Nassif X, Tinsley CR. INSERM U411, Faculté de Médecine Necker, Paris, France.

Neisseria meningitidis (Nm) and *N. gonorrhoeae* (Ng) are human pathogens which are responsible for dramatically different diseases. Furthermore these human pathogens are very closely related to each other and also *N. lactamica* (Nl). Unlike meningococcus and gonococcus, this latter species is a commensal. Hence DNA sequences specific to these pathogens may be implicated in their ability to cause disease. Using subtractive hybridization we have identified sequences of DNA specific for Nm and Ng, and these sequences have been mapped on the chromosome of both Nm and Ng. However in order to identify the precise bounderies of the DNA sequences encompassing these subtractive clones and to improve the detection of all pathogenic neisseria-specific regions, an approach using DNA array was developed.

Based on the total genome sequence of *N. meningitidis* Z2491, we designed oligonucleotides to amplify fragments of 1 kb, corresponding to the entire chromosome. All of these amplicons were spotted in duplicate, representing 4150 spots, on a nylon membrane of 11 x 7 cm. This high density membrane has been initially hybridized with a ^{33}P-labelled probe composed of total chromosome of Nm strains Z2491 and MC58, Ng strain FA1090 and Nl strains 8064 and 9064. The intensity of the signal from each spot was quantified using "XdotsReader" (COSE) and compared first with an internal control and then between strains on different membranes.

Comparison of the patterns will be presented which has made possible the detection of regions specific for pathogenic *Neisseria*. In contrast we did not find large pathogenicity islands.

#305

GENETIC CHARACTERISATION OF PILIN GLYCOSYLATION IN *NEISSERIA MENINGITIDIS*

Power PM, Roddam LF, Berrington A, Dieckelmann M, Srikhanta Y, Jennings MP
Depart. of Microbiology and Parasitology, The University of Queensland, St Lucia, Queensland, 4072, Australia.

Objective: To identify and characterise genes involved in pilin glycosylation and to survey a collection of strains and clinical isolates for their presence.

Design: Several potential pilin glycosylation genes were identified from the group B and group A genome sequencing projects. The role of these genes in pilin glycosylation was examined by the creation of insertional mutations in various strains and the subsequent analysis of these mutants by several methods. A collection of strains and clinical specimens was surveyed by Southern hybridization, sequence analysis and immunological and structural techniques to determine the presence of the genes and the glycan structure expressed

Results:
We identified several genes involved in Neisserial pilin glycosylation. The best characterised of these so far are *pglB*, *pglC* and *pglD* (Power *et al* 2000 *Microbiology* v146 967-79) The potential role of the identified genes in the glycosylation of pilin was examined by the creation of insertional mutants. Analysis of the pilin of the mutants by gel migration revealed differential migration when compared to wildtype. The pilin subunit gel migration observed in the *pglB,C,D* mutants is also different from other recently characterised pilin glycosylation mutants (*galE* and *pgl4* (Jennings *et al* 1998 *Molecular Microbiology* v 29 975-984). Furthermore, both a terminal-galactose specific stain, and antiserum specific for the C311#3 trisaccharide, failed to react with pilin from the *pglB*, *pglC*, *pglD* and *galE* mutants. These differences suggest that *pglB,C,D* play roles in the addition and biosynthesis of the O-linked trisaccharide attached to pilin. The structure of the glycan modification is currently being analysed

A survey of *N. meningitidis* strains, with known glycosylation structures, found that *pglB*, *pglC*, and *pglD* are present in all. Of 70 clinical isolates only 31 were found to express type I pilin. The pilin of only one of these isolates was found to be modified by a trisaccharide structure and one by a truncated sugar.

Conclusions:

- We have confirmed the role of three genes and identified several others that are involved in the biosynthetic pathway of pilin glycosylation and are able to propose a model for this biosynthesis
- Our analysis of a large clinical isolates collection revealed that the C311#3 trisaccharide is only expressed in a limited number of isolates

#306

OVEREXPRESSION OR DELETION OF THE CELL DIVISION INHIBITOR MinC FROM *NEISSERIA GONORRHOEAE* CAUSES GRAM NEGATIVE COCCI TO LYSE

Ramirez-Arcos S[1], Szeto J[1], Beveridge TJ[2], Ridsdale A[3], and Dillon JR[1]. Dept. Biochem/ Microbiol/ Immunol, University of Ottawa, Ottawa, ON[1]; Dept. Microbiol, University of Guelph, Guelph, ON[2]; Loeb Health Research Institute, Ottawa, ON[3].

Objective: To determine the role of MinC from *Neisseria gonorrhoeae* (*Ng*) in cell division. Gonococcal and round *E. coli rodA* cells have been used as model systems since both divide in alternative perpendicular planes. In rod-shaped bacteria, MinC binds to the cell poles, inhibiting septum formation, and allowing division to occur at the middle.
Design: A MinC homologue was identified in *N. gonorrhoeae* and its biological function has been investigated using genetic, biochemical and microscopic methods.
Results: *N. gonorrhoeae* CH811 *minC* was insertionally inactivated to produce *Ng* CSRC1. Western Blot analysis of CSRC1 confirmed that MinC was not produced. Electron microscopy indicated grossly abnormal cell division and cell lysis in CSRC1. In addition, this strain displayed decreased viability. Complementation of the $minC_{Ng}$ mutant by integrating a *minC*-6XHis tag fusion at the *proAB* locus restored a wild-type phenotype for viability and protein expression. $MinC_{Ng}$ was functional in other genera. Overexpression in wild-type *E. coli* cells induced filamentation and in round *E. coli rodA* induced enlargement of the cells leading to lysis. Deconvolution microscopy indicated that $MinC_{Ng}$ tagged to GFP was localized at opposite ends of *E. coli rodA* cells.
Conclusions: MinC from *N. gonorrhoeae* acts as a cell division inhibitor maintaining proper division in Gram negative cocci which makes it an alternative target to find new antimicrobial compounds.

#307

INVESTIGATION OF POTENTIALLY PHASE VARIABLE GENES IDENTIFIED IN *N. MENINGITIDIS* (Nm) STRAIN MC58

Nigel J Saunders, Alex C Jeffries, Kathy Makepeace, Derek W Hood & E Richard Moxon
Institute of Molecular Medicine, The University of Oxford

An analysis of the simple nucleotide repeats in the strain MC58 genome sequence revealed 65 potentially phase variable genes. A subset of 21 of these genes was selected to cover the functional categories inferred by homology comparisons and the types and lengths of repeats. The presence or absence of these genes and the length of the associated repeat tracts were investigated in a set of 12 Nm strains and one *N. lactamica* strain. The 21 genes were found to be present in the majority of strains with variation in the length of the repeat tracts apparent in PCR products from some strains. In addition, a homologue of a glycosyl transferase that was associated with a heptomeric repeat composed of 34 copies of (AAACAAC) was selected for specific investigation. The presence or absence of this gene and the length of the repeat tracts were investigated in a set of 35 Nm strains, 7 *N. gonorrhoeae* strains and 7 *N. lactamica* strain. The gene (NMB0624) was also amplified by PCR and engineered to produce constructs where the reading frame would be constitutively translated ('ON') or disrupted ('OFF'). The 'ON' construct was engineered by removing the potentially unstable repeats from this gene and inserting a kanamycin cassette upstream of the predicted promoter region. For the 'OFF' construct, a kanamycin cassette was inserted to replace the first 50% of the reading frame including the repeats. These constructs were transformed into strains MC58 cut 2 and MC58 cut 3, which express L8 and L3,7,9 LPS phenotypes respectively, to replace the original gene. The LPS phenotype of these transformants was assessed by gel electrophoresis. No phenotype change was seen in the LPS when comparing it to LPS from the parent strains. It seems likely that this transferase directs a glycosylation of another cell surface molecule. *In vitro* and *in vivo* studies are underway to assess the likely function of the gene and its influence upon the biology and pathogenesis of Nm.

#308

ANALYSIS OF TRANSPOSASE GENES IN THE COMPLETE GENOME SEQUENCE OF *NEISSERIA MENINGITIDIS*

Saunders, N. J., Jeffries, A. C. & Moxon, E. R.
Institute of Molecular Medicine, The University of Oxford

In microbial genomes, multiple copies of specific transposons can act as sites of homologous recombination leading to chromosomal rearrangements. This together with gene inactivation through transposons inserting into coding and promoter regions can lead to significant affects on the phenotype of an organism.

We have searched the *N. meningitidis* serogroup B strain MC58 genome sequence for transposase (Tpase) genes, both complete and remnant, and compared the types of transposon families present and genetic context of each Tpase sequence with the serogroup A strain Z2491 genome sequence.

A total of 13 complete and 42 degenerate Tpase genes were identified in the group B genome. Tpases from the following transposon families are present; IS3, IS5, IS30, IS110. Two further unclassified transposon families are represented; IS1016 and a family first identified in *Synechocystis* sp. The same transposon families are represented in the group A genome. In addition the group A genome contains three degenerate Tpase sequences that are most similar to an unclassified transposon family found in *Streptococcus pneumoniae* and *Synechocystis* sp. but which is not found in the group B strain.

The largest group (14) of intact Tpases in the group B genome are most closely related to IS4351 (IS30 family). In the group A genome only six IS4351 Tpases are present of which only two are located in the same genetic context as those in the group B genome. This suggests that the remaining 12 group B IS4351 elements are the result of transposition after the divergence of these two serogroups.

To our knowledge this is the largest number of Tpases that have been identified in a microbial genome to date. The significant differences in numbers and genetic context of the Tpase genes in these two closely related neisserial species suggests a significant role for mobile elements in determining their genome structures.

#309

THE ROLE OF IRON LIMITATION IN RECOMBINATION LEVELS OF *Neisseria Gonorrhoeae*

Eric V. Sechman, Carla D. Serkin, and H.S. Seifert, Department of Microbiology-Immunology, Northwestern University, 303 E Chicago Ave. Searle 6-497, Chicago IL 60611.

The type IV pilus of *N. gonorrhoeae* is a major virulence determinant of this obligate human pathogen. Antigenic variation of the pilus results from high frequency gene conversion events and occurs when a segment from one of many silent pilin loci (*pilS*) recombines into the pilin expression locus (*pilE*). The RecA protein is required for pilus variation, as are RecO and RecQ, two proteins which are part of a RecF-like pathway. The RecBCD pathway, however, is not required. Since environmental conditions regulate many bacterial processes, we examined whether different environmental conditions would alter the frequency on pilin antigenic variation. Iron limitation was only condition that had a significant effect.

Limitation of available iron during Gc growth resulted in an increased frequency of pilin variation. Further analysis revealed that this effect was not pilin specific; levels of DNA repair and DNA transformation were also increased by iron limitation. In addition, RecA protein levels of iron starved gonococci were not altered, indicating another protein is mediating the elevated recombination levels. At present, the only identified gonococcal iron-responsive regulatory protein is Fur (ferric uptake regulator). Studies using a variant containing a missense mutation in *fur* still demonstrated the high rate of recombination when grown in iron limiting conditions. We are currently searching for regulators and/or effectors of the increased frequency of recombination when gonococci are iron starved. Since *N. gonorrhoeae* is likely exposed to conditions of low iron within the human host, the elevated recombination we detect under iron limiting conditions may more accurately indicate the level of pilin variation during an infection, rather than recombination levels measured in iron replete conditions. It is also likely that Gc experience microenvironments with different amounts of iron. This suggests that regulation in response to iron by Gc is not simply "on or off." It is more plausible that there are various degrees of induction of iron responsive genes/systems. Studies have begun to determine if different levels of iron or iron limitation result in different increases in recombination levels. Additionally, we are examining the expression of other iron responsive genes under various levels of iron.

#310

THE SIGNAL PEPTIDE SEQUENCE OF GNA33: A ROLE IN REGULATION OF GENE EXPRESSION?

Serruto D, Arico' B, Pizza M, Grandi G, Rappuoli R, Galeotti CL
IRIS, Chiron S.p.A., Siena, Italy.

In a genome-wide screening for *Neisseria meningitidis* serogroup B antigens (Aricò et al., Abstract submitted) it was found that different forms of GNA33, a lipoprotein with homology to the *E. coli* murein transglycosylase MltA, were expressed in a heterologous system (T7 expression system in *E. coli*) at considerably different levels. The three forms analysed differ due to the presence or absence of a signal peptide sequence. The GNA33 ORF devoid of its signal peptide sequence gave the highest expression level, while the full-length form was poorly expressed. Substitution of the homologous signal peptide sequence with one derived from another lipoprotein resulted in higher expression of GNA33.

In an attempt to increase the level of expression of the "native" forms, the *E. coli* strains expressing GNA33 were transformed with plasmids containing a gene coding for an inhibitor that specifically reduces the basal level of the T7 RNA polymerase. Northern and Western blot analyses showed that the two forms, that differ only in the signal peptide sequence, behaved in opposite ways. Inhibition of T7 RNA polymerase gave a significant increase of GNA33 with its own signal peptide sequence, while the level of GNA33 with the heterologous signal peptide was reduced, as expected. This result suggests that, in addition to its role in directing the protein to the correct cellular localization, this signal peptide sequence may contribute to the regulation of GNA33 expression.

We are now analysing the influence of this signal peptide sequence on transcription/translation of a reporter gene.

#311

A GENE FAMILY WITH SIMILARITY TO THE *Moraxella* Piv SYSTEM EXISTS IN *Neisseria gonorrhoeae*

Skaar, E. and Seifert, H.S., Department of Microbiology-Immunology. Northwestern Medical School. Chicago, IL 60611.

Neisseria gonorrhoeae is an obligate human pathogen that has acquired multiple factors to effectively infect and survive in its host. One of these virulence factors, the pilus, plays a vital role in the establishment of infection. The gonococcal pilus is composed primarily of pilin protein monomers. These pilin monomers are capable of undergoing antigenic variation due to a non-reciprocal homologous recombination between silent copies of pilin information (*pilS*) and the expressed pilin locus (*pilE*). It is currently known that these recombination events are RecA-, RecO, and RecQ-dependent, and independent of the RecBCD pathway. For a number of reasons, it is possible that an as yet unidentified site-specific recombinase may also be involved in the recombination events responsible for antigenic variation of the pilus.

Moraxella, like *Neisseria*, is a member of the family *Neisseriaceae*, and capable of variation of its pili. *Moraxella* species are able to switch between production of antigenically distinct pili through the inversion of a 2.1-kb DNA segment in the 3' end of the pilin structural genes. This inversion is mediated exclusively by the site-specific recombinase Piv. Recently, several open reading frames have been identified in the chromosome of *N. gonorrhoeae* with substantial amino acid similarity to Piv from *Moraxella sp* (1). Initially, sequence surrounding each *piv*NG copy was analyzed for characteristics of insertion sequence (IS) elements. Extensive sequence analysis of most of the *piv*NG copies and surrounding regions has not revealed any IS element signatures, however we have found that each *piv*NG copy analyzed to date contains the amino acid residues shown to be necessary for Piv mediated inversion of *Moraxella* DNA in *E. coli*. Reverse transcription experiments demonstrate that at least one *piv*NG copy is transcribed. Currently, insertional inactivation of several of these open reading frames is being carried out to determine if PivNG has any role in the recombination reactions of the gonococcus such as those involved in replication, transformation, DNA repair, and antigenic variation. Completion of these experiments will determine the role, if any, of this gene family in *N. gonorrhoeae*.

1. Carrick C.S., and J.A.M. Fyfe, and J.K. Davies. *Neisseria gonorrhoeae* contains multiple copies of a gene that may encode a site-specific recombinase and is associated with DNA rearrangements. Gene 220 (1998) 21-29.

#312

A PUTATIVE PHASE VARIABLE GENE IN THE DIVISION CELL WALL CLUSTER OF PATHOGENIC *NEISSERIA* REQUIRED FOR NATURAL COMPETENCE IN *N. GONORRHOEAE*.

Snyder, L. A.[1], Saunders, N. J.[2] and Shafer, W. M.[1,3]
[1] Emory University School of Medicine, Atlanta, GA; [2]University of Oxford, UK; [3]VA Medical Center, Decatur, GA.

A cluster of eighteen open reading frames (ORFs), fifteen of which are homologous to genes involved in division and cell wall synthesis has been identified in *Neisseria gonorrhoeae* and *Neisseria meningitidis*. The three additional ORFs, located in the central region of the cluster, are not homologous to DCW-related genes present in other bacterial species. Analysis of the *N. meningitidis* strain MC58 genome for foreign DNA suggests that these additional ORFs have not been acquired by recent horizontal exchange, indicating that they are a long-standing, integral part of the neisserial DCW gene cluster. RT-PCR analysis of RNA extracted from *N. gonorrhoeae* strain FA19 confirmed that all three ORFs are transcribed in gonococci. One of these ORFs (*dca; d*ivision cell wall cluster *c*ompetence *a*ssociated gene), located between *murE* and *murF*, was studied in detail and found to be essential for competence in gonococci but not in the meningococcal strains tested. Computer analysis predicts that the *dca*-encoded protein is an inner membrane protein similar to hypothetical proteins produced by other Gram-negative bacteria. In some meningococcal strains *dca* is prematurely terminated following a homopolymeric tract (HPT) of Gs, the length of which differs between isolates of *N. meningitidis*, suggesting that *dca* is phase variable in this species. A non-polar deletion and insertional mutation in the *dca* gene of *N. gonorrhoeae* strain FA19 and strain FA1090 abrogated the ability of either to be transformed with chromosomal DNA. In contrast, this mutation in the *dca* gene of *N.* meningitidis strain NMB and 0929 did not impair transformation with chromosomal DNA.

#313

IDENTIFICATION OF *recX*, A NOVEL GENE INVOLVED IN RECOMBINATION IN *Neisseria gonorrhoeae*. Stohl, EA and Seifert, HS. Department of Microbiology and Immunology, Northwestern Medical School, Chicago, IL.

In *Neisseria gonorrhoeae* (Gc), the RecA protein is necessary for DNA repair, DNA transformation, and pilus antigenic and phase variation. Certain additional cellular factors are specifically required for each of these recombination processes, including Gc homologues of *E. coli* RecF and RecBCD pathway genes. Nearly all bacteria assayed, including *E. coli* and Gc, contain a yet-uncharacterized gene, *recX*, that is hypothesized to regulate *recA* through an unknown mechanism. To investigate the possible role of Gc *recX* in RecA-mediated processes, we disrupted the *recX* gene in *N. gonorrhoeae*. Mutants showed decreases in pilus phase variation, DNA transformation, and DNA repair ability as compared to wild type. We were able to complement all of these deficiencies of the mutants by supplying a functional copy of *recX in trans*, demonstrating a role for *recX* in recombination. Wild-type and mutant strains showed identical levels of RecA protein, suggesting that RecX does not function by regulating levels of RecA.

#314

MinD IS IMPLICATED IN MAINTAINING PROPER CELL DIVISION IN GRAM NEGATIVE COCCI

Szeto J[1], Ramirez-Arcos S[1], Li H [1], Dillon, JR[1]. Dept. Biochemistry, Microbiology, Immunology, University of Ottawa, Ottawa, ON[1].

Objective: To characterize the role of MinD in cell division of Gram negative cocci. *Neisseria gonorrhoeae* (Ng) and round *Escherichia coli* cells have been used as models to study $MinD_{Ng}$ since they divide in a similar way.
Design: Molecular biological, biochemical, and microscopy techniques were employed to study MinD function.
Results: *N. gonorrhoeae* has a MinD homologue (29.6 KDa) that shares 73% identity to MinD from *E. coli*. Alignment of MinD from different bacteria revealed several highly conserved regions, including an ATP-binding cassette. Purified $MinD_{Ng}$ was shown to bind the ATP analog, FBSA. Using a dot blot assay, purified $MinD_{Ng}$ was shown to interact with purified $MinC_{Ng}$. A *minD*$_{Ng}$ insertional mutant, CJSD1, was created from *N. gonorrhoeae* CH811, and displayed markedly aberrant division. Growth of CJSD1 in liquid media was severely compromised. Western blot analysis confirmed the absence of $MinD_{Ng}$ in the mutant. Overexpression of $MinD_{Ng}$ in a coccal *E. coli* (*rodA*) strain, which divides in perpendicularly alternating planes similar to *N. gonorrhoeae*, resulted in significant enlargement of the cells. $MinD_{Ng}$ expression studies in various rod-shaped *E. coli min* mutants demonstrate that the protein is involved in cell division site selection and that it can function in conjunction with the native *E. coli* MinC protein.
Conclusions: $MinD_{Ng}$ acts in cell division inhibition and is required for proper division in coccal bacteria such as *N. gonorrhoeae*.

#315

GENOME COMPARISON IN THE SEARCH FOR NEW VIRUENCE DETERMINANTS

Tinsley CR, Perrin A, Nassif, X. INSERM U411. Faculté de Médecine Necker, Paris, France.

In contrast to their close biochemical and genetic relatedness the species *Neisseria meningitidis*, *N. gonorrhoeae* and *N. lactamica* express strikingly different pathogenic potentials. Their high degree of overall genetic homology make genomic subtraction an attractive approach to search for the genes which may determine these differences in virulence.

We have undertaken genetic comparisons of the meningococcus, the gonococcus and *lactamica*, using genomic subtraction, a technique which compares genomes either aided by or in the absence of whole genome sequences. In an earlier work we had performed a physical subtraction to clone sequences specific to *N. meningitidis* and absent from *N. gonorrhoeae* in the search for genes which might explain their differential pathogenicity. Recently we have performed genomic subtractions of *N. meningitidis* and *N. gonorrhoeae* with *N. lactamica*, in order to distinguish chromosomal sequences common to the pathogens and absent from the commensal species, and hence implicated in determining the virulence of the meningococcus and gonococcus. In order to determine the chromosomal regions surrounding these pathogen-specific sequences we have used gene array technology, which has been made possible by the availability of the meningococcal and gonococcal genome sequence data. Membranes were fabricated supporting arrays of DNA spots of PCR-ampified genes of the whole *N. meningitidis* chromosome. Using whole, ^{33}P-labelled chromosome of meningococcus Z2491 and of *N. lactamica* as probe we have defined the extent of the pathogen-specific regions.

We present the genomic analysis of the specific chromosomal regions and preliminary results of biological investigations of mutations in the genes differentially present in the pathogenic *Neisseria* species. In conclusion the physical methods of genome subtraction and comparison outlined above are an easy and effective way to identify potential virulence factors in pathogenic microorganisms.

#316

THE ROLE OF THE BASE EXCISION REPAIR PATHWAY IN MENINGOCOCCAL GENOME MAINTENANCE

Tønjum T, Davidsen T, Bjørås M, Seeberg E. Institute of Microbiology, National Hospital, Oslo, Norway

Objective: To determine the role of the DNA glycosylases of the base excision repair pathway in meningococci. The DNA of the meningococcus is highly recombinogenic and has an increased spontaneous mutation rate, representing an unusual challenge for DNA repair mechanisms in genome maintenance.

Design: Based on homology to known genes, we have identified meningococcal genes encoding DNA glycosylases. PCR cloning and mutagenesis were performed to compare expression profiles. The clones in *E. coli* were analyzed by several biochemical and biological assays. Meningococcal wildtype and single and double mutant strains were constructed and compared with regard to mutagenicity and survival under varying conditions.

Results: We have cloned and expressed the genes encoding the DNA glycosylases Nth, Fpg and MutY from meningococci. The DNA glycosylase mutants displayed a decreased survival rate and an elevated spontaneous mutation rate compared to the wild type strain.

Conclusions: This is the first report on the expression of meningococcal Nth, Fpg and MutY. We have shown that meningococcal DNA glycosylases clearly play a role in genome maintenance, alone and in their interactions with other DNA repair mechanisms.

#317

CONSTRUCTION AND EVALUATION OF A RECA MUTANT IN NEISSERIA MENINGITIDIS.

[1]Turner G.A, [1]Zang Barber L, [1]Winzer K, [1]McDowell PW, [2]M[c]Lean L, [3]Gorringe A. and [1]Baldwin T.J. [1]Institute of Infection and Immunity, Queens Medical Centre, Nottingham.
[2]Cantab Pharmaceutical, Cambridge Science Park, Cambridge.
[3]CAMR, Porton Down, Salisbury.

Background: *Neisseria meningitidis* is naturally competent, and can incorporate foreign DNA into its chromosome by homologous recombination. This process may be responsible for the variability seen between meningococcal isolates. The *rec*A gene is essential for DNA repair, recombination and pilin variation in some bacteria.

Design: The *rec*A homologue from *Neisseria meningitidis* strain B:16:B6 was isolated and a deletion/insertion mutation constructed by inverse PCR and insertion of a kanamycin resistance cassette within the coding sequence. The mutation was introduced back into the *Neisseria meningitidis* chromosome by natural transformation and kanamycin resistance selection.

Results: Analysis of the mutant by PCR, showed a 1.9 kb fragment compared to 823 bp for the parental WT. Homologous recombination in the mutant was compared to the parental WT strain by introducing an erythromycin tagged *aroB* gene and determining the transformation efficiency. No transformants were obtained for the mutant whereas the parental strain showed high levels of transformation. UV sensitivity of the mutant strain compared to the wild type strain was demonstrated by exposure to UV light at time intervals of 0 to 120 seconds. The viability of the mutant strain was reduced by 20% at 40 seconds and 100% viability loss was observed at 60 and 120 seconds. The wild type strain was 100% viable at all UV exposure times. Preliminary animal studies suggest that the *Neisseria meningitidis rec*A mutant is not attenuated, which is shown on another poster.

Conclusions: The *Neisseria meningitidis rec*A mutant is deficient in recombination activity and DNA repair mechanisms. It is unable to repair mutations by homologous recombination using exogenously added DNA and is extremely UV sensitive.

#318

CONTROL OF CAPSULE EXPRESSION OF NEISSERIA MENINGITIDIS BY THE CAPSULE PROMOTOR REGION

von Loewenich FD, Dümig M, Frosch M, Wintermeyer E,
Institut für Hygiene und Mikrobiologie, Universität Würzburg, Germany

Objective: Analysis of the intergenic promotor region seperating the capsule biosynthesis operon (*siaA-D*) and the capsule transport operon (*ctrA-D*).

Design: We looked for sequence variation in the promotor region of 42 menigococcal isolates of different serogroups. To determine the promotor activity of the respective intergenic regions, we performed transcriptional and translational fusions with the *lacZ* gene integrated in the chromosome of *Neisseria meningitidis*. Additionally we determined the promotor relevant regions by a sequential deletion analysis

Results Sequence variations were found mainly in a repeated region preceding the *siaA* start codon This sequence variations had no effect on ß-galactosidase activity Correlation between sequence variation and serogroup was not detected Different in vitro growth conditions such as temperature, glucose concentration, osmolarity and iron concentration did also not influence ß-galatosidase activity However, the deletion of the repeated region preceding the *siaA* start codon lead to a up to threefold higher ß-galactosidase activity compared with the full length contruct, suggesting that the respective region may be involved in capsule regulation

Conclusions: Under in vitro conditions, neither sequence variation nor variation of different environmental factors seem to influence the activity of the capsule promotor. A deletion analysis of the intergenic region revealed, that the repeated region preceding the *siaA* start codon has a negative influence on ß-galactosidase activity. We are currently investigating if this is due to binding of a regulator.

#319

CLONING AND EXPRESSION OF *tspA* AND CONSTRUCTION OF AN ISOGENIC DELETION MUTANT.

Wells DB, Wooldridge KG, and Ala'Aldeen DAA.

Meningococcal Research Group, Division of Microbiology, University of Nottingham, UK.

We have previously reported the identification of TspA, a major CD4+ T-cell and B-cell stimulating *Neisseria*-specific antigen [1]. The complete gene (2,761bp) was reconstructed with reference to the meningococcal genomic sequence (Sanger) and amplified. Expression of the N-terminal 1kb of *tspA* proved to be too toxic for *E.coli* preventing straight forward cloning or T-tailing into various vectors, including pUC19, pGEM and pCRII. The entire gene was eventually cloned into pQE30 but only in the presence of a constitutively expressed *lacZ* repressor (pREP4).

For mutagenesis, the N-terminal 887bp of *tspA* was amplified along with an upstream 1.7kb containing the putative promotor region. A TspA isogenic deletion mutant has been constructed with the gene being disrupted by insertion of an antibiotic cassette.

Full characterisation of *tspA* and the meningococcal isogenic mutant will be presented.

Reference:
1. Kizil, G *et al.*, *Infect Immun* 1999; 67 (7): 3533-3541.

#320

A CELL-CELL SIGNALLING SYSTEM IN *NEISSERIA MENIGITIDIS*

Winzer K, Blackely D, Green A, Hardie K, Barber L, McDowell PW, Turner G. and Baldwin TJ. Institute of Infection and Immunity, Queen's Medical Centre, University of Nottingham, Nottingham, UK

Background: Many bacteria communicate via diffusible signal molecules termed autoinducers, which regulate multiple target genes in a cell density-dependent manner. This type of cell-cell-signalling, termed 'quorum sensing', regulates diverse physiological processes, including virulence. *luxS*, a new gene responsible for signal molecule production, has recently been identified in *Vibrio harveyi*, where it controls the production of an autoinducer of unknown structure, termed 'AI-2' (1). Analysis of the genome sequences of *N. gonorrhoea* FA1090 and *N. meningitidis* Z2491 (1), as well as *N. meningitidis* MC58 revealed the presence of *luxS* homologues.

Results: We have studied the function of *luxS* in *N. meningitidis* to obtain further evidence for cell-cell signalling in pathogenic *Neisseria* and its significance in colonisation/infection. Spent culture supernatants of various *N. meningitidis* strains were tested for autoinducer activity using a biosensor, *V. harveyi* BB170 (2), which responds specifically to AI-2. The supernatants were capable of activating BB170, albeit not to the same extent as a *V. harveyi* positive control. Insertional inactivation of *luxS* in *N. meningitidis* B16:B6 resulted in a loss of autoinducer activity in culture supernatants. However, preliminary comparisons of the *luxS* mutant and wild type strains revealed no major changes in protein profiles or cell morphology under various *in vitro* conditions.

Conclusions: *luxS* directs the production of an AI-2 type autoinducer in pathogenic *Neisseria*, but the phenotypes regulated by quorum sensing still need to be identified.

1. Surette *et al.* (1999) Proc. Natl. Acad. Sci. USA 96:1639-1644
2. Bassler *et al.* (1994) Mol. Microbiol. 13:273-286

#321

USE OF PROTEOMICS TO IDENTIFY GENES REGULATED BY THE 2CR *BASRS* IN PATHOGENIC NEISSERIA

Zaleski, A1, Scheffler, N. K2, Preston, A3, Matt Binicker1, Gibson BW2, Apicella MA1. 1Dept. of Microbiology University of Iowa, Iowa City, IA. 2 Dept. of Pharmaceutical Chemistry University of California San Francisco, San Francisco, CA and the College of Veterinary Medicine, Cambridge, England3

The *N. gonorrhoeae* genome contains a 2 component regulatory homolog of the *E. coli basRS*. We have generated an erythromycin-insertion mutation in the Strain 1291 *basS* homolog. 2D gel analysis of 35S methionine-cysteine labeled *N. gonorrhoeae* strain 1291 and strain 1291 *basS* revealed significant differences in the degree of expression and presence of specific proteins. Autoradiographs derived from these gels were analyzed the BioImage 6.0.1 2D gel analysis program. Using a coomassie stained 2D gel of *basS*, spots were selected based on increased expression in the mutant, cut from the gels and subjected to trypsin digestion. Mass spectrometry analysis of the tryptic fragments of these proteins was performed. The resulting fragments were compared with a virtual tryptic digest of the Meningococcal strain Z2491 database using the MSFit program from Protein Prospector. These were AtpC (10/34 fragments, covering 95% of the protein), NusG (15/29 fragments, covering 86% of the protein), Gpm (13/18 fragments, covering 53% of the protein), EtfB (5/12 fragments, covering 34% of the protein) and MinD (6/15 fragments, covering 48% of the protein).

N. gonorrhoeae has a limited number of regions with homology to 2 component regulators. Using proteomics we have identified 5 proteins which appear to be upregulated in *N. gonorrhoeae* strain1291 *basS*. Studies are underway to confirm these finding using reporter constructs in these genes within the chromosome of the gonococcus.

#322

GENETIC BASIS OF PHASE VARIATION AND STABILITY OF LIPOOLIGOSACCHARIDE EXPRESSION IN TWO L8-IMMUNOTYPE STRAINS OF *NEISSERIA MENINGITIDIS*

Zhu P, Klutch M, Tsai C-M. Divisions of bacterial and viral products, Center for Biologics Evaluation and Research, FDA, Rockville, MD 20852 USA

Phase variation and stability of lipooligosaccharide (LOS) expression were observed in two *N. meningitidis* strains, M978 and A1, both with the same L8 LOS immunotype. Strain M978 produced hypervariable LOS profiles on SDS-PAGE under different growth conditions whereas strain A1 did not change its profile. In order to understand the genetic difference of the LOS phase variation and stability between the two strains, the *lgt* locus encoding glycosyltransferases responsible for the biosynthesis of the α-chain of LOS was amplified by PCR and the nucleotide sequences were determined. The *lgt* genetic organization of the two strains was different and was also different from the three *lgt* loci known in *N. meningitidis* (strains 126E, MC58 and Z2491). Strain M978 possessed four genes in the order of *lgtC*, *lgtA*, *lgtB*, *lgtE1* and an additional truncated *lgtA/B* at the beginning, which had a potential ability to make L1, L3 and L8 LOS. Strain A1 contained only two genes of *lgtA* and *lgtE2* in the same position but *lgtA* was inactivated by a frame shift, thus it expressed only L8 LOS. The homology between *lgtE1* and *lgtE2* in the two strains was 64% at the 320 bp region of 3'-end and the sequence differences probably reflect the specificity of their galactosyltransferase products for recognition of the inner core acceptor. The expression pattern of *lgt* genes in M978 is being investigated by RNA analysis. The data suggest that both the organization and expression of *lgt* gene cluster are involved in regulation of LOS diversity. The stability in expression of L8 LOS, resulting from limited components at *lgt* locus and the frame shift, renders strain A1 a better reference strain for the L8 immunotype.

#222

NEISSERIA MENINGITIDIS SEROGROUP W135 IN THE GLOBAL VILLAGE

Popovic T[1], Sacchi CT[1], Reeeves MW[1], Whitney AM[1], Al-Hamdan N[2] Caugant D[3], Noble CA[1], Ajello GW[1], Mayer LW[1], Lingappa J[1], Rosenstein NE[1], Mostashari F[1,4], Bendana N[5], Alonso J-M[6] and Taha M-K[6].

[1] Center for Disease Control and Prevention, Atlanta, GA, [2]Saudi Arabia Field Epidemiology Training Program, Rhiad, Saudi Arabia, [3]National Institute of Public Health, Oslo, Norway, [4]New York City Department of Health, New York, NY, [5]California Department of Health, Los Angeles, CA, [6]Institut Pasteur, Paris, France.

Objective: Over 300 laboratory confirmed cases of meningococcal disease caused by *N. meningitidis* serogroup W135 (NMSW135) among Hajj 2000 pilgrims/close contacts have been reported worldwide. To evaluate relatedness of these isolates to some of the major meningococcal clonal groups, extensive molecular characterization was performed on NMSW135 outbreak-associated (OA) isolates from the US (n=5) France (4), Saudi Arabia (3), and on 28 NMSW135 isolates collected from 1993-2000 in the US, Gambia, Mali, Algeria, Canada and Indonesia.

Results: All tested OA isolates were of porA VR type P1,5,2, had identical 16S rRNA gene sequences, identical PFGE (*Nhe*I) profiles, and by multilocus-enzyme electrophoresis (MEE) belonged to a ET-927 more closely related to the ET-37 complex than to any other major clonal group. Eight isolates from Gambia, Mali, Indonesia and Canada (1995-97) shared all these molecular markers with the OA isolates. NMSW135 isolates previously identified in the US, as well as those isolated at the same time as the OA isolates but with no epidemiologic link to the current outbreak, had clearly distinct molecular markers which allowed for the easy differentiation of OA from other NMSW135 isolates.

Conclusion: NMSWW135 associated with this year's Hajj outbreak are not entirely new. Our data demonstrated that isolates with these specific molecular markers have been in circulation in human populations for at least several years in different parts of the world, and at the same time show the utility of molecular subtyping for rapid and precise identification of isolates associated with epidemic situations.

#223

SEXUAL ISOLATION IN *NEISSERIA MENINGITIDIS*

Claus H, Stoevesandt J, Frosch M, Vogel U. Institute for Hygiene and Microbiology, University of Wuerzburg, Germany

Objective: To identify genes, which are differentially distributed among hypervirulent clonal groupings of meningococci.

Design: Representational difference analysis (RDA) and plasmid isolation were performed. Strains from our own collection as well as from the collections of D. Caugant (Oslo), M. Achtman (Berlin), and the National Reference Center for Meningococci in Heidelberg were used for the study.

Results: By RDA, a variety of DNA fragments were isolated, which were found to be characteristic for distinct clonal lineages of *N. meningitidis*, i.e. the ET-5 complex, the ET-37 complex, and the subgroup IV-1. Among the fragments were several restriction-modification systems and phage associated genes. The differential distribution was a stable characteristic of the clonal groupings. Plasmid analysis of clonal groupings of meningococci allowed the isolation of a novel meningococcal plasmid, pJS-B, which was restricted to the ET-37 complex and the cluster A4.

Conclusions: Our analysis is fitting into previous observations concerning the differential distribution of *opcA* and *porB* genes. The results demonstrate that despite of frequent horizontal gene transfer there is sexual isolation of a variety of hypervirulent clonal groupings. We will discuss the inhibition of horizontal gene transfer by the action of differentially distributed restriction-modification systems.

#224

MULTILOCUS SEQUENCE TYPING OF 104 SEROGROUP A *NEISSERIA MENINGITIDIS* STRAINS ISOLATED IN AFRICA BETWEEN 1988-1999: WILL THE CLONAL EXPANSION OF ST5 BE REPLACED BY THE CLONAL EXPANSION OF ST7?

NICOLAS P., DECOUSSET L., RIGLET V., CASTELLI P., BLANCHET G.

Unité du méningocoque, Institut de Médecine Tropicale du Service de Santé des Armées, Centre Collaborateur OMS, Parc du Pharo BP 46 13998 Marseille Armées, France.

Objective: MLST (Multilocus Sequence Typing), a new typing method, will probably become the reference technique for global epidemiology of some bacteria. For *Neisseria meningitidis*, MLST compares the sequences of 7 loci belonging to 7 housekeeping genes, to the different existing alleles on the MLST website of meningococcus (http://mlst.zoo.ox.ac.uk). That permits to characterize one strain by its sequence type (ST). The first data published in 1998 by Maiden showed a good congruence between MLST and multilocus enzyme electrophoresis (MLEE, the former reference technique). The aim of this work was to check MLST for the characterization of 104 serogroup A *Neisseria meningitidis* isolated in Africa between 1988 and 1999, in order to determine (i) the feasibility of the technique and (ii) which sequence types were circulating in some African countries during this period.

Design: 104 strains, isolated in different African countries (Algeria, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Guinea Bissau, Ivory Coast, Mali, Niger, Senegal, Sudan and Zaire) between 1988 and 1999, received at the WHO collaborating center in Marseilles were included in this study. Many of these strains were isolated during epidemics, others were isolated during endemic periods. All the 104 strains were characterized by grouping, typing and subtyping, and MLST.

Results: 103 out of 104 strains were serogroup A:4 P1.9 *Neisseria meningitidis*. All the strains were characterized by 7 alleles corresponding to the 7 loci and by their sequence type. One hundred out of 104 strains belonged to ST5 or ST7. Four strains showed one locus that was different from the consensus, and were classified in others sequence types.

Conclusions: ST5 strains were responsible of 1988 Chad outbreak and of most of African cases and outbreaks analyzed in our laboratory until 1996. As ST5 continued its expansion towards West and was the cause of outbreaks in Senegal and in Guinea Bissau in 98 and 99, ST7, a new clone, closely related to ST5, emerged. The first ST7 strain isolated in Africa, analyzed in our laboratory, was one strain isolated in Algeria in 1995. ST7 strains were then isolated in Cameroon and Chad in 97, in Zaire in 98, Niger and Sudan in 99. ST 5 and ST 7 are closely related clones with only one locus different: pgm1 for ST5 and pgm19 for ST7. Between the two alleles there are 47 base pair differences. This difference is probably due to recombination as this phenomenon is very frequent in *Neisseria meningitidis*. Few data are available, however, they seem indicate us that, country after country, we probably observe the beginning of clonal replacement of ST5 strains by ST7. Why? if direct selection were to play a major role, variations in cell surface epitopes should have happened. This is not the case as the surface epitopes studied in this work such as serogroup, type, subtype, are the same for ST5 and ST7. Occasional genetic variants are lost during most phases of clonal reduction. But here, the clonal replacement occured by amplification of a genetic variant and subsequent population are uniform for the new variant. This is the concept of sequential bottleneck which allow variation of genotypes independently of selective pressure. The importance of the outbreaks in Sudan, Chad, can infer that ST7 is as a virulent clone as ST5.

#225

SURVEILLANCE OF PATHOGENIC STRAINS OF *NEISSERIA MENINGITIDIS* IN VICTORIA, AUSTRALIA, HAS REVEALED THE EMERGENCE OF A NOVEL STRAIN (C:2a:P1.7b,4; ST11) WITH A MARKER FOR THE "ET15" CLONE

Griffith JM[1], Zaia A[1], Hogg GG[1], Li H[1], Tribe DE[2], Taylor, KN[3], Robinson, PM[3]

[1]Public Health Laboratory (Microbiological Diagnostic Unit) and [2]Microbiology & Immunology Dept, University of Melbourne, [3]Communicable Diseases, Dept of Human Services, Victoria, Australia

From 1992 to 1998, between 60 to 90 cases of invasive meningococcal disease were reported each year. Of the 475 culture-positive cases, 454 isolates were submitted to MDU. Generally, serogroup B strains were isolated at least twice as often as serogroup group C strains (275B:124C). By 1996, the most commonly identified phenotype was B:4:P1.4 etc (25% that year). Most of those isolates examined so far have been **ST 41** (common in W. Europe) or related sequence types; a few have been **ST 42** (common in New Zealand).

In 1999, a significant change occurred. The number of cases rose to 141, of which 94 were culture-positive. PCR testing confirmed a further 19 cases. Almost half of the isolates belonged to serogroup C. One serogroup C clone was responsible for 16% of all culture-positive cases that year. This clone had a higher than usual case-fatality rate (20%) and the mean age of cases was 30.5 years. DNA subtyping identified it as **a novel recombinant - C:2a:P1.7b,4 (ST11)**. The P1.7b,4 porA (identical over 538 base pairs to that of Lineage III strains) may be an advantage to the pathogen by having a low immunogenicity. Furthermore, the ***fum* C locus** of this strain is identical by sequencing to that of the **ET15 clone** (as distinct from ET37) which caused serious problems in Canada.

Conclusion: Of **significant public health concern** is the demonstration of a novel serogroup C recombinant , with an altered surface antigen (P1.7b,4) and bearing a marker for the ET 15 clone.

#226

GEOGRAPHIC CLUSTERING OF MENINGOCOCCAL CLONES AS DETERMINED BY FAFLP PATTERNS DURING A PROTRACTED OUTBREAK OF SEROGROUP C MENINGOCOCCAL DISEASE.

Neal Keith R, Goulding Jonathan N, Hookey John V, Stanley John, Olver Will, Ala'Aldeen Dlawer AA, Arnold Catherine.
Meningococcal reaserach Group University of Nottingham, Molecular Biology Unit, VRD, Central PHL, London, UK

Introduction – Between April 1997 and August 1999, 12 cases of serogroup C meningococcal disease occurred in a rural village with a population of 1600, a rate of over 250 cases per 100,000 for a three year period. Antibiotics were administered to over 95% of the resident population in August 1998 and naso-pharyngeal swabs were taken from 1242 residents at one main antibiotic session.
Methods – Meningococci were recovered and characterised using standard methods. The carriage rates of meningococci and *N. lactamica* were consistent with other UK carriage studies.
Fluorescent Amplified Fragment Length Polymorphism (FAFLP), a PCR-based genome technique that generates a specific profile for each bacterial clone was used to investigate the genetic epidemiology of 60 isolated from the swabbing in 1998 and 1 disease isolate from 1997. The software package Neighbour (Phylip) was used to construct a tree by successive clustering using an average-linkage method of clustering.
Results – The disease isolate was very similar to the serogroup C organisms carried in the population one year later. Organisms from the same street and part of the same part of the village were more likely to be similar than randomly selected isolates. No evidence of clustering was seen by school class for pupils aged 4-11.
Conclusion – Similar strains were much more likely to be found within the same street, in particular neighbouring houses. This suggests contact tracing of in the public health management of meningococcal disease should not be necessarily restricted to household contacts.

#323

CLASSIFICATION TREES AND LOGISTIC REGRESSION APPLIED TO PROGNOSTIC STUDIES: A COMPARISON USING MENINGOCOCCAL DISEASE AS AN EXAMPLE

Werneck GL[1,3], Carvalho DM[1], Barroso DE[2], Cooks EF[3], Walker AM[3]. [1]Federal University of Rio de Janeiro, Brazil; [2]Oswaldo Cruz Institute, Brazil; [3]Harvard School of Public Health, U.S.A.

Objective: This study compares the ability of multiple logistic regression (MLR) and classification and regression trees (CART) to discriminate between fatal and non-fatal meningococcal disease cases (MD) in a cohort of children.

Design: During 1989-1990 a total of 829 MD cases (<16 years) were admitted to the Infectious Diseases State Institute São Sebastião in the city of Rio de Janeiro, Brazil. Medical and epidemiological surveillance records were reviewed. We used MLR and CART to identify the most important independent prognostic factors to death in MD. The predicted probability of death for each individual was estimated from each model, and then compared to the actual outcomes.

Results: Variables associated with death in the MLR model and the best CART obtained include seizures, diarrhoea, shock, focal neurological sign, and residence outside Rio de Janeiro city. Age above 1 year, neck stiffness, and longer duration of disease were associated with a lower risk of death.

Conclusions: The two prognostic models generated are statistically equivalent. However, the graphical display of the results from a tree is easier to understand and is straightforward to apply in clinical settings.

#324

AUTOMATION OF THE POLYMERASE CHAIN REACTION FOR THE LABORATORY CONFIRMATION OF MENINGOCOCCAL DISEASE

Clarke SC, Diggle MA and Edwards GFS. Scottish Meningococcus and Pneumococcus Reference Laboratory, North Glasgow University Hospital NHS Trust, Glasgow, UK.

Objective: To automate the procedure involved for the laboratory diagnosis of meningococcal disease (MD) using the polymerase chain reaction (PCR).
Design: A prospective study was performed to evaluate the procedure necessary for PCR automation to enable the rapid and accurate laboratory confirmation of meningococcal disease. One thousand sera received from patients with clinically-suspected MD were investigated. The results of the PCR tests were compared with clinical information, meningococcal antibody testing and blood culture isolates.
Results: One thousand sera were tested for the presence of IS1106 and *por*A by PCR using an automated liquid handling system. The system automated all the liquid handling steps required for setting up the PCR reaction, automated the thermocycling procedure, and automated the gel-loading operation. In addition, the procedure was less labour intensive, accurate and highly reproducible. The method required very few consumable items and was therefore very cost-effective. It was shown that both methods were highly effective when automated for the confirmation of MD. However, the *por*A method was less sensitive but at the same time more useful as it could be used for additional DNA sequencing analysis and therefore provide a subtype of the infecting organism.
Conclusions: Automated PCR is a cost-effective method for the laboratory confirmation of MD when a high-throughput is required. The method is less labour-intensive than the equivalent manual procedure and is less prone to errors associated with liquid handling.

#325

LABORATORY CONFIRMATION OF *NEISSERIA MENINGITIDIS*: PAST, PRESENT AND FUTURE

Diggle MA, Clarke SC and Edwards GFS. Scottish Meningococcus & Pneumococcus Reference Laboratory (SMPRL), North Glasgow University Hospital NHS Trust, Glasgow, UK.

Objective: To highlight the constant evolution of techniques required for the laboratory confirmation of meningococcal isolates and future prospects for the role of new technologies in a National Reference Laboratory.
Design: A retrospective and prospective overview of the traditional techniques and the more recent molecular advances used in the laboratory confirmation of meningococcal disease (MD).
Overview: The Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) provides a national service for the laboratory confirmation of meningococcal and pneumococcal disease in Scotland. Within this service, MD is confirmed by antigen, culture and serological methods. The ability for the SMPRL to type micro-organisms to a sub-species level plays an essential role in the diagnosis, treatment and control of infection. Traditionally, the differentiation of micro-organisms has involved analysis of phenotypic markers such as capsule and outer membrane proteins using latex agglutination, co-agglutination and ELISA methods. However, these methods are not universally applicable to all circumstances presented to the SMPRL. Recent developments in DNA analysis, together with the natural limitations of phenotypic methods, have resulted in a natural evolution towards genotypic procedures. These are based on DNA analysis, such as PCR for IS*1106*, *siaD*, *ctrA* and *porA*, and more recently the incorporation of an automated multi-locus sequence typing (MLST) service for the full identification of meningococcal isolates in Scotland.
Conclusions: The potential application of recent genotypic methods indicate that there is a trend towards the use of DNA sequencing as a typing tool, along with other methods such as DNA chip technology. These raise the issue of the long-term provision of reference facilities because such methods could provide a service for typing all human pathogens.

#326

REDUCED CASE FATALITY RATE OF MENINGOCOCCAL DISEASE AFTER PUBLIC INFORMATION ABOUT THE EARLY SIGNS OF MENINGOCOCCAL SEPTICEMIA

Halstensen A, Aase I, Bjørnevik AT, Smith I.
Institute of Medicine, University of Bergen, Norway.

Objective: Public information about meningococcal disease often focus on the clinical signs of meningitis, which rarely kills these patients. In contrast, patients with meningococcal septicemia have a high case fatality rate (CFR). The aim of the present study was to examine the effect of simple public information about the early clinical signs of meningococcal septicemia on the CFR of meningococcal disease.

Design: The core information to the public were: During the first 24 hours of unexplained fever, especially in children and teenagers, please look for skin rash or skin bleedings at regular intervals (2-4 hours) even during the night. In the period 1986-91 colour pictures of petechial rash and the "glass test" were frequently shown in the newspapers and on television. CFRs during 1976-2000 were recorded, and a questionnaire about disease knowledge were sent to all surviving patients admitted during 1982-83 and 1992-93 and/or their closest relatives.

Results: CFR fell from 8.5% during 1976-84 to 3.9% during 1985-93 ($P=0.05$). During 35 months of 1990-94 none of 92 patients died ($P \leq 0.05$). Knowledge of skin rash as an early sign increased from 37% among those admitted during 1982-83 to 96% during 1992-93 ($P<0.001$) The time from onset of the disease to admission were shorter during the last period and the patients had less petechiae on admission. However, during 1994-99, the CFR increased to 25%. This increase has several explanations, but we suspect less public information to be part of it.

Conclusions: Simplified and regularly repeated information to the public about the early signs of meningococcal septicemia is necessary to reduce CFR of meningococcal disease. Colour prints of petechiae in newspapers are most efficient.

#327

PURULENT MENINGOCOCCAL PERICARDITIS ASSOCIATED WITH *NEISSERIA MENINGITIDIS* MLST ST11, C:2a:P1.4.

Jelfs, J[1,2], Hughes BR[1], Gosbell, IB[1,2].
[1]Department of Microbiology and Infectious Diseases, SWAPS, Liverpool, NSW. Australia.
[2]Faculty of Medicine, University of New South Wales, Kensington, NSW Australia.

A previously healthy 41-year-old male presented to hospital with a purpuric rash, septic shock and multi-organ failure. The chest x-ray revealed a small globular heart. The electrocardiogram showed ST segment elevation. The patient received antibiotics and inotropes with slow improvement. *N. meningitidis* was isolated from blood cultures. An EDTA blood sample was positive for *N. meningitidis* serogroup C by PCR. The patient had several echocardiograms, which initially revealed pericardial effusion with no tamponade but later showed a large pericardial effusion with tamponade. 830mL of straw coloured fluid was aspirated. Culture of this fluid was negative but PCR was positive for *N. meningitidis* serogroup C. The patient also developed bilateral pleural effusions which were drained and found to be both culture and PCR negative for *N. meningitidis*. The bilateral pleural effusions and pericardial effusion persisted. Echocardiography revealed dense material in the pericardial sac and on pericardiotomy the pericardium was grossly abnormal being 3cm thick with a buttery exudate. Culture of the pericardial tissue was negative. After surgery the patient recovered fully. The strain isolated, C:2a:P1.4, had not been detected previously in NSW and was found to belong to MLST ST11. In NSW Serogroup C MD had been associated with predominantly C:2a:P1.5, ET-37 complex (ET-15) and the P1.4 phenotype commonly associated with Lineage III (B:4:P1.4). Meningococcal pericarditis has been described infrequently in the literature and invasive MD associated with the phenotype C:2a:P1.4 is rare. The patient described here had not only an unusual clinical presentation but MD associated with an unusual strain of *N. meningitidis* belonging to MLST ST11 with an unusual *por*A genotype P1.7b,4.

#328

INTRODUCTION OF PCR DIAGNOSIS OF MENINGOCOCCAL INVASIVE DISEASE IN THE CZECH REPUBLIC

Kalmusova J, Pavlikova V and Kriz P. National Reference Laboratory for Meningococcal Infections, National Institute of Public Health, Prague, Czech Republic.

Objective: To introduce a further non-cultural method for diagnosis of invasive meningococcal disease (IMD) in addition to the currently used non-cultural methods, i.e. latex agglutination and serology.

Design: A polymerase chain reaction (PCR)-based diagnostic assay for the identification of *Neisseria meningitidis* was introduced in our laboratory in 1999. The PCR method of Frosch and Zambardi was modified and standardised for our conditions. Sensitivity and specifity of the PCR method were assessed in 195 samples of cerebrospinal fluid (CSF) Gen *siaD* was amplified for serogroup B and serogroup C (oligonucleotides 98-19, 98-20, 98-17 and 98-18 kindly provided by Dr. Taha, Institut Pasteur , Paris)

Results In the whole collection of 195 samples investigated true-positive PCR results for *N. meningitidis* were found in 55 samples, true-negative PCR results in 123 samples, false-positive PCR results in 5 samples and false-negative PCR results in 12 samples The sensitivity and specificity of the PCR method for the diagnosis of IMD from CSF were calculated and reached 82.1% and 96.1%, respectively.

Conclusions The PCR method for the non-cultural diagnosis of invasive meningococcal disease was introduced and serogroups B and C can be identified. The relatively low sensitivity of the PCR method used indicates that an extended PCR assay for the identification of *N. meningitidis* species should be introduced before its wide use for diagnostic purposes in practice.

#329

MULTILOCUS SEQUENCE TYPING MADE IN THE CZECH REPUBLIC - FIRST RESULTS

Kriz P[1], Kalmusova J[1], Felsberg J[2], Pavlikova V[1], Musilek M[1].
National Reference Laboratory for Meningococcal Infections, National Institute of Public Health, Prague, Czech Republic,
[2]Institute of Microbiology, Czech Academy of Science, Prague, Czech Republic.

Objective: Multilocus sequence typing (MLST) of unusual pheno- and/or genotypes of *Neisseria meningitidis* strains isolated from patients with invasive meningococcal disease in the Czech Republic.

Design: During the last three decades a large number of *Neisseria meningitidis* strains isolated in the Czech Republic from patients with invasive meningococcal disease was collected and analyzed using Whole-Cell ELISA and multilocus enzyme electrophoresis. We found that the Czech meningococcal population is distinct different from those in western countries These differencies could be the result of the isolation of the human population, and consequently the microbial population, during the previous four decades up to the late 1980's as a consequence of the cold war Our project is focused on meningococcal strains with unusual phenotypes and ET-types to be investigated by MLST among which identification of new sequence types (STs) is expected

Results: First results confirm our working hypothesis: six strains were analysed by MLST and for three of them, new STs were described.

Conclusions: The genotypes of the Czech meningococcal population started to be investigated by a new, advanced method, the MLST. The first results are suggestive of the genetic dissimilarity of the Czech and the western meningococcal populations.

#330

Neisseria gonorrhoeae infection among the CSW with STDs and HIV and their management in Big Cities in Indonesia.

Matulessy PF., Pattiradjawane FM, Simanjuntak M, Lohy J; ESG.NH-FKUKI, Christian University of Indonesia. PALMA-Foundation; JEN-IEN, Jakarta, Indonesia.

During the last ten years, health programs in Indonesia have not given priority to the prevention and control of STDs and HIV/AIDS.

Objectives: to find out the prevalence of Neisseria gonorrhoeae infection among the CSWs with STDs and the impact to their health, and to find out what the best solution to prevent them.

Methods: using LCR test, physical examination & interview to measure the prevalence of STDs; ELISA Pool test to screen HIV among the CWSs in 3 big Cities in Indonesia.

Results: The prevalence of Gonorrhea is 28.89%, Chlamydia is 32.06% and both Chlamydia with Gonorrhea is 44.13% from 511 CSWs and 74 STD patients. Less than 20% of respondents suffered from the FOUR Symptoms like Vaginal discharge, Dysuria, Genital lessions, and Lower Abdominal Pain. 46.9% of the CSWs who suffered from any synptoms used to do self-treatment. Only 8.1% of patients received a good quality of STD care, using the PIs developed by WHO/GPA. There is no correlation between the high prevalence of Gonorrhea or other STDs and the quality of assurance of case management and the knowledge of CSWs or STD patients, because they do not use frequently the facilities of the STD Clinics or Condom used regularly.

Conclusion: Mixed infection was the highest prevalence from the STDs infection among the CSWs which suffered different Symptoms. Improvement of management quality and CIE program to develop a comprehensive approach for STDs and HIV protection.

#331

AN EVALUATION OF FLUORESCENT AMPLIFIED-FRAGMENT LENGTH POLYMORPHISM (FAFLP) AS A TYPING TOOL FOR *N. GONORRHOEAE.*

Palmer HM[1] & Arnold CA[2].

1 Gonococcus Reference Unit, Genitourinary Infections Reference Laboratory, Public Health Laboratory, Bristol Royal Infirmary, Bristol BS2 8HW UK

2 Central Public Health Laboratory, London NW9 5HT UK

High resolution methods for typing *N. gonorrhoeae* are required for outbreak analysis and the study of genetic relatedness of strains. FAFLP is a genome fingerprinting technique which generates accurately sized fragment patterns that can be stored in a database. The aim of this project was to investigate the resolving power of FAFLP when applied to *N. gonorrhoeae*. We analysed a collection of approximately 60 strains containing representatives from six serovars and five auxotypes, including strains known to be epidemiologically related and others that are unrelated. All strains were typed using FAFLP and the related strains were also opa-typed (a highly discriminatory PCR-RFLP method). FAFLP was reproducible after serial subculture of strains, and from duplicate DNA preparations. Epidemiologically unrelated strains of the auxotypes arginine- hypoxanthine- uracil-requiring (AHU) and proline-requiring, arginine-requiring not satisfied by ornithine, uracil-requiring ($PA^{\circ}U$) were grouped as two distinct FAFLP clusters. Also, strains from sexual contacts and strains from a local outbreak formed distinct FAFLP clusters, and this discrimination was similar to that achieved by opa-typing.

FAFLP offers a high level discrimination between strains of *N. gonorrhoeae* based on whole genome fingerprints that can be stored in a database. As such it is a valuable tool for identifying relationships between strains that form part of large surveillance projects.

#332

PRACTICAL USE OF NUCLEIC ACID TECNIQUES FOR DIFFERENTIAL DIAGNOSIS OF MENINGITIS IN MOSCOW

Shipulin GA, Tyutyunnik EN, Shipulina OY, Platonova OV, Koroleva IS, Guiver M, Kaczmarski EB, Platonov AE. Central Research Institute of Epidemiology, Moscow, Russia; Moscow State University for Medi-cine and Dentistry; Meningococcal Reference Unit, Manchester, UK.

A set of PCR-based diagnostic assays was applied to the CSF samples obtained from 218 patients with bacterial and aseptic meningitis in Moscow. The set included 16S RNA gene-specific assays for Neisseria, Streptococcus, and Haemophilus, assays for serogroup A, B, and C meningococci using *mynA* and *siaD* genes, assays for enteroviruses (EV) and human herpes viruses (HHV6, HSV1/2, VZV, CMV, EBV). 97, 43, and 12 samples were found to be positive for Neisseria, Streptococcus, and Haemophilus DNA. Of these, 34, 28, and 8 were culture-positive, respectively. Serogroup-specific assays were slightly less sensitive, so group A, B or C meningococcal genes were identified in 44, 30, and 14 CSF samples. 13 samples from 35 patients with aseptic meningitis were EV-positive, suggesting enteroviral etiology, whereas other viruses were found occasionally (HHV6, HSV1/2, VZV, CMV, EBV in 2, 2, 0, 0, 1 samples, respectively). The presence of viral (EBV, HHV, or CMV) DNA in 16 CSF samples from patients with bacterial meningitis might indicate chronic viral infection. In total 70 of 183 bacterial meningitis cases were diagnosed by culture and PCR while 82 cases more were confirmed by PCR alone. This represents a substantial improvement in diagnostic capabilities. It is planned to examine these samples using *N.meningitidis ctrA* assay, pneumolysin-specific assay, and *H.influenzae* type b *bexA* assay that will evaluate the relative sensitivity and specificity of different approaches.

#333

THE QUANTITATION OF GENOME COPIES OF *N.MENINGITIDIS* IN THE CEREBROSPINAL FLUID (CSF) OF PATIENTS WITH MENINGITIS IN RELATION TO THE CLINICAL AND LABORATORY FINDINGS

Guiver M, Platonov AE, Tyutyunnik EN, Shipulina OY, Platonova OV, Shipulin GA, Kaczmarski EB.
Central Research Institute of Epidemiology, Moscow, Russia; Moscow State University for Medicine and Dentistry; Meningococcal Reference Unit, Manchester, UK.

The number of genome copies was determined, using Taqman with primers to *ctrA* gene, in CSF samples from 36 patients with meningococcal meningitis and compared to the clinical presentation and laboratory characteristics of disease. The number of copies varied from 1/µl (detection limit) to $7.3*10^6$ /µl (median value = $1.1*10^4$ /µl) and correlated significantly with a) outcome of disease (two non-survivors had the greatest number of copies); b) severity of disease; c) altered level of consciousness and duration of meningeal syndrome; d) pathological CSF findings (low glucose and high protein levels, high proportion of neutrophils). Interestingly, there was a negative correlation between the number of copies and such signs of systemic inflammatory response as high temperature, tachycardia and hypotension on admission. Higher number of copies was associated with positive latex agglutination tests but not with positive culture. The number of copies did not depend on the serogroup of meningococcus and pre-hospital antibiotic treatment. Taken together, these data demonstrate that quantitative estimates of the amount of meningococcal DNA in the CSF sample may serve as a prognostic factor and correlate with the severity of meningitis.

#334

REDUCTION IN MORTALITY FROM INVASIVE MENINGOCOCCAL DISEASE IN THE INTENSIVE CARE UNIT: DEVELOPMENT OF AN EMERGENCY TREATMENT ALGORITHM

Pollard AJ, Mehta N, Coen P, Nadel S, Galassini R, Morrison A, Britto J, Habibi P, Booy R, Levin M. Dept. of Paediatrics, St Mary's Hospital, London, UK

Objective: To outline the changes in case fatality rate from meningococcal disease (MD) in a specialist paediatric intensive care unit and describe the management plan that has been developed for such patients.
Background: The case fatality rate for children with MD is 10% overall, rising to 20-50% for those with shock. In 1992 we developed a specialist paediatric intensive care unit (PICU), linked to a research group, concentrating on children with life-threatening infection, using mobile intensive care to transfer these children from local hospitals. We also disseminated information about the disease to local secondary healthcare providers through lectures and telephone advice and, in 1998, developed an algorithm for the early management of children with MD that described our practice. This algorithm was distributed to emergency staff, intensivists and paediatricians throughout the UK.
Design: 543 admissions to PICU with MD from 1992-9 were studied. Data on severity of disease was collected via the Pediatric Risk of Mortality score (PRISM). Logistic regression analysis was used to correct for clinical severity with death as the outcome and year of admission as the primary exposure. The algorithm was developed as a consensus amongst our intensive care staff based on evidence wherever possible as described previously (Pollard *et al*, Arch Dis. Child. 1999).
Results: The case fatality rate fell from 24% in 1992-3 (predicted mortality 31%, n=45) to 12% in 1994-6 (predicted 27%, n=177) and to 4% in 1997-9 (predicted 28%, n=321). The overall improvement in odds of death from disease was 50.5% per year (95% CI 39 to 66%; $p<0.001$).
Conclusions: We have observed very low case fatality rates from MD amongst children with an expected high mortality in our PICU. However, we cannot attribute these findings solely to specialist intensive care but believe that by directing the initial management by local healthcare providers through telephone advice and education we have been able to have an unprecedented impact on the fatality from the disease in our region. We have developed emergency treatment guidelines based on our experience of managing >500 patients with the disease and the revised 2000 edition of the algorithm is presented here.

#335

IDENTIFICATION OF A HIGHLY CONSERVED PROTEIN MARKER AND ITS USE IN THE DEVELOPMENT OF A RAPID IMMUNOASSAY FOR *NEISSERIA GONORRHOEAE* (GC)

Svanas G[1], Brien J[1], Hopkins D[1], Knaus R[1], Maynard J[1], Keith A[1], Boisen M[1], Frisch L[1], Etoh T[2], Judd R[3]. 1BioStar, Inc., Boulder, CO, [2]Asahi Chemical Company, Fuji City, Japan and [3]Univ. of Montana, Missoula, MT

Previous GC immunoassays have focused on the detection of porin proteins in endocervical specimens. The inter-strain variability of this protein led to the necessity of using mixtures of monoclonal antibodies, often paired with polyclonal antibodies. Inevitably, balancing the antibody mixture proved to be very difficult.

A new GC assay target protein, the ribosomal protein L7/L12, avoids these problems encountered with the porin protein-based assays. A single pair of complementary monoclonal antibodies produced against recombinant L7/L12 protein were used to establish a prototype immunoassay in the BioStar® Optical Immunoassay (OIA®) format. The assay can be performed in 22 minutes using either female endocervical swab or male urine as the sample matrix.

Analytically, the limit of detection of the assay for the recombinant protein from GC was found to be approximately 3 pg protein/assay. The corresponding limit of detection for intact GC cells was approximately 350 GC cells/assay. A preliminary clinical study was performed on symptomatic male urine samples. Two milliliters of urine were concentrated in a prototype urine filtration device (UFD) and then assayed using the new kit, with the results compared to amplified probe testing. Based on a very limited set of samples, the preliminary sensitivity was 18/22 (82%), and specificity was 37/37 (100%).
Conclusion: A new protein target for GC immunoassay has been identified that simplifies immunoassay design and offers the opportunity for rapid diagnosis of GC infections in men and women.

INDEX OF AUTHORS

**asterisk indicates principal author*

INDEX OF AUTHORS

INDEX OF AUTHORS

INDEX OF AUTHORS

INDEX OF AUTHORS

INDEX OF AUTHORS

INDEX OF AUTHORS