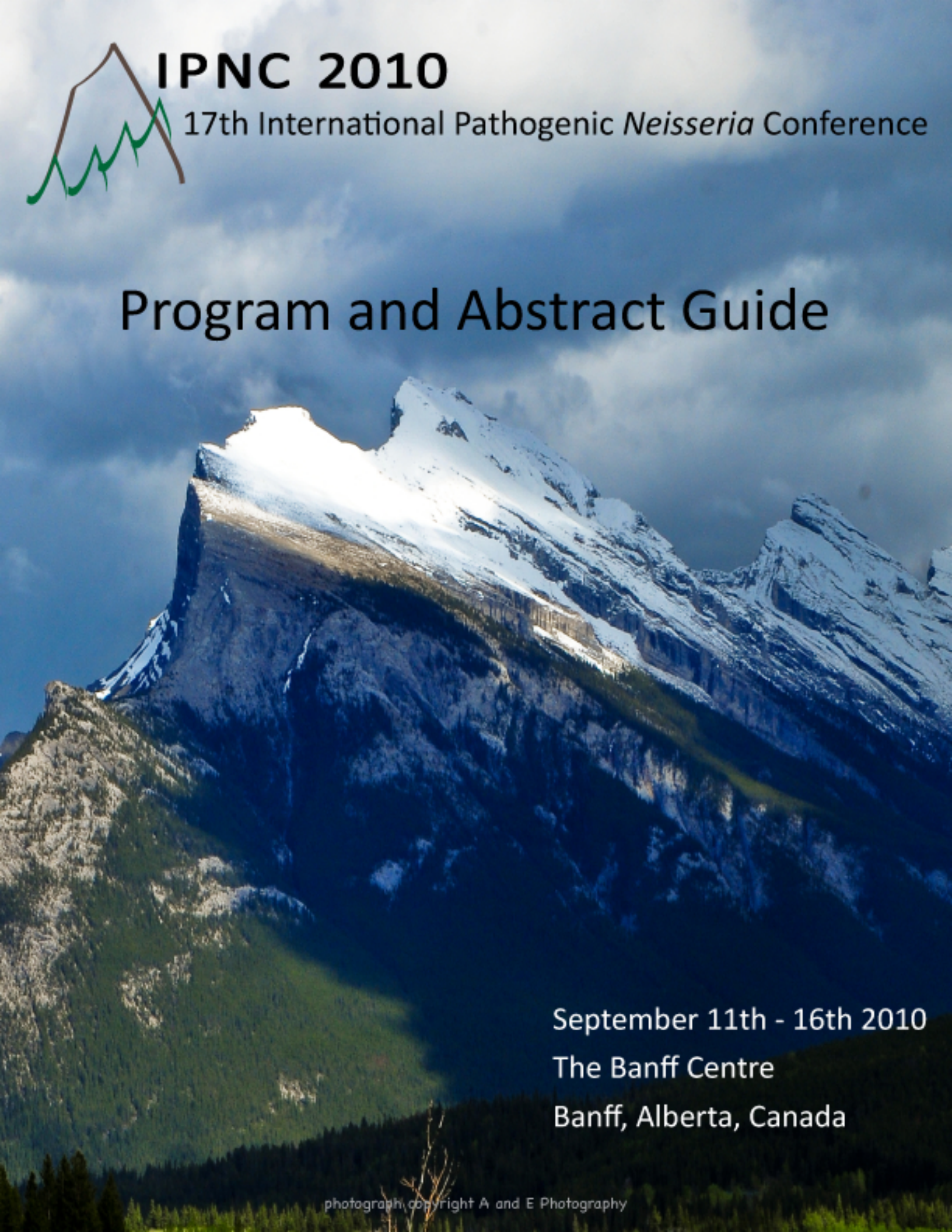


IPNC 2010

17th International Pathogenic *Neisseria* Conference

Program and Abstract Guide

September 11th - 16th 2010

The Banff Centre

Banff, Alberta, Canada

CONTENTS

THE HISTORY OF THE INTERNATIONAL PATHOGENIC *NEISSERIA* CONFERENCE

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, however the first official International Pathogenic *Neisseria* Conference was held in San Francisco, California in 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. Danielsson.

Third International Pathogenic *Neisseria* Conference 1982
Montreal, Canada
Chair: I.WDeVoe

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 Mountain, 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.I. Conde-Glez.

Ninth International Pathogenic *Neisseria* Conference 1994
Winchester, England
Chairs: M.C.J. Maiden and I Feavers.

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

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

Twelfth International Pathogenic *Neisseria* Conference 2000
Galveston, Texas, USA
Chairs: F. Sparling and P. Rice.

Thirteenth International Pathogenic *Neisseria* Conference 2002
Oslo, Norway
Chair: E. Wedege

Fourteenth International Pathogenic *Neisseria* Conference 2004
Milwaukee, Wisconsin, USA
Chairs: M.A. Apicella and H. Seifert

Fifteenth International Pathogenic *Neisseria* Conference 2006
Cairns, North Queensland, Australia
Chairs: J. Davies and M. Jennings

Sixteenth International Pathogenic *Nesseria* Conference 2008
Rotterdam, The Netherlands
Chairs: L. van Alphen, P. van der Lay and G. van den Dobbelsteen

Seventeenth International Pathogenic *Neisseria* Conference 2010
Banff, Alberta, Canada
Chairs: Dr. Tony Schryvers and Dr. Scott Gray-Owen

IPNC 2010
International Pathogenic Neisseria Conference

A warm welcome to The 17th International Pathogenic *Neisseria* Conference in scenic Banff, Alberta, Canada, the heart of the Canadian Rockies! We hope that in addition to participating in the scientific sessions, you take the opportunity to explore the scenery in this UNESCO World Heritage Site and enjoy the charm of this small mountain community. The secluded, yet accessible venue at the Banff Centre is an ideal setting for exchanging ideas during the scientific sessions and during the buffet meals with a panoramic view of the mountain valley. The selected afternoon breaks will provide the opportunity to continue discussions with your colleagues while exploring nature's wonders. The IPNC covers a broad range of topics ranging from Neisserial genetics, surface structures, and host-pathogen interactions, to epidemiology and vaccine development. We hope that the program will enable you to satisfy your appetite for the latest developments in the field and carry on discussions while taking in the awe-inspiring surroundings.

We would like to thank the Local and Scientific Organizing Committees and greatly appreciate the contributions of those who contributed to reviewing abstracts and contributing to the scientific program.

This meeting would not have been possible without generous sponsorship from Alberta Innovates-Health Solutions (Albert Heritage Foundation for Medical Research), the Canadian Institutes of Health Research, the National Institutes of Health, GlaxoSmithKline, Pfizer, Novartis, and Sanofi Pasteur.

Enjoy the conference!

Tony Schryvers
Scott Gray-Owen
Co-Chairs, IPNC 2010 Banff

IPNC 2010 CONFERENCE ORGANIZERS

SCIENTIFIC ORGANIZING COMMITTEE

Dr. Tony Schryvers
(University of Calgary, Canada)
Dr. Scott Gray-Owen
(University of Toronto, Canada)
Dr. Lee Wetzler
(Boston University School of Medicine, United States of America)
Dr. Caroline Genco
(Boston University School of Medicine, United States of America)
Dr. Rolando Pajon-Feyt
(Children's Hospital Oakland Research Institute, United States of America)

LOCAL ORGANIZING COMMITTEE

Dr. Tony Schryvers
(University of Calgary, Canada)
Dr. Jaime Kaufman
(University of Calgary, Canada)
Ms. Julia Trangeled
(University of Calgary, Canada)

IPNC 2010 ABSTRACT REVIEW COMMITTEES

Trainee Travel Awards

Caroline Genco (Boston University School of Medicine, United States of America)
Scott Gray-Owen (University of Toronto, Canada)
Jaime Kaufman (University of Calgary, Canada)
Tony Schryvers (University of Calgary, Canada)
Lee Wetzler (Boston University School of Medicine, United States of America)

Antibiotic Resistance

Chair: **Jo-Anne Dillon** (University of Saskatchewan, Canada)
Adele Benzaken (Alfredo da Matta Foundation, Brazil)
Cathy Ison (Heath Protection Agency Centre for Infections, United Kingdom)
David Lewis (STI Reference Centre, South Africa)
Mingmin Liao (University of Saskatchewan, Canada)
Shu-ichi Nakayama (National Institute of Infectious Diseases, Japan)
Lai-King Ng (World Health Organization, Switzerland)
David Trees (Centers for Disease Control, United States of America)
Magnus Unemo (Örebro University Hospital, Örebro, Sweden)

Cellular Microbiology

Chair: **Jennifer Edwards** (Research Institute @ Nationwide Children's/Ohio State University, United States of America)
Alison Criss (University of Virginia, United States of America)
Paola Massari (Boston University, United States of America)
Thomas Rudel (University of Würzburg, Germany)
Mumtaz Virji (University of Bristol, United Kingdom)

Epidemiology

Chair: **Julie Bettinger** (Vaccine Evaluation Center, BC Children's Hospital, Canada)
Dominique Caugant (Norwegian Institute of Public Health, Norway)
Lee Harrison (University of Pittsburgh, United States of America)
Keswadee Lapphra (Vaccine Evaluation Center, BC Children's Hospital, Canada)
Martin Maiden (University of Oxford, United Kingdom)
Raymond Tsang (National Microbiology Laboratory, Canada)

Genetics, Physiology, and Metabolism

Chair: **Daniel Stein** (University of Maryland, United States of America)
Jeff Cole (University of Birmingham, United Kingdom)
Stuart Hill (Northern Illinois University, United States of America)
Chris Tang (Imperial College, United Kingdom)
Tone Tonjum (University of Oslo, Norway)

Host Response, Immunology, and Experimental Therapy

Chair: **Lee Wetzler** (Boston University School of Medicine, United States of America)
Dlawer (Del) Ala'Aldeen (University of Nottingham, United Kingdom)
Ann Jerse (Uniformed Services University, United States of America)

Host and Pathogen Genomics and Gene Expression

Chair: **Caroline Genco** (Boston University School of Medicine, United States of America)
Ginny Clark (University of Rochester, United States of America)
Matthias Frosch (University of Wuerzburg, Germany)
Nigel Saunders (University of Oxford, United Kingdom)

Surface Structures

Chairs: **Joanne Lemieux** (University of Alberta, Canada) and **Trevor Moraes** (University of Toronto, Canada)
Andrew Cox (National Research Council, Canada)

Pre-Clinical Vaccinology

Chair: **Rolando Pajon-Feyt** (Children's Hospital Oakland Research Institute, United States of America)
Ian Feavers (National Institute for Biological Standards and Control, United Kingdom)
Dan Granoff (Children's Hospital Oakland Research Institute, United States of America)
Andrew Pollard (University of Oxford, United Kingdom)

Clinical Vaccinology

Chair: **Andrew Gorringe** (Health Protection Agency, United Kingdom)
Einar Rosenqvist (Norwegian Institute of public Health, Norway)
David Scheifele (University of British Columbia, Canada)
Wendell Zollinger (Walter Reed Army Institute of Research, United States of America)

Vaccine Discussion Panel

Mike Apicella (University of Iowa, United States of America)
Ian Feavers (National Institute for Biological Standards and Control, United Kingdom)
Bob Hancock (University of British Columbia, Canada)
John Heckels (University of Southampton, United Kingdom)
Marc LaForce (PATH, United States of America)
Dave Scheifele (University of British Columbia, Canada)
Lee Wetzler (Boston University School of Medicine, United States of America)

Igor Stojiljkovic Memorial Award

Igor Stojiljkovic, (MD, PhD), a dear friend and colleague to many, passed away on October 10,2003 after a two year heroic battle with brain cancer. Igor is remembered fondly as one of the bright young stars in *Neisseria* research. In memory of Igor, a memorial scholarship fund through the Emory University School of Medicine was initiated. This fund is used exclusively to provide travel funds to young trainees (graduate students, post-doctoral fellows and medical fellows in training) so that they can attend bacterial pathogenesis meetings such as the International Pathogenic *Neisseria* Conference (IPNC).

IPNC 2010 would like to thank the following for their generous contributions:

PLATINUM SPONSORS

GlaxoSmithKline
Pfizer

GOLD SPONSORS

Novartis
Sanofi Pasteur
Canadian Institutes for Health Research
National Institutes of Health
Alberta Innovates-Health Solutions

Invited Speakers Funded By:

Keynote Presentation - Dr. Robert Hancock

Bob Hancock is a Professor of Microbiology & Immunology at UBC and is a Canada Research Chair. He was the founding Scientific Director of the Canadian Bacterial Diseases Network and currently heads the UBC Centre for Microbial Diseases and Immunity Research. His research interests include the investigation of small cationic peptides from nature as antimicrobials and modulators of innate immunity, the development of novel treatments for antibiotic resistant infections based on these templates, the systems biology of innate immunity and the bacterial pathogen *Pseudomonas aeruginosa*, and antibiotic uptake and resistance. He has published more than 500 papers and reviews, is an ISI highly cited author in Microbiology with more than 22,000 citations, and has 36 patents awarded. He has won many awards, including the Aventis Pharmaceuticals Award in 2003 and Canada's three top prizes for Health Research. In 2001 he was inducted as an Officer of the Order of Canada. He is co-Founder of Inimex Pharmaceuticals Inc, BioWest Therapeutic Inc, and the Centre for Drug Research and Development, and has served as a Scientific Advisory Board Member or consultant with 27 Biotech and Pharmaceutical Companies. Bob Hancock is a participant on two large multinational Grand Challenges in Global Health Research grants aimed at delivering antimicrobial therapies and effective vaccines to developing countries.

Invited Speaker - Dr. Natalie Strynadka

Dr. Strynadka uses x-ray crystallography and other biophysical analysis tools to study the structure and function of proteins that play key roles in antibiotic resistance and bacterial pathogenicity. Her group specifically targets bacterial membrane assemblies involved in protein transport and cell-wall synthesis. She is currently a professor in the Department of Biochemistry and Molecular Biology at the University of British Columbia in Vancouver, Canada. She has been named a Medical Research Council of Canada Scholar, a Canadian Institute of Health Research Scientist, a Burroughs Wellcome New Investigator in the Pharmacological Sciences, a Michael Smith Foundation for Health Research Senior Scholar, and a UBC Distinguished University Scholar. She has also received the Merck Frosst Prize, the UBC Killiam Research Prize, a Killam National Fellowship, the Steacie Prize and is a multiple awardee of the HHMI International Scholar program. She is a fellow of the Royal Society of Canada.

Invited Speaker - Dr. Michael Surette

Dr. Surette is a professor in the Departments of Microbiology and Infectious Diseases and Biochemistry and Molecular Biology in the Faculty of Medicine at the University of Calgary. His research focuses on how bacteria respond to their environment and regulate gene expression. For most bacteria it is other microbes that dominate their environment. For pathogens, these other microbes are usually the normal microbiota of the human host (the human microbiome). The research in Dr. Surette's lab investigates the role of normal microbiota -pathogen interactions in health and disease in the area of respiratory infections. The lab has primarily focused on chronic airway infections in cystic fibrosis (CF). A re-examination of the microbiology of CF airways from polymicrobial perspective on these infections has lead to identification of overlooked pathogens in airway disease such as the *Streptococcus milleri* group. In addition, work with animal models has identified bacteria considered part of the normal microbiota of the upper airways that act synergistically with traditional airway pathogens to accelerate disease. These ***synergens*** may be avirulent or even beneficial on their own and consequently easily overlooked as contributors to airway disease. More recent studies in the lab indicate that the findings in CF airway disease extend to other respiratory infections. Understanding these infections and improved treatment requires a comprehensive understanding of the underlying polymicrobial infections. The lab continues to examine the nature of microbe-microbe interactions as well as to examine the composition of the human microbiome and airway infection using culture-dependent and culture-independent techniques. Dr. Surette holds a Canada Research Chair in Bacterial Gene Expression and is an Alberta Heritage Foundation for Medical Research Scientist.

Invited Speaker - Dr. Brian Coombes

Dr. Brian Coombes is an Assistant Professor and Associate Chair in the Department of Biochemistry and Biomedical Sciences at McMaster University in Hamilton, Ontario. Since 2006, he has led an independent research laboratory that has made groundbreaking discoveries in the areas of host-pathogen evolution, including the mechanisms of genome architecture, regulation, host selection and immunity, and on fundamental mechanisms of bacterial secretion systems, which are widespread colonization factors in the bacterial world. Dr. Coombes has received 5 career awards, including the CIHR New Investigator Award, the Early Researcher Award from the Ontario Ministry of Research and Innovation, the Most Promising Scientist Award from the Public Health Agency of Canada, the Young Investigator Award from the American Society for Microbiology and the 2010 Boehringer Ingelheim Young Investigator Award in the Biological Sciences. His research is supported by grants from the Canadian Institutes of Health Research, the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation and the Crohn's and Colitis Foundation of Canada.

Invited Speaker - Dr. Paul Kubes

Dr. Paul Kubes began his research as a graduate student at Queen's University in 1984 in the area of Cardiovascular Physiology. In 1988 he moved to Louisiana State University (LSU) Medical Center to start a post-doctoral fellowship trying to understand why there is excessive inflammation associated with heart attacks and strokes. Dr. Kubes took a position as Assistant Professor in 1991 at the University of Calgary and continued to investigate the mechanisms leading to white cell recruitment in cardiovascular disorders. Dr. Kubes and his team identified that an endogenously produced gas, nitric oxide, functions to reduce leukocyte recruitment. This work has subsequently branched out into areas of infection and autoimmunity and he was one of the first to use inhaled NO as a potential therapy. Dr Kubes was appointed as the Snyder Research Chair in Critical Care Medicine and has used the money to develop and operate a translational laboratory that supports critical care clinical trials with molecular lab tests. In collaboration with a number of critical care physicians, numerous discoveries about the biology of the immune system in sepsis have been published in Nature Medicine, Nature Immunology, Immunity and Journal of Experimental Medicine. More recently his focus presently is imaging the host responses to infections with particular interest in how the immune system deals with pathogens in blood or Intravascular Immunity.

Dr. Kubes is presently a full professor at the University of Calgary's Faculty of Medicine. In addition to this, he is also an AHFMR scientist, a Canada Research Chair, and the founding Director of the new Institute of Infection, Immunity and Inflammation. Together with a group of nine other professors at the University of Calgary, and with the help of a substantial grant from the Canadian Institutes of Health Research, he has also created a training program geared towards elucidating the cellular, molecular, and physiologic mechanisms of infectious and immune disease and a clinical program entitled the AHFMR Alberta Sepsis Network. Dr. Kubes sits on national and international grant panels and presently sits on numerous editorial boards including *The Journal of Clinical Investigation and Journal of Experimental Medicine.*

INFORMATION

Registration

Max Bell Building, Central Foyer (see map)

Saturday, September 11, 2010
2:00 – 8:00 PM
This is the primary registration day and everyone is encouraged to register on this day if possible

Sunday, September 12, 2010
8:00 AM – 12:30 PM

Monday, September 13, 2010
8:00 AM – 12:00 PM

Tuesday, September 14, 2010
8:00 AM – 12:00 PM

The Registration Desk is where you will receive the following important items:

- Name Tag – **PLEASE NOTE THAT YOU WILL NEED TO WEAR YOUR NAME TAG TO GET INTO THE SESSIONS, SOCIAL FUNCTIONS AND FOR ALL THE MEALS. THIS IS ALSO FOR SECURITY PURPOSES.**

- Your banquet and drink tickets. These will be placed in your name tag – PLEASE CHECK THAT YOU HAVE THEM. Drink tickets can be used at the Welcome Reception or at any of the poster sessions throughout the week. A cash bar will be available for additional beverages.

- Conference/Abstract Book – This will contain all the information about the conference, including venue, floor plans, schedule for the conference, abstracts and poster information.

- Portfolio – A zippered portfolio with writing pad/pen and space for your conference/ abstract book and other papers.

STUDENTS SHOULD REMEMBER TO BRING THEIR STUDENT ID TO THE REGISTRATION DESK.

Posters

Set-up

Saturday, September 11, 2010

2:00 – 10:00 PM

The Kinnear Centre, KC 101, KC 201, KC 203 (see map)

Take-down

Thursday, September 16, 2010

7:00 AM - 12:00 PM latest

General poster information

All scientific posters will be displayed in **The Kinnear Centre** for Creation and Innovation (KCCI) Building at The Banff Centre. Delegates will have the opportunity to view posters at their leisure on Sunday evening following the Keynote address, during all breaks and meal times, as well as during the formal poster sessions on Monday-Wednesday evenings from 8:00-10:00pm.

Poster Discussion Session

Note: All posters must be removed by presenting authors by noon on Thursday, September 16th, 2010. There is no guarantee that uncollected posters will be available following this time.

During the Poster Session, presenters will be assigned as follows:
Sunday, September 12, 2010 – 8:00-10:00pm **Poster Viewing** (Cash Bar)
Monday, September 13, 2010 – 8:00-10:00pm **Odd-numbered posters** (Cash Bar)
Tuesday, September 14, 2010 – 8:00-10:00pm **Even-numbered posters** (Cash Bar)
Wednesday, September 15, 2010 – 8:00-10:00pm **All posters-casual** (Cash Bar)

This format will allow presenting authors to take part in poster sessions for other presenting authors within their same topic area. Your poster number will determine which day you will be required to be standing next to your poster for presentation and informal discussions.

Posters are expected to be on display in their designated rooms from Saturday, September 11th to the end of the poster session on Wednesday, September 15th.

Social Program

Saturday, September 11, 2010
WELCOME RECEPTION
7:00 – 10:00 PM
The Kinnear Centre, KC 303 + KC 300 Galleria
Food and refreshments will be served and a cash bar will be available
Buses will be running constantly from 1:30 – 10:30 PM from Inns of Banff and Hidden Ridge Resort to and from The Banff Centre

Thursday, September 16, 2010
BANQUET
7:00 – 11:00 pm
Brewsters MountView Barbeque, Banff
6:00 – 6.45 PM – Buses pick up guests at The Banff Centre, Inns of Banff and Hidden Ridge Resort
7:00 PM – Welcome, entertainment, snacks (outdoors, weather permitting)
7:30 PM – Dinner and live music, followed by dancing
This is a unique Canadian western experience, just minutes from downtown Banff
Dress: Casual or western wear
Admission: Banquet ticket
Additional tickets: CAD 100 - To be pre-purchased by Monday, September 13 at 12 noon.

Free Time

Monday, September 13, 2010
1:30 – 4:00 PM
Lunch served from 12:30 – 1:30PM

Tuesday, September 14, 2010
12:30 – 7:00 PM
Lunch and dinner at leisure

Wednesday, September 15, 2010
1:30 – 4:00 PM
Lunch served from 12:30 – 1:30PM

Optional sightseeing tours – Books tours online from the IPNC website or in person at the Discover Banff desk situated at the IPNC Registration Desk, Sat and Sun.

Local Hiking/Walking

Banff boasts more hiking trails than any other mountain national park in the world. There are too many to mention, but you may want to check out the following hike which is within easy walking distance of the venue.

Tunnel Mountain (St. Julian Rd to Tunnel Mountain Summit – 2.3 km, 300 m elevation)
This is a short, relatively easy hike from just behind The Banff Centre. Excellent views at the top looking over the town of Banff and the Bow Valley. One of the oldest trails in the park.
You should also visit the **Bow Falls** nearby and **Cave and Basin** Centennial Centre, the birthplace of Canada's oldest national park, a National Historic Site with boardwalk interpretive trails, cycle and walking trails. The **Hoodoos**, on Tunnel Mountain Road, is well worth a visit and has great views.

Check out the IPNC 2010 website for links to hiking trails and maps, or visit the Banff Visitor Centre, 317 Banff Ave. Tel. 403 762 8421.

Meals

All meals will be held in the Vistas Dining Room, Sally Borden Building (see map)

Sunday, September 12, 2010
Breakfast: 7:00 – 8:45 AM
Lunch: 12:30 – 1:30 PM
Dinner: 5:00 – 7:00 PM

Monday, September 13, 2010
Breakfast: 7:00 – 9:00 AM
Lunch: 12:30 – 1:30 PM
Dinner: 5::30 – 7:00 PM

Tuesday, September 14, 2010
Breakfast: 7:00 – 9:00 AM

Wednesday, September 15, 2010
Breakfast: 7:00 – 9:00 AM
Lunch: 12:30 – 1:30 PM
Dinner: 5:30 – 7:00 PM

Thursday, September 16, 2010
Breakfast: 7:00 – 9:00 AM
Lunch: 11:30 – 1:30 PM

Friday, September 17, 2010
Breakfast: 7:00 – 9:30 AM

Buses

Buses will run between Inns of Banff, Hidden Ridge Resort and The Banff Centre on the following dates/times:

Saturday, Sep. 11
1.30 – 10.30 pm

Monday, Sep 13
6.30 – 9.30 am
1.00 – 4.00 pm
8.00 – 11.00 pm

Wed. Sep. 15
6.30 – 9.30 am
1.00 – 4.00 pm
8.00 – 11.00 pm

Fri. Sep. 17
6.30 – 9.30 am

Sunday, Sep. 12
6.30 – 9.30 am
8.00 – 11.00 pm

Tuesday, Sep. 14
6.30 – 9.30 am
12.00 – 3.00 pm
6.00 – 10.30 pm

Thu. Sep 16
6.30 – 9.30 pm
3.30 – 6.30 pm

General Information

The Banff Centre

The Banff Centre is located on St-Julien Road, a gradual climb up the side of Tunnel Mountain. The Banff Centre is located on the east side of Banff, four blocks from Banff Avenue. The town can be accessed by foot three ways from the Centre grounds: St-Julien Road, Buffalo Street, and the Ken Madsen Path to town. It is approximately a 10 minute walk downhill to town. The return trip is longer as it is uphill.

Life at The Banff Centre is informal; casual clothing is the norm. Come prepared to enjoy the many outdoor recreational opportunities available in Banff National Park, or indoors at The Centre's Sally Borden Recreation Facility. A variety of outdoor and recreational equipment is available on a rental basis through the Sally Borden Building or rental firms in downtown Banff.

Smoking

The Banff Centre is a smoke-free work environment. Smoking is permitted in specific locations only.

Parking

There is a car park next to the Professional Development Centre for daily parking and there is some parking available on the road outside the building. However, visitor parking at The Banff Centre is limited. Please obverse the No Parking signs. Vehicles outside of designated areas will be towed. Guests at The Banff Centre will receive complimentary parking in the parkade.

Fitness Facilities

Guests at The Banff Centre have access to the Sally Borden Fitness and Recreation centre, upon presentation of their room key. The facility includes a 25 metre swimming pool, saunas, whirlpool, exercise room, squash courts, indoor running track and on-site massage therapists.
Delegates staying at off-site accommodations may also use the facility upon request – please see the Front Desk in the Professional Development Centre.

Disabled Access

There are paths, roadways or ramps that provide access to and throughout most buildings. However, The Centre, built on the side of a mountain, does present challenges to those with limited mobility. On the sixth floor of Lloyd Hall, there is a phone equipped with an amplifier handset for the hearing impaired. Also, there are phones located throughout the campus that are wheelchair accessible.

Business Centre

The Business Centre, located at the front desk of the Professional Development Centre (PDC), handles requests for faxing, photocopying, and computer use. External and internal calls are fielded through the Centre’s switchboard here.

Banking

An ATM banking machine is located by the front desk of the Professional Development Centre (PDC), for cash withdrawals only.
In the Town of Banff there are several banks.

Messages

Conference delegates wishing to leave messages for colleagues, can leave a note on a notice board at the Registration Desk. It is the delegates' responsibility to check and pick up messages.

Contact :

The Banff Centre
107 Tunnel Mountain Drive
Box 1020
Banff, Alberta T1L 1H5
Telephone 403.762.6100
www.banffcentre.ca

Medical

There are several doctors and dentists in the town of Banff, and the hospital provides a 24-hour emergency service.

Banff National Park Pass

Please be advised that The Banff Centre is located within the Banff National Park. There is a Park Pass fee for staying within a national park and if you have not already stopped at the Banff East Gate on your way into Banff to buy a Park Pass, you can purchase one at the Banff Information Centre. If you are travelling and parking within the park, your vehicle may be checked for a Pass.

Car and Cycle Rental

Car rental is available in Banff.
Avis – 403 762 3622. Enterprise – 403 760 3599. Budget – 403 762 4565. Dollar – 403 760 3881. Hertz – 403 762 2027. Thifty – 403 760 3599

There are several places in Banff to rent bicycles, one of which is the Inns of Banff where some of our delegates are staying.

The Banff Centre Campus Map

Front Desk Reception

Check in for guests staying at The Banff Centre

Kinnear Centre

Welcome Reception

Evening Sessions

Posters

Sally Borden Building

Meals

Recreation Facilities

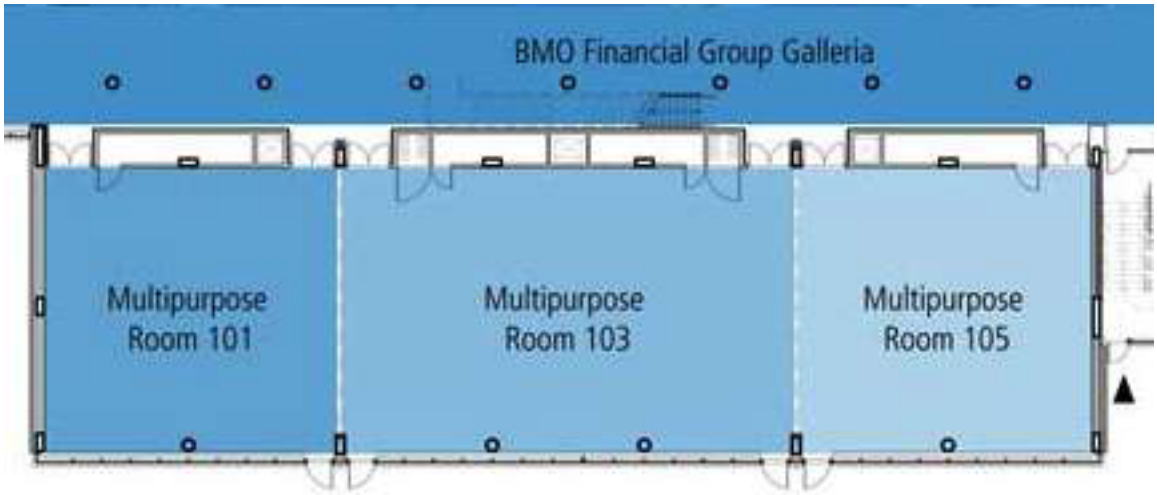
Max Bell Building

Conference Registration

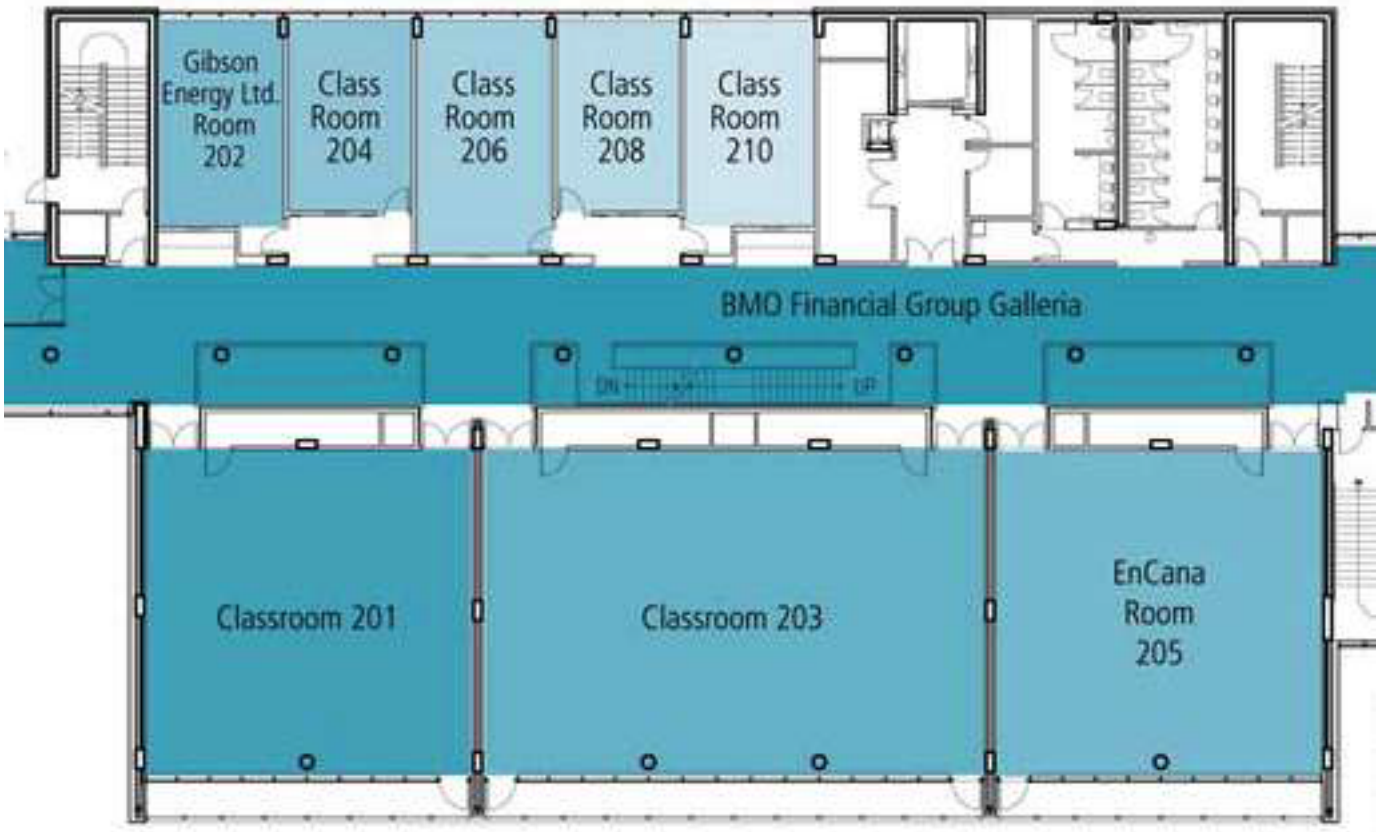
Day Sessions

Kinnear Centre Floor Plan

Ground Floor

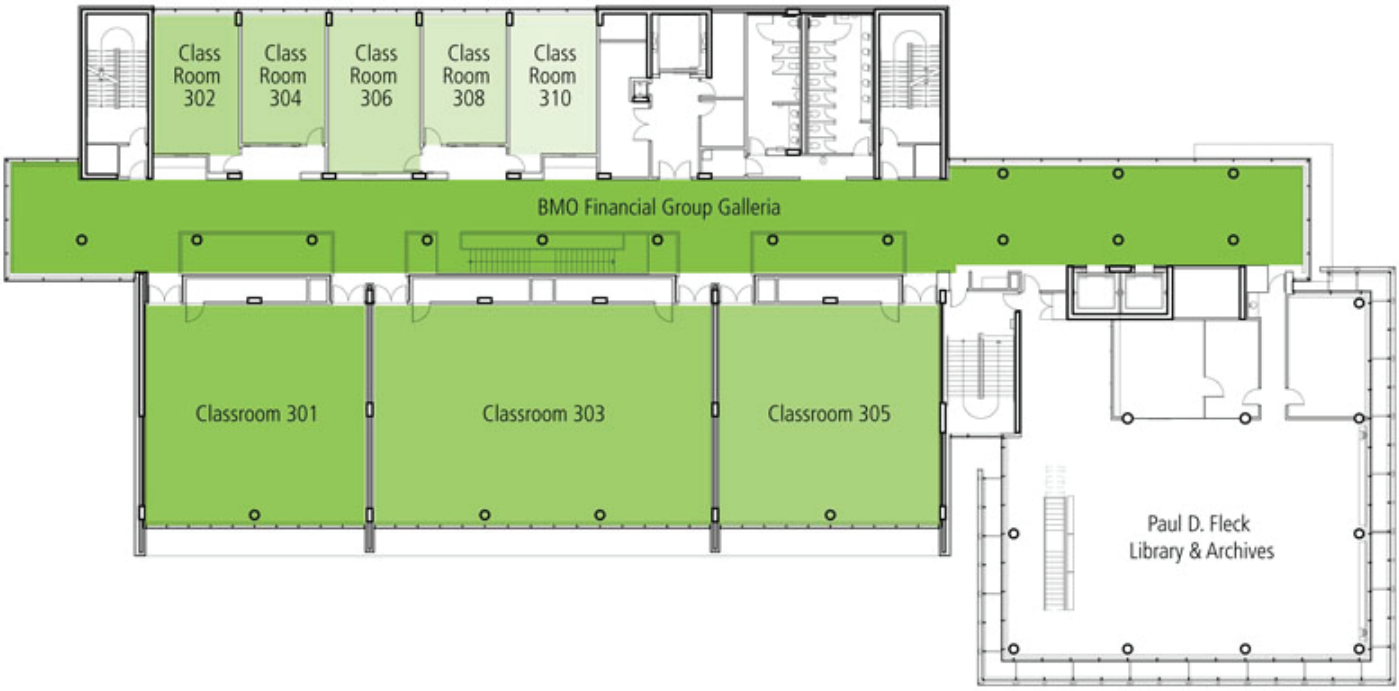


Second Floor

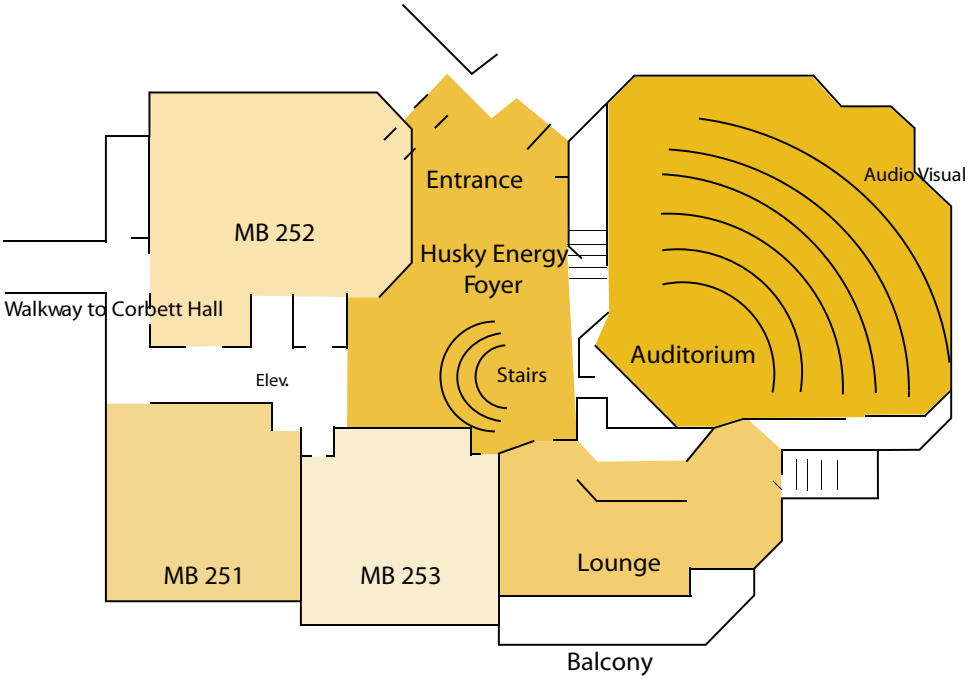


Kinnear Centre Floor Plan

Third Floor



Max Bell Building Floor Plan



PROGRAM OVERVIEW

Saturday, September 11, 2010

2:00-8:00pm	Registration	Max Bell Foyer
2:00-10:00pm	Poster Set-up	KC101/103, 201/203
7:00-10:00pm	*Welcome Reception*	*KC303 & 300 Galleria*

Sunday, September 12, 2010

7:00-8:45am	*Breakfast Buffet*	*Vistas Dining Room*
8:45-9:00am	Introductory Remarks	Max Bell Auditorium
9:00-12:20pm	Vaccine Workshop	Max Bell Auditorium
12:20-1:30pm	*Lunch Buffet*	*Vistas Dining Room*
1:30-4:40pm	Vaccine Workshop	Max Bell Auditorium
5:00-7:00pm	*Dinner Buffet*	*Vistas Dining Room*
7:00-8:00pm	Keynote Presentation	Max Bell Auditorium
8:00-10:00pm	Poster Viewing (cash bar)	KC101/103, 201/203

Monday, September 13, 2010

7:00-9:00am	*Breakfast Buffet*	*Vistas Dining Room*
9:00-12:00pm	Surface Structures	Max Bell Auditorium
12:20-1:30pm	*Lunch Buffet*	*Vistas Dining Room*
1:30-4:00pm	Free time	
4:00-5:30pm	Epidemiology	Max Bell Auditorium
5:30-7:00pm	*Dinner Buffet*	*Vistas Dining Room*
7:00-8:00pm	Parallel Sessions	KC205, 301, 303, 305
8:00-10:00pm	Poster Sessions (cash bar)	KC101/103, 201/203

Tuesday, September 14, 2010

7:00-9:00am	*Breakfast Buffet*	*Vistas Dining Room*
9:00 -12:20pm	Host Response, Immunology & Experimental Therapeutics	Max Bell Auditorium
12:20-7:00pm	Free Time	
7:00-8:00pm	Parallel Sessions	KC205, 301, 303, 305
8:00-10:00pm	Poster Sessions (cash bar)	KC101/103, 201/203

PROGRAM OVERVIEW

Wednesday, September 15, 2010

7:00-9:00am	*Breakfast Buffet*	*Vistas Dining Room*
9:00-11:00am	Host & Pathogen Genomics & Gene Expression	Max Bell Auditorium
11:20-12:20pm	Cellular Biology	Max Bell Auditorium
12:20-1:30pm	*Lunch Buffet*	*Vistas Dining Room*
1:30-4:00pm	Free time	
4:00-5:20pm	Cellular Biology	Max Bell Auditorium
5:30-7:00pm	*Dinner Buffet*	*Vistas Dining Room*
7:00-8:00pm	Parallel Sessions	KC205, 301, 303, 305
8:00-10:00pm	Poster Sessions Casual (cash bar)	KC101/103, 201/203

Thursday, September 16, 2010

7:00-9:00am	*Breakfast Buffet*	*Vistas Dining Room*
9:00-12:20pm	Genetics, Physiology & Metabolism	Max Bell Auditorium
12:20-1:30pm	*Lunch Buffet*	*Vistas Dining Room*
1:30-2:10pm	Antibiotic Resistance	Max Bell Auditorium
2:10-4:50pm	Vaccinology	Max Bell Auditorium
4:50-5:00pm	Closing Remarks	Max Bell Auditorium
7:00*pm-12:00am	*Banquet*	*MountView Barbeque*

*Buses will leave for MountView BBQ starting at 6:00pm

Friday, September 17, 2010

7:00-9:30am	*Breakfast Buffet*	*Vistas Dining Room*

PROGRAM SCHEDULE

Saturday, September 11, 2010

TIME	EVENT	LOCATION
2:00-10:00pm	Poster Set-up	KCCI Building (KC101/103; KC 201/203)
2:00-8:00pm	Registration*	Max Bell Foyer
7:00-10:00pm	WELCOME RECEPTION	KC303 and KC300 Galleria

***Registration will also be available on Sunday September 12th from 8:00am-1:30pm, and Monday September 13th and Tuesday September 14th from 8:00am-12:00pm**

Sunday, September 12, 2010

MAIN SESSION

TIME	EVENT	PRESENTER
7:00-8:45am	*BREAKFAST BUFFET*	
8:45-9:00am	Introductory Remarks	Tony Schryvers and Lee Wetzler
9:00-12:20pm	**VACCINE WORKSHOP**	**CHAIR: LEE WETZLER**
9:00-10:30am	***Factors to consider in developing meningococcal conjugate vaccines***	***GlaxoSmithKline***
9:00-9:05am	Welcome and Introduction	Dr. Mailman and Dr. Poolman
9:05-9:25am	Conjugate immunology including carrier protein related immune interferences	Dr. Poolman
9:25-9:45am	Immunological and safety aspects to consider for meningococcal conjugate vaccines	Dr. Miller
9:45-10:05am	The UK experience with meningococcal vaccines conjugated to different carrier proteins	Dr. Findlow
10:05-10:30am	Discussion and Q&A	Dr. Mailman and Dr. Poolman
10:30-11:00am	*COFFEE BREAK*	

Sunday, September 12, 2010

TIME	TITLE	PRESENTER
11:00-12:20pm	***Should prevention of meningococcal disease be a priority?***	***Pfizer***
11:00-11:05am	Introduction and Objectives	Dr. Andrew Pollard
11:05-11:20am	Meningococcal Epidemiology and Vaccine Experiences-Global and US	Dr. Lee Harrison
11:20-11:35am	Meningococcal Epidemiology and Vaccine Experiences-Canada	Dr. Julie Bettinger
11:35-11:50am	Meningococcal Epidemiology and Vaccine Experiences-Spain	Dr. Julio Vasquez
11:50-12:05pm	Meningococcal Epidemiology and Vaccine Experiences-UK	Dr. Andrew Pollard
12:05-12:20pm	Panel Discussion and Summary	Dr. Andrew Pollard
12:20-1:30pm	*LUNCH BUFFET*	
1:30-4:40pm	**VACCINE WORKSHOP**	**CHAIR: LEE WETZLER**
1:30-2:00pm	Early Clinical Development of a Novel, Multicomponent Meningococcal Serogroup B Vaccine (4CMenB)	Dr. Peter Dull
2:00-2:30pm	Meningococcal Biology and Vaccines: ten years of genomics and reverse vaccinology	Dr. Rino Rappuoli
2:30-2:50pm	Estimating effectiveness for *Neisseria meningitidis* serogroup B (MnB) vaccine candidates composed of non-serogroup specific antigens	Dr. Kathrin Jansen
2:50-3:10pm	Safety & immunogenicity of serogroup B *Neisseria meningitidis* (MnB) rLP2086 vaccine in adults and adolescent subjects: overview of 3 clinical trials	Dr. Peter Richmond
3:10-3:40pm	*COFFEE BREAK*	
3:40-4:40pm	Vaccine Panel Discussion	
5:00-7:00pm	*DINNER BUFFET*	
7:00-8:00pm	**Keynote Presentation: Systems Biology of Host Pathogen Interactions - Development of New Anti-Infective Strategies**	**Dr. Bob Hancock**
8:00-10:00pm	Poster Viewing (Cash Bar)	KC101/103; 201/203

Monday September 13, 2010
MAIN SESSION

TIME	TITLE	PRESENTER
7:00-9:00am	*BREAKFAST BUFFET*	
9:00-12:20pm	**SURFACE STRUCTURES**	**CHAIR: JOANNE LEMIEUX and TONE TONJUM**
9:00-9:40am	Targeting the glycosyltransferase and transpeptidase steps in peptidoglycan synthesis for structure-based antibiotic discovery	Natalie Strynadka
9:40-10:00am	Biochemical and biophysical analysis indicates conformation plays an important role in the binding of hfH and antibodies to the fHBP of *N.meningitidis*	Gary Zlotnick
10:00-10:20am	Twitching Motility by Scattered Pili Creates Distinct Motility Types	Jens Eriksson
10:20-10:40am	Accessibility to pili-linked phosphorylcholine of *N. meningitidis* is influence by phase-variation of the pili-linked glycan, and both factors are required for efficient cell association via the PAF receptor on human airway epithelial cells	Michael Jennings
10:40-11:00am	*COFFEE BREAK*	
11:00-11:20am	Structural insights into Transferrin Binding Protein B, TbpB	Trevor Moraes
11:20-11:40am	Mass Shift Perturbation for Structure/Conformation Analyses: Concepts and Applications)	Dave Schriemer
11:40-12:00pm	Expression of de-N-acetyl sialic acid containing polysialic acid in normal and diseased human peripheral blood and tissues	Lindsay Taylor Steirer
12:00-12:20pm	Structural insight into solute transport and immunological recognition of outer membrane protein PorB during Neisserial pathogenesis	Mikio Tanabe
12:20-1:30pm	*LUNCH BUFFET*	
1:30-4:00pm	*BREAK*	

Monday September 13, 2010

MAIN SESSION

TIME	TITLE	PRESENTER
4:00-5:20pm	**EPIDEMIOLOGY**	**CHAIR: JULIE BETTINGER**
4:00-4:20pm	Modeling the cost-effectiveness of new meningococcal vaccines in England	Hannah Christensen
4:20-4:40pm	Characterisation of *fhbp, nhba, nadA, porA* and sequence type in meningococcal group B case isolates collected in England and Wales in the epidemiological year 2007/2008, and the potential coverage of an investigational group B vaccine	Jamie Findlow
4:40-5:00pm	Interim Analysis of the Effectiveness of Quadrivalent Meningococcal Conjugate Vaccine (MenACWY-D): A Matched Case-Control Study	Amanda Cohn
5:00-5:20pm	Emergence of *Neisseria meningitidis* serogroup X meningitis in Togo and Burkina Faso before introduction of a monovalent meningococcal conjugate vaccine against serogroup A	Judith Mueller
5:20-7:00pm	*BUFFET DINNER*	
7:00-8:00pm	PARALLEL EVENING SESSIONS	KCCI Building
8:00-10:00pm	POSTER SESSION-SEE SCHEDULE	KC101/103; 201/203

PARALLEL EVENING SESSION 1-KC205

TIME	TITLE	PRESENTER
7:00-8:00pm	**EPIDEMIOLOGY**	**CHAIR: LEE HARRISON**
7:00-7:20pm	Baseline meningococcal carriage in Burkina Faso before introduction of a meningococcal serogroup A conjugate vaccine	Paul Kristiansen
7:20-7:40pm	Optimal molecular characterisation of meningococci in conjunction with enhanced molecular surveillance of the vaccine candidate, fHBP	Jay Lucidarme
7:40-8:00pm	Changes in the carriage of serogroup Y meningococci amongst university students in the United Kingdom	Dlawer Ala'Aldeen
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	ODD NUMBERED POSTERS PRESENT

Monday September 13, 2010

PARALLEL EVENING SESSION 2-KC301

TIME	TITLE	PRESENTER
7:00-8:00pm	**GENETICS, PHYSIOLOGY AND HOST METABOLISM**	**CHAIR: STUART HILL**
7:00-7:20pm	Complex network of interactions involved in base excision repair in *Neisseria meningitidis*	Krzysztofa Nagorska
7:20-7:40pm	The Gonococcal biofilm matrix contains DNA and an endogenous nuclease controls its incorporation	Michael Apicella
7:40-8:00pm	*In vitro* biofilm formation as a model for meningococcal carriage and transmission	Martin Lappann
8:00-10:00pm	POSTERS **KC101/103, 201/203**	ODD NUMBERED POSTERS PRESENT

PARALLEL EVENING SESSION 3-KC303

TIME	TITLE	PRESENTER
7:00-8:00pm	**HOST RESPONSE, IMMUNOLOGY, AND EXPERIMENTAL THERAPY**	**CHAIR: ROBIN INGALLS**
7:00-7:20pm	Alternative pathway activation on *Neisseria meningitidis* reveals paradoxically enhanced C3 deposition on encapsulated groups W-135 and Y strains	Sanjay Ram
7:20-7:40pm	Human airway epithelial cell responses to *N. lactamica* and to purified Nlac porin	Paola Massari
7:40-8:00pm	*N. lactamica* attenuates TLR-2 dependent epithelial inflammatory responses by reducing nuclear Nf-*kappa*B activity using PPAR-*gamma*	Keith Page
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	ODD NUMBERED POSTERS PRESENT

Monday September 13, 2010
PARALLEL EVENING SESSION 4-KC305

TIME	TITLE	PRESENTER
7:00-8:00pm	**SURFACE STRUCTURES**	**CHAIR: TREVOR MORAES**
7:00-7:20pm	*Neisseria meningitidis* NHBA, a heparin-binding protein that induces protective immunity in humans	Davide Serruto
7:20-7:40pm	Using lipo-oligosaccharide modification to generate a novel vaccine candidate to protect against serogroup B meningococcal disease	Hannah Jones
7:40-8:00pm	The Gonococcal Pilin Glycan Mediates Primary Cervical Epithelial Cell Challenge	Jennifer Edwards
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	ODD NUMBERED POSTERS PRESENT

Tuesday, September 14, 2010
MAIN SESSION

TIME	TITLE	PRESENTER
7:00-9:00am	*BREAKFAST BUFFET*	
9:00-12:20am	**HOST RESPONSE, IMMUNOLOGY AND EXPERIMENTAL THERAPEUTICS**	**CHAIR: DEL ALA'ALDEEN and ANN JERSE**
9:00-9:40am	The war between immunity and bacteria in blood vessels	Paul Kubes
9:40-10:00am	Naturally occurring Lipid A variants among meningococcal carriage and disease isolates	Peter van der Ley
10:00-10:20am	TLR2-dependent induction of pro-inflammatory cytokines and chemokines by *N. meningitidis* PorB	Lee Wetzler
10:20-10:40am	Detection of *Neisseria gonorrhoeae* by the cytosolic Nod receptors	Nikolaos Mavrogiorgos
10:40-11:00am	*COFFEE BREAK*	
11:00-11:20am	*Neisseria gonorrhoeae* exploits TGF-β to elicit innate host responses and suppress adaptive immunity in a murine model of genital tract infection	Michael Russell
11:20-11:40am	CEACAM-humanized mice a model for *N. meningitidis* nasopharyngeal colonization	Kay Johswich

Tuesday, September 14, 2010

MAIN SESSION

TIME	TITLE	PRESENTER
11:40-12:00pm	Enhanced bacteremia and decreased antibody passive protective activity in human factor H transgenic rats challenged with encapsulated strains of *Neisseria meningitides*	David Vu
12:00-12:20pm	Gonococcal infection is enhanced in mice transgenic for Human C4b-binding protein (C4BP)	Jutamas Shaughnessy
12:20-7:00pm	*BREAK*	
7:00-8:00pm	PARALLEL EVENING SESSIONS	
8:00-10:00PM	POSTER SESSION-SEE SCHEDULE	KC101/103; 201/203

PARALLEL EVENING SESSION 1-KC205

TIME	TITLE	PRESENTER
7:00-8:00pm	**SURFACE STRUCTURES**	**CHAIR: TONY SCHRYVERS**
7:00-7:20pm	Structural Studies of the Lactoferrin Binding Proteins from *Neisseria meningitidis*	Joanne Lemieux
7:20-7:40pm	*Neisseria gonorrhoeae* Type IV pili change structure under force	Nicolas Biais
7:40-8:00pm	The Role of Lipooligosaccharide in Triggering Invasion of *Neisseria meningitidis* into Epithelial Cells	Stephanie Bell
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	EVEN NUMBERED POSTERS PRESENT

PARALLEL EVENING SESSION 2-KC301

TIME	TITLE	PRESENTER
7:00-8:00pm	**HOST AND PATHOGEN GENOMICS AND GENE EXPRESSION**	**CHAIR: CAROLINE GENCO**
7:00-7:20pm	Differences in iron status and MtrA dominate stable physiological differences between gonococcal strains MS11 and FA1090	Nigel Saunders
7:20-7:40pm	Genome Sequencing Reveals Widespread Virulence Gene Exchange Among Human *Neisseria* Species	Nathan Weyand

Tuesday, September 14, 2010

PARALLEL EVENING SESSION 2-KC301

TIME	TITLE	PRESENTER
7:40-8:00pm	Pangenomic anatomy of a species: *Neisseria meningitidis* population structure and dynamics	Duccio Medini
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	EVEN NUMBERED POSTERS PRESENT

PARALLEL EVENING SESSION 3-KC303

TIME	TITLE	PRESENTER
7:00-8:00pm	**VACCINOLOGY**	**CHAIR: ANDREW POLLARD**
7:00-7:20pm	A critical threshold of meningococcal fHbp expression is required for increased breadth of protective antibodies elicited by native outer membrane vesicle (NOMV) vaccines prepared from mutants	Oliver Koeberling
7:20-7:40pm	Antibody persistence of group A meningococcal conjugate vaccine (MenAfriVac ^{TM}) in 1-29 years old subjects from Africa and India	Simonetta Viviani
7:40-8:00pm	Cooperative meningococcal serum bactericidal activity (SBA) between vaccine-induced human antibodies to factor H binding protein (fHbp) and *Neisseria* Heparin binding antigen (NHba)	Dan Granoff
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	EVEN NUMBERED POSTERS PRESENT

PARALLEL EVENING SESSION 4-KC305

TIME	TITLE	PRESENTER
7:00-8:00pm	**HOST RESPONSE, IMMUNOLOGY, AND EXPERIMENTAL THERAPY**	**CHAIR: SCOTT GRAY-OWEN**
7:00-7:20pm	Protective and Immunoregulatory Role of Toll-like Receptor 4 in Experimental Gonococcal Infection of Female Mice	Mathanraj Packiam
7:20-7:40pm	Gonococcal resistance to neutrophil clearance	Alison Criss

Tuesday, September 14, 2010
PARALLEL EVENING SESSION 4-KC305

TIME	TITLE	PRESENTER
7:40-8:00pm	Human Complement Computer Model (HCCM): An Agent Based System Implementation of the Classic, Alternative and MBL Complement Pathways	Carey Gingras
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	EVEN NUMBERED POSTERS PRESENT

Wednesday, September 15, 2010
MAIN SESSION

TIME	TITLE	PRESENTER
7:00-9:00am	BREAKFAST BUFFET	
9:00-11:00am	**HOST AND PATHOGEN GENOMICS AND GENE EXPRESSION**	**CHAIR: JOHN DAVIES**
9:00-9:40am	Revisiting respiratory infections: Lessons from the CF airway microbiome	Mike Surette
9:40-10:00am	Mechanisms of neisserial transformation and the evolution of sexual reproduction	Ole Ambur
10:00-10:20am	*Neisseria* population genomics: integrating whole genome data with multi locus approaches to epidemiology and population biology	Martin Maiden
10:20-10:40am	Differential genome expression of serogroup B meningococci under *in vivo* mimicking conditions	Christoph Schoen
10:40-11:00am	Analysis of Novel Fur-Mediated Global Regulatory Circuits in the Pathogenic *Neisseria*	Caroline Genco
11:00-11:20am	*COFFEE BREAK*	
11:20-12:20pm	**CELLULAR MICROBIOLOGY**	**CHAIR: JENNIFER EDWARDS**
11:20-11:40am	*Neisseria meningitidis* Hsf (NhhA) interacts directly with human vitronectin: the interplay between meningococcal Hsf and Opc in host cell adhesion and serum resistance	Mumtaz Virji
11:40-12:00pm	*Neisseria meningitidis* hijack the β2-adrenoceptor/β-arrestin pathway to open and traverse brain microvasculature endothelium	Mathieu Coureuil

Wednesday, September 15, 2010

MAIN SESSION

TIME	TITLE	PRESENTER
12:00-12:20pm	Gonococcal Cervical and Amniochorionic Infections as Potential Factors Contributing to Adverse Pregnancy Outcomes	Emily Butler
12:20-1:30pm	*LUNCH BUFFET*	
1:30-4:00pm	*BREAK*	
4:00-5:20pm	**CELLULAR MICROBIOLOGY**	**CHAIR: ALISON CRISS**
4:00-4:20pm	Decoy receptor CEACAM3 elicits Opa protein-dependent neutrophil responses and inflammation typical of symptomatic gonococcal infection in transgenic mice	Scott Gray-Owen
4:20-4:40pm	A conserved domain of *Neisseria meningitidis* TspB/Orf6 has Ig-binding activity	Maike Muller
4:40-5:00pm	Olfactory nerve - a novel invasion route of *Neisseria meningitidis* to reach the meninges	Hong Sjölinder
5:00-5:20pm	Cell-contact induced posttranslational modification of type IV pilin triggers *Neisseria meningitidis* propagation and dissemination	Guillaume Dumenil
5:30-7:00pm	*BUFFET DINNER*	
7:00-8:00pm	PARALLEL EVENING SESSIONS	
8:00-10:00PM	POSTER SESSION-SEE SCHEDULE	KC101/103; 201/203

PARALLEL EVENING SESSION 1-KC205

TIME	TITLE	PRESENTER
7:00-8:00pm	**CELLULAR MICROBIOLOGY**	**CHAIR: MUMTAZ VIRJI**
7:00-7:20pm	*Neisseria* adhere to the uropod of human polymorphonuclear cells, suggesting a novel pilus dependent pathway to reach subepithelium	Niklas Söderholm
7:20-7:40pm	*Neisseria meningitidis* induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8	Alexandra Schubert-Unkmeir
7:40-8:00pm	*Neisseria gonorrhoeae* induce transactivation of EGFR for their invasion	Karen Swanson
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	

Wednesday, September 15, 2010

PARALLEL EVENING SESSION 2-KC301

TIME	TITLE	PRESENTER
7:00-8:00pm	**ANTIBIOTIC RESISTANCE**	**CHAIR: MAGNUS UNEMO**
7:00-7:20pm	Impact of Multiple Antibiotic Resistance Mutations on *Neisseria gonorrhoeae* Growth and Fitness *in vivo* and Selection for Compensatory Mutations during Infection	Ann Jerse
7:20-7:40pm	Molecular Mechanism of MtrR Multidrug Recognition and Binding	Richard Brennan
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	

PARALLEL EVENING SESSION 3-KC303

TIME	TITLE	PRESENTER
7:00-8:00pm	**HOST RESPONSE, IMMUNOLOGY, AND EXPERIMENTAL THERAPY**	**CHAIR: SANJAY RAM**
7:00-7:20pm	Characterization of antibodies in human serum against group B *Neisseria meningitidis* that block complement-dependent bactericidal activity	Ray Dutta
7:20-7:40pm	*Neisseria meningitidis* type C polysaccharide mediated unresponsiveness of B cells to BAFF and APRIL is responsible for its weak immunogenicity	Mustafa Akkoyunlu
7:40-8:00pm	Human innate-like memory B cells potently respond to infection by pathogenic and commensal *Neisseria*	Nancy So
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	

PARALLEL EVENING SESSION 4-KC305

TIME	TITLE	PRESENTER
7:00-8:00pm	**EPIDEMIOLOGY**	**CHAIR: DOMINIQUE CAUGANT**
7:00-7:20pm	Novel Gonococcal Multilocus Sequencing Typing (MLST) Scheme Suitable for Short and Long-Term Molecular Epidemiology Studies	Sinisa Vidovic
7:20-7:40pm	Potential Impact of Serogroup B Vaccines: Prevalence of candidate vaccine antigens among invasive *Neisseria meningitidis* isolates in the United States	Amanda Cohn

Wednesday, September 15, 2010
PARALLEL EVENING SESSION 4-KC305

TIME	TITLE	PRESENTER
7:40-8:00pm	Factor H binding protein (fHbp) sequence variants among epidemic meningococcal strains from Africa: Implications for development of a broadly protective outer membrane vesicle (OMV) vaccine	Rolando Pajon Feyt
8:00-10:00pm	POSTERS **(KC101/103, 201/203)**	

Thursday, September 16, 2010
MAIN SESSION

TIME	TITLE	PRESENTER
7:00-9:00am	*BREAKFAST BUFFET*	
9:00-12:20am	**GENETICS, PHYSIOLOGY, AND METABOLISM**	**CHAIR: DAN STEIN and HANK SEIFERT**
9:00-9:40am	Adaptation of regulatory systems for bacterial pathogenesis	Brian Coombes
9:40-10:00am	The transcriptional regulator FarR contributes to divergent host niche adaptation by adopting different functions in *N. meningitidis* and *N. gonorrhoeae*	Oliver Kurzai
10:00-10:20am	The NmlR regulon and stress defense in *Neisseria gonorrhoeae*	Alastair McEwan
10:20-10:40am	Unraveling small RNA Mediated Post Transcriptional Control Mechanisms in the Pathogenic *Neisseria*	Ryan McClure
10:40-11:00am	*COFFEE BREAK*	
11:00-11:20am	*Neisseria gonorrhoeae* responses and defenses against reactive oxygen species	Hank Seifert
11:20-11:40am	Biochemical characterization of TraI, the relaxase involved in DNA secretion by *Neisseria gonorrhoeae*	Joseph Dillard
11:40-12:00pm	Probing the functions of O-linked protein glycosylation in *Neisseria gonorrhoeae*	Åshild Vik
12:00-12:20pm	Organization of the electron transfer pathways to oxygen and nitrite in the obligate human pathogen, *Neisseria gonorrhoeae*: how are electrons transferred to the outer membrane?	Amanda Hopper
12:20-1:30pm	*LUNCH BUFFET*	

Thursday, September 16, 2010
MAIN SESSION

TIME	TITLE	PRESENTER
1:30-2:10pm	**ANTIBIOTIC RESISTANCE**	**CHAIR: JO-ANNE DILLON**
1:30-1:50pm	Identification and Characterization of a Transcriptional Regulatory Circuit that Modulates Expression of the Mtr Efflux System in *Neisseria gonorrhoeae*	William Shafer
1:50-2:10pm	Molecular and structural analysis of gonococcal resistance to expanded-spectrum cephalosporins mediated by mosaic *penA* alleles: role of epistatic mutations	Robert Nicholas
2:10-4:50pm	**VACCINOLOGY**	**CHAIR: JOHN HECKELS and ANDREW GORRINGE**
2:10-2:30pm	Introduction of a new Group A meningococcal conjugate vaccine (MenAfriVac) in the African meningitis belt	Francois Marc LaForce
2:30-2:50pm	Optimisation of schedules for meningococcal serogroup C conjugate vaccines driven by new immunogenicity and epidemiological data	Ray Borrow
2:50-3:10pm	Age-Related Immune Responses Following *Neisseria meningitidis* serogroup C Conjugate Vaccination in the Netherlands: a pre- and post-vaccination survey	Fiona van der Klis
3:10-3:30pm	*COFFEE BREAK*	
3:30-3:50pm	Binding to complement factor H decreases protective serum antibody responses of human fH transgenic mice immunized with recombinant meningococcal factor H binding protein vaccine	Peter Beernink
3:50-4:10pm	Evaluation of the adjuvant effects of novel meningococcal detoxified lipopolysaccharide structures formulated in native outer membrane vesicles	Ojas Mehta
4:10-4:30pm	Using population biology to inform vaccine design	Carina Brehony
4:30-4:50pm	Immunoproteomics and pan-meningococcal vaccine antigen discovery	John Heckels
4:50-5:00pm	Closing Remarks	Tony Schryvers
7:00*-12:00am	BANQUET (buses will leave for BBQ at 6pm)	MountView BBQ

IPNC 2010
International Pathogenic Neisseria Conference

ABSTRACTS

VW01

Early Clinical Development of a Novel, Multicomponent Meningococcal Serogroup B Vaccine (4CMenB)

Peter M Dull, Mariagrazia Pizza, Daniela Toneatto, Lisa DeTora, Ellen Ypma, Annett Kleinschmidt, Alan Kimura
Novartis Vaccines and Diagnostics, United States of America

Background: Reverse vaccinology identified proteins included in a recombinant meningococcal serogroup B vaccine (rMenB) that has been evaluated alone or as a multicomponent formulation with New Zealand Strain 98/254 outer membrane vesicles (4CMenB).

Methods: In completed and ongoing studies, 4CMenB was administered to adults (phase 1), infants and toddlers (phase 2 and 3) in clinical trials as 3- and 4-dose schedules with rMenB, meningococcal conjugate vaccine, and placebo controls. Immunogenicity of 4CMenB and rMenB was assessed via serum bactericidal assays using exogenous human complement (hSBA) against three serogroup B indicator strains (5/99, NZ98/254 and H44/76). Solicited injection site and systemic reactions were recorded for 7 days after each vaccination. Adverse events were evaluated for 6 months after final vaccinations.

Results: Immunogenicity, assessed by hSBA titers, was observed in all study populations; rMenB was somewhat less immunogenic compared with 4CMenB. A high percentage of vaccinees had seroresponse after 2 doses of 4CMenB; immunogenicity outcomes were similar following 3 or 4 doses. Both rMenB and 4CMenB were more reactogenic at the injection site than placebo or meningococcal conjugate or polysaccharide vaccine controls. Most 4CMenB recipients reported injection-site pain after the first vaccination; fewer reported pain with subsequent doses or a booster. Solicited reactions were generally mild to moderate and self-limited. Updated data will be presented.

Conclusions: A multicomponent meningococcal serogroup B vaccine, 4CMenB, had a favorable immunogenicity and tolerability profile supporting further study in phase 3.

VW02

Meningococcal Biology and Vaccines: ten years of genomics and reverse vaccinology

Rino Rappuoli
Novartis Vaccines and Diagnostics, Italy

The genome sequencing of Neisseria meningitidis opened a new era in meningococcal research and brought new hope to the development of a serogroup B vaccine. Novel antigens discovered using the genome-based approach to vaccine development, called reverse vaccinology, are part of a vaccine that is now at the end of phase III clinical trials. Comparative analysis of more than 10 meningococcal genomes has given us a greater insight into the biology, structure and dynamics of the meningococcal population, allowing us to define the core and dispensable genes. We have also identified novel virulence factors and restriction modification systems. The discovery of previously unknown antigens has generated new challenges, because the presence, expression and sequence variability of these antigens are not completely aligned with classical typing systems. Therefore, we propose a new typing system as a basis for epidemiological studies and evaluation of vaccine coverage. Functional analysis has revealed the role of these novel antigens in virulence, adhesion and invasion, and has provided a greater understanding of the molecular mechanisms of meningococcal pathogenesis. Perhaps the most widely-examined protein discovered by reverse vaccinology is factor H binding protein (fHbp), a protein that explains in part how the bacterium survives in humans, but poorly in animals used as models of infection. Interestingly, in N. gonorrhoeae factor H is bound by a different protein, PorB, suggesting that fHbp may have an additional function. We examined alternative roles of fHbp and discovered what we believe is the primary function of this protein.

VW03

Estimating effectiveness for *Neisseria meningitidis* serogroup B (MnB) vaccine candidates composed of non-serogroup specific antigens

Kathrin U. Jansen, Emilio A. Emini, Annaliesa S. Anderson, Thomas R. Jones, Shannon L. Harris, Ellen Murphy, Susan K. Hoiseth

Pfizer Vaccine Research, Pearl River, NY

Objective: Vaccine efficacy for prevention of invasive MnB disease (IMBD) will be determined by assessing the vaccine's ability to elicit serum bactericidal antibodies. Evaluating breadth of coverage for vaccine candidates poses unique challenges for vaccines not designed to elicit antibodies directed to the serogroup-defining capsular polysaccharide. Therefore, systematic and extensive surveys of vaccine antigen diversity and expression are required, alongside comprehensive and unbiased SBA analyses of sera from vaccinees using MnB isolates that reflect IMBD diversity. Methods: We systematically collected and analyzed >1900 IMBD isolates from the US, Europe and other countries, and conducted comprehensive analyses of vaccine antigen presence and diversity for fHBP, PorA,and NadA vaccine antigens. MLST was used to describe epidemiological diversity and to examine potential dependencies between MLST and vaccine antigen expression. SBA analyses for >100 diverse IMBD isolates were conducted. Results and Conclusions: Multiple factors were found to contribute to estimation of vaccine efficacy by SBA. Specifically, • fhbp and porA genes were present in all and nad A genes in ~34% of IMBD isolates tested. • The nada gene was present predominantly in isolates also expressing the B24 fHBP variant. • Nad A showed overall less sequence conservation compared to fHBP or PorA. • fHBP and Por A variants, but not Nad A variants, were found to be distributed across the MLST types; • Vaccine antigen expression, important for SBA response, varied among IMBD isolates. These factors should be taken into consideration when assessing the potential efficacy coverage of protein-based MnB vaccines against IMBD.

VW04

Safety & immunogenicity of serogroup B *Neisseria meningitidis* (MnB) rLP2086 vaccine in adults and adolescent subjects: overview of 3 clinical trials

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Objectives: MnB causes a large proportion of invasive meningococcal disease, but no licensed vaccine with broad protection is yet available. The safety and immune responses from recent Phase I–II trials of a recombinant investigational MnB vaccine with antigenic components from subfamilies A and B of meningococcal factor-H binding protein (rLP2086) are assessed.

Methods: Adult subjects received 120 µg of bivalent rLP2086 vaccine at 0, 1, and 6 months in study 1, and escalating doses (60; 120; 200 µg) in study 2. In study 3, adolescents received escalating doses of vaccine at 0, 2, and 6–9 months. Immunogenicity for subfamily A and B MnB strains was assessed by serum bactericidal assay (SBA) titers 1 month after vaccine doses 2 and 3 using human complement. Subjects recorded local and systemic adverse events for 7 days post-vaccinations in e-diaries.

Results: Following two 120-µg doses, 90.9% of adolescents and 74.5% of adults (study 1) achieved SBA titers >/=4 for subfamily A; 86.4% and 69.6%, respectively, for subfamily B. After dose 3, 94.3% and 94.1% of adults had SBA titers >/=4 for subfamilies A and B, respectively. Vaccinations were generally well-tolerated, with mainly mild to moderate local and systemic reactions. No subject had fever >40°C. No clinically-significant laboratory abnormalities were noted.

Conclusions: In these clinical studies, bivalent rLP2086 vaccine elicited SBA titers >/=4 against MnB subfamily A and B strains in a high proportion of adults and adolescents. No significant safety concerns were identified. These data support further development of rLP2086 vaccine.

OM01

Biochemical and biophysical analysis indicates conformation plays an important role in the binding of hfH and antibodies to the fHBP of *N.meningitidis*

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Objectives: The tertiary structure of bacterial factor H binding protein (fHBP) subfamily B variants has been determined both in solution by NMR and by co-crystalization with its primary ligand, human factor H (hfH). We have used peptides, isolated domains, and thermally denatured fHBP to explore the role that conformation plays in binding of hfH and to distinguish monoclonal antibodies (mAbs) recognizing linear or conformational epitopes.

Methods: Several fHBP variants from both subfamilies A and B have been characterized using differential scanning calorimetry to assess domain organization and surface plasmon resonance to determine relative affinity for both hfH and several mAbs. Western ligand blot analysis with hfH utilized both lipidated and non-lipidated variants to determine the importance of the tri-Pam-Cys N-terminal modification on renaturation and hfH binding following SDS-PAGE.

Results and Conclusions:

1. Protein variants from both fHBP A and B subfamilies are composed of two independently folded domains, of which the C-domain is more thermostabile.
2. Prolonged heating at high temperatures can produce a soluble, denatured fHBP that is useful in discriminating between conformational and. linear epitopes.
3. The binding of hfH and several mAbs is sensitive to conformational changes in fHBP.
4. The N-terminal tri-Pam-Cys modification enables the fHBP to more readily refold following western blots enabling the recognition of conformational determinants by hfH and mAbs.
5. Western blot analysis cannot be used for assessing the binding ability of fHBP to bind to hFH or mAbs when the protein is not in its native form with the tri-pam-Cys modification.

OM02

Twitching Motility by Scattered Pili Creates Distinct Motility Types.

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Background: Type IV pili mediate initial attachment of pathogenic Neisseria to host epithelial cells, twitching motility, DNA uptake, and biofilm formation. Twitching motility of Neisseria is dependent on the retraction of type IV pili powered by the ATPase PilT. In this work, we analyzed twitching motility patterns by individual bacteria.

Objectives:

1. Determine the speed and characteristics of twitching motility in a number of well-characterised Neisseria strains and piliated mutants.
2. Visualize pili and study single bacteria undergoing twitching motility in vivo.
3. Determine the number of individual pili contributing to twitching motility.
4. Build a theoretical model for bacterial twitching motility coupled to pilus retraction.

Methods: Live cell microscopy, automated particle tracking, total internal reflection fluorescence (TIRF)-laser illumination.

Conclusions: The average speed of twitching motility on a solid surface for N. gonorrhoeae (GC) strains was 0.8-1.1 µm/s, which was significantly lower than the average speed of any N. meningitidis (NM) strain at 1.3-1.7 µm/s. Speed of twitching motility was independent of antigenic variation in the major pilus subunit PilE for GC, or capsular serotype for NM. We predict that a few pili retract simultaneously during twitching motility, by studying how the distributions of velocities from particle tracking experiments deviate from the normal distribution. We confirmed this prediction by direct visualization of pili undergoing twitching motility, and identified two types of twitching motility, one encompassing the action of a few long Tfp filaments, and the other involving one short pilus and one or two long pilus fibers.

OM03

Accessibility to pili-linked phosphorylcholine of *N. meningitidis* is influence by phase-variation of the pili-linked glycan, and both factors are required for efficient cell association via the PAF receptor on human airway epithelial cells

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Pili of pathogenic Neisseria are major virulence factors associated with adhesion, twitching motility, autoaggregation and DNA transformation. Pili of N. meningitidis are post-translationally modified by several different modifications including the addition of phosphorylcholine (ChoP) and a glycan. Previous work has shown that expression of both the pilin-linked ChoP and glycan are phase-variable (subject to high frequency reversible on/off switching of expression). The current study reports the location of ChoP on the C-terminus of N. meningitidis pilin, and how the accessibility of ChoP is affected by changes to the pilin amino acid sequence and by changes to the structure of the pilin-linked glycan due to phase variation. We also confirm a key role for the pilin-linked glycan and ChoP in adherence to 16HBE14 human bronchial epithelial cells via the platelet activating factor receptor.

OM04

Structural insights into Transferrin Binding Protein B, TbpB

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The Neisseriaceae and Pasteurellacea families of Pathogenic bacteria acquire iron directly from the host iron binding glycoprotein, transferrin (Tf), in a process mediated by surface receptor proteins that directly bind host Tf, extract the iron, and transport it across the outer membrane. The bacterial Tf receptor is comprised of a surface exposed lipoprotein, Tf binding protein B (TbpB) and an integral outer membrane protein, Tf binding protein A (TbpA) both of which are essential for survival in the host.

The surface exposed lipoprotein, TbpB, is essential for colonization of a host. Taken together with it high affinity interaction with a host protein, TbpB provides a potential target for vaccine development.

Here we report the 1.98 Å resolution structure of TbpB, providing insights into the mechanism of Tf binding and the role of TbpB. A model for the complex of TbpB bound to Tf is proposed. Mutation of a single surface exposed Phe residue on TbpB within the predicted interface completely abolishes binding to Tf, suggesting that the TbpB N-lobe comprises the sole high affinity-binding region for Tf. (Moraes et al. 2009 Molecular Cell)

Interestingly the proposed interaction surface between TbpB and Tf is not highly conserved, thus structures of additional TbpBs were obtained to gain insight into a common binding determinant that explains this high affinity interaction.

OM05

Mass Shift Perturbation for Structure/Conformation Analyses: Concepts and Applications

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The success of rational drug design and vaccine development is dependent on the availability of high-resolution protein structures. Extending structure determination to large protein complexes (under native conditions) will open up new therapeutic target space and advance a molecular-level understanding of complex systems. Currently, there is no single technology available for “building” high resolution models of protein systems, in both static and dynamic representations. Structural mass spectrometry represents an interesting alternative to conventional structural methods for such purposes. This presentation will introduce a Mass-Shift Perturbation (MSP) method, patterned after a popular NMR technique, which can be use to map binding domains and conformational changes in high molecular weight protein systems using sub-microgram amounts of protein. To present an objective assessment of its current state of development, MSP applications in the mapping of transferrin-receptor interactions will be described.

OM06

Expression of de-N-acetyl sialic acid containing polysialic acid in normal and diseased human peripheral blood and tissues

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Objective: Vaccines based on de-N-acetyl sialic acid (ie. neuraminic acid)-containing polysialic acid (NeuPSA) elicit antibodies that mediate bactericidal activity against Neisseria meningitidis group B. In this study, we evaluated NeuPSA epitope expression in human peripheral blood cells and tissues since little is known about NeuPSA expression in humans.

Methods: Peripheral blood cells and formalin-fixed tissue specimens from all major human organs were analyzed for anti-NeuPSA reactivity with two mAbs by flow cytometry and immunohistochemisty. The mAbs recognized different NeuPSA epitopes. Specificity of binding to tissues and cells was demonstrated by inhibition with soluble NeuPSA. Irrelevant, isotype-matched mAbs were additional controls. NeuPSA expression also was evaluated in a human lymphoblastic tumor cell line and in several solid primary tumors.

Results: Leukocytes and all tissues examined exhibited some anti-NeuPSA mAb reactivity. NeuPSA epitope expression correlated with known polysialytransferase mRNA levels. The antigens recognized by anti-NeuPSA mAbs were largely expressed intracellularly, and located in the cytoplasm or nucleus. Tumors exhibited considerably greater staining than the corresponding normal tissues. A subset of leukocytes and the lymphoblastic tumor cells also expressed cell surface antigens recognized by the mAbs. Furthermore, these cells were susceptible to anti-NeuPSA antibody-dependent cytotoxic (ADC) activity.

Conclusion: Widespread anti-NeuPSA reactivity with human tissues and the ability of anti-NeuPSA mAbs to mediate ADC activity raises questions about the use of NeuPSA-based vaccines for the control of meningococcal disease. Surface expression of NeuPSA antigens by meningococci, peripheral blood cells, and malignant tissues suggests an unrecognized role for NeuPSA in immune modulation.

OM07

Structural insight into solute transport and immunological recognition of outer membrane protein PorB during Neisserial pathogenesis

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Objectives: PorB is the second most prevalent outer membrane protein in Neisseria meningitidis. PorB is required for neisserial pathogenesis and bacterial survival, but is also able to elicit a Toll-like receptor mediated host immune response. During infection, PorB likely binds host cell mitochondrial ATP, which is thought to be important for mitochondrial cellular apoptosis. To understand the unusual characteristics of PorB, we have tried to determined the x-ray crystal structure of class 2 PorB.

Methods and Results: We were able to obtain the structures of substrate-free and substrate-bound form of PorB. Structural analysis and co-crystallization studies suggest three distinct putative solute translocation pathways through the central channel pore. One pathway transports anions, one transports cations, and one facilitates the specific uptake of sugars. Co-crystallization with the ATP analog AMP-PNP suggests that binding of nucleotides regulates these translocation pathways both by partial occlusion of the pore and by restricting the motion of a putative voltage gating loop. PorB, which is located on the surface of N. meningitidis, can be recognized by receptors of the host innate immune system. Features of PorB suggest that Toll-like receptor mediated recognition of outer membrane proteins may be initiated by a non-specific electrostatic attraction.

OM08 *Igor Stojiljkovic Memorial Award Winner**

Modelling the cost-effectiveness of new meningococcal vaccines in England

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Objectives: New meningococcal vaccines able to protect against serogroup B disease are expected to go to licensure shortly. Vaccine policy makers will soon be faced with a decision about if and how to introduce these vaccines. This study uses a cohort model to predict the potential impact of introducing a new meningococcal vaccine in England.

Methods: A probabilistic age structured cohort model of meningococcal disease and vaccination was developed, following a hypothetical 2008 birth cohort over their lifetime (100 years). Epidemiological parameters and the costs of meningococcal disease and vaccination to the health service were estimated from available data; future costs and benefits were discounted back to 2008 (HM Treasury recommendations). A number of routine and catch-up vaccination strategies were simulated. Vaccines were assumed to provide direct protection only.

Results: Preliminary results from the baseline model (vaccination at 2,3,4+12 months of age, 75% effective vaccine coverage, 24 months average protection following booster, £40 per vaccine dose) indicate introduction of an infant routine schedule is highly unlikely to be cost effective (>£100,000 per QALY gained). Catch-up campaigns in 1-4 or 1-17 year olds are also unlikely to be cost-effective. The results are sensitive to changes in disease incidence, proportion of cases with sequelae, vaccine cost and vaccine duration of protection.

Conclusion: Introduction of the new meningococcal vaccines is unlikely to be cost-effective in England, given current disease levels. Models allowing for herd immunity may reach different conclusions, however it is currently unknown whether these new vaccines will provide protection against carriage.

OM09

Characterisation of *fhbp, nhba, nadA, porA* and sequence type in meningococcal group B case isolates collected in England and Wales in the epidemiological year 2007/2008, and the potential coverage of an investigational group B vaccine.

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Background: Effective glycoconjugate vaccines have been developed to prevent invasive disease of meningococcal capsular groups A, C, Y and W135. This approach is not possible for the polysialic acid capsule of group B (MenB) as it is identical to sugars on human cells. Outer membrane vesicle (OMV) vaccines can provide protection against clonal epidemics (corresponding to a single PorA protein) but provide little cross-protection to diverse MenB strains. More recently, an investigational recombinant protein vaccine was developed containing factor H-binding protein (fHBP), Neisserial Heparin-binding antigen (NHBA) and Neisserial adhesin A (NadA). The proteins were formulated with OMVs containing the P1.4 PorA (4CMenB) and the vaccine has proven safe and immunogenic in Phase I and II trials. As MenB currently accounts for approximately 90% of disease in England and Wales we investigated the potential coverage of the 4CMenB vaccine.

Methods: All MenB case isolates received at the Health Protection Agency Meningococcal Reference Unit (n = 535) from the epidemiological year 2007/2008 were genetically characterised with respect to sequence type, fHBP, NHBA, NadA and PorA.

Results and Discussion: Preliminary results demonstrated that all isolates possessed alleles for NHBA and 18% of isolates also harboured nadA alleles. For PorA, 20% of isolates possesed the vaccine PorA protein P1.4 and for fHBP, 70% of isolates harboured variant 1 proteins as contained within the vaccine. Based upon genotypic data and potential cross-reactivity, the 4CMenB vaccine has the potential to protect against a significant proportion of MenB disease in England and Wales.

OM10

Interim Analysis of the Effectiveness of Quadrivalent Meningococcal Conjugate Vaccine (MenACWY-D): A Matched Case-Control Study

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Background: In 2005, a quadrivalent (serogroups A, C, Y, and W-135) meningococcal conjugate vaccine (MenACWYD) was licensed in the United States and recommended for adolescents. We initiated a case-control study in January 2006 to evaluate vaccine effectiveness (VE).

Methods: Cases of meningococcal disease (serogroups A, C, Y, or W-135) in persons aged ≥11 years and born on or after January 1, 1986 were identified through Active Bacterial Core surveillance (ABCs) and MeningNet sites. Serogroup was confirmed at the state and CDC laboratory. Controls were matched to cases by age and geographic area. We calculated the matched odds ratio for vaccination using conditional logistic regression, controlling for underlying conditions (cancer, complement deficiency, immune deficiency, kidney disease, diabetes, sickle cell, asplenia). VE was calculated as one minus the adjusted matched odds ratio times 100%.

Results: As of June 1, 2010, 81 cases and 125 controls were enrolled. Serogroup distribution among cases was 63 (50%) C, 53 (46%) Y, and 4 (3%) W-135. Thirteen (16%) cases and 40 (32%) controls were vaccinated with MenACWYD. The VE is 75% (95% CI 17-93) in healthy adolescents. Serogroup specific VE estimates and time since vaccination will be presented.

Conclusions: This initial estimate of MenACWYD VE is similar to meningococcal polysaccharide vaccine, but is lower than published estimates for monovalent meningococcal C conjugate vaccine (MenC). These VE estimates will inform future decisions to improve the US vaccination program.

OM11

Emergence of *Neisseria meningitidis* serogroup X meningitis in Togo and Burkina Faso before introduction of a monovalent meningococcal conjugate vaccine against serogroup A

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Meningococcal serogroup X (NmX) causes sporadic meningitis and occasional outbreaks in the African meningitis belt. We describe recent NmX epidemiology in Togo and Burkina Faso.

Data were collected from a) sentinel surveillance in Togo during 2006-2009, in western Burkina Faso during 2009-2010 and in central, northern and eastern Burkina Faso during 2010; b) population-based surveillance in Bobo-Dioulasso, Burkina Faso during 2007-2009 and c) outbreak investigations using a mobile microbiological laboratory throughout Burkina Faso during 2007-2010. All cerebrospinal fluid (CSF) samples were analysed by multiplex PCR and, when possible, by culture and latex agglutination.

In Togo, 1295 CSF samples were analysed. Per year we identified 0, 91, 21, and 1 NmX cases during 2006-2009, representing 0%, 29.5%, 43.8%, and 12.5% of all Nm cases, respectively. During 2007, 83% of NmX cases came from a single district where cumulative seasonal NmX incidence was 33/100,000 and no NmA occurred. In Burkina Faso, the surveillance system detected one NmX during 2006-2007 (1.3% of all Nm cases), none during 2008 and 7 during 2009 (30.4% of Nm). NmA occurred sporadically during 2009 and 2010. During 2010, 215 cases of NmX were PCR-confirmed, concentrated in north-western, south-western and eastern districts and representing 73% of all PCR-confirmed cases. In Seguénéga district (North West), cumulative seasonal NmX incidence reached 129/100,000.

NmX meningitis moved from sporadic to epidemic occurrence with numerous outbreaks. Outbreaks only occurred in the context of low NmA incidence. These data underline the need for NmX vaccine development or serogroup-independent prevention measures.

OM12

Naturally occurring Lipid A variants among meningococcal carriage and disease isolates

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An important trigger of the pro-inflammatory cascade accompanying meningococcal disease is lipopolysaccharide (LPS). Recently, we found that ca. 9% of meningococcal isolates from adult patients with meningococcal meningitis make a less active LPS with underacylated lipid A due to lpxL1 mutations. Infection with these strains caused less coagulopathy compared to wild-type meningococci due to reduced activation of Toll-like receptor 4. Impact of lipid A variants in carriage and other invasive meningococcal syndromes is currently unknown.

Methods and Results: Two large cohorts of 448 disease and 822 carrier meningococcal isolates were screened for IL-6 induction. Twenty-nine (6.5%) lipid A variant strains were identified among the disease isolates, all having lpxL1 mutations. Of the 45 lipid A variants found among carrier isolates, 96% belonged to clonal complex 23. Among the other carrier strains lipid A variants were scarce (0.3%). Clinical characteristics of the disease isolates were extracted from hospital records. Infections with lipid A variant strains were associated with older age (19.3 vs. 5.9 years of wild-type infected patients, p=0.007). Patients over 5 years of age infected with lipid A variant strains developed less meningitis or shock (OR 0.17; 95% CI 0.06-0.51), and had lower intensive care admittance (0.20; 95% CI 0.06-0.68).

Conclusions: Among carriage isolates lipid A mutations occur only very rarely, with the exception of one single clonal complex. By contrast, in adults with meningococcal disease lipid A variation is common and associated with less severe disease. These findings suggest that lpxL1 mutations have an important role in immune evasion.

OM13

TLR2-dependent induction of pro-inflammatory cytokines and chemokines by *N. meningitidis* PorB

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Meningococcal bacteremia is correlated with systemic release of a number of pro-inflammatory mediators. These mediators are thought to modulate induction of most systemic inflammatory responses during Neisserial infections. Toll-like receptor 2 (TLR2) plays a pivotal role in the production of a number of these cytokines and chemokines, including TNF- , IL-1 , IL-6, IL-12, IL-8, and their induction. Expression of these pro-inflammatory mediators is regulated by NF-κB activation, which is directly dependent on TLR2 signaling, and can be initiated by the meningococcal porin, PorB. PorB is a TLR2 ligand, signaling through the TLR2/TLR1 hetero-dimer and requiring MyD88 for inducing cell activation. For these reasons, the role of TLR2 in the induction of a panel of pro-inflammatory cytokines and chemokines by PorB was investigated. PorB was a potent inducer of pro-inflammatory cytokines, including IL-6, TNF-α and IL-12, in primary mouse immune cells, and its effect was TLR2 dependent. This data is in agreement with the TLR2-dependent cell activation studies in epithelial cells reporter systems. Interestingly the level of induction of these cytokines is as great or greater than more common TLR2 ligands (i.e. PAM3CSK4, MALP, etc.) and the role of PorB in the inflammatory process induced by meningococci may be underappreciated.

OM14

Detection of *Neisseria gonorrhoeae* by the cytosolic Nod receptors

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Nucleotide-binding Oligomerization Domain (NOD) 1 and NOD2 are members of the Nod-like receptor (NLR) family that activate innate immune signaling pathways by recognizing specific fragments of the bacterial cell wall component peptidoglycan (PGN). Neisseria species are unique among Gram negative bacteria in that they turn over large amounts of PGN during logarithmic growth. In this study we examined the ability of these receptors to recognize N. gonorrhoeae, and activate specific proinflammatory signaling pathways. We found that both live gonococci (GC) and lysates of GC, as well as conditioned medium from GC grown to mid-logarithmic phase growth, were capable of activating an NF-κB driven luciferase reporter construct when cotransfected with either human Nod1 or Nod2. Similar preparations were also capable of inducing polyubiquitination of the Nod-specific adaptor Rip2 in both HeLa cells and human PBMC, a signaling pathway that is specific for Nod activation and independent of Toll like receptors (TLRs). Using mouse macrophages derived from mice with defined deletions of either Nod1 or Nod2, we confirmed the role of Nod2 in directing Rip2 polyubiquitination in response to GC. However, we found murine Nod1, unlike human Nod1, was not activated by GC preparations. While GC is known to be a potent stimulator of both TLR4 and TLR2, we found Nod2 deficient macrophages were abrogated in their ability to upregulate TNF-α in response to GC compared to wild type macrophages, suggesting that the collective inflammatory response to GC is regulated by a combination of TLRs and NLRs.

OM15

Neisseria gonorrhoeae exploits TGF-β to elicit innate host responses and suppress adaptive immunity in a murine model of genital tract infection

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We propose that N. gonorrhoeae elicits Th17-driven responses that recruit innate defense mechanisms, which gonococci can partially survive, and concomitantly suppresses Th1/Th2-dependent adaptive responses that might eliminate the infection, and have tested this hypothesis in vitro and in vivo in a murine model. N. gonorrhoeae induced Th17-associated but not Th1/Th2-associated cytokines, and TGF-β, in spleen cells in vitro, and in genital tract tissue ex vivo. IL-17 production depended on gonococcal LOS acting through TLR4. Vaginal gonococcal infection of mice in which IL-17 was blocked or which were IL-17RA-deficient was prolonged. TGF-β production in mononuclear cell cultures was enhanced by N. gonorrhoeae dependent on Opa expression, and antibody blockade of TGF-β allowed Th1 and Th2 responses to emerge. Treatment of mice with anti-TGF-β antibody during gonococcal challenge shortened the duration of infection without affecting the neutrophil influx, and permitted the development of Th1/Th2 responses. Subsequent secondary challenge resulted in more rapid elimination of the infection than in control animals, suggesting that alleviation of suppression of specific anti-gonococcal responses permits the generation of protective immunity. Thus N. gonorrhoeae exploits and enhances the naturally TGF-β–rich environment of the genital tract to elicit responses that are favorable to its own survival. Blockade of IL-17–dependent responses significantly prolongs infection in vivo, whereas blockade of TGF-β reverses suppression of adaptive immune responses. Further elucidation of the crucial role of Th17 and regulatory T cells in the immune response to N. gonorrhoeae will have a profound impact on comprehending immunity to this infection.

OM16

CEACAM-humanized mice as a model for *N. meningitidis* nasopharyngeal colonization

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It is established in vitro that opacity-associated (Opa) proteins of Neisseria meningitidis bind to carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family members. However, the relevance of this interaction for carriage or invasive disease remains elusive. Here, we demonstrate successful colonization of CEACAM-humanized mice with pathogenic N. meningitidis, indicating the importance of the Opa/CEACAM interaction for human carriage and disease.

Mice expressing either human CEACAM1 (Tg418) or human CEACAM3,CEACAM5 and CEACAM6 (CEABAC) and their according WT littermates (FvB background) were infected intranasally with N. meningitidis MC58. Live bacteria were sampled from the nasopharynx (retrograde lavage, swabbing of nasal cavities) and the lung. Tg418 mice were colonized for up to 7 days after infection, whereas WT mice and CEABAC mice yielded no viable bacteria past day 1. Gentamycin binding assays revealed that all four Opa proteins of N. meningitidis MC58 bind specifically to CEACAM1, thus explaining no sustained colonization of CEABAC mice. However, when CEABAC mice were infected with CEACAM5 binding N. meningitidis isolates, bacteria could be recovered from both, transgenic and WT littermates, 3 days after infection.

In mice depleted of neutrophils using the Gr1-specific monoclonal antibody RB6-8C5, bacterial counts were significantly enhanced and live bacteria were found for up to seven days (WT), or even fourteen days (CEABAC, Tg-418) after infection, indicating a dominant role for neutrophils in clearance of nasopharyngeal colonization. Upon intraperitoneal infection to mimic invasive disease, no differences in mouse survival or bacteremia were observed between either Tg418 and WT, or CEABAC and WT mice, suggesting a role of CEACAM mainly in colonization in these mouse models.

OM17

Enhanced bacteremia and decreased antibody passive protective activity in human factor H transgenic rats challenged with encapsulated strains of *Neisseria meningitidis*

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Background: In wild-type rats, complement factor H does not bind to N. meningitidis, and most meningococcal strains are rapidly cleared from blood. We investigated whether expression of human fH by transgenic rats affects antibody passive protective activity against meningococcal bacteremia.

Results: Six hours after IP challenge of 17 day-old rats with ~1,000 CFU, 5/7 transgenic rats given group B strain H44/76, 4/7 given group B strain NZ98/254, and 5/7 given group C strain 4243 had bacteremia, compared with 0/3, 0/3 and 1/3 wild-type littermates (14/21 vs. 1/9, $p<0.02$). In subsequent experiments, 7 day-old transgenic (n=8) or wild-type (n=14) rats were challenged with ~1,000 CFU of strain 4243. Twenty-hours later, the geometric mean CFU/ml in blood of transgenic rats (≥1,000,000) was 20- to 100-fold higher than wild-type rats ($p<0.01$). Additional transgenic or wild-type rats were pre-treated with stored sera from adolescents immunized three years earlier with ACYW-135 polysaccharide-protein conjugate vaccine. Three of 4 sera with bactericidal titers <1:4 (human complement), which in previous experiments had conferred passive protection in wild-type rats against strain 4243, conferred protection against 4243 in the wild-type rats (≥100-fold decrease in CFU/ml) but not in transgenic rats (0/4). In contrast, 5 of 6 sera with bactericidal titers ≥1:4, which previously had conferred passive protection in wild-type rats, conferred protection in transgenic rats.

Conclusions: Binding of human fH enhances meningococcal virulence, even for strains such as 4243 that can cause bacteremia in wildtype rats. In transgenic rats, human fH also decreases antibody passive protective activity by non-bactericidal sera.

OM18

Gonococcal infection is enhanced in mice transgenic for Human C4b-binding protein (C4BP)

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Background: Gonorrhea is restricted to humans. Species-specificity embodies binding to gonococci of C4b-binding protein (C4BP), a human classical complement pathway regulator; non-human C4BP does not bind to gonococci and results in unimpeded killing of N. gonorrhoeae. We investigated whether mice transgenic for human C4BP (Tg) could maintain higher vaginal bacterial loads and for longer duration.

Methods and Results: Estrogen-treated Tg (n=10) and wild-type (Wt, n=10) female BALB/c mice were inoculated intravaginally with 3 x 105 CFU of N. gonorrhoeae strain FA1090 that binds human, but not mouse, C4BP. On day 1 after inoculation, 7/10 Tg and 5/10 Wt mice remained colonized with N. gonorrhoeae; there was no difference in vaginal bacterial loads (mean ± SE, CFU/100 µl) between the two groups (4.0 ± 2.0 x 104 in Tg and 8.0 ± 6.3 x 104 in Wt). On day 4, bacterial loads in 4 Tg mice that remained infected were four logs higher than in 3 Wt mice (4.3 ± 4.0 x 104 in Tg vs. 8.0 ± 8.0 x 100 in Wt). On day 7, 4 Tg mice 4 remained infected (2.7 ± 2.7 x 104 CFU/100 µl); no Wt mice were infected. On day 14, 3 Tg mice remained infected (4.5 ± 2.6 x 103 CFU/100 µl). Kaplan-Meier analysis using log-rank testing indicated that tg mice remained infected longer than Wt mice ($p=0.048$).

Conclusions: These results underscore the importance of complement evasion in gonococcal pathogenesis and may also lead to a more relevant experimental model of human gonococcal infection.

OM19

Mechanisms of neisserial transformation and the evolution of sexual reproduction

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Reproductive barriers exist in all domains of life which engage in some form of sexual reproduction. Members of the genus Neisseria are competent for transformation and have evolved features that favour uptake of homologous DNA. Discrimination between self and alien DNA is achieved by a specific 10-12 nucleotides long DNA Uptake Sequence (DUS) which must be present in the incoming DNA for efficient uptake. We seek to explore the proximate and ultimate mechanisms driving neisserial transformation.

We have studied the transforming potential, the genomic distribution and conservation of DUS in Neisseria and other species. We relate the remarkable conservation of the DUS sequence itself to transforming ability. In a deconstructive approach we were able to map the contribution of each individual nucleotide of the DUS in transformation assays. We have also tested novel components of the transformation machine for their DNA binding activity in vitro and in vivo and have designated roles to their activities in the route for DNA during transformation.

The accumulation of DUS in the core genome is a tell-tale of neisserial evolution. The study of DUS dynamics has proven an excellent model for the study of genome maintenance far beyond the realm of bacteria. We employ and develop current models for the complex transformation process which consists of many components that are promising targets for intervention. We argue that both transformation and sexual reproduction evolved for their common ability to reassemble beneficial alleles from a gene-pool which is under constant diversification by spontaneous mutations.

OM20

Neisseria population genomics: integrating whole genome data with multi locus approaches to epidemiology and population biology.

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Pathogenesis has emerged in the genus Neisseria on several occasions, but is yet to be fully understood as it is a complex polygenic trait. Comparative whole genome studies of multiple bacterial isolates with defined phenotypes provides a powerful approach to this question, but such studies present challenges in integrating and analysing large complex datasets. We propose a scalable isolate-centric approach to whole genome analysis and have developed and implemented Internet-based software that realises this paradigm (Bacterial Isolate Genome Sequence Database, BIGSdb). Isolate provenance and phenotype are linked to sequence data, which may range from a single gene fragments, through multilocus sequence data and partial genome assemblies, to a whole closed genomes. The sequences are queried using curated reference datasets for defined genetic loci, enabling rapid discovery and characterisation of genetic variation and its association with phenotype. Loci can be grouped into schemes with unlimited numbers of members to reveal higher order structure, including schemes for typing (including MLST and antigen typing), and metabolic processes (including antibiotic resistance). BIGSdb is now running the Neisseria reference and isolate databases (http://pubmlst.org/neisseria/), which currently include provenance and genotypic data for over 17000 isolates, including 22 complete genomes. It is also being used to analyse the genomes of 200 N. meningitidis isolates from 'Next Generation' sequencing platforms. There are no practical limits to the number of bacterial genomes, loci, and schemes that can be accommodated within BIGSdb, which is available as a resource for community annotation and investigation of phenotypic and genotypic variation.

OM21

Differential genome expression of serogroup B meningococci under *in vivo* mimicking conditions

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In this study, we analyzed the changes in the transcriptional pattern in the invasive meningococcal serogroup B strain MC58 (ST-74) as well as in the serogroup B carriage strain alpha522 (ST-35) which are geno- and phenotypcially very similar to each other except for their epidemiology.

As animal models covering all aspects of meningococcal carriage and disease are still lacking, we established an in vitro system mimicking the conditions encountered by the bacteria on their route from the human nasopharynx to the human meninges and allowing for the preparation of sufficient amounts of bacterial RNA for further analyses. Accordingly, the two strains MC58 and alpha522 were exposed to human saliva representing the nasopharyngeal niche, human whole blood representing the blood stream niche, and human cerebrospinal fluid representative of the meningeal niche. Consecutively, total RNA from the two strains after exposure to the various human materials were isolated, and oligonucleotide-based whole genome microarrays together with ultra-deep sequencing of cDNAs using the Illumina/Solexa technology (RNA-seq) and with whole-genome shotgun sequencing of the alpha522 genome using a combined 454/Sanger sequencing approach were used to characterize the transcriptomes in both strains to also identify changes in the expression of small non-coding RNAs.

Our results indicate that these strains display a dramatically altered expression pattern in response to stress conditions encountered within the human host. The results of the microarray and RNA-seq analyses which reveal a niche specific and strain specific transcriptional response will be discussed.

OM22

Analysis of Novel Fur-Mediated Global Regulatory Circuits in the Pathogenic *Neisseria*

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We have established that in the pathogenic Neisseria the ferric uptake regulatory protein (Fur) functions as a global regulatory protein as both a repressor and activator of gene transcription. Whereas the repressive mechanisms of Fur have been thoroughly investigated, the mechanisms of direct and indirect -activation by Fur have not been elucidated. In this study we have defined 3 mechanisms that contribute to Fur mediated activation of gene transcription in the pathogenic Neisseria. Fur mediated direct activation was established by direct binding of Fur to the putative promoter regions of a subset of Fur activated genes. However this analysis revealed that less than 50% of the genes, which contained Fur boxes, bound Fur in vitro indicating that additional regulatory circuits exist which function in a Fur mediated indirect mechanism of control. We subsequently identified a Fur mediated indirect mechanism that was mediated via the repression of a novel gonococcal repressor. This Fur activated regulatory protein (farP) was demonstrated to control expression of a number of genes required for gonococcal invasion of female epithelial cells. Finally we further defined a post-transcriptional mechanism of Fur mediated indirect activation that was mediated by regulatory small RNA molecules (sRNAs). Regulation via NrrF, the first fully characterized iron and Fur regulated Neisseria sRNA was shown to occur independently of the cofactor RNA-binding protein (Hfq). Collectively our studies have established that in the pathogenic Neisseria Fur functions as an activator of transcription through both direct and indirect mechanisms involving novel regulatory cascades.

OM23

Neisseria meningitidis Hsf (NhhA) interacts directly with human vitronectin: the interplay between meningococcal Hsf and Opc in host cell adhesion and serum resistance

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Recently, we have investigated two key functions of meningococcal outer membrane proteins, that of sequestering complement regulatory molecules which leads to serum resistance and that of binding to cellular receptors which enables them to cross human cellular barriers in the nasopharynx and at the vascular interface. In this study, we observed that meningococcal Hsf (Msf for brevity) imparts vitronectin (Vn) binding property to the bacterium. In addition, Opc and Msf utilise vitronectin in normal human serum (NHS) to achieve complementary as well as exclusive functions. Using synthetic Vn peptides, we have delineated the Msf binding regions of vitronectin. As with Opc, this interaction prolongs the survival of meningococci in NHS by inhibiting C9 polymerisation and terminal complement complex insertion into the Msf-containing bacterial membrane. Thus, when mixtures of phenotypes are exposed to NHS, those expressing Vn-binding proteins demonstrate a striking ability to resist killing over those lacking their expression. However, it appears that Msf and Opc are not equally efficient in cellular adhesion and invasion via binding to Vn. The data imply that vitronectin binding may be an important properly for the survival of the pathogen in in vivo environments for which the bacterium has evolved a number of distinct adhesion mechanisms. The aim of this presentation is to describe the novel mechanisms of interactions at the molecular level and the functional characteristics of the outer membrane proteins as well as the host components that are manipulated to enable survival and barrier penetration.

OM24

Neisseria meningitidis hijack the β2-adrenoceptor/β-arrestin pathway to open and traverse brain microvasculature endothelium

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The tropism of N. meningitidis for the meninges reveals the efficiency with which the interactions between this pathogen and the brain microvasculature modify the BBB function.

Adhesion of the meningococcus is mediated by type IV pili that induce a localized remodeling of the sub cortical cytoskeleton, leading to the formation of endothelial membrane protrusions that anchor bacterial colonies at the endoluminal face of the endothelial cell membrane, allowing a better resistance to blood flow. N. meningitidis is also able to recruit the polarity complex Par3/Par6/aPKC that re-routes junctional molecules at the site of bacterial cell interaction thus opening a paracellular route for bacteria to cross the endothelial barrier.

The signaling receptor activated by the pathogen remained unknown. We report that N. meningitidis specifically stimulates a β2-adrenoceptor β-arrestin signaling pathway in human brain endothelial cells (hCMEC/D3), which ultimately traps β-arrestin interacting partners, such as cytoskeletal and junctional proteins under bacterial colonies. These molecules are progressively depleted from endothelial cell junctions resulting in anatomical gaps likely used by bacteria to penetrate into tissues.

Pharmacological activation of β2-adrenoceptors with specific agonists that induce their endocytosis prevents signaling events downstream of N. meningitidis adhesion and inhibits bacterial crossing of endothelial cell monolayers. These results reveal a novel strategy used by a pathogen for hijacking host cell signaling machineries.

OM25

Gonococcal Cervical and Amniochorionic Infections as Potential Factors Contributing to Adverse Pregnancy Outcomes.

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Infection of the amnion and chorion (i.e. amniochorionic) membranes or fluid occurs in up to 80% of women experiencing preterm, adverse pregnancy outcomes (APO). Consistent with the predominantly asymptomatic nature of gonococcal cervicitis, infection of the amniochorionic membranes also typically results in sub-clinical or asymptomatic disease. Regardless, antibiotic therapy usually is not associated with improved pregnancy outcomes. Maternal gonorrhea increases a woman's risk for APO by 6.5-fold. Although invasion and/or transcytosis of the amniochorionic membranes by gonococci, in vivo, is inferred by the ability to isolate bacteria from these tissues, and from amniotic fluid, gonococcal adherence to and/or to invasion of the amniochorion has not been directly examined. Therefore, we investigated gonococcal infection under conditions reflecting normal human pregnancy and by using primary epithelial cells derived from the human cervix (i. e., pex cells) and amniochorionic membranes (i.e., pace cells; primary amniochorion epithelial). A further objective was to establish whether gonococcal infection directly stimulated a host response(s) compatible with those responses defined for human parturition. Comparative, quantitative, infection assays indicated that gonococci do indeed adhere to and invade pace cells. Additionally, using a variety of techniques, we demonstrate that N. gonorrhoeae infection of pex and pace cells elicits the differential production of nitric oxide and complement proteins, as well as the specific matrix metalloproteases and cytokines that are thought to participate in triggering human parturition. Hence, we provide the first evidence to indicate a direct potential link between gonococcal infection and the induction of APO.

OM26

Decoy receptor CEACAM3 elicits Opa protein-dependent neutrophil responses and inflammation typical of symptomatic gonococcal infection in transgenic mice

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Since Neisserial Opa proteins have a remarkable selectivity for human CEACAM receptors, we have employed transgenic mice expressing various combinations of human CEACAM1, CEACAM3, CEACAM5 and/or CEACAM6, each of which are differentially expressed on various cell types. Human CEACAM1 and CEACAM5 transgenic mice can be effectively colonized by both N. meningitidis and N. gonorrhoeae, with bacterial loads and durations of infections significantly greater than wild type mice, confirming a central role for Opa-CEACAM interactions in vivo. Using a combination of primary cells and transfected cell lines, we have established that neisserial Opa protein binding to CEACAM1 elicits a phosphatase cascade that effectively opposes normal activating signals downstream of the T cell receptor, suppressing normal adaptive immunity. Evolutionarily, humans have responded by acquiring a molecular mimic of CEACAMs on neutrophils, our most potently bactericidal cell type. This protein, known as CEACAM3, contains the bacterial-binding domain of CEACAM1 linked to a cytoplasmic sequence that elicits a potent activation cascade which promotes bacterial engulfment and killing. Neutrophils from transgenic mice expressing human CEACAM3 demonstrate that this receptor is necessary and sufficient for the phagocyte's overzealous response to Neisseria, leading to enhanced inflammation to these bacteria in vivo. While all neisserial strains encode Opa variants that bind to a variety of CEACAM receptors, analysis of fresh isolates from primary human specimens indicates that there is a selection for Opa variants that bind CEACAM1 and CEACAM5 but not CEACAM3 in vivo, consistent with the divergent effects of these CEACAMs on neisserial infection.

OM27

A conserved domain of *Neisseria meningitidis* TspB/Orf6 has Ig-binding activity

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Objectives: Gram-positive bacteria such as S. pyogenes express Ig-binding proteins that are important for pathogenesis. We have observed similar activity in some N. meningitidis (Nm) strains. This study aimed to identify the cell surface molecules mediating Ig binding.

Methods: Meningococci were cultured in chemically defined medium supplemented with 5% human serum. Expression of Ig-binding activity was measured by flow cytometry. After washing, cellular proteins were solubilized and IgG-binding molecules were purified by Protein G affinity selection and separated by SDS-PAGE. Individual proteins were identified by MALDI-TOF mass finger print.

Results: Strains from groups A, B, C, W135, X and Y expressed Ig-binding activity that was increased when bacteria were selected for survival in human complement. An Ig-binding protein was identified as T and B cell stimulating protein B (TspB)/Orf6, which is encoded by a gene that has been correlated with increased risk of invasive meningococcal disease. Sequence analysis of TspB identified four distinct structural regions. A region consisting of a conserved β-sheet domain was cloned and expressed in E. coli. By ELISA, the purified recombinant protein had Ig-binding activity, with a preference for human Ig and possible specificity for Fc region glycans. The recombinant protein formed distinctive polymeric structures whose presence was associated with Ig-binding activity.

Conclusion: Nm strains from several capsular groups can bind Ig and a conserved domain of TspB/Orf6 can mediate Ig-binding. The Ig-binding activity expressed by Nm strains was correlated with survival in human complement and, therefore, may contribute to immune evasion.

OM28

Olfactory nerve - a novel invasion route of *Neisseria meningitidis* to reach the meninges

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Neisseria meningitidis is a human-specific pathogen with capacity to cause septic shock and meningitis. It has been hypothesized that invasion of the central nervous system (CNS) is a complication of a bacteremic condition. In this study, we aimed to characterize the invasion route of N. meningitidis to the CNS. Using an intranasally challenged mouse disease model, we found that twenty percent of the mice developed lethal meningitis even though no bacteria could be detected in blood. Upon bacterial infection, inflammatory lesions and redistribution of intracellular junctional protein N-cadherin were observed at the nasal epithelial mucosa, especially at the olfactory epithelium, which is functionally and anatomically connected to the CNS. Bacteria were detected in the submucosa of the olfactory epithelium, along olfactory nerves in the cribriform plate, at the olfactory bulb and subsequently at the meninges and subarachnoid space. Even without systemic inflammation, the local immune response in the brain was actively induced upon bacterial invasion. Furthermore, our data suggest that a threshold level of bacteremia is required for the development of meningococcal sepsis. Taken together, N. meningitidis is able to pass directly from nasopharynx to meninges through the olfactory nerve system without detectable bloodstream dissemination. Inflammation-induced damage and depletion of intracellular junctional proteins of the host cells could be a general mechanism used by N. meningitidis during dissemination.

OM29

Cell-contact induced posttranslational modification of type IV pilin triggers *Neisseria meningitidis* propagation and dissemination

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The Gram-negative bacterium Neisseria meningitidis is an asymptomatic colonizer of the throat of 10-30% of the human population but throat colonization is also the port of entry to the blood (septicemia) and then the brain (meningitis). Colonization is mediated by filamentous organelles referred to as type IV pili (T4P), which allow the formation of bacterial aggregates associated with host cells. We show that proliferation of N. meningitidis in contact with host cells increases the transcription of a bacterial gene encoding a transferase that adds phosphoglycerol onto T4P. This unusual posttranslational modification (PTM) releases T4P-dependent contacts between bacteria. This cell-contact induced PTM thus allows propagation of the bacterium to new hosts but also migration across the epithelium, a prerequisite for dissemination and invasive disease.

OM30

The transcriptional regulator FarR contributes to divergent host niche adaptation by adopting different functions in *N. meningitidis* and *N. gonorrhoeae*

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In Neisseria gonorrhoeae, the transcriptional regulator FarR is involved in regulation of the FarAB operon and fatty acid resistance. We have shown, that the meningococcal homologue NmFarR (98% sequence identity) acts as a repressor of the adhesin nadA, which has most likely been acquired by horizontal gene transfer and is not found in gonococci. In this study, the divergent functions of FarR in the two pathogenic Neisseria species were examined. We tested the susceptibility of 23 meningococcal isolates against long-chain fatty acids, proving that meningococci are generally highly resistant, with the exception of some SGY strains. Using lipopolysaccharide (LPS)-truncated mutant strains as well as a farAB deleted strain, we show that intact LPS structure as well as presence of the farAB operon are required for primary fatty acid resistance of meningococci. The sensitivity of the serogroup Y strains is due to naturally occurring mutations within the lpxL1 gene, responsible for addition of the sixth acyl chain to lipid A and not related to NmFarR. The regulon of NmFarR was examined by microarray analyses, confirming that nadA is the main effector of FarR. Alterations of the FarR binding site within the nadA promoter are sufficient to induce transcription of nadA. The NmFarAB operon is not part of the NmFarR regulon. Consequently the transcriptional regulator FarR has adopted different functions in the two pathogenic Neisseriae: In N. gonorrhoeae it provides a means of inducible fatty acid resistance whereas in N. meningitidis it contributes to the balance between adhesin expression and immune evasion.

OM31

The NmlR regulon and stress defense in *Neisseria gonorrhoeae*

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Recently, we identified a regulon of Neisseria gonorrhoeae that is under the control of the Neisserial merR-like Regulator (NmlR). Phenotypic characterization of mutants and biochemical characterization of proteins has been used to determine the role of the three functional genes under NmlR control: trxB tencodes a thioredoxin reductase, copA, encoding a putative copper-efflux ATPase and estD, encoding an esterase. A trxB mutant was more sensitive to killing by nitric oxide compared to wild-type gonococcus and was unable to grow under microaerophilic conditions in the presence of nitrite. A copA mutant was shown to be more sensitive to killing by copper ions, confirming its role in removal of copper ions from the cytoplasm. EstD is a serine esterase is able to hydrolyse a variety of esters but we have shown that it does not metabolize derivatives of S-nitrosoglutathione. The trxB and estD mutants show reduced ability to form a biofilm on human cervical epithelial cells and have reduced survival inside cervical epithelial cells. In contrast, the copA mutant forms a stable biofilm but is affected in intracellular survival. We have purified NmlR and its DNA-binding properties have been characterized. All four cysteines in the NmlR protein are reduced but glutathionylation at C71 abolishes promoter binding. This suggests that NmlR may sense oxidized or nitrosated glutathione intermediates and activate expression of genes involved in protection against the effects of nitrosative stress.

OM32

Unraveling small RNA Mediated Post Transcriptional Control Mechanisms in the Pathogenic *Neisseria*

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A number of pathogenic bacteria utilize regulatory small RNA molecules (sRNAs) to post-transcriptionally regulate gene expression. sRNAs act by binding to mRNA targets through short regions of complementarity and act to augment transcription either positively or negatively. Nrrf, the only fully characterized sRNA known in Neisseria, is an iron and Fur-regulated molecule expressed under low iron conditions which acts to down regulate stability and translation of the succinate dehydrogenase subunits sdhA and sdhC. In the majority of Gram-negative bacteria sRNA-mediated regulation requires a cofactor RNA-binding protein (Hfq) for proper gene regulation and stabilization. In this study we demonstrated that the stability of Nrrf, as well as the regulation of sdhC and sdhA in vivo, was unaltered in the hfq- mutant. Despite Nrrf's independence of Hfq in vivo, Nrrf was unable to form a complex with sdhC in vitro in the absence of any cofactor as demonstrated by EMSA, suggesting the existence of an unidentified cofactor. Global sequence analysis was further utilized to identify novel sRNA molecules in N. gonorrhoeae. This has led to the discovery of a new sRNA. Subsequent work has shown that this sRNA is downregulated in a fur- mutant suggesting that Fur drives expression of this sRNA through either direct or indirect means. Taken together these studies have established unique aspects of sRNA-mediated control of gene expression in the pathogenic Neisseria.

OM33

Neisseria gonorrhoeae responses and defenses against reactive oxygen species

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The Neisseria are not regularly exposed to chemicals or UV light but are regularly exposed to reactive oxygen (ROS) and nitrogen species (RNS). We postulate that the long existence of the Neisseria in humans has focused their response and repair capabilities to the agents encountered in vivo.

A H2O2-induced gene product, NG1427, has similarity to the SOS response regulator LexA. Similarly to LexA, NG1427 undergoes RecA-mediated autoproteolysis both in vitro and in Neisseria cells treated with non-oxidative DNA damaging agents. NG1427 controls a three gene regulon, however, NG1427 regulon de-repression is RecA-independent following hydrogen peroxide treatment. NG1427 specific binding to DNA is abrogated by site-directed mutation, or thiol-modification of the single cysteine residue encoded in NG1427. We conclude that NG1427 uses thiol-sensing of ROS and RNS to control gene expression without requiring a level of DNA damage necessary to stop DNA replication.

The Neisseria phrB gene product is orthologous to light-activated photolyases, which directly remove cytobutane dimers formed by UV light. Since none of the Neisseria are regularly exposed to visible or UV light, we asked whether this gene encodes a photolyase or supplied an alternate function. We show that PhrB is not a photolyase but is required for maintaining proper DNA supercoiling in the cell and that a phrB mutant is very sensitive to ROS that alter the level of supercoiling within Neisseria and very resistant to nalidixic acid. While not a topoisomerase, PhrB is required to maintain proper supercoiling density to resist ROS activities.

OM34

Biochemical characterization of TraI, the relaxase involved in DNA secretion by *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae secretes chromosomal DNA into the surrounding milieu as the bacteria grow. The secreted DNA is single-stranded and active in the transformation of other gonococci in the population. To better understand the process of DNA secretion we are characterizing the relaxase, TraI, and its interaction with the origin of transfer, oriT. TraI is an unusual relaxase in that it has an N-terminal region that facilitates interactions with the membrane, and certain motifs found in characterized relaxases are absent or highly divergent in gonococcal TraI. Overexpression of TraI generated mostly insoluble protein, but overexpression of a central portion generated a protein that was active after refolding. In cleavage assays, truncated TraI was able to relax supercoiled plasmid DNA that carried the putative oriT, but not plasmid lacking oriT. Cleavage was dependent on the presence of Mg++. Unexpectedly, TraI did not remain bound to the cleaved DNA, and precipitation of the protein from the nicking reaction did not precipitate the cleaved DNA. Genetic experiments localized the oriT to a 150bp region located near the traI gene. The oriT region includes an inverted repeat sequence. Sequencing of the DNA products of the cleavage reaction identified the putative nic site, and localized it to the inverted repeat. Since nuclease susceptibility experiments showed that DNA secreted from gonococci is protected at the 5' end, these data suggest that TraI cleaves the DNA and remains non-covalently bound or that another protein binds the DNA prior to secretion.

OM35

Probing the functions of O-linked protein glycosylation in *Neisseria gonorrhoeae*

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Protein glycosylation is now well recognized in bacterial pathogens and symbionts of man. The prevalence of bacterial protein glycosylation strongly suggests that these systems are advantageous and impact on fitness.

We have shown that Neisseria gonorrhoeae and other neisserial species express a general O-linked glycosylation system that targets many proteins. The majority of these glycoproteins are predicted to be localized to the periplasm, whereas the most abundant glycoprotein is PilE, the protein subunit of type IV pili.

While studying gonococcal pilus biogenesis, we observed that expression of certain pilE alleles inhibited bacterial growth when co-expressed with wild-type pilin. Realizing that this system might provide a sensitive means to identify factors impacting on the efficacy of pilin subunit interactions, we examined the effects of altering glycosylation status and disrupting pilus dynamics (assembly and retraction) and found that all of these affected the growth arrest phenotype. As both pilin-pilin and pilin-assembly/retraction component interactions takes place in the cytoplasmic membrane, protein glycosylation appears to play a role in intracellular processes occuring at the inner membrane. This hypothesis is consistent with the glycosylation status of other inner membrane-localized proteins that are unlikely to be trafficked to the cell surface.

OM36

Organization of the electron transfer pathways to oxygen and nitrite in the obligate human pathogen, *Neisseria gonorrhoeae*: how are electrons transferred to the outer membrane?

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Neisseria gonorrhoeae inhabits an oxygen-limited environment. Two of its eight c-type cytochromes, cytochromes c4 and c5, form a bifurcated electron transfer pathway to the single cytochrome oxidase, cbb3 (1). When oxygen is limiting, the gonococcus reduces nitrite to NO catalysed by the copper-containing nitrite reductase, AniA, which is an outer membrane lipoprotein. Mutants defective in cytochromes c2, c4, c5, c2 and c4, or c2 and c5 all reduce nitrite, though more slowly than the parent, implying the existence of multiple electron transfer pathways to AniA. CcoP is one of four subunits constituting the cytochrome cbb3 terminal oxidase. Surprisingly, unlike CcoP in many other bacteria, CcoP from gonococci and commensal neisseria contains three rather than two haem-binding motifs. Mutants defective in this motif reduce nitrite at only 50% of the rate of the parent, providing the first published report implicating a cytochrome oxidase subunit in electron transfer to a nitrite reductase (2). Three other outer membrane redox proteins are the gonococcal azurin, Laz, cytochrome c peroxidase, Ccp, and the NO-binding protein, cytochrome c'. Unlike azurins in other bacteria, gonococcal Laz is not thought to transfer electrons to AniA. A model of how electrons might be transferred to these outer membrane redox proteins from the electron transfer chain in the cytoplasmic membrane will be presented.

1. Li et al. (2010) Journal of Bacteriology 192: 2395-2406.
2. Hopper et al. (2009) FEMS Microbiology Letters 301: 232-240.

OM37

Identification and Characterization of a Transcriptional Regulatory Circuit that Modulates Expression of the Mtr Efflux System in *Neisseria gonorrhoeae*

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The MtrC-MtrD-MtrE efflux pump of Neisseria gonorrhoeae exports both classical antibiotics and certain host-derived antimicrobial agents that bathe mucosal surfaces and its level can impact in vivo fitness. We examined the cis- and trans-acting factors that modulate mtrCDE expression. In strain FA19, the cis-acting elements consist of a 13 bp inverted repeat sequence within the promoter for mtrR, which encodes a DNA-binding protein that represses mtrCDE, and a silent promoter for mtrCDE transcription that becomes active due to a point mutation located 120 bp upstream of mtrCDE; this point mutation is present in strain MS11. In contrast to the normal promoter, we found that the presence of MtrR enhances mtrCDE expression from this promoter. Since MtrR determines levels of the MtrC-MtrD-MtrE pump, we determined how mtrR expression is controlled and now report a regulatory circuit that dampens mtrR expression in strain FA19. This circuit involves two DNA-binding proteins that influence mtrCDE (MtrA and MpeR, respectively), a two-component regulatory system (MisR/MisS) that senses the human antimicrobial peptide LL-37, and a third component involving the levels of free iron. We determined that MpeR can dampen mtrR expression under iron-limited conditions, a finding that is consistent with the observation that mpeR expression is repressed by Fur+iron. mpeR expression is also subject to regulation by MtrA. Hence, conditions likely encountered by gonococci in vivo (e.g., presence of host defense compounds and iron-limitation) influence transcriptional regulatory processes that modulate levels of the MtrC-MtrD-MtrE efflux pump and this may influence survival of gonococci during infection.

OM38

Molecular and structural analysis of gonococcal resistance to expanded-spectrum cephalosporins mediated by mosaic *penA* alleles: role of epistatic mutations

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Mutations in penicillin-binding protein 2 (PBP 2) encoded by mosaic penA alleles are crucial for intermediate resistance in Neisseria gonorrhoeae to the expanded-spectrum cephalosporins ceftriaxone and cefixime. Of the ~60 mutations present in mosaic alleles, G545S, I312M, and V316T have been suggested to be responsible for conferring increased resistance, particularly to cefixime. However, the Minimum Inhibitory Concentrations (MICs) of penicillin, ceftriaxone, and cefixime for a wild type strain (FA19) with a wild type penA gene containing these three mutations increased only 1.5-, 1.5-, and 3.5-fold, respectively. In contrast, when these three mutations in a mosaic penA allele (penA35) were reverted back to wild type and the gene transformed into FA19, the MICs of the three antibiotics were reduced to near wild type levels. Thus, these three mutations display epistasis, such that their capacity to increase resistance to β-lactam antibiotics is dependent on the presence of other mutations in the mosaic alleles. We also identified an additional mutation (N512Y) that contributes to resistance to expanded-spectrum cephalosporins. Lastly, we investigated the effects of a mutation (A501V) currently found only in non-mosaic penA alleles on resistance to expanded-spectrum cephalosporins, under the expectation that this mutation may soon appear in mosaic alleles. When the penA35-A501V allele was transformed into FA6140, a chromosomally mediated, penicillin-resistant isolate, the MICs of ceftriaxone and cefixime (0.5 and 1.2 μg/ml, respectively) increased to levels above their breakpoint (>0.25 μg/ml). The structural mechanisms of these mutations are examined in light of the recently published structure of PBP 2.

OM39

Introduction of a new Group A meningococcal conjugate vaccine (MenAfriVac) in the African meningitis belt

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Epidemic meningitis due to Group A Neisseria meningitidis remains an important public health problem in Africa. Reactive vaccination campaigns with meningococcal polysaccharide vaccines have not resolved this problem. With Gates Foundation support the Meningitis Vaccine Project was created in 2001 as a partnership between PATH and WHO with the specific goal of eliminating Group A meningococcal epidemics through the development, testing, licensure and introduction of a new and affordable (< $US 0.50 per dose) MenA conjugate vaccine. MenAfriVac, a new MenA conjugate vaccine has been developed through an innovative public/private partnership and manufactured at Serum Institute of India using a conjugation method originally discovered at CBER/FDA, Bethesda. Clinical trials in India and Africa have shown the vaccine to be as safe and immunologically superior to a Group A polysaccharide vaccine. In December 2009 the vaccine was granted market authorization by the Drugs Controller General of India and WHO prequalification is expected in June 2010. Introduction of the MenA conjugate vaccine in large "catch up" vaccination campaigns in 1-29 year olds is planned in Burkina Faso, Mali and Niger in Q4 2010. The campaigns are expected to generate herd immunity. Over the next decade about 250 million persons are targeted to receive this vaccine and over 1 million cases of Group A meningococcal meningitis and well over 100,000 deaths are expected to be prevented. Funds are available for the 2010-2011 campaigns in Burkina Faso, Mali and Niger but identifying new funds to support introduction in other African countries will be challenging.

OM40

Optimisation of schedules for meningococcal serogroup C conjugate vaccines driven by new immunogenicity and epidemiological data

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Background: In 1999, in the UK, MCC vaccines, from 3 different manufacturer's, were introduced at 2,3,4 months of age with a single dose for children 1 to 18 years. In 2006 this changed to 3, 4, 12 months of age. Recent data have demonstrated antibody persistence following the 12 month booster dose was surprisingly poor, suggesting further booster doses may be required.Also, that 2 of the 3 MCC vaccines were sufficiently immunogenic to be used as a single priming dose in infancy with a booster at 12 months.

Methods: A randomised study was undertaken with one MCC-TT and one MCC-CRM197 vaccine in 146 infants immunised at 3 months of age with a booster of combined MCC/*Haemophilus* influenzae type b at 12 months.

Results: The serum bactericidal antibody (SBA) geometric mean titre (GMT) one month following a single dose of MCC-TT or MCC-CRM 197 was 214.7 (95% CI156.8-294.0) and 100.6 (95% CI 70.4 to 143.7) with 100% and 95.7% of infants with SBA titres ≥ 8, respectively. Pre-booster, antibody levels had declined and 1 month post Menitorix booster, SBA GMTs rose to 2251.0 (95% 1546.2-3276.9) and 399.1 (95% CI 263.8-603.9) for children primed with MCC-TT and MCC-CRM 197 respectively.

Discussion: Enhanced surveillance is continuing in the UK and has identified three '2+1' vaccine failures being reported during the winter of 2009/10. Modelling has shown that the herd effects seen in the UK is likely to last until 2014/15, thus there is no urgent need for further boosters to be introduced though further novel schedules will be discussed.

OM41

Age-Related Immune Responses Following *Neisseria meningitidis* serogroup C Conjugate Vaccination in the Netherlands: a pre- and post-vaccination survey

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Objective: In 2002 a MenC conjugate (MenCC) vaccination was introduced at the age of 14 months and a mass catch-up campaign was performed targeting individuals aged between 1 and 18 years. We determined age-related immune responses before and after introduction of the MenCC vaccine.

Methods: In two population-based studies, established in pre- and post-vaccination periods, polysaccharide-specific IgG, IgM, IgG subclasses and avidity were determined by a multiplex immunoassay. Also, in a subset MenC-specific serum bactericidal antibody titers were determined.

Results: Overall SBA seroprevalence was 22% and 45% in the pre- and post-vaccination period, respectively. SBA titers and PS-specific IgG show an age-specific trend, with the highest antibody persistence in the oldest vaccinated age-groups. SBA seroprevalence is not significantly different between the pre- and post-vaccination periods in unvaccinated adult groups, whereas the MenC PS-specific antibodies are. In all immunized age-groups higher levels of IgG1 compared to IgG2 were observed, while naturally derived immunity was mainly restricted to the IgG2 subclass. An age-related increase in IgM levels was observed, correlating with the persistence of IgG antibodies with age. Noteworthy, the increase in IgG2 correlated with a reduced IgG-avidity with age.

Conclusions: MenCC vaccination induced higher IgG levels compared to natural exposure, but only older vaccinated age-groups seem to benefit from antibody persistence. Due to mass vaccination, MenC circulation probably decreased, resulting in lower IgG titers in the unvaccinated older age-groups, posing them at risk if MenC starts re-circulating. The humoral MenCC vaccine response appeared to be a mixture of both TD and TI responses.

OM42

A mutant meningococcal factor H binding protein that eliminates factor H binding enhances protective antibody responses

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Factor H binding protein (fHbp) is part of two meningococcal group B vaccines being investigated in humans. The protein specifically binds the human complement inhibitor, factor H (fH). When humans are immunized, but not mice or most non-human primates, the vaccine binds fH. To determine the effect of human fH on antibody responses to fHbp, we immunized human fH transgenic or wild-type mice with recombinant fHbp vaccine. Control transgenic and wild-type mice were immunized with a meningococcal group C polysaccharide diphtheria-CRM197 conjugate vaccine that did not bind fH. There were no significant differences in the respective serum IgG anti-capsular or anti-CRM antibody titers, or bactericidal antibody responses ($P>0.5$). There also were no significant differences in the respective IgG or bactericidal responses to a control mutant fHbp vaccine with one amino acid substitution, which did not bind fH ($P>0.5$). In contrast, transgenic mice immunized in two studies with a fHbp vaccine that bound human fH had 2- to 3-fold lower IgG anti-fHbp responses than wild-type mice ($P\leq0.05$), and 3- to 8-fold lower bactericidal responses ($P\leq0.05$). The serum human fH concentrations of the transgenic mice correlated inversely with serum bactericidal responses to the fHbp vaccine that bound fH ($r= -0.68$, $P=0.01$), but not with responses to the mutant vaccine that did not bind fH ($r=+0.17$; $P=0.58$). Thus, the lower antibody responses of transgenic mice immunized with the vaccine that bound human fH resulted from fH binding, and mutant fHbp molecules that do not bind fH may be superior vaccine candidates in humans.

OM43

Evaluation of the adjuvant effects of novel meningococcal detoxified lipopolysaccharide structures formulated in native outer membrane vesicles

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Background and Objectives: Inactivation of lpxL1 in Neisseria meningitidis results in the loss of an acyl chain within the lipid A moiety of lipopolysaccharide (LPS). Penta-acylated LPS shows lowered endotoxicity whilst retaining the adjuvant properties of hexa-acylated LPS. This detoxification allows safe delivery of native outer membrane vesicles (nOMVs) with abundant LPS. Secondly, structural differences within the oligosaccharide moiety of LPS affect dendritic cell stimulation (Steeghs L. et al. Cell Microbiol. 2006:8(2);316-25).

Therefore, we investigated the adjuvant properties of native OMVs with various glycoforms of penta-acylated LPS when co-administered with recombinant PorA P1.7-2,4 in mice.

Methods: We investigated N. meningitidis strain H44/76 (B:15:P1.7,16:L3,7,9) and three derived mutants expressing LPS with decreasing lengths of the oligosaccharide core, respectively H44/76-lgtB, H44/76-lgtE and H44/76-icsB. The lpxL1 gene was inactivated and nOMVs produced from these mutants were co-administered with PorA P1.7-2,4 in mice. IgG levels were measured using an ELISA against nOMVs produced from the meningococcal strain BZ198 (B:15:P1.7-2,4). The serum bactericidal activity was measured against BZ198 using 0.5% human complement and sera at a concentration of 8%.

Results: Mice immunised with nOMVs from H44/76-lgtB-lpxL1 and PorA P1.7-2,4 showed the largest IgG response, approximately six-fold greater than mice immunized with Alhydrogel as an adjuvant and PorA P1.7-2,4. Only antibodies elicited using nOMVs from H44/76-lgtB-lpxL1 were found to be bactericidal.

Conclusion: Native OMVs with penta-acylated lgtB LPS was the most effective adjuvant. Our results warrant further investigation of penta-acylated lgtB LPS as a promising adjuvant to recombinant protein vaccines.

OM44

Using population biology to inform vaccine design

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Background: PorA and FetA are meningococcal vaccine candidates due to their natural presence in OMVs, expression in most meningococci, immunogenicity, including their ability to elicit bactericidal antibodies, and their structured antigenic diversity. Structuring manifests in a relatively small number of PorA and FetA types found amongst most disease isolates. The association of antigenic epitopes appears in a non-random non-overlapping fashion and there is also some association of types with clonal complex. A comprehensive collection of meningococcal disease isolates from the years 2000-2002 from 18 European countries were sequence typed for PorA and FetA VRs and by MLST. These sequence data were merged with epidemiological information (serogroup, age, sex) collected separately for the isolates by the EMEC/EU-IBIS project. These data formed the basis upon which a vaccine recipe could be formulated which gave as wide coverage as possible in the EUMenNet dataset.

Results and Summary: There were strain type (serogroup, PorA, FetA, ST) data available for 2456 isolates. A multivalent vaccine formulation that corresponded to the most common antigens representing the main hyperinvasive lineages associated with disease in Europe (ST-11, ST-32, ST-41/44, ST-8 and ST-269 complexes) was devised. These antigens are also found in stable associations with each other so the most common PorA/FetA strain types in the recipe were non-overlapping. The vaccine recipe contained four each of PorA and FetA antigens. Together, these antigens made up 78.8% of the disease isolates in the EU-MenNet dataset which is also the theoretical coverage attained by a cocktail vaccine containing them.

OM45

Immunoproteomics and pan-meningococcal vaccine antigen discovery

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Objective: To use proteomics and immunoproteomics to identify conserved meningococcal antigens that represent potential vaccine antigens and to test their ability to induce serum bactericidal activity against a wide range of heterologous meningococci.

Methods & Results: Outer membrane preparations from meningococci were subjected to proteomic analysis using GeLC-MS/MS, revealing an unexpected extent of protein diversity. The outer membranes were subject to immunoproteomic analysis using sera from individuals taken before and after colonization with serogroup B meningococci. In this way it was possible to identify antigens that were associated with the development of increased serum bacteridical activity against heterologous strains which always followed colonization. These methods produced a catalogue of antigens which had not previously been studied for their vaccine potential. Bioinformatic analysis was then used to prioritize the antigens for further study.

One such protein of ~30KDa was identified as a potential virulence antigen which showed ≥98% homology between meningococci of different serogroups, serotype and subtypes. The 30K protein was cloned into *E.coli* using the PRSET vector and expressed with a His tag to facilitate purification by nickel affinity chromatography. The purified protein was incorporated into human compatible vaccine formulations and used for immunisation of mice. The resulting antisera recognized the 30K protein on western blot and reacted strongly in immunofluorescence with meningococcal cells. Most importantly the antisera showed high levels of bactericidal activity against homologous and heterologous meningococcal strains.

Conclusions: Proteomic methods have facilitated the identification of novel proteins with the potential to induce a cross-protective immune response against serogroup B meningococci. One such protein induces a bactericidal immune response which targets an antigen that is highly conserved across meningococcal serogroups, serotypes and subtypes.

OE01

Baseline meningococcal carriage in Burkina Faso before introduction of a meningococcal serogroup A conjugate vaccine

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The serogroup A Neisseria meningitidis conjugate vaccine, MenAfriVac, has the potential to confer herd immunity by reducing carriage prevalence of epidemic strains. To measure this potential effect we initiated a meningococcal carriage study to determine the baseline carriage rate before vaccine introduction in the 1-29 years old African population in Burkina Faso, eligible for the first introduction of the vaccine.

A multiple cross-sectional carriage study was implemented in one urban and two rural districts in Burkina Faso in 2009. Every 3 months, oropharyngeal samples were collected from > 5000 randomly selected individuals within a 4-week period. Isolation and identification of the meningococci from 20,326 samples were performed by national laboratories in Burkina Faso. Confirmation and further strain characterization, including genogrouping, multilocus sequence typing, and porA/fetA sequencing, were performed at the Norwegian Institute of Public Health.

The overall carriage prevalence was 3.98%. Serogroup Y dominated (2.28%), followed by serogroups X (0.44%) and A (0.39%). Carriage prevalence was highest in the rural districts and in the dry season, but serogroup

distribution also varied by district. A total of 29 sequence types and 51 porA/fetA combinations were identified, The dominant strain was serogroup Y, ST-4375, P1.5-1,2-2;F5-8, belonging to ST-23 complex (47%). All serogroup A isolates were ST-2859, P1.20,9;F3-1.

Meningococcal carriage rates and particularly serogroup A were low in the 1-29 population in Burkina Faso in 2009, a year with relatively little disease. Nonetheless, the sample size should be sufficient to detect a 50% reduction of serogroup A carriage following vaccine introduction.

OE02

Optimal molecular characterisation of meningococci in conjunction with enhanced molecular surveillance of the vaccine candidate, fHBP.

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Objective: Current recommendations for molecular typing of meningococci include (i) MLST, to guide national and international disease management, whilst facilitating studies of population biology and evolution, and (ii) sequence analysis of porA variable regions (VRs) 1 and 2, and the fetA VR, for monitoring antigenic distribution and investigating potential outbreaks. porB genotyping is recommended for additional resolution as required. Several broadly cross-reactive investigational vaccines (at or approaching phase III clinical trials) incorporate factor H binding protein (fHBP). Enhanced surveillance of fhbp would place additional financial and labour pressures on reference laboratories. This study investigates the optimal and most efficient molecular typing schemes for routine meningococcal characterisation and the investigation of potential outbreaks, in conjunction with the enhanced surveillance of fhbp.

Methods: Invasive disease isolates received by the Meningococcal Reference Unit between July 2007 and June 2008 (n=613) were characterised in terms of capsular group, porA, fetA VR, fhbp and MLST.

Results: Capsular grouping and porA genosubtyping left several large unresolved blocks of isolates, comprising up to 17.5% of the total isolates studied. fetA and fhbp provided comparable, but limited, degrees of additional resolution, and partially complemented one-another in this respect. MLST afforded markedly superior resolution overall.

Discussion: Enhanced surveillance of fhbp may serve to substitute for routine fetA VR typing as a means for monitoring antigenic distribution, thus reducing costs. MLST constitutes the optimal scheme for investigating outbreaks where a) typing data are unavailable for the index case or b) the index case falls within a known, large group:porA block.

OE03

Changes in the carriage of serogroup Y meningococci amongst university students in the United Kingdom

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Objective: In 1997, 14% of first year students registering at Nottingham University were meningococcal carriers. This increased to 31% by November and remained at similar levels throughout the academic year*. Amongst serogroupable isolates, serogroup B was most commonly detected**. To determine whether the dynamics of carriage had changed, we performed epidemiological studies between November 2008 and December 2009 in students at Nottingham University.

Methods: Pharyngeal swabs were obtained, cultured on selective media and meningococci characterized using molecular techniques.

Results: In November 2008, 47% of first year students were carriers, which rose to 62% in May 2009. 44% of carriers in November were colonised with serogroup Y strains; only 12% with serogroup B strains. This distribution was maintained throughout the 08/09 academic year. In the following academic year, 23% of first year students were carriers in September which increased to >55% in December. Carriage rates were also high in second and third year students in September (34% and 39%, respectively), but did not show a significant change in December.

Carriage of serogroup Y strains again dominated; notably an increase in serogroup Y carriage in first year students from 13% (September) to 44% (December) of isolates was detected.

Conclusions: Carriage rates were higher than those previously reported. High carriage rates for serogroup Y strains suggest a shift in meningococcal population structure and lack of immunity to serogroup Y strains. We postulate that vaccination of UK students with a serogroup Y conjugate vaccine should be considered.

*BMJ 2000;320:846-9.
**J Clin Microbiol 2000;38:2311-6.

OE04

Complex network of interactions involved in base excision repair in *Neisseria meningitidis*

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Base excision repair (BER) plays a vital role in repairing DNA damaged by oxidative stress. While BER has been extensively studied in model organisms such as Escherichia coli, little is known about the role and integration of BER enzymes in obligate human pathogen, such as Neisseria meningitidis (Nm), which are continually subjected to oxidative stress as a component of the innate immune system. We have investigated BER in the meningococcus through a combination of genetic, biochemical, and structural studies.

Here we examined the role of the bi-functional DNA glycosylases, formamidopyrimidine-DNA glycosylase (MutM) and endonuclease III (Nth), and show that these enzymes can compensate for the absence of each other in N. meningitidis as they have overlapping substrate specificities. We also studied the regulation of these BER enzymes under different conditions, and confirmed that Nm lacks an SOS response. Furthermore we defined the interaction between MutM and Nth with NApe and NExo, other enzymes involved in BER pathways. Our findings reveal novel enzymatic functions which are essential for completion of BER and provide insights into the interplay between different enzymes which leads to successful DNA repair, via a network of interactions rather than distinct pathways.

OE05

The Gonococcal biofilm matrix contains DNA and an endogenous nuclease controls its incorporation.

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Neisseria gonorrhoeae is a gram-negative diplococcus bacterium and a strict human pathogen. N. gonorrhoeae can form biofilms within the female urogenital tract with one of the components of the biofilm matrix being bacterial outer membranes. Through the use of confocal microscopy and electron microscopy, we now have microscopic and enzymatic evidence indicating that DNA also makes up a substantial portion of the N. gonorrhoeae biofilm matrix. When DNase I is added to established N. gonorrhoeae biofilms, the biofilm structure is rapidly degraded. We have identified a nuclease (nuc) in the N. gonorrhoeae genome in a previously unannotated gene with homology to the secreted Staphylococcus aureus thermonuclease. We have expressed the N. gonorrhoeae nuclease homolog in E. coli and demonstrated its ability to digest DNA. Cell extracts of the wildtype gonococci exhibit nuclease activity while a nuc deletion mutant has no nuclease activity. Nuclease activity could not be detected in 1000x concentrated culture supernates after four, eight and 24 hours of growth of the wildtype strain. The Nuc enzyme is heat stable and requires divalent cations for activity. The nuc mutant forms biofilms of significantly greater thickness than the parent strain and also contains significantly more DNA in the biofilm matrix. Chromosomal complementation of the mutation results in restoration of the wildtype biofilm phenotype. We hypothesize that the nuclease plays a role in remodeling of the gonococcal biofilm and modulates release of gonococci from the biofilm.

OE06

*Igor Stojiljkovic Memorial Award Winner**

In vitro biofilm formation as a model for meningococcal carriage and transmission

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Biofilm formation by Neisseria meningitidis serves as a model for asymptomatic nasopharyngeal carriage (reviewed by Neil and Apicella, 2010; Lappann and Vogel, 2010). The inhibitory effect of the polysaccharide capsule, the role of pili, and the changes of the transcriptome during biofilm growth have been investigated recently. In the present study, the impact of extracellular DNA (eDNA) on biofilm formation was investigated. eDNA proved to be the key factor for initial biofilm formation of most, but not all clonal lineages and enhanced fitness in mixed biofilm experiments. Interestingly, during the course of biofilm formation the importance of proteins for biofilm integrity increases (unpublished). eDNA utilization in early biofilms and autolysis served to categorize the meningococcal population into spreaders and settlers by inference from molecular epidemiological data (Lappann et al., 2010). To further unravel the adaptation of meningococci to the unique biofilm state, we conducted proteome and transcriptome analyses. The studies revealed that meningococcal biofilms undergo metabolic adaptation, e.g. by reduced expression of enzymes of the citrate cycle. Interestingly, alterations of expression of several proteins including the outer membrane protein Opc suggest reduction of AsnC regulator binding, most likely due to reduced availability of nutrients. Oxidative stress response genes were up-regulated (bacterioferritin A/B and MntC). MntC mutation specifically impaired biofilm formation. We hypothesize that biofilm formation might strengthen meningococci to cope with oxidative stress elicited by the host immune response. Transcription of genes involved in pilus assembly and functionality was down-regulated which correlated with reduced piliation and motility.

OE07

Alternative pathway activation on *Neisseria meningitidis* reveals paradoxically enhanced C3 deposition on encapsulated groups W-135 and Y strains

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Capsular polysaccharide (CPS) is an important meningococcal virulence factor. The molecular basis of regulation of the alternative complement pathway (AP) by meningococci is unclear and formed the basis for this investigation. Encapsulated groups A, B, C, W-135 and Y strains and their isogenic unencapsulated counterparts were studied; all strains lacked both identified factor H (fH; AP inhibitor) ligands (fHbp and NspA) and lipooligosaccharide (LOS) sialylation, which minimized confounding by membrane-bound fH or LOS sialic acid. C2-depleted serum was used to selectively activate the AP. Consistent with previous studies, groups B and C CPS expression inhibited C3 deposition. However, the amount of C3a generated (a measure of C3 activation) by encapsulated and unencapsulated groups B and C strains were similar, suggesting that these CPSs blocked C3 deposition. Expression of group A CPS had no effect on C3 deposition or C3a generation. Paradoxically, marked C3 deposition on, and C3a generation by, encapsulated W-135 and Y strains occurred as early as 10 min; at this early time point minimal C3 activation and deposition occurred with unencapsulated mutants. W-135 and Y CPSs themselves served as a site for C3 deposition, which was confirmed using immobilized purified W-135 and Y CPSs. Purified CPSs bind to unencapsulated meningococci and, surface-bound W-135 and Y CPSs simulated findings with naturally encapsulated strains. These data highlight the heterogeneity of AP activation on the different meningococcal serogroups and may affect the pathogenicity of the different serogroups, in particular in certain complement–deficiency states where groups W-135 and Y strains predominate.

OE08

Human airway epithelial cell responses to *N. lactamica* and to purified Nlac porin.

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Neisseria lactamica colonizes the human nasopharynx but is not usually associated with disease. Upon colonization of the human airway epithelium, bacterial outer membrane components interaction with host cell receptors, such as Toll-like receptors (TLRs), influence activation of local and systemic host defense mechanisms. In particular, TLR2 recognizes the PorB porin, but the mechanism(s) of this interaction are unknown. A recent analysis of the crystal structure of meningococcal PorB has suggested a potential mechanism of electrostatic interaction with TLR2. Although porins are the major Neisserial OMPs and are expressed regardless of organism's pathogenicity, there is significant sequence variability within surface-exposed loop regions among strains.

Nlac PorB is also recognized by TLR2 and induces TLR2-dependent cell activation. However, its binding specificity for TLR2 in vitro and its interaction with human airway epithelial cells is dissimilar from that of meningococcal PorB. Furthermore, Nlac PorB induces only a low and transient TLR2-dependent pro-inflammatory cytokine secretion and a modest TLR2 up-regulation over time in human airway epithelial cells. Analysis of the surface-exposed regions of Nlac PorB reveals significant residues difference in some surface-exposed loops which might be crucial for Nlac PorB interaction with TLR2 and subsequent induction of cellular responses. A similar effect is observed in response to whole N. lactamica organisms in human airway epithelial cells. Regulation of TLR2 expression mitigates exacerbation of human airway epithelium inflammation induced by bacterial ligands from commensal organisms. This could be a potential negative feedback mechanism against local inflammation arising following colonization with N. lactamica.

OE09

*Igor Stojiljkovic Memorial Award Winner**

N. lactamica attenuates TLR-2 dependent epithelial inflammatory responses by reducing nuclear Nf-kappaB activity using PPAR-gamma

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Our objective was to investigate the mechanism and specificity of innate attenuation elicited by commensal Neisseria in nasopharyngeal derived epithelial cells.

Commensal N. lactamica (Nl), N. polysaccharea (Np) and N. cinerea (Nc) were compared for their ability to suppress nasopharyngeal epithelial inflammatory responses to pathogenic Neisseria meningitidis serogroup B (NmB). The specificity of Nl attenuation was examined against pro-inflammatory TLR ligands (LPS, Pam3Cys, LTA); cytokines (IL-1beta and TNF-alpha) and pathogenic S. pneumoniae (Sp). Cytokine mRNA levels, nuclear Nf-kappaB activity and pro-inflammatory cytokines/chemokines (in the presence and absence of PPAR-gamma agonist) were quantified by qPCR and ELISA and used to elucidate the mechanism of attenuation.

Of the commensal Neisseriae, only Nl significantly attenuated inflammatory IL-6 and RANTES responses to NmB, reaching levels of up to 75% suppression. Nl was found to significantly attenuate responses to stimulation with TLR-2 ligand Pam3Cys, pro-inflammatory cytokines and Sp. Nl was unable to attenuate responses to LPS and our Detroit cells were unresponsive to LTA. NmB-induced nuclear Nf-kappaB activity was significantly reduced by Nl, as were mRNA levels of IL-6, IL-8 and RANTES. Cytokine attenuation was released in the presence of PPAR-gamma agonist, suggesting a role for PPAR-gamma in the reduction of nuclear Nf-kappaB activity.

We have demonstrated for the first time that the ability commensal Neisseria to attenuate mucosal inflammation is restricted to N. lactamica. The pathway of attenuation is evident through TLR-2, IL-1alpha and TNF-alpha signalling, and involves suppression of nuclear Nf-kappaB activity, by a mechanism involving the nuclear to cytoplasmic shuttle, PPAR gamma.

OE10

Neisseria meningitidis NHBA, a heparin-binding protein that induces protective immunity in humans

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Neisserial Heparin Binding Antigen (NHBA, also named GNA2132) is a surface-exposed lipoprotein of Neisseria meningitidis (Nm) expressed by genetically diverse strains. NHBA induced bactericidal antibodies in animal models and is a component of a multicomponent protein-based vaccine. Direct evidence that NHBA induces bactericidal antibodies in humans is lacking.

To investigate whether NHBA is expressed in vivo during infection we analyzed 22 patients convalescing after meningococcal disease. All of the sera tested recognized NHBA protein suggesting that it is immunogenic during disease.

To examine whether NHBA contributes to the immunogenicity of the multicomponent vaccine, we constructed a target meningococcal strain susceptible only to anti-NHBA antibodies. In a serum bactericidal assay, sera from adults immunized with the test vaccine killed the target strain, indicating that NHBA induced bactericidal antibodies in humans.

Biochemical, genetic and cellular approaches were applied to understand the function of the protein. We proved that the protein is able to bind heparin in vitro through a conserved Arg-rich region. In the absence of the bacterial capsule, heparin binding mediated by NHBA is associated with increased survival of Nm in human serum and might facilitate adherence to host tissues by binding to glycosaminoglycans. In order to prove the potential role of NHBA in host-cell interaction, we generated nhba deletion mutants in a panel of Nm strains and they are currently under characterization using an adhesion assay.

Our results suggest that NHBA is an important protective antigen involved in the pathogenesis of Nm.

OE11

Using lipo-oligosaccharide modification to generate a novel vaccine candidate to protect against serogroup B meningococcal disease

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Objectives: To develop a natural outer membrane vesicle (NOMV) vaccine, with a modified LOS structure, designed to facilitate presentation of a broad range of meningococcal antigens and to optimise stimulation of the immune system with minimal toxicity.

Results: We engineered an unencapsulated strain of Neisseria meningitidis that expresses pentacylated lipid A (lpxL1), conferring reduced toxicity, and lgtB oligosaccharide structure that targets meningococcal epitopes to antigen presenting cells (siaD/lgtB/lpxL1). Single and double deletion mutants (siaD/lpxL1, siaD/lgtB, siaD) were used as comparators. NOMVs were isolated and characterised.

The immunogenicity of the NOMVs was assessed using a murine model. All the NOMVs induced good IgG antibody production and serum bactericidal activity (SBA) was observed against the homologous strain. SBA was also observed against an isogenic porA deficient mutant showing that the NOMVs induce bactericidal antibodies against bacterial components other than PorA.

The inflammatory properties of the NOMVs were assessed using a human dendritic cell (DC) in vitro assay. All NOMV preparations induced DC maturation and production of TNFα, IL-1β, IL-6, and IL-10, although those expressing lpxL1 were less potent stimulators than NOMV expressing wild type lipid A. NOMV with the lgtB/lpxL1 structure were significantly more potent stimulators of DC than NOMV with lpxL1 and wild type oligosaccharide, suggesting that this lgtB modification enhances the stimulatory capacity of pentacylated lipid A.

Discussion: The ability of the siaD/lpxL1/lgtB NOMV preparation to effectively induce humoral responses in mice and stimulate human DC maturation and cytokine responses makes this a vaccine candidate worthy of further study

OE12

The Gonococcal Pilin Glycan Mediates Primary Cervical Epithelial Cell Challenge.

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Expression of Type IV pili by Neisseria gonorrhoeae plays a critical role in mediating adherence to human epithelial cells. Gonococcal pilin is modified with an O-linked glycan, which may be present as a di- or monosaccharide because of phase-variation of select pilin glycosylation genes. It is accepted that bacterial proteins may be glycosylated; less clear is how the protein glycan may mediate virulence. Using primary, human, cervical epithelial (i. e. pex) cells, we now provide evidence to indicate that the pilin glycan mediates productive cervical infection. These data are in contrast to our parallel works in which the pilin glycan and the phosphorylcholine substitution both play a role in mediating the adherence of Neisseria meningitidis to host epithelial cells. In this regard, pilin glycan-deficient mutant gonococci exhibited an early hyper-adhesive phenotype but were attenuated in their ability to invade pex cells. Our data further indicate that the pilin glycan was required for gonococci to bind to the I-domain region of complement receptor 3, which is naturally expressed by pex cells. Comparative, quantitative, infection assays revealed that mutant gonococci lacking the pilin glycan did not bind to the I-domain when it is in a closed, low-affinity, conformation and cannot induce an active conformation to complement receptor 3 during pex cell challenge. To our knowledge, these are the first data to directly demonstrate how a protein-associated bacterial glycan may contribute to pathogenesis.

OE13

Structural Studies of the Lactoferrin Binding Proteins from *Neisseria meningitidis*

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Neisseria meningitides are host-restricted respiratory pathogens that cause a spectrum of infections including meningitis. Currently most vaccines against these pathogens are based on the immunogenicity of the capsular polysaccharides. However, capsular polysaccharides of N. meningitides group B, the most virulent group of these bacteria are poorly immunogenic. An alternative approach is to use protein antigens widely presented on the bacterial cell surface.

Lactoferrin binding proteins (Lbp) are bacterial conserved outer-membrane proteins responsible for the iron uptake directly from host lactoferrin (Lf). LbpB is considered an attractive candidate for vaccine development due to its essential role in bacteria survival. Lbp consists of two proteins: LbpA, an integral outer-membrane protein; and LbpB, a bi-lobed peripheral membrane protein which assemble into an oligomeric complex. Protein crystallography studies of both proteins should provide molecular details of the Lf binding determinants on Lbp and the structure of iron-binding site which is essential for vaccine design.

Various LbpB constructs including intact proteins, N-lobes, C-lobes from different strains of N. meningitides were generated. Purification was optimized with general chromatography columns. Crystals of LbpB N-lobe diffracting to 2.15 Å were obtained and the structure of the protein was solved using TbpB (Transferrin-binding protein) as a molecular replacement search model. Future experiments include crystallization of the intact LbpB and the iron receptor LbpA. Structural information for both proteins (LbpA and LbpB) will be used for vaccine development.

OE14

Neisseria gonorrhoeae Type IV pili change structure under force

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Neisseria Type IV Pili (Tfp) are long, thin, helical polymers extending away from the cell surface and are thus often the first line of interaction with the environment and among the most important factors of colonization. While the structure of Neisseria gonorrhoeae Tfp has been defined by conventional structural techniques, it remains difficult to explain the wide spectrum of functions associated with Tfp including: twitching motility, DNA uptake, human cell infectivity and immunogenic properties. Using a combination of optical and magnetic tweezers, AFM and molecular combing, we uncover a new force-induced quaternary structure of the Neisseria gonorrhoeae Tfp. We demonstrate that Tfp subjected to approximately 100 pN of force will transition into a new conformation. The new structure is roughly 3 times longer and 40% narrower than the original structure. Upon release of the force, the Tfp fiber regains its original form, indicating a reversible transition. Equally important, we show that the force-induced conformation exposes hidden epitopes previously buried in the Tfp fiber. We postulate that this transition provides a means for Neisseria gonorrhoeae to maintain attachment to its host while withstanding intermittent forces encountered in the environment. Besides, the existence of a force dependent polymorphism in Tfp opens a new door for the understanding of Tfp functions and immunogenicity.

OE15

The Role of Lipooligosaccharide in Triggering Invasion of *Neisseria meningitidis* into Epithelial Cells

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Invasion into host cells by meningococci is initiated by the opacity protein (Opa) engagement of the carcinoembryonic antigen cell adhesion molecules (CEACAMs). However, in ST-32 strain MC58 this process is inhibited by the expression of capsule and long lipooligosaccharide (LOS) structures. ST-8 strain NMB naturally expresses non-phase variable capsule and long chain LOS. Nonetheless, ST-8 strain NMB invades Detroit nasopharyngeal cells 10-fold better than ST-32 strain MC58. One feature that is present in strain NMB, but absent in MC58 is regulated control of the composition of the LOS structure by the contact dependent two component system regulator, MisRS. We investigated whether the LOS inner core composition could impact on attachment or invasion into host epithelial cells.

Removal of O-3 glucose and O-6 PEA groups from the LOS inner core alone or in combination resulted in 18-, 4- and 9-fold increases in invasion respectively. Removal of the capsule and sialylation of the terminal LOS resulted in further increases in invasiveness. The increase in invasiveness as a result of changes in the LOS inner core remained when tested in Opa minus, encapsulated backgrounds. Lastly the effect of LOS structure on meningococcal invasiveness was shown to be absent when a different cell line was utilized.

Therefore, we propose a second model for invasion of N. meningitidis into host cells whereby the LOS structure is a ligand for an unknown receptor. This interaction is a regulated event specific to ST-8 strains expressing L2/L4 LOS which is absent in other hypervirulent lineages of meningococci.

OE16

Differences in iron status and MtrA dominate stable physiological differences between gonococcal strains MS11 and FA1090

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An integrated gene complement, genome sequencing, phase variable status, and comparative transcriptomics strategy has been used to compare N. gonorrhoeae strains FA1090 and MS11. This identifies wide-ranging stable physiological differences that are reflected in differential expression of around a third of the common / core genes. The combined approach enables phase varied, positively regulated, reactively induced, and selected phenotypes to be distinguished, and the integrated function of the cell to be approached in a way that was not previously possible.

A central feature of the differences between these two strains is in iron responses and acquisition (which are not necessarily attributed to the presence of the GGI in MS11). This includes differential expression of ABC iron binding transporters, the presence of alternate tonB-dependent receptors, sulphate uptake, and differential expression of recognizable components of the iron restriction / replete fur-dependent responses. In addition, the differences between the physiological status of these cells provides a potential explanation for the more stable expression of Opa and Pilus in strain FA1090, and several surface-associated properties. There are also wide-ranging direct and indirect effects that are attributable to a point mutation within mtrA in FA1090, with influences that extend through reactive changes in mtrR and farR.

This 'systems biology' approach reveals a nature and scale of differences between the strains that highlights the limited informational value of 'single level' studies, demonstrates a powerful approach for the investigation of the underlying differences between strains, and which highlights the inter-dependence of the 'core' and 'non-core' genomes.

OE17

Genome Sequencing Reveals Widespread Virulence Gene Exchange Among Human *Neisseria* Species

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Commensal bacteria comprise a large part of the microbial world, playing important roles in human development, health and disease. However, little is known about the genomic content of commensals or how related they are to their pathogenic counterparts. The genus Neisseria, containing both commensal and pathogenic species, provides an excellent opportunity to study these issues. We undertook a comprehensive sequencing and analysis of human commensal and pathogenic Neisseria genomes. Commensals have an extensive repertoire of virulence alleles, a large fraction of which has been exchanged among Neisseria species. Commensals also have the genetic capacity to donate DNA to, and take up DNA from, other Neisseria. Our findings strongly suggest that commensal Neisseria serve as reservoirs of virulence alleles, and that they engage extensively in genetic exchange.

OE18

Pangenomic anatomy of a species: *Neisseria meningitidis* population structure and dynamics

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N. meningitidis population is structured into groups of evolutionarily related strains, named clonal complexes in the MLST framework, and is characterized by a significant degree of homologous recombination. Consistency of MLST predictions at the whole genome level, origin of clonal complexes and mechanisms responsible for their coexistence with a panmictic background, are largely debated. For a systematic analysis, a meningococcal pangenomic sampling was conducted, comprising fifteen new and five public genomes spanning five serogroups and ten clonal complexes.

Analysis of the core genome confirmed MLST complexes and revealed robust phylogenetic relationships between them. Therefore we propose a “phylogenetic clade” concept for this species, that may include more clonal complexes. Bayesian reconstruction of the positional dynamics in the eleven genomes sequenced to closure, shows that specific chromosomal rearrangements with a potential impact on the host-pathogen interaction can be associated with the clades identified.

Conversely, in the dispensable genome the phylogenetic signal tends to be obscured by horizontal DNA exchange. Distribution of insertion sequences is substantially unrelated to the phylogeny of the species. However, the presence/absence pattern of twenty-two putative restriction/modification (RM) systems, all identified in the dispensable genome, accurately reconstructs the core-genome clades. Every clade is associated with a unique combination of RM-systems, and gene-conversion events identified by in-silico analysis between clades are significantly shorter than within clades, a typical signature of cleavage mechanisms acting on exogenous DNA during transformation.

Results suggest that chromosomal rearrangements and differential restriction barriers are key to the structure and dynamics of this population.

OE19

A critical threshold of meningococcal fHbp expression is required for increased breadth of protective antibodies elicited by native outer membrane vesicle (NOMV) vaccines prepared from mutants

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Objective: To develop improved group B vaccines, we prepared NOMVs (not detergent-treated) from mutants with attenuated endotoxin activity (ΔLpxL1) and over-expressed fHbp. Immunized mice developed broad bactericidal (SBA) responses but the amount of over-expressed fHbp required for optimal immunogenicity was unclear, and the relative contribution of antibodies directed at LPS or fHbp to SBA is controversial.

Methods: We prepared NOMV vaccines from ΔLpxL1 mutants of strain H44/76 with different levels of fHbp. One had 10-fold increased fHbp (10x) compared to wildtype 1X-fHbp expression, while a second mutant had 3x-fHbp. Control vaccines included NOMV from ΔfHbp mutants, and recombinant fHbp.

Results: The NOMV vaccines with 3x- or 10x-fHbp expression elicited 1.8-fold and 6-fold higher IgG anti-fHbp antibodies, respectively, compared to NOMV vaccine with 1X-fHbp. All vaccines elicited high SBA titers against the homologous vaccine strain. However, against three strains with heterologous PorA, the 10x-fHbp NOMV elicited higher SBA responses ($P<0.05$) against all three, as compared to rfHbp or the NOMV vaccine with 1X-fHbp, while the NOMV with 3x-fHbp gave higher SBA ($P<0.05$) to only one strain. Against PorA heterologous strains, SBA was directed primarily at fHbp as inferred from complete loss of SBA after adsorption of anti-fHbp antibodies, and SBA titers <1:10 in sera from mice immunized with NOMV from ΔfHbp mutants.

Conclusion: A critical threshold of increased fHbp expression in the NOMV is required for broad SBA responses. As evidence that NOMV vaccines from mutants may be effective in humans, immunized infant rhesus monkeys developed broad SBA responses (reported separately)

OE20

Antibody persistence of group A meningococcal conjugate vaccine (MenAfriVac ™) in 1-29 years old subjects from Africa and India

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The Meningitis Vaccine Project (MVP) was funded in 2001 as a partnership between WHO and PATH to eliminate meningococcal epidemics in Sub-Saharan Africa through accelerated development and introduction of meningococcal group A conjugate vaccine. The new MenA conjugate vaccine (PsA-TT) (SIIL Menafrivac ™), is licensed in India and prequalified by WHO. Menafrivac will be primarly used for mass vaccination campaigns in the African meningitis belt countries as a single dose in 1-29 years old population. We report here antibody persistence in subjects vaccinated at 1-29 years of age in Africa and India.

Methods: Subjects vaccinated at 12-24 months were followed up for two years after primary immunization and approximately one year after booster. Subjects vaccinated at 2-29 years were followed up for one year.

Results: Two years after primary vaccination, children vaccinated with PsA-TT followed by Hib had higher serum bactericidal antibody rSBA GMT (1314 95% CI 875-1972 vs 545 95% CI 311-958) and showed a higher proportion of subjects with rSBA ≥ 1:128 (97% 95% CI 89-100 vs 88% 95% CI 78-95) than those who received PsACWY vaccine followed by Hib. Fourteen months after booster subjects who had received 2 doses of PsA-TT had rSBA GMTs of 2721 (95% CI 1960-3777) vs 1314 (95% CI 875-1972) of those who had PsA-TT followed by Hib. The proportion of subjects with rSBA ≥ 1:128 was the same in both groups (98% - 95% CI 91-100).

One year after immunization 2-29 years old African subjects in PsA-TT group had higher rSBA GMTs than those in PsACWY group (2889 -95% CI 2643-3159 - vs 922- 95% CI 743-1143) as well as a higher proportion of subjects with rSBA ≥ 1:128 (99% - 95% CI 98-100) vs those in the PsACWY group (93% - 95% CI 90-96). Similar trends were observed for anti-PsA IgG GMCs and proportion ≥ 2 μg/ml. Results similar to those in African subjects were obtained in 2-10 years old Indian children

Conclusion: Two years after immunization with one dose of Menafrivac 12-23 months old African children had sustained antibody titres and these titres persisted better than in those vaccinated with PsACWY vaccine. Similar trends were observed in 2 -29 years old African subjects and 2-10 years old Indian subjects one year after immunization

In African children aged 12-23 months two doses of PsA-TT administered 10 months apart had no significant advantage over one dose.

OE21

Cooperative meningococcal serum bactericidal activity (SBA) between vaccine-induced human antibodies to factor H binding protein (fHbp) and *Neisseria* Heparin binding antigen (NHba)

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Three meningococcal antigens, fHbp, NadA and NHba (GNA2132), individually elicited SBA in mice. A vaccine with all three recombinant antigens elicited SBA in humans. The protective role of antibodies to individual antigens is unknown. Sera from 6 adults immunized with 3 doses of vaccine (no OMV) were adsorbed with immunoadsorbents containing fHbp, NHba, or recombinant human albumin (negative control). Adequacy of adsorption was monitored by ELISA. SBA (human complement) was measured against strain H44/76 with low NHba and high expression of fHbp variant 1 (that matched the vaccine v.1 antigen); and 3 strains with high NHba expression and fHbp v.2 mismatched to the vaccine fHbp. None of the strains had NadA. All six adults developed ≥4-fold SBA titer increases against strain H44/76 (GMT <1:4 baseline, to 1:149). Adsorption of anti-NHba antibodies had no effect on SBA titers against H44/76, which decreased >90% after adsorption of anti-fHbp v.1 antibodies. Four subjects showed ≥4-fold SBA titer increases against 3 strains with fHbp v.2 (GMT<1:4 to 1:6 baseline, to 1:20-1:41). The majority of the SBA was directed at fHbp in one subject, against NHba in another subject, while adsorption of either anti-fHbp or anti-NHba antibody removed the majority of SBA in two other subjects. In all four subjects, titers after adsorption of both antibodies ranged from <1:4 to 1:6. Conclusion: Nearly all of the protective antibodies against H44/76 were directed at fHbp v.1. Against strains with fHbp v.2, cross-reactive anti-fHbp v.1 antibodies cooperated with anti-NHba antibody to elicit SBA.

OE22

Protective and Immunoregulatory Role of Toll-like Receptor 4 in Experimental Gonococcal Infection of Female Mice

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The role of Neisseria gonorrhoeae endotoxin in the mucosal immune response to infection is unclear because epithelial cells of the lower reproductive tract may be deficient in TLR4 and the associated protein MD2. Here we investigated the contribution of TLR4-mediated signaling events during experimental murine gonococcal infection by comparing the colonization load and proinflammatory response in normal BALB/c and C.C3-tlr4LPS-d/J mice, which carry the Tlr4 Lpsd mutation that renders them unresponsive to endotoxin. We found no difference in the duration of infection in the two mouse strains; however, a significant difference in the colonization load reproducibly occurred on days 4-7 of infection with ~1-2 logs more bacteria recovered from C.C3-tlr4LPS-d/J mice compared to BALB/c mice ($p < 0.007$, repeated measures ANOVA). Interestingly, this time period corresponded to an influx of polymorphonuclear leukocytes (PMNs) that was significantly greater in C.C3-tlr4LPS-d/J mice (~40-60% PMNs/100 vaginal cells) compared to BALB/c mice (~5-10% PMNs/100 vaginal cells) ($p<0.001$). Studies with TLR2 ligands and bone marrow-derived macrophages showed TLR2 expression in the BALB/c-Lpsd strain was not significantly altered compared to BALB/c mice, and thus the increased PMN response in C.C3-tlr4LPS-d/J mice is more likely due to increased colonization rather than dysregulated signaling through TLR2. We also found that transcripts for RORGT, STAT3, IL-6, IL-17A, IL-21, IL-22 were highly upregulated in vaginal washes from in infected BALB/c versus C.C3-tlr4LPS-d/J mice. We conclude that TLR4 plays a protective role in controlling N. gonorrhoeae replication in vivo and is linked to the induction of IL17 responses.

OE23

Gonococcal resistance to neutrophil clearance

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Acute symptomatic infection with Neisseria gonorrhoeae generates a potent neutrophil-driven inflammatory response; however, gonorrheal neutrophilic exudates contain viable, culturable bacteria. The mechanisms used by N. gonorrhoeae to resist neutrophil clearance have been elusive but are beginning to be identified. We are exploring the molecular and cellular interactions between exponential-phase N. gonorrhoeae and adherent, chemokine-primed, primary human neutrophils. Under these conditions, neutrophils internalize N. gonorrhoeae, including unopsonized, Opa-negative bacteria. Fluorescence-based bacterial viability assays show that intact, viable bacteria are present at the neutrophil surface and inside phagosomes. Numbers of viable intracellular bacteria increase over time, suggestive of intracellular replication. To date we have identified three mechanisms that support N. gonorrhoeae resistance to neutrophil clearance. 1) Neutrophil antimicrobial activities are housed in cytoplasmic granules that deliver their contents to sites of bacterial attachment and entry. Our results indicate that granules are incompletely mobilized during N. gonorrhoeae infection, suggesting the bacteria may actively redirect neutrophil membrane trafficking. 2) N. gonorrhoeae possesses gene products (ngo1686, recN) that protect from killing by neutrophil-derived oxidants as well as neutrophil non-oxidative components such as LL-37 antimicrobial peptide and bactericidal/permeability increasing protein. 3) N. gonorrhoeae disarms neutrophil oxidative killing by suppressing cellular production of reactive oxygen species, a process requiring live, translationally-active bacteria and bacteria-cell contact. These findings emphasize the diverse and complementary approaches used by N. gonorrhoeae to survive the host inflammatory response.

OE24

Human Complement Computer Model (HCCM): An Agent Based System Implementation of the Classic, Alternative and MBL Complement Pathways

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The Complement system is the major effector in humeral immunity and far reaching in ability to affect other biological systems. In addition to a direct roll in the removal and destruction of pathogens, the Complement system can also invoke responses from a diversity of other important biological systems. These include cell activation, metabolism, signaling, growth, reproduction and differentiation.

HCCM utilizes an Agent Based System (ABS) approach to model Complement proteins and pathogen, within a simulated physics environment. The ABS approach is uniquely different from other modeling techniques in that a defining characteristic is no central control. Agents (proteins) are autonomous within the experimental environment and generally have a simple set of rules that govern their interaction with other agents and the environment. The conceptual framework afforded by ABS models make them well suited for illuminating emergent behaviour and provides intuitive tuning of localized interactions.

HCCM models the majority of the active fluid component proteins (including repressors) from the Classic, Alternative, MBL Complement pathways and represents over 75 different protein/complexes. It features a simulated physics environment, force implemented Brownian motion and a calibrated representation of a 3D interphase between solution proteins and hydrophobic pathogen surfaces due to solvent interactions. The model can be configured to run all the pathways at once, separately or any custom protein population.

Eventually modeling tools like HCCM and others, will be used not only as a Complement pathway explorative tool, but also more broadly, generate further understanding how these pathway mechanisms contribute to individual immunity.

OE25

Neisseria adhere to the uropod of human polymorphonuclear cells, suggesting a novel pilus dependent pathway to reach subepithelium

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Pathogenic Neisseria do not only survive inside polymorphonuclear cells (PMNs), they can also adhere to PMNs. By live-cell microscopy and SEM we show that microcolonies of both piliated, Opa-negative N. gonorrhoeae and piliated N. meningitidis adhere to the uropod of up to 25% of the freshly isolated human PMNs, thereby hiding at the rear from the phagocytic activity at the pseudopod. The adherence is specific to Neisseria since other bacteria including the Type IV expressing P. aeruginosa do not adhere to the uropod. The pilus receptor CD46 is most likely not involved since no colocalisation of CD46 is seen adjacent the adhered microcolony.

Further, this adherence is dependent on the bacterial capacity to form microcolonies and the expression of Type IV pili, since a PilT mutant shows high number of adherence, while a non-piliated phenotype does not. The uropod adherence is dependent on PilC, since PilC1 and PilC2 mutant meningococci reduce the number of PMNs carrying a microcolony at the uropod. Migration assays indicate that PMNs carrying a microcolony at the uropod are still fully motile and active and able to migrate between human epithelial cells.

We suggest a novel mechanism for the bacteria to hitchhike at the uropod of PMNs to reach subepithelium, thereby facilitating the spread of the pathogen.

OE26

Neisseria meningitidis induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8

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A crucial step in the pathogenesis of bacterial meningitis is the disturbance of cerebral microvascular endothelial function, resulting in blood-brain barrier (BBB) breakdown. Matrix metalloproteinases (MMPs) have been implicated in BBB damage in bacterial meningitis in several studies.

Here we show that infection of human brain microvascular endothelial cells (HBMEC) with Neisseria meningitidis induced an increase of permeability at prolonged time of infection. By investigating the mechanism of permeability changes we discovered an unexpected connection between pathogen-induced MMP secretion and disruption of cell-cell connections. A detailed analysis revealed that MMP-8 was involved in the proteolytic cleavage of the tight junction protein occludin, resulting in its disappearance from the cell periphery as demonstrated by immunofluorescence microscopy analysis and cleavage to a lower-sized 50-kDa protein in infected HBMEC. Abrogation of MMP-8 activity by specific inhibitors as well as transfection with MMP-8 siRNA abolished production of the cleavage fragment and occludin remained attached to the cell periphery. In addition, MMP-8 affected cell adherence to the underlying matrix. Injury of the HBMEC monolayer suggested the requirement of direct cell contact because no detachment was observed when bacteria were placed above a transwell membrane or when bacterial supernatant was directly added to cells. Inhibition of MMP-8 partially prevented detachment of infected HBMEC and restored BBB permeability. Together, we established that MMP-8 activity plays a crucial role in disassembly of cell junction components and cell adhesion during meningococcal infection.

OE27

Neisseria gonorrhoeae induce transactivation of EGFR for their invasion

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted infection gonorrhea, adheres to and invades genital epithelial cells. Here, we investigate host components that are used by the bacteria for their entry into epithelial cells. We found that gonococcal microcolony formation on the surface of two genital epithelial cell lines, HEC-1-B and ME180, caused redistribution of both epidermal growth factor receptor (EGFR) and ErbB2, a related family member. Both EGFR and ErbB2 were translocated from the basolateral to the apical membrane in polarized HEC-1-B cells and concentrated under the microcolonies. Gonococcal infection increased EGFR and ErbB2 phosphorylation, indicating activation of the receptors. Kinase inhibitors of EGFR and ErbB2 inhibited and increased bacterial invasion, respectively, without affecting gonococcal adherence or the recruitment of EGFR and ErbB2 to the microcolonies. A monoclonal antibody that blocks EGF binding to EGFR had no effect on the ability of gonococci to adhere to epithelial cells, but reduced their invasion by 50%, suggesting that gonococci do not interact directly with EGFR. EGFR ligands are shed from the cell surface via cleavage by matrix metalloproteinases (MMP). Inhibiting ligand shedding by the MMP inhibitor, Batimastat, or removal of MMPs via heparin washes both reduced gonococcal invasion without altering their adherence. These data indicate that N. gonorrhoeae modulates the activity and cellular distribution of host EGFR and ErbB2, leading to their invasion. This EGFR activation does not appear to be due to direct gonococcal binding to EGFR, but instead by its transactivation by gonococcal induced increases in EGFR ligands.

OE28

Impact of Multiple Antibiotic Resistance Mutations on *Neisseria gonorrhoeae* Growth and Fitness *in vivo* and Selection for Compensatory Mutations during Infection

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Antibiotic resistance is generally accompanied by fitness loss due to the effect of resistance mutations on microbial growth and physiology. We recently reported that gonococci with clinically relevant resistance to macrolides due to mtrR locus mutations have a fitness advantage during murine genital tract infection. Here we tested the fitness of gonococcal mutants in gyrase A (gyrA91/95) [intermediate ciprofloxacin resistance (CipR)] or both GyrA and ParC (parC86) (high level CipR) with and without an mtrR-56 mutation. Acquisition of each mutation resulted in a step-wise reduction in growth rate with the gyrA91/95,parC86 and gyrA91/95,parC86,mtrR-56 mutants exhibiting the slowest generation times. However, following intravaginal inoculation of mice, the gyrA91/95 and gyrA91/95,mtr-56 mutants exhibited increased fitness relative to wild-type and mtrR-56 mutant bacteria, respectively. In contrast, gyrA91/95,parC86 mutants (high CipR) were less fit in vivo. Competition between intermediate and high level CipR mutants confirmed the fitness advantage conferred by mutation of gyrA alone. Finally, in one mouse, high level CipR isolates out-competed intermediate CipR mutants. These CipR isolates grew markedly faster in vitro and thus appear to have compensatory mutations. We conclude gyrA mutations may contribute to the spread of CipR N. gonorrhoeae. Further acquisition of a parC mutation abrogates this fitness advantage perhaps via increased energy expenditure; however, compensatory mutations occur during infection that maintain high level CipR without a fitness cost. We are currently analyzing gene expression in the gyrA mutant to identify factors responsible for the fitness advantage conferred by an altered GyrA subunit.

OE29

Molecular Mechanism of MtrR Multidrug Recognition and Binding

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Expulsion of drugs and cytotoxins from the cell by efflux pumps is a fundamental mechanism of antibiotic resistance and a major contributor to bacterial multidrug resistance. The multidrug efflux pump encoded by the mtrCDE genes of Neisseria gonorrhoeae confers resistance to a wide range of structurally dissimilar compounds, which include host antimicrobials and prescribed antibiotics. Expression of the mtrCDE genes is negatively regulated by MtrR. MtrR, a TetR family transcription regulator, binds to an inverted repeat located in the mtrC promoter to repress transcription. MtrR senses increased intracellular concentrations of diverse cytotoxins and responds by relieving repression of the mtrC promoter thereby allowing the production of additional MtrCDE pumps to remove the threat. Although cytotoxin-dependent induction of mtrCDE is clear, physiologically relevant inducers that bind directly to MtrR have yet to be identified unequivocally and characterized. Here we shall describe the crystal structure of MtrR bound to a fortuitous inducer to 2.3 Å resolution. MtrR has a typical TetR family fold with an N-terminal three-helix DNA-binding domain and a C-terminal six-helix drug binding/dimerization domain. As anticipated the ligand binds in a large internal pocket formed by the C-terminal domain helices. Using the chemical structure of this ligand as a guide, we identified potential physiologically relevant inducers. Their interactions with MtrR were characterized both in vitro and in vivo and strongly support two as bona fide inducers. High-resolution structures of MtrR bound to these natural inducers revealed its mechanism of inducer recognition and binding and will be described.

OE30

Whole-genome sequencing of a *Neisseria gonorrhoeae* isolate with decreased susceptibility/increased resistance to extended-spectrum cephalosporins

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The emergence of decreased susceptibility/increased resistance to extended-spectrum cephalosporins in Neisseria gonorrhoeae is a major concern globally. In this study, an N. gonorrhoeae isolate with decreased susceptibility to oral extended-spectrum cephalosporins (cefpodoxime MIC=1.0 mg/L; cefixime MIC=0.25 mg/L) was whole-genome sequenced. The isolate was cultured in 2008 in San Francisco, USA from a patient with possible treatment failure (subsequent positive culture with matching genotype) and possessed a mosaic penA gene (SF-A; Pandori. AAC. 2009). Sequencing was performed using Illumina Genome Analyzer II platform. The genome sequence of the NCCP11945 strain (Chung. J Bacteriol. 2008) was used as a scaffold, resulting in 90 contigs covering 2,029,064 bp (91%; >150×coverage) of the genome. Interestingly, over half of the genetic divergence between these strains appeared to have occurred via horizontal transfer events with other Neisseria strains (gonococci and other neisserial species). Two genes, opa and txf, acquired from non-gonococcal Neisseria species were identified. These genes were screened for in penA mosaic strains (n=114) and non-mosaic strains (n=169) from USA and Europe. The opa gene was detected in most (82%), and mainly all recent, penA mosaic isolates, however, not in non-mosaic isolates. Screening for the penA mosaic, NG-MAST sequence type and the horizontally transferred opa gene identified one penA mosaic strain (ST1407), and its evolved genetically highly similar subtypes, spreading in Europe and North America. These findings have important implications for tracking of strains with decreased susceptibility/resistance worldwide and identification of mutations responsible for decreased susceptibility/resistance to extended-spectrum cephalosporins.

OE31

Characterization of antibodies in human serum against group B *Neisseria meningitidis* that block complement-dependent bactericidal activity

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The classical pathway of complement (C) is essential for both naturally acquired and vaccine-induced immunity against Neisseria meningitidis. Qualitative and/or quantitative differences in anti-meningococcal Abs in serum contribute to variations in C-dependent bactericidal activity among individuals. IgG isolated from select individuals could block killing of group B meningococci by otherwise bactericidal human sera, and reduced efficacy of mAbs against fHbp, a leading group B protein vaccine candidate. Ligand overlay immunoblots revealed that these blocking IgGs were directed against a meningococcal lipoprotein called H.8 that is composed almost entirely of pentapeptide 'AAEAP' repeats. Killing of meningococci in reactions containing bactericidal mAbs and human blocking IgG was restored when blocking Ab was diverted away from bacteria using either synthetic peptides corresponding to H.8 or a non-blocking murine anti-H.8 mAb. Further, deleting H.8 or another meningococcal protein that contains N-terminal "imperfect" AAEAP repeats called lipid-modified azurin (Laz) from target organisms abrogated blocking. F(ab)2 generated from blocking IgG lost blocking activity, suggesting that blocking required Fc. Blocking required IgG glycosylation; deglycosylation of blocking IgG with PNGase eliminated blocking potential. Classical pathway activation was inhibited by anti-H.8 human IgG; C4 deposition mediated by an anti-fHbp mAb was reduced by intact, but not by deglycosylated, blocking IgG. In conclusion, we have identified a function for H.8 as a novel meningococcal target for blocking Ab. Blocking Ab may reduce the efficacy of fHb-based meningococcal vaccines. We also propose that outer membrane vesicle-containing meningococcal vaccines may be more efficacious if purged of subversive epitopes such as H.8.

OE32

Neisseria meningitidis type C polysaccharide mediated unresponsiveness of B cells to BAFF and APRIL is responsible for its weak immunogenicity

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BAFF system molecules, especially TACI are essential for the development of antibody responses against bacterial capsular polysaccharides (CPS). Here, we sought to investigate whether meningococcal type C CPS (MCPS) has an effect on BAFF system molecules.

Pre-incubation of purified Balb-c mouse B cells with MCPS for 24 hrs or injection of MCPS into Balb-c mice rendered B cells unresponsive to BAFF or APRIL mediated Ig secretion, plasma cell generation, and proliferation. Correlating with this observation, TACI was strongly downregulated on all B cell subsets of MCPS exposed B cells. Interestingly, the prototype TI-II Ag, NP-ficoll did not manifest a suppressive effect on B cells. Investigation of BAFF mediated signaling demonstrated that MCPS inhibits both the classical and alternative NF-kappaB pathways. Staining of MCPS exposed B cells with Annexin-V suggested that the suppressive effect of MCPS may be due to its apoptotic effect on B cells. Previously, we had shown that CpG strongly upregulates TACI expression and sensitizes B cells to BAFF or APRIL induced Ig secretion. We therefore tested whether CpG would ablate the inhibitory effect of MCPS. The results showed that CpG reversed MCPS mediated TACI downregulation but was only partially able to sensitize B cells to BAFF or APRIL mediated Ig secretion. Supporting these in vitro results, CpG was not able to augment antibody response against MCPS when mice were immunized with a CpG containing MCPS vaccine. Taken together, CPS mediated suppression of B cells appears to be the reason for the poor immunogenicity of CPS vaccines.

OE33

Human innate-like memory B cells potently respond to infection by pathogenic and commensal *Neisseria*.

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted infection gonorrhea, enters the sub-epithelial space where sentinel immune cells are known to reside. Despite direct contact with immune cells, gonococcal infection elicits low levels of specific immunoglobulin and re-infection with the same serovar can occur, suggesting that protective immunity is not induced. This prompted us to consider whether infection might directly influence B cell function. In stark contrast to infection with other gram-negative bacteria, we observed that gonococcal infection caused prolonged viability of primary human B cells and elicited both robust activation and vigorous proliferation in the absence of T cells. Upon further analyses, we observed the specific expansion of memory B cells, including the innate-like memory population, upon exposure to a variety of neisserial species, including pathogenic N. gonorrhoeae and Neisseria meningiditis, and commensal Neisseria sp. These innate-like memory cells are reported to confer protection against a diverse array of bacteria and viruses through the production of low affinity, broadly-reactive IgM without inducing immunologic memory. Although N. gonorrhoeae infection produced a small amount of gonococci-specific IgM, IgM specific for memory recall (tetanus toxoid) and irrelevent (keyhole limpet hemocyanin) antigens were also produced, indicative of a broad polyspecific immunoglobulin response. Considering that T cell function is inhibited upon gonococcal infection, this T-independent response may contribute to both an unfocused adaptive response and the absence of immunologic memory to this important human pathogen.

OE34

Novel Gonococcal Multilocus Sequencing Typing (MLST) Scheme Suitable for Short and Long-Term Molecular Epidemiology Studies

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Objective: Neisseria gonorrhoeae is the causative agent of gonorrhea. Presently there is no vaccine against N. gonorrhoeae and therefore accurate information on gonococcal transmission plays a crucial role for interventions designed to limit the spread of the infection. The objective of this study is the introduction of a gonococcal MLST typing scheme for molecular epidemiological studies.

Methods: A panel of 80 N. gonorrhoeae isolates from six geographic locations worldwide was used in this study. Initially, a pool of ten housekeeping genes was selected. In parallel, porB typing analysis was performed using the same set of isolates. MEGA4 was used to construct phylogenetic trees from concatenated sequences of the housekeeping genes and individual porB sequences.

Results: Based on empirical validation of ten housekeeping genes the following 7 loci pyrD, glnA, fumC, gdh, adk, abcZ and gnd were selected for the final MLST scheme. The MLST resolved the examined gonococcal population into 41 STs (0.51 ST/isolates). Although there was a significant degree of consistency between MLST and porB analysis, a most notable difference was that the most numerous Asian cluster in the porB tree, which comprised six isolates, was resolved into a monophyletic cluster consisting of 5 lineages in the MLST tree. Another important difference between the MLST tree and the porB tree was a discordant topology observed among North American isolates. Using epidemiological data it was found that the MLST scheme was able to capture circulating strains of N. gonorrhoeae from North America while porB analysis failed to do so.

OE35

Potential Impact of Serogroup B Vaccines: Prevalence of candidate vaccine antigens among invasive *Neisseria meningitidis* isolates in the United States

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Introduction: Serogroup B protein antigen vaccines in development, including a bivalent and 4-valent vaccine, may protect broadly against meningococcal disease.

Methods: Cases were identified through active, population-based surveillance (ABCs) from 9 states. Incidence rates were calculated using U.S. census data, standardizing for race and age group. Serogroup B isolates from 2000-2008 (n=650) and non-B isolates from 2006-2008 (n=246) were assayed for fHbp by sequence analysis and presence/absence of nadA by PCR. nhbA was sequenced in 381 randomly selected isolates. Results were weighted for Oregon, which had hyperendemic B rates during this period.

Results: During 2000-2008, 1726 cases were identified; 42% B, 24% C, 27% Y, and 6% other serogroups/non-groupable. Average annual U.S. incidence was 0.34 per 100,000. Among B isolates, 61% were fHbp B/v1 (subfamily/variant) and 39% A/v2-3. Among C and Y isolates, 40% and 3% were fHbp B/v1, respectively. Proportions of fHbp subfamily/variant varied by age group and ABCs site; no linear trend was observed over time. NadA gene was detected in 42% of B isolates, but in only 9% of fHbp A/v2-3 isolates. Additionally, 62% C and 4% Y isolates contained nadA. In the subset assessed, nhbA was present in 100% B and 98% non-B isolates.

Conclusions: Two subfamily/variants fHbp antigens, contained in the bivalent vaccine, are present in a high proportion of U.S. strains. Addition of NadA to a subfamily/variant B/v1 fHbp antigen, contained in the 4-valent vaccine, does not substantially increase the proportion of strains covered. However nhbA and porA, may improve coverage by the 4-valent vaccine.

OE36

Factor H binding protein (fHbp) sequence variants among epidemic meningococcal strains from Africa: Implications for development of a broadly protective outer membrane vesicle (OMV) vaccine

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Background. Meningococcal epidemics in Africa are caused by serogroup A, W-135, or X strains. Native OMV vaccines from mutants with over-expressed fHbp are promising for prevention of serogroup B disease. For a broadly protective OMV vaccine for Africa, selecting PorA and fHbp variants is critical.
Methods. Genes encoding fHbp and PorA variable regions (VR) 1 and 2 of 124 case isolates from 19 African countries were sequenced.
Results. All 31 serogroup A isolates, 21 of 22 X isolates, and 18 of 53 W-135 isolates had fHbp modular group I. fHbps of serogroup A isolates (1963-2007) had 99.6% amino acid identity to each other, and 95% and 93% identity to modular group I fHbps of W-135 and X strains, respectively. Of 35 remaining W- 135 isolates, 27 had fHbp modular group III (>99% identity to each other). Anti-fHbp modular group III antiserum (fHbp ID 22) was bactericidal (human complement) against W-135 strains with low, medium or high expression of modular group III fHbp. Anti-fHbp modular group I antisera (IDs 4, 9 or 74) are being tested for activity against serogroup A, W-135 and X isolates with modular group I fHbp. Since 1999, PorA VR types of all A strains were P1.20,9; all but five W-135 isolates, P1.5,2; and all but four X isolates, P1.19,26.
Conclusions. A recombinant OMV vaccine from mutants expressing three PorA types, and two modular group I and one modular group III fHbp, could elicit protection against nearly all serogroup A, X and W-135 isolates causing disease in Africa.

P001

Quinolone resistance among *Neisseria gonorrhoeae* isolates in India: Detection of quinolone determining region mutations

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Background and objectives: Quinolones have a broad spectrum of antimicrobial activity and are widely used for treatment of gonorrhea. A dramatic increase in the number of reported quinolone resistant Neisseria gonorrhoeae isolates in India and worldwide prompted us to investigate the pattern of mutation in gyr A and par C in these isolates.
Methods: Fifty four Neisseria gonorrhoeae clinical isolates were collected from patients attending sexually transmitted disease clinics [34-Delhi, 13-Pune, 5-Mumbai and 2-Nagpur] during 2006-2009. The minimum inhibitory concentration {MIC} of quinolones for these isolates was determined by agar dilution and E-test method. Mutation patterns of the gyr A and par C were analysed.
Results: All the strains were resistant to ciprofloxacin, ofloxacin and norfloxacin with 83% strains showed resistance to gatifloxacin, 94.4% to lomefloxacin and 98% to enoxacin. Sequencing analysis of gyr A and parC revealed that all resistant isolates had dual mutation at S91F, D95 G/N, Y58F in gyrase region, Some isolates had extra mutation within par C either of E91 G/K, D86N, S87R , M59I, S88F, L61M. The MICs level of these isolates were significantly associated ($p<0.01$) with the mutation profile in the gyr A and parC .
Conclusion: Mutation in S91F and D95 G/N in gyr A combined with E91G in par C was the most commonly observed mutation in quinolone resistant Neisseria gonorrhoeae isolates. D95G mutation was most prevalent in strains isolated from Delhi while D95N was prevalent in strains isolated from Pune. This mutation pattern was associated with a high level of quinolone resistant {MIC>8 ug/ml} which can be marker for quinolone resistance in India.

P002

MpeR is a transcriptional regulator of the *mtr* locus in *Neisseria gonorrhoeae*

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MpeR is an AraC-like DNA-binding protein in gonococci that has the capacity to transcriptionally repress expression of two genes in the mtr locus along with other genes outside of this region. We found that at the mid-logarithmic phase of growth, MpeR can repress expression of mtrF, which encodes an accessory protein of the MtrC-MtrD-MtrE efflux pump that is required for high level resistance of gonococci to antimicrobial substrates of the pump. In contrast, at late-logarithmic phase, MpeR represses mtrR, which encodes the transcriptional repressor of the mtrCDE operon in strain FA19. We determined that loss of mpeR by insertional mutagenesis resulted in enhanced expression of both mtrF and mtrR; wild type levels of expression of both genes were restored by complementation. Expression of mtrR was also dependent on the level of free iron and this regulation required MpeR. This finding is consistent with an earlier report that mpeR expression is controlled by Fur+iron. The DNA-binding property of MpeR was examined using mtrF as a model target. We obtained evidence that MpeR can bind in a specific manner to the DNA sequence upstream of mtrF. Moreover, in preliminary experiments, we found that MpeR bound preferentially to single strand DNA within the target mtrF DNA. Given that mpeR expression is subject to control by the levels of free iron, we propose that the regulatory action of MpeR on genes within the mtr locus (e.g., mtrF and mtrR) is relevant to the physiology of gonococci during infection.

P003

Identification of a point mutation that generates a novel promoter for *mtrCDE* transcription in gonococci

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The gonococcus utilizes multiple mechanisms to resist both antibiotics and host antimicrobial agents involved in innate immune defense, including active efflux of these toxic compounds by four known efflux pumps. The MtrC-MtrD-MtrE multidrug efflux pump, a member of the resistance-nodulation-division family of efflux pumps, is a well-characterized system that serves to transport structurally diverse hydrophobic antimicrobial agents out of the gonococcus. In strain FA19, the mtrCDE operon is tightly regulated by both a repressor, MtrR, and an activator, MtrA. MtrR is a TetR family transcriptional regulator, and the mtrR gene lies 250 base pairs upstream and is transcribed divergently from the mtrCDE operon. Previous research has shown that mutations in the mtrR coding region and in the mtrR-mtrCDE intergenic region increase levels of gonococcal antibiotic resistance and in vivo fitness. Recently, a C to T transition mutation 120 base pairs upstream of the mtrC start codon, termed mtr120, was identified in strain MS11 and found to confer high levels of antimicrobial resistance when introduced into strain FA19. We now report that this mutation results in a consensus -10 element and that its presence generates a novel promoter for mtrCDE transcription. Interestingly, this newly generated promoter was found to be stronger than the primary promoter and, unlike the primary promoter, does not appear to be subject to MtrR repression or MtrA activation. We propose that cis-acting mutations can develop in gonococci that significantly alter the regulation of the mtrCDE operon and result in increased resistance to antimicrobials.

P004

Impact of Commonly Isolated *gyrA* and *parC* Mutations in Fluoroquiniolone Resistant *Neisseria gonorrhoeae* on Microbial Fitness

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Background: Fluoroquinolone resistance in Neisseria gonorrhoeae (GC) occurs via mutations in the gyrA gene or gyrA, parC genes. Ciprofloxacin resistant (CipR) strains often also carry mtrR locus mutations, which increase resistance to macrolides and penicillin G via overexpression of a multidrug resistance efflux pump. Recently, we reported that mtrR locus mutants outcompeted wild type GC in a mouse infection model. Based on the rapid spread of CipR GC strains, we hypothesized that gyrA and/or gyrA, parC mutations confer a fitness advantage in vivo.

Methods: Mutant gyrA and parC sequences were PCR-amplified from a CipR GC strain that produces GyrA (Ser91Phe and Asp95Gly) and ParC (Asp86Asn), and were introduced into wild type GC strain FA19 (CipS) and its isogenic mutant KH15 (mtrR).

Results: The gyrA mutants AK1 (gyrA) and AK11 (gyrA, mtrR) were ~2-fold more resistant to Cip than parent strains FA19 and KH15. Cip resistance was increased 6-fold in mutants AK2 (gyrA, parC) and AK12 (gyrA, parC, mtrR). All mutants showed a growth disadvantage when tested in vitro. When tested in vivo, however, mutants AK1 and AK11 were 10- to 100-fold more fit relative to the respective parent strains. Mutants AK2 and AK12 were slightly attenuated in vivo.

Conclusion: We conclude that gyrA mutations with or without an mtrR locus mutation may contribute to the spread of CipR GC due to an unidentified fitness advantage. This fitness advantage is lost, however, upon acquisition of higher level CipR with a parC mutation perhaps due to the resultant increased energy expenditure.

P005

Molecular mechanisms for increased cephalosporin MICs in *Neisseria gonorrhoeae*: The search for "Gene X"?

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Objective: To investigate the molecular mechanisms of cephalosporin resistance by using an isogenic pair of gonococcal isolates, one of which had an induced elevated MIC to cephalosporins.

Methods: A mutant exhibiting higher MIC values was selected on GC agar containing 3ug/ml cefpodoxime. Transformation studies were performed to determine if the increased MIC phenotype could be transferred to other gonococcal isolates. Genes suspected to be involved in cephalosporin resistance were examined in both isolates by standard or whole genome sequencing.

Results: Upon selection, strain SPN284 demonstrated an elevated MIC to cefpodoxime. The MIC values of this mutant, SPN284 (3-1), for cephalosporins were found to have increased at least 4-fold (>8 ug/ml for cefpodoxime and 1.0 ug/ml for ceftriaxone). Sequencing analysis demonstrated no differences in sequences of penA, pilQ, and porB between SPN284 and SPN284 (3-1). DNA isolated from SPN284 (3-1) was able to transform other gonococcal isolates that contained a mosaic penA to elevated ceftriaxone MICs at a frequency of 1 X 10-3. The MIC of the transformants to ceftriaxone was 0.5 ug/ml. Identical experiments performed with strains that lacked the mosaic penA gene yielded no such transformants.

Conclusion: N. gonorrhoeae SPN284 (3-1), that possessed an elevated MIC to cephalosporins selected in vitro did not contain altered penA, pilQ, and porB genes as compared to SPN284. SPN284 (3-1) DNA was able to transform gonococcal strains to elevated MICs at a frequency that is equivalent to a single gene event. Additional analysis of the genome sequence data is underway.

P006

The MisR/MisS two-component regulatory system modulates *mtrCDE* expression in gonococci

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Transcription of the mtrCDEF efflux pump-encoding locus in Neisseria gonorrhoeae is controlled by stand alone regulators such as MtrR, MtrA, and MpeR. The MtrC-MtrD-MtrE efflux pump can recognize structurally diverse hydrophobic antimicrobial agents, including the human antimicrobial peptide LL-37. An earlier study (Lee et al., 16th International Pathogenic Neisseria Conference, Rotterdam, The Netherlands), determined that growth of strain FA19 in the presence of a sub-lethal level of LL-37 (0.1x MIC) resulted in increased resistance of gonococci to this host defense peptide and elevated production of the MisR/MisS two component regulatory system proteins. This work also showed that loss of the MisS sensor kinase due to mutation in the misRS operon resulted in increased expression of mtrR, which encodes a transcriptional repressor of the mtrCDE efflux pump operon in strain FA19. In this study, we used a mtrC-lacZ translational fusion to study expression of mtrCDE in misR and misS mutants of strain FA19. We found that loss of MisR (the response regulator) or MisS (the sensor kinase) resulted in decreased expression of mtrC-lacZ. For both mutants, the wild type level of mtrC-lacZ expression was restored when the wild type gene was expressed ectopically. Based on our earlier studies and the results obtained herein, we propose that the capacity of MisR/MisS to regulate mtrCDE is likely linked to its regulation of mtrR. Accordingly, this regulatory scheme could influence levels of gonococcal susceptibility to antimicrobial agents that are substrates of the MtrC-MtrD-MtrE efflux pump when gonococci are exposed to sub-lethal levels of such compounds.

P007

Correlation of Penicillin Susceptibility Testing Using Etest and Broth Microdilution with *penA* Mutations in *Neisseria meningitidis*

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Increasing antimicrobial resistance in Neisseria meningitidis (Nm) is a public health concern. Mutations in the penA gene have been associated with increased MICs for penicillin and may lead to resistance against third-generation cephalosporins, which are used for empiric treatment of meningitis. Susceptibility testing for penicillin is often done by Etest or broth microdilution (BM), but there are limited data on how accurately these test results correlate to mutations in the penA gene that increase MICs. We tested all Nm isolates received in 2004 and 2008 by a population-based surveillance system, Active Bacterial Core Surveillance (ABCs), for penicillin susceptibility using BM and Etest (n=306). Penicillin non-susceptibility, defined as an intermediate or resistant MIC using breakpoints established by the Clinical and Laboratory Standards Institute, was compared with the presence or absence of mutations in a 402-nucleotide region of the penA gene associated with increased MICs. By Etest, 97 isolates (32%) were penicillin-intermediate and 9 (3%) were penicillin-resistant. By BM, 20 isolates (7%) were penicillin-intermediate and 4 (1%) were penicillin-resistant. Non-susceptible results by Etest were 93% sensitive (95CI 76.50–99.12) and 71% specific (95CI 65.51–76.47) in predicting the presence of one or more of these penA mutations. For BM, the sensitivity and specificity was 64% (95CI 44.07–81.36) and 98% (95CI 95.36–99.20), respectively. Using the current breakpoints, the sensitivity of Etest makes it useful as a screening tool to identify isolates for further characterization, while the more specific and validated BM assay is optimal for surveillance and clinical decision making.

P008

Genomic analysis of chromosomally mediated penicillin-resistant strains of *Neisseria gonorrhoeae*

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Chromosomally mediated resistance to penicillin is complex and requires at least five resistance determinants. Four of these determinants have been identified at the molecular level: penA (mutations in penicillin-binding protein 2 [PBP 2]), mtrR (overexpression of the MtrC-MtrD-MtrE efflux pump), penB (mutations in PorB1b), and ponA (mutation in PBP 1). These determinants can be readily transferred from a penicillin-resistant donor (FA6140) to a susceptible recipient strain (FA19) by homologous transformation. However, despite repeated attempts, transformation to high-level penicillin resistance equivalent to the donor strain has not been achieved, suggesting that one or more additional determinants are required to reach donor levels of resistance but cannot be transferred by homologous transformation. To investigate potential mechanisms that might explain the lack of transformation, we compared the recent genome sequences of two penicillin-resistant strains, FA6140 and 35/02, with the sequences of two susceptible strains, FA19 and FA1090, using Mauve genome alignment software and reciprocal BLAST searches. We observed significant genomic rearrangements in resistant strains compared to sensitive strains, which may contribute to the difficulty in transforming penicillin-sensitive strains to donor levels of resistance. Interestingly, we identified 67 open reading frames present in FA6140 and 35/02 but absent in FA19 and FA1090, highlighting the significant differences between penicillin-resistant and -susceptible strains of N. gonorrhoeae. We are currently in the process of expressing these 67 orfs in FA19 containing the four known determinants (FA19 penA mtrR penB ponA) and also inactivating them in FA6140 to identify any genes that alter resistance to β-lactam antibiotics.

P009

Emergence and characteristics of *Neisseria gonorrhoeae* with decreased susceptibility/resistance to extended-spectrum cephalosporins in Sweden

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Neisseria gonorrhoeae has developed resistance to most therapeutic antimicrobials introduced; this has left extended-spectrum cephalosporins (ESCs) as the sole antimicrobials recommended in many regions worldwide. Most worrying, treatment failures with oral ESCs are reported, especially from the WHO Western Pacific Region (WPR), and the susceptibility to all ESCs (oral and injectable) is decreasing globally.

The aims were to examine the emergence, spread, and phenotypic and genetic characteristics of N. gonorrhoeae strains with decreased susceptibility/resistance to ESCs in Sweden.

All available Swedish isolates, with exposure of infection in many countries worldwide, during 1998-2009 displaying a “decreased susceptibility” to cefixime and/or ceftriaxone (MIC >=0.032 mg/L; n=338) were examined using antibiograms, full-length porB gene sequencing, Neisseria gonorrhoeae multi-antigen sequence typing (NG-MAST), and sequencing of ESC resistance determinants (penA, mtrR and porB [penB alteration]).

penA mosaic alleles and A501V alteration in penA were detected in 23% and 10%, respectively, of the isolates, and in increasing prevalence over the years. mtrR and penB resistance determinants were present in 96% and 95%, respectively, of these isolates with penA mosaic allele or A501V. Moreover, among these isolates 37 NG-MAST STs were detected, with ST1407 (n=29), ST1103 (n=9) and ST3378 (n=8) being most prevalent.

In conclusion, both penA mosaic allele and A501V alteration, together with mtrR and penB, are important for decreased susceptibility/resistance to ESCs. Genetic typing revealed a spread and increasing prevalence globally of gonococcal isolates, many belonging to the same evolving clone, with decreased susceptibility/resistance to ESCs; from the WHO WPR to East- and West-Europe, USA, etc.

P010

Antimicrobial Resistance Continues To Threaten Treatment for Gonorrhea in Canada

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Objective: Canada conducts surveillance of ciprofloxacin, cefixime, and ceftriaxone susceptibilities in Neisseria gonorrhoeae strains to support development of treatment guidelines.

Methods: N. gonorrhoeae strains were isolated or collected by Canadian provincial public health laboratories. Auxotype, plasmid profiles and MICs for penicillin, spectinomycin, tetracycline, erythromycin, ceftriaxone, ciprofloxacin, cefixime, and azithromycin using agar dilution were determined.

Results: From 2001-2007, 36497 strains of N. gonorrhoeae were tested by laboratories across Canada. Of these, 7843 resistant strains were characterized by the National Microbiology Laboratory. During this period, an increase in the detection of ciprofloxacin resistant strains, cipR, (MIC value ≥ 1.0 mg/L) was observed. In 2001, only 130 (2.9%) of the strains tested nationally (n=4498) were found to be resistant to ciprofloxacin, increasing to 6.2% (n=4018) in 2004, and 1291 or 30.3% (n=4261) by 2007. The MICs for ceftriaxone and cefixime are gradually increasing. In 2000, 0.5% of strains had ceftriaxone MIC=0.063, increasing to 20.9% by 2007. Cefixime MICs=0.063 µg/ml increased from 0.5% of all strains in 2003 to 7.6% by 2007. Two strains had a cefixime MIC of 0.5µg/ml, classified as reduced-susceptibility. The highest MIC for ceftriaxone was 0.25µg/ml (n=1).

Conclusions: Ciprofloxacin resistance in N. gonorrhoeae within Canada has increased to a level where ciprofloxacin is no longer an appropriate empiric treatment option. The primary treatment for gonococcal infection is a single dose of ceftriaxone (125 mg IM) or cefixime (400mg PO). The MICs for both ceftriaxone and cefixime have increased over time but all are clinically susceptible.

P011

LpxC inhibitors as a novel class of antibiotics against *Neisseria gonorrhoeae*

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The recent emergence of intermediate resistance of gonococcal isolates to expanded-spectrum cephalosporins (cephI strains) suggests that it is a matter of when, not if, these antibiotics become obsolete in the treatment of gonococcal infections. Because of dearth of antibiotics currently available with proven activity against N. gonorrhoeae, there is an urgent need to develop new antibiotics to be able to continue to treat infections into the foreseeable future. To this end, we are investigating inhibitors of LpxC (UDP-3-O-(acyl)-N-acetylglucosamine deacetylase), the zinc-dependent enzyme that catalyzes the deacetylation of UDP-3-O-(R-3-hydroxyacyl)-N-acetyl-glucosamine in lipid A biosynthesis, as a novel class of antibiotics against N. gonorrhoeae. Although N. meningitidis has been shown to be viable following disruption of lipid A biosynthesis, the same is not true for N. gonorrhoeae, suggesting that these inhibitors can be exploited to develop effective anti-gonococcal antibiotics. To test this hypothesis, we evaluated potent inhibitors of E. coli LpxC for their ability to inhibit the growth of N. gonorrhoeae. Our results indicate that these compounds inhibit the growth of FA19, with MICs in the low µM range, and appear to be less affected by the presence of chromosomally mediated resistance determinants than existing antibiotics. This observation is consistent with their novel mode of action. Thus, LpxC inhibitors are a promising class of compounds for the development of new antibiotics to treat gonococcal infections.

P012

Identification of genes required for host colonization in *Neisseria meningitidis*

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Objectives: Our objective was to establish an epithelial cell culture model in a continuous flow system in order to identify genes required during the colonization process.

Methods: Our laboratory constructed an ordered library of 4548 signature-tagged mutants (STM) among which 3625 transposon insertions have been mapped. We selected and transferred 1000 of these mutations in the capsulated strain 8013 (serogroup C). The 1000 mutants were analyzed for their inability to survive in a colonization model which consists of T84 epithelial cell line cultured in IBIDI* microslides under continuous flow system.

Results: Using PCR-based signature-tagged mutagenesis screening method, we screened approximately 1000 mutants in our colonization model. We identified 190 potentially attenuated candidates. To rule out the possibility that in some of these candidates, the phenotype observed was due to a phase variation event, we transformed the 190 mutations into the parental strain and tested them a second time in the colonization model. We identified 63 candidates. Mutants displaying a significant growth defect in cell cuture medium were discarded. Among the interrupted genes of the 30 remaining mutants, we found genes encoding putative transporters, transcription factors, proteins involved in stress protection and many conserved hypothetical proteins with unknown functions. As expected, the screen also identified several pil mutants well-known to be adhesion defective.

Conclusions: We are currently testing 600 additional mutants and individual testing of all the attenuated mutants is underway to further characterize the genes required for colonization of the host by Nm.

P013

A cell-type specific response to *Neisseria meningitidis* adhesion

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Before getting access to the subarachnoidal space, Neisseria meningitidis (Nm) has to cross two barriers: an epithelial barrier, the oropharynx mucosa, and an endothelial monolayer, the blood brain barrier.

Interaction of piliated capsulated Nm with both epithelial and endothelial cells leads to a common feature: the formation of a cortical plaque composed of several transmembrane proteins and actin. Onto endothelial cells, this signaling is responsible for a disruption of the cell-cell junctions and a subsequent disruption of the endothelial barrier. On the other hand, several reports have shown that Nm is able to cross an epithelial cell monolayer without disrupting the integrity of the intercellular junctions, thus suggesting that the consequences of the bacterial cell interactions is different onto both endothelial and epithelial cells.

We previously showed that the bacterial colonies induce the recruitment of the polarity complex Par3/Par6/PKCx. This complex is responsible for the recruitment of several cell-cell junction proteins underneath the colonies, thus leading to the depletion of junctional component at cell-cell contact and then the opening of the paracellular route.

The aim of this work was to explore the response induced by the interaction of Nm with epithelial cells. We showed using various epithelial cell lines that Nm does not recruit efficiently the polarity complex (Par3/Par6) or the components of the cell-cell junctions (p120-catenin, cadherin, catenin, ZO1/2, claudin) underneath the microcolonies.

Our results confirmed that meningococcal signaling onto epithelial and endothelial cells is different, thus precluding of an interaction with different receptors.

P014

The moonlighting protein fructose bisphosphate aldolase of *Neisseria meningitidis*: Surface localisation and role in cell adhesion

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Objective: Neisseria meningitidis is an obligate human nasopharyngeal commensal, which has the capacity to cause life-threatening meningitis and septicemia. Fructose-1, 6-bisphosphate aldolases (FBA) are cytoplasmic glycolytic enzymes, which despite lacking identifiable secretion signals, have also been found localized to the surface of several bacteria where they bind host molecules and exhibit non-glycolytic functions. The aim of this study was to determine whether FBA is surface exposed in meningococci and can influence the interaction between meningococci and host cells.

Methods: cbbA knockout and complemented derivatives of N. meningitidis MC58 were generated. Sub-cellular fractionation and flow cytometry were used to investigate outer membrane localisation and surface exposure of FBA. The wild-type, mutant and complemented strains were compared in association assays using human brain microvascular endothelial (HBME) or larynx carcinoma (HEp-2) cells.

Results: Despite lacking a recognisable export signal, cell fractionation experiments showed that meningococcal FBA is localized both to the cytoplasm and the outer membrane. Flow cytometry further demonstrated that outer membrane-localized FBA was accessible to FBA-specific antibodies. An FBA-deficient mutant was not affected in its ability to grow in vitro, but showed a significant reduction in adhesion to HBME and HEp-2 cells compared to its isogenic parent and complemented derivative.

Conclusion: FBA is a surface-exposed protein that is required for optimal adhesion of meningococci to host cells.

P015

TspA interacting proteins of *Neisseria meningitidis*

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Objective: Neisseria meningitidis is a commensal bacterium of the human nasopharynx. Occasionally, it gains access to the bloodstream and causes septicaemia and subsequently meningitis. T-cell stimulating protein A (TspA) is an immunogenic, conserved, T-cell and B-cell stimulating protein of N. meningitidis that is required for optimal adhesion to human cells. The role of TspA in the adhesion process is thought to be indirect since it is predicted that the N-terminus is localised to the periplasmic space with an inner membrane-spanning domain linking it to a cytoplasmic C-terminal domain); the aim of this study was to identify proteins within the meningococcal envelope that interact with TspA.

Methods: Four overlapping recombinant fragments of TspA were expressed and purified as fusion proteins with the pGEX-2T-encoded glutathione-S-transferase protein. A receptor activity-directed affinity tagging protocol was employed to identify TspA-interacting meningococcal proteins. Interactions between TspA and candidate proteins were investigated further using ELISA and surface plasmon resonance.

Results: Four putative TspA-interacting proteins were identified: PilQ and PilT (components of the type IV pilus machinery); the major outer membrane protein, PorA, and the protein chaperone, ClpB. Furthermore, the portion of TspA responsible for interaction with PorA was confirmed to be the N-terminus.

Conclusions: Periplasmic domains of TspA interact with several outer membrane proteins which have a key role in meningococcal pathogenesis and adhesion to host cells.

P016

Attenuated virulence of cell division mutants of *Neisseria gonorrhoeae*

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Objective: To study the contribution of minC, minD and oxyR to the virulence of Neisseria gonorrhoeae.

Materials and Methods: Isogenic minC, minD and oxyR mutants of wild-type N. gonorrhoea (Ng CH811) were constructed by insertional inactivation. Adherence/invasion assays and scanning/transmission electron microscopy were performed on urethral epithelial cells (THUEC) infected with wild-type and mutants. Cytokine release in infected cells was estimated by ELISA. Opa and pilin levels in wild-type and mutants were determined by western blot. Mass-spectrometry and silver-staining revealed chemical structure and levels of lipooligosaccharide (LOS) respectively.

Results: The minC, minD and oxyR mutants formed defective microcolonies. Compared to wild-type, the mutants, which had aberrant shapes, showed reduced adherence to and invasion of epithelial cells. However, IL6 and IL8 release levels induced by wild-type and mutants in urethral epithelial cells were similar. The Opa, pilin and LOS levels in mutants were unchanged relative to wild-type. LOS extracted from mutants and wild-type had identical chemical structure.

Conclusion: The decreased adherence and invasion of minC, minD and oxyR mutants cannot be explained by the levels of Opa, pilin and LOS. But quantitative and qualitative similarity of LOS between wild-type and mutants does explain similar IL6 and IL8 release levels induced by N. gonorrhoeae in urethral epithelial cells. The abnormal morphology of min and oxyR mutants may explain their defective microcolony formation which contributes to their significantly reduced adherence to epithelial cells. In all, our results demonstrate the significance of cytokinesis genes i.e. minC, minD and oxyR in N. gonorrhoeae virulence.

P017

Opa expression hinders dissemination of *Neisseria gonorrhoeae* into tissues

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It is generally accepted that Opa-expressing gonococci are more virulent than Opa-negative gonococci, due to the increased ability of Opa-expressing strains to invade into various human cells. However, to cause disseminated disease, the bacteria must cross an epithelial layer to enter into tissue. We created and characterized a derivative of MS11 (MS11delta opa) that had the coding sequence for all Opa proteins deleted. When grown in broth, this strain rarely formed clumps of greater than four bacteria. While piliated MS11delta opa adhered to ME180 cells and polarized T84 cells with the same frequency as WT MS11, the microcolonies that they formed appeared distinct from those of WT MS11. MS11Opa+ formed large microcolonies while MS11delta opa was observed as disperse diplococci and small clusters. The data indicated that these strains produced distinctly different interactions on the epithelial cells. We found that MS11Δopa was able to readily traverse polarized monolayers of T84 cells in less than 6 hours and had a higher transmigration frequency than WT strain. Most of these traversed bacteria appeared to remain adherent to the basolateral surface of epithelial cells. Since these traversed adherent bacteria lacked Opa and had already shed their pili, this suggests that the organisms possess additional adhesins. Taken in toto, the data support a model of infection where Opa-expressing gonococci are better at invading into cells, while Opa-negative gonococci are better at transmigrating across an epithelial barrier. This suggests that Opa-expressing gonococci would be less likely to disseminate from the site of infection.

P018

Genome-wide RNAi screen to identify host cell factors involved in *Neisseria gonorrhoeae* infection

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Objective. Type IV pili (Tfp) constitute the key factors for mediating the initial attachment of pathogenic Neisseria species to human cells. Previous work has identified the tip-located PilC protein as the receptor binding protein [Rudel et al., 1995]. However, the nature of the cellular receptor(s) required for this species-specific interaction remains enigmatic [Kirchner and Meyer, 2005a]. Preliminary characterisation of receptor properties suggests the surface receptor is a protein rather than glycostructures [Kirchner and Meyer, 2005b]. We initiated a genome-wide loss-of-function (RNAi) screen in host cells to elucidate the nature and identity of the N. gonorrhoeae pilus receptor, and additional factors involved in the infection process.

Methods and results. The genome-wide RNAi loss-of-function analysis is carried out in 384-well format using a robotic screening platform (Beckman). ME-180 cells, a human cervical epithelial cancer cell line, are transfected using HiPerFect reagent and siRNAs (genome-wide siRNA Library, V1.0: 3 siRNAs per gene; both QIAGEN). After three days of incubation, cells are infected with a piliated, Opa-negative and GFP expressing N. gonorrhoeae strain (derivative of MS11F3 strain) for three hours. Subsequently, non-adherent bacteria are removed from cells. The read-out of fixed cells is based on automated fluorescence microscopy (Olympus). We monitor (i) the attachment of N. gonorrhoeae, (ii) the formation of microcolonies, (iii) the recruitment of the actin cytoskeleton underneath microcolonies and (iv) the invasion rate. The combined data analysis will further clarify the interplay between epithelial host cells and N. gonorrhoeae during infection.

P019

Neisseria meningitidis and *Neisseria gonorrhoeae* cause transient decrease of barrier integrity in cell monolayers of primary human umbilical vein endothelial cells (HUVEC)

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Objective and methods. N. gonorrhoeae (Ngo) and N. meningitidis (Nme) share numerous virulence factors, namely type IV pili (Tfp) and Opa proteins. Both Ngo and Nme cross epithelial cell monolayers by transcytosis without affecting barrier integrity [Merz et al., 1996; Wang et al., 1998 and 2008]. Recent work has suggested a paracellular route for crossing endothelial monolayers through recruitment and/ or destruction of cell-cell-contact forming proteins [Coureuil et al., 2009; Schubert-Unkmeir et al., 2010]. However, the overall picture remains incomplete. To further elucidate the underlying molecular mechanisms we assess an in vitro infection model using primary HUVECs in combination with CellZscope (nanoAnalytics), an automated cell monitoring system. This system allows determination of alterations in the transendothelial electrical resistance (TEER) of the polarised cell monolayers upon infection.

Results. Preliminary results show that both Ngo and Nme may cause a specific, temporal loosening of the tight cell-cell contacts of HUVEC monolayers starting approximately 2 h p.i., reaching a minimum at 9-10 h p.i. and fully recovering around 46 h p.i. Tfp are dispensable for this process as ΔpilE1/2 mutants expressing Opa proteins also induced a decrease of TEER. Moreover, Opa-negative selected ΔpilE1/2 mutants exhibited a 3 h delay in their effect on TEER, which was correlated with an intensive Opa-switch. We hypothesize that Tfp establish the first contact to the host cell, then Opa proteins induce a loss in barrier integrity, allowing the paracellular crossing of endothelial cell monolayers. Further work now focuses on the involved signalling network.

P020

***Neisseria meningitidis* NadA and Eukaryotic Hsp90 Interaction: Biochemical and Functional Analysis**

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NadA (Neisseria adhesin A), a novel antigen proposed as a vaccine candidate against N. meningitidis serogroup B (Pizza et al., 2000; Comanducci et al., 2002; Giuliani et al., 2006), has been shown to mediate bacterial adhesion to and invasion of epithelial cells (Capecchi et al., 2005).

The identification of cellular host receptors/interactors for NadA, and the description of the cell response following the interaction with meningococcus, represents a chance to gain insights into the infection process.

While searching for NadA receptors/partners on Chang epithelial cells we identified Hsp90, an essential molecular chaperone with ATPase activity which holds key roles in several cellular processes and with possible implication in host-pathogen interactions.

Hsp90 and other proteins belonging to the same family have been recently described to interact with bacterial proteins and to trigger signaling pathways within eukaryotic cells in the context of bacterial attachment and penetration into host cells (Jin et al., 2003; Cabanes et al., 2005; Rechner et al., 2007).

We demonstrated that the interaction between the meningococcal antigen and Hsp90 is strictly dependent upon the ATP/ADP driven chaperone conformation.

We further investigated the influence of Hsp90 in NadA-mediated bacterial interaction with epithelial cells and we found that the modulation of Hsp90 chaperone activity and its level of expression affect NadA-mediated properties.

P021

Integrin-mediated invasion of *Neisseria meningitidis* into human cells requires Src family protein tyrosine kinases

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N. meningitidis is able to invade eukaryotic cells by indirectly (via fibronectin or vitronectin) engaging β1 integrin-containing host receptors.

In this study we identified intracellular signals involved in integrin-initiated internalization of N. meningitidis. We could show that the host cell cytoskeleton and Src family protein tyrosine kinases (PTKs) are essential to mediate N. meningitidis invasion. Src PTKs are activated in response to Opc expressing N. meningitidis, but not in response to an isogenic opc-deficient mutant strain. In addition, inhibition of Src PTK activity by the general tyrosine kinase inhibitor genistein and the specific Src inhibitor PP2 reduced Opc-mediated invasion into human brain microvascular endothelial (HBMEC) and human embryonic kidney (HEK 293T) cells up to 90 %. Moreover, overexpression of the cellular Src antagonist C-terminal Src kinase (CSK) significantly reduced N. meningitidis invasion. Importantly, Src PTK-deficient fibroblasts were impaired in their ability to internalize N. meningitidis and showed reduced phosphorylation of the cytoskeleton and decreased development of stress fibers.

These data demonstrate that the Src family protein tyrosine kinases, in particular the Src protein, are important signal proteins, which are responsible for the transfer of signals from activated integrins to the cytoskeleton and thus mediate uptake of N. meningitidis into human cells.

P022

Presenilin/gamma-Secretase Dependent Processing of CD46 During *Neisseria* Infection Modulates Invasion

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CD46 is a type I transmembrane protein with complement regulatory functions that is targeted by numerous viruses and bacteria. CD46 has signaling and receptor properties in immune and nonimmune cells, many of which are dependent on the expression of cytoplasmic tail (cyt) isoforms cyt1 or cyt2. We recently demonstrated that CD46-cyt1 and CD46-cyt2 are substrates for presenilin/gamma-secretase (PS/gS), an endogenous protease complex that regulates many important signaling proteins through proteolytic processing. Both, Neisseria gonorrhoeae and Neisseria meningitidis, stimulate PS/gS processing of CD46-cyt1 and CD46-cyt2.

Objectives: The goal of this study was to determine the function of CD46 proteolysis during infection.

Aim1: Establish if inhibition of PS/gS influences Neisseria infection.

Aim2: Establish the role of PS/gS-dependent CD46 processing during infection.

Methods: Site directed mutagenesis was used to mutate putative PS/gS cleavage sites within the transmembrane segment of CD46. Gentamicin protection assays were performed in the presence of PS/gS inhibitors and in cell lines stably expressing noncleavable CD46 expression constructs. Ng strain MS11 (P+, Opa-nonexpressing) was used for infection studies.

Results: PS/gS inhibitors promote bacterial invasion. We identified several amino acid residues in the CD46 transmembrane sequence that block proteolytic processing by PS/gS. Noncleavable CD46-cyt1 expression constructs were found to promote invasion.

Conclusions: Our data supports the hypothesis that CD46 proteolysis modulates invasion during Neisseria infection of epithelial cells. Blocking CD46-cyt1 cleavage by site directed mutagenesis or use of PS/gS inhibitors promote higher levels of invasion by N. gonorrhoeae.

P023

Role of a *Neisseria gonorrhoeae* glycoprotease in modulating bacterial invasiveness and Type IV pili dynamics

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Clinical observations suggest that Neisseria gonorrhoeae (Ng) can persist on the mucosa in a quiescent state. Symptomatic infection may be due to a shift in the delicate balance between quiescence and virulence. We report on a putative protease in Ng with homology to the O-Sialoglycoprotein endopeptidase (Gcp) that is secreted by virulent strains of Mannheimia haemolytica. We characterized the Ng Gcp and explored its role in Ng infectivity. We demonstrate that the Ng gcp gene is transcriptionally active. Immunoblot analyses of Ng cultures using a monoclonal antibody raised to the closely related M. haemolytica Gcp confirmed the presence of a 37-kDa protein, consistent with the mass predicted by the Ng gcp sequence. Transposon mutagenesis of Ng gcp resulted in a loss of the 37-kDa protein. The Ng gcp mutant is more invasive than the wt strain in infection studies of A431 epithelial cells. Inducing gcp expression in the mutant with isopropyl-beta-D-thiogalactopyranoside partially reduced invasiveness, demonstrating that Gcp modulates bacterial entry into the host. Force measurement studies suggest that Gcp alters Type IV pili dynamics to influence bacterial invasiveness.

P024

Neisseria gonorrhoeae inhibits apoptosis in granulocytic HL-60 cells

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Neisseria gonorrhoeae (Gc) interacts extensively with polymorphonuclear leukocytes (PMNs) during infection, and actively modulates PMN functions in vitro. In order to examine the effect of bacterial infection on apoptosis in PMNs, we established a cell culture model for the Gc-PMN interaction using HL-60 myeloid leukemia cells differentiated down the granulocytic pathway. Differentiation of HL-60 cells with dimethylformamide resulted in cells which exhibited bactericidal activity and were able to mount an oxidative burst. Infection of differentiated HL-60 cells with Gc resulted in the survival and expansion of bacteria in association with the cells, and live Gc actively inhibited the oxidative burst induced by both PMA and opsonized Staphylococcus aureus, suggesting that differentiated HL-60 cells can recapitulate previously described aspects of the Gc-PMN interaction. To determine whether Gc infection exerts a pro- or anti-apoptotic effect in HL-60 cells, differentiated cells were infected with Gc and treated with the apoptosis inducers staurosporine (STS) and TRAIL. Infection of HL-60 cells with Gc alone did not induce apoptotic signalling as measured by DNA fragmentation and caspase-3 activity. However, pre-infection of HL-60 cells that were subsequently treated with either STS or TRAIL resulted in the inhibition of apoptosis induced by both stimuli, indicating that Gc is able to inhibit both intrinsic and extrinsic apoptotic signalling. These data show that the HL-60 cell line can be useful for modeling Gc-PMN interactions, and suggest that Gc may actively inhibit apoptosis in infected PMNs.

P025

Neisseria gonorrhoeae alters key cell cycle regulators, which can be restored by *Lactobacillus*

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Long-term colonisation, such as Lactobacillus adherence and asymptomatic infections with Neisseria gonorrhoeae, affects the host target cells in various ways. In this study we have investigated the target cell cycle progression and division upon adhesion with Neisseria gonorrhoeae and different Lactobacillus strains alone as well as in co-colonisation.

We used different lactobacilli strains isolated from healthy human individuals and the piliated N. gonorrhoeae MS11 strain. ME-180 cervical carcinoma cells served as host cells. We studied the effects of bacterial colonisation on host cell cycle progression by live cell microscopy and proliferation assay, determined cell phase profile by flow cytometry as well as shifts in expression levels of cell cycle regulatory genes by q-PCR.

We show that Neisseria gonorrhoeae decelerates the epithelial cell cycle and induces a small accumulation of cells in G2. None of the Lactobacillus strains tested could negate the reduced cell proliferation. In fact, three out of four Lactobacillus strains by them self hampered host cell proliferation, with cells partly sequestered in G1 phase. However, during a gonococcal infection key regulators such as MAD2L2 and TP53, that safeguard the genome integrity, were compromised but the expression of these genes could be restored depending on which Lactobacillus strain was present.

P026

Polynucleotide phosphorylase (PNPase) a regulator of virulence in *Neisseria meningitis*?

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In different bacteria polynucleotide phosphorylase (PNPase) is a subunit of the RNA degradosome complex taking part in RNA maturation, acting as a 3′ to 5′ phosphorolytic exonuclease. Changing the stability of RNA transcripts is one mechanism by which bacteria regulate gene expression in order to adapt to environmental changes.

In this project we investigate the role of PNPase in regulating the virulence of Neisseria meningitidis. We report an increased ability to adhere to the human hypopharyngeal epithelial cell line FaDu for the Δpnp deletion mutant in comparison with wt (wild type) bacteria. Also mutational inactivation of the PNPase resulted in an increased resistance to killing by normal human serum.

Combined, our results support the idea that in Neisseria meningitidis PNPase is a regulator of virulence genes and bacterial fitness during infection.

P027

Neisseria gonorrhoeae survival inside primary human neutrophils

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Infection with Neisseria gonorrhoeae (Gc) is characterized by a potent polymorphonuclear leukocyte (PMN, neutrophil)-driven inflammatory response. PMNs are the first responders to bacterial infection and are capable of phagocytosing and killing a variety of microorganisms. Despite the diverse repertoire of mechanisms used by PMNs to kill microbes, viable Gc can be cultured from the purulent exudates of human gonorrhea samples, indicating Gc are resistant to some PMN killing. We have developed an assay using adherent PMNs followed by exposure to bacterial viability dyes to monitor Gc survival during infection of primary human PMNs. Results with this assay show that a subset of intracellular and extracellular Gc associated with PMNs remain viable over time. PMNs possess primary and secondary granules, which during normal phagosome maturation fuse with the phagosome to form a phagolysosome in which bacteria are exposed to oxidative and non-oxidative antimicrobial components. One hypothesis to explain Gc survival inside PMNs is that Gc affects maturation of the phagosome into a degradative phagolysosome. In support of this hypothesis, we have demonstrated via immunofluorescence microscopy that Gc-containing phagosomes display reduced granule fusion compared to serum-opsonized zymosan phagosomes in primary human PMNs. These results, suggest that intracellular survival of Gc may be due to altered phagosome maturation. Our current studies will determine how Gc modulate phagosome maturation to avoid antimicrobial agents and survive in the presence of PMNs, which is vital for the persistence of this important human pathogen.

P028

*Lactobacilli i*nterference with *Neisseria meningitidis* adhesion to host cells

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The normal microbiota prevents colonization of pathogenic bacteria and represents an important first line of defence. Lactobacilli belong to the normal microbiota and colonize various parts of the body. These bacteria are common inhabitants of the mucus membranes in the oral-pharyngeal tract. We investigate the influence of lactobacilli on N. meningitidis adhesion to pharyngeal epithelial cells. The capacity to interfere with meningococcal adhesion to host cells differed among Lactobacillus strains. Thus, the composition of the Lactobacillus flora may play an important role in regulating the colonization of N. meningitidis. Lactobacillus mediated inhibition was dependent on live lactobacilli as well as living host cells. Microarray experiments showed that epithelial cells incubated with an inhibitory Lactobacillus strain had a different gene expression profile, in comparison to epithelial cells incubated with a non-inhibitory Lactobacillus isolate. This data suggest that certain Lactobacillus strains prevent meningococcal attachment by affecting host cell signalling pathways.

P029

Estrogen And Progesterone Increase *Neisseria Gonorrhoeae* Transmigration Across Polarized Genital Epithelial Cells

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Neisseria gonorrhoeae (GC) can transmigrate across epithelial barriers, which may lead to more complex diseases i.e. pelvic inflammatory disease (PID) and disseminated gonococcal infection (DGI). The epithelial barrier consists of a polarized epithelial monolayer sealed by apical junctional complexes. Both the function of epithelial barrier and GC infection in the genital tissue of women have been shown to associate with the sex hormone cycle. We have investigated the role of the apical junctional complex in gonococcal transmigration across polarized genital epithelial cells and the effect of the female sex hormones, estrogen and progesterone, on this transmigration process. Using confocal microscopy, we found that live GC, but not gentamicin-killed GC, preferentially localized at the epithelial apical junction, marked by ZO-1, and induce redistribution of the apical junctional proteins, ZO-1 and E-cadherin. Transmigration assays, showed that under steroid hormone-free conditions, GC transmigrated across polarized HEC-1-B cells at an extremely low rate in a time dependent manner. Disrupting the junctional complex with the calcium chelator, EGTA, increased GC transmigration. Exposure of HEC-1-B cells to the sex hormones, estrogen and progesterone, caused an increase in gonococcal transmigration comparable to that observed in EGTA-treated cells. Moreover, hormone treatment enhanced GC induced redistribution of junctional proteins. These results suggest that GC target the apical junctional complex of polarized epithelial cells and work in synergy with sex hormones to affect the apical junction, thereby facilitating GC transmigration across the host epithelium.

P030

Factor H facilitates adherence of *Neisseria gonorrhoeae* to complement receptor 3 on eukaryotic cells

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Neisseria gonorrhoeae can engage human complement receptor 3 (CR3) directly or through surface-bound iC3b. Factor H (fH) bound to bacteria facilitates conversion of C3b to iC3b and binds directly to CR3 on professional phagocytes. Certain non-professional phagocytes, including primary cervical epithelial cells, express CR3. We hypothesized that fH could bridge bacteria to CR3 and facilitate gonococcal association with host cells. Specificity of the fH-CR3 interaction was confirmed using human CR3-transfected CHO (CHO/CR3) cells. Using recombinant proteins that comprised contiguous fH domains fused to murine Fc, we identified two regions in fH (comprises 20 short consensus repeat (SCR) domains) that bound to CR3; SCRs 18-20, bound strongly while weaker binding occurred through SCRs 6-10. Both regions also bound to an unsialylated PorB.1A strain 252 of N. gonorrhoeae. Accordingly, fH-related protein 1 (CFHR1) (comprises fH SCRs 18-20 plus two dissimilar N-terminal SCRs) bound to CHO-CR3 and to unsialylated strain 252. A variant of fH called fH-like protein-1 (FHL-1) (contains fH SCRs 1-7) bound to gonococci, but minimally to CHO-CR3 cells. An SCR 6-20 protein construct enhanced binding of unsialylated PorB.1A gonococci to CHO-CR3. However, a construct that contained only the apparently relevant SCRs (6-7 and 18-20) bound to CHO-CR3 and to gonococci separately, but did not enhance bacteria-CR3 interactions, suggesting that the intervening SCRs (8-17) may have imparted a configurational and spatial requirement for fH to bridge gonococci to CR3 on eukaryotic cells. These results indicate adherence between fH-coated gonococci and CR3 and may provide a means for gonococci to gain sanctuary into non-professional phagocytes.

P031

*Igor Stojiljkovic Memorial Award Winner**

Meningococcal Vitronectin Binding Phenotypes: Sialylation, Serum Resistance and Cellular Interactions

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Neisseria meningitidis (Nm) Opc protein binds to human vitronectin (Vn) by an unusual mechanism that involves the sulphated tyrosines of the activated form of human vitronectin. This interaction enables it to target human endothelial cell integrins leading to cellular invasion (Sa E Cunha et al, PLoS Pathogens, 2010). In addition, we have shown that Vn targeting enables Nm to control the insertion of C5b-9 terminal membrane attach complex (MAC). We have been interested in Opc as a model protein capable of mediating these two important biological functions and because Opc is present in Nm clonal complexes with the predilection for meningitis. In current studies, we have investigated how bacterial cell surface sialylation differentiates its serum resistance properties from cellular interactions mediated by Opc-Vn interactions. Sialylation, either LPS or capsule expression neither hinder bacterial binding of human Vn nor its acquired serum resistance through binding to activated vitronectin, the well known inhibitor of C9 polymerisation and MAC insertion. In contrast, surface sialic acids hinder Vn-mediated interactions of Nm with human endothelial integrins. This clearly demonstrates that Nm surface sialic acids function as barriers at the cell-bound receptor but not at the soluble receptor levels at least as far as the Vn-Opc mediated interactions are concerned. We have also established the critical level for Opc expression in Vn binding and cellular interactions. As the biological effects of Vn may be of significance during meningococcal dissemination, the studies imply that Opc and Opc-like Vn-binding molecules could have significant pathogenic potentials during blood dissemination.

P032

Molecular and cellular interactions of *Neisseria meningitidis* Opc adhesin and human cytoskeleton protein α actinin

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We have recently shown that Opc expressing N.meningitidis (Nm) binds to the cytoskeletal protein α actinin (Sa E Cunha, et al., Cellular Microbiology, 2009). In non-muscle cells, α-actinin occurs in 2 isoforms (1 & 4) which play important roles in stress fiber formation, cell adhesion and regulation of cell shape and motility, as well as in the control of distinct cellular signaling pathways. Thus interactions with α actinin may help Nm to manipulate the cytoskeleton for traversal of cellular barriers and perhaps also influence cellular signalling processes. In this study we provide evidence for differential effects of Nm infection on cellular distribution of α-actinin. In order to assess the significance of Nm/α-actinin interactions, we have used recombinant technology and live imaging/confocal microscopy to investigate Nm binding to α-actinin1 vs. 4 and the downstream consequences of these interactions. Using recombinant fragments of α-actinin4, specific binding of Nm to N-terminal but not C-terminal domains have been established. Relative affinities of Opc expressing Nm to various isoforms of α-actinin are currently being investigated. Previously, we have shown colocalisation of Nm with α-actinin by confocal microscopy. Our recent studies have shown that both α-actinin1 & 4 colocalise with Opc expressing N.meningitidis. Comparison of α-actinin localisation in infected and uninfected cells has shown the distribution of α actinin4 to alter from its normal pattern to increased localization in the nucleus after infection. By using live imaging, we have also observed that Nm is able to move inside human target cells; the role of α actinin in intracellular movement is under investigation.

P033

Anti-fHBP antibodies elicited after immunization with a recombinant fHBP vaccine candidate (rLP2086) can displace human Factor H from the surface of Serogroup B Meningococci

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Objective: The N meningitides serogroup B (MnB) factor H binding protein (fHBP) is a vaccine candidate currently in clinical trials for prevention of invasive disease. fHBPs bind human Factor H (hfH), a negative regulator of the alternative complement pathway. Immunization with fHBPs induces functional antibodies that kill meningococci directly through complement-mediated and antibody-dependent lysis engaging the classical pathway of the complement system. However, bacterial clearance could be potentially enhanced if anti-fHBP antibodies also inhibited hfH binding to the bacteria, thereby increasing bacterial vulnerability to attack by the alternative complement pathway. We evaluated the cell surface interactions between hfH and anti- fHBP antibodies using diverse invasive MnB clinical isolates.

Methods: Binding of hfH to meningococci and hfH competition of anti-fHBP antibody binding were examined using flow cytometry. Purified hfH was used at physiological concentrations. Forty diverse clinical MnB isolates were evaluated for hfH binding and fHBP expression. The effect of hfH binding on serum bactericidal activity by anti-fHBP antibody was also determined.

Results and Conclusion: hfH bound to all 40 MnB strains tested, irrespective of fHBP variant type. hfH binding did not prevent bactericidal antibodies from binding to fHBP on the bacteria even in the presence of physiological concentrations of hfH. Furthemore, anti-fHBP sera did prevent hfH from binding to fHBP. Collectively, these data provide evidence that functional antibodies generated after immunization with the rLP2086 candidate vaccine should provide protection against N. meningitidis serogroup B by enlisting both the classical and alternative pathways of the host's complement system.

P034

Specific contribution of individual CEACAMs for *Neisserial* transcytosis through polarized epithelial cells

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Neisseria gonorrhoeae is a gram-negative, sexually transmitted pathogen that causes gonorrhoea in humans. Upon entering the urogenital tract, the neisserial outer membrane colony opacity-associated "Opa" proteins interact with human CEACAM receptors on the apical surface of mucosal epithelium. This results in uptake, transcytosis and release of viable bacteria into the lamina propria. Three different CEACAMs – CEACAM1, CEACAM5 and CEACAM6 – are coexpressed by epithelial cells. CEACAMs belong to the Immunoglobulin (Ig) superfamily. Each contain an Ig variable region-like N terminal domain which is required for bacterial binding, followed by up to six Ig constant region-like domains, and are anchored to the plasma membrane via a transmembrane domain (CEACAM1) or a glycosylphosphatidylinositol (GPI) anchor (CEACAM5 and CEACAM6). Previous studies in non-polarized HeLa cells transfected with individual CEACAMs suggest different mechanisms for gonococcal uptake by CEACAM1 versus CEACAM5 and CEACAM6. However, the morphology and signalling in these cells do not reflect that of polarized epithelial monolayers occurring in vivo. We have generated murine polarized epithelial models which express individual human CEACAMs. When expressed in isolation, each CEACAM is capable of allowing engulfment of Opa-expressing gonococci when the transfected cells are maintained in a non-polarized state. Despite their normal function in the control of cell growth and differentiation, the transfected cell lines each form polarized epithelial monolayers in transwell dishes. These models are currently being used to assess the relative contribution of individual CEACAMs during gonococcal transcytosis the epithelial barrier, thereby defining their role in neisserial infection and disease.

P035

Exploring the evolutionary processes which shaped the diversity of the FadL like protein in the Neisseria species

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The molecular diversity of a novel Neisseria meningitidis antigen, encoded by the ORF NMB0088 of MC58 (OmpP1 or FadL) was assessed in a panel of 64 diverse meningococcal strains. The panel consisted of strains belonging to different serogroups, serotypes, serosubtypes and sequence types, of different clinical sources and countries of isolation. Based on the sequence variability of the protein, the FadL like protein has been divided into four variant groups in the meningococcus. Antigen variants were associated with specific serogroups and MLST clonal complexes. Relationships among sequences were determined using distance-based methods and maximum-likelihood and Bayesian analyses were used to compare the selection pressures acting on the encoded protein. The meningococcal sequences were also compared with those of the related surface protein in non-pathogenic commensal Neisseria species to investigate potential horizontal gene transfer. Three variable regions (VRs) were identified which are predicted to be located in the outer membrane loops of the protein. The diversity in the shortest VR appears to be a result of immunoselective pressure. The majority of the variability in the other VRs was due to recombination among existing alleles from the same or related species that resulted in a discrete mosaic structure in the meningococcal population. In general, the population structuring observed based on the FadL like membrane protein indicates it is under intermediate immune selection. However, the emergence of a new sub-variant among hyperinvasive lineages, highlights the plasticity of the ORF NMB0088 and the ability of the meningococcus to diversify in response to selective pressure.

P036

Update on the Incidence of Meningococcal Invasive Infections in Canada, IMPACT 2002-2009

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Objectives: To update disease epidemiology since universal meningococcal conjugate C infant immunization programs were started across Canada.

Methods: Active population based surveillance was conducted across Canada by the 12 centers of the Immunization Monitoring Program, Active (IMPACT) for hospital admissions in all ages related to Neisseria meningitidis from January 2002 – December 2009. Case definition required the isolation of meningococcus or positive PCR test from a sterile site. Incidence rates are reported per 100,000.

Results: A total of 665 cases were reported; 357 in children (<20 years) and 308 in adults. Annually, cases ranged from 39 to 58 in children and 32 to 57 in adults. Incidence ranged from 0.63 to 0.42, with a peak in 2007, driven by serogroup B in select provinces. The highest rates were in children (1.30) in 2007. Overall 54 cases (8.1%) resulted in death (annual range 2 to 10). Mortality was higher in adults than children (12.3% vs. 4.5%).

From 2006-2009, serogroup B caused the majority of invasive meningococcal disease in Canada with an incidence ranging from 0.21 to 0.36. From a pre-vaccination rate of 0.23, serogroup C occurred at a rate of 0.08, similar to serogroup Y infections (0.07), which remained unchanged. Serogroup W135 remained infrequent at a rate of 0.01 to 0.06.

Conclusion: Serogroup C infections now occur with the same lower frequency as serogroup Y infections. Currently, Serogroup B has the highest incidence in Canada. Although 2007 saw an increase in the incidence of serogroup B cases, this does not appear to be sustained.

P037

Prevalence of Factor H Binding Protein (fHBP) Variants in *N. meningitidis* Carriage Isolates

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Objectives: N. meningitidis serogroup B (MnB) fHBP is undergoing clinical trials as a vaccine candidate for prevention of invasive disease. A comprehensive, systematic survey of fHBP sequences in invasive MnB isolates revealed that fHBP variants segregate into two subfamilies, A and B, and that 12 variants cover 82% of invasive isolates*. The study described here elucidates fHBP sequence diversity in carriage isolates.

Methods: Pharyngeal swabs were obtained from 190 University students 1 month after the beginning of term and at two subsequent time points during the winter of 2008/2009. Positive cultures were evaluated for fHBP, MLST, PorA-type and serogroup.

Results: The carriage rate was 47% at the first sampling time point. Predominant serogroups were Y(45%), 29E/Z(31%) and B(12%). Of 23 subjects positive at time 0 who returned for sampling at both subsequent time points, 14 carried the same strain for 4 months while the remainder carried different strains at the later time points or became carriage-negative. fHBP sequences in carriage isolates were similar to those identified previously in invasive serogroup B isolates. Three fHBP variants accounted for 65% of strains. Only two new variants were identified but these did not represent a new branch on the fHBP phylogenetic tree. Several fHBP variants found commonly in invasive MnB strains were also prevalent in the MnY and 29E carriage isolates.

Conclusion: fHBP was present in all strains isolated from healthy subjects. fHBP sequences in carriage isolates were similar to those identified previously in invasive MnB isolates.

*Murphy et al. JID(2009)200:379-389

P038

Evaluation of a rapid test for the detection of *Nesseria meningitidis* group B in human serum and whole blood using a novel affinity biosensor

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Serogroup B Nesseria meningitidis (MenB) is the leading cause of meningococcal disease in developed countries. Diagnosing meningococcal disease early provides important information for initial treatment. Currently, PCR techniques are widely used to detect meningococcal DNA, but can take one or more working day to perform. The aim of this study was to evaluate a rapid test for MenB using ELISHA (ELectro-ImmunointerfaceS Heterodoxical Approach) immunosensor technology, which is based on electrochemical biosensors that directly measure the antibody-antigen binding reaction. A specific IgM antibody for MenB was used to construct the biosensors. Two meningococcal strains serogroup C11 (MenC, negative analyte control) and MenB NZ 98/254 were heat-killed and spiked at concentrations of 0, 100, 10,000 and 1,000,000 organisms.ml-1 into PBS (Phosphate Buffered Saline), serum and whole blood samples respectively. Measurements were performed by applying 100µL of sample to the immunosensor surface and incubating for 20 minutes at room temperature. Alternating current impedance scans of a minimum of 2 repeats were then performed between frequencies of 1000Hz to 1Hz using a Perkin-Elmer VersaStat V3 potentiostat. Calibration curves were obtained by subtraction of the responses for specific (MenB IgM) and non-specific (anti-digoxin) electrodes, thereby eliminating non-specific adsorption of sample components. The responses of the immunosensors exposed to NZ 98/254 were found to increase with increasing concentration in PBS, serum and whole blood and no cross-reactivity of the immunosensors was found with MenC strain. The limit of detection was found to be as low as 10 organisms in PBS, serum and whole blood.

P039

NadA sequence variants among meningococcal case isolates from Sub-Saharan Africa

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Background. NadA is a surface adhesin and part of a promising experimental vaccine for prevention of meningococcal serogroup B disease. NadA could be a useful addition to a recombinant protein and/or outer membrane vesicle-based vaccine for Africa targeting epidemic serogroup A, W135 and X isolates.

Methods. We investigated nadA and fetA alleles, and sequence types (ST) of 60 case isolates from 16 countries in Sub-Saharan Africa.

Results. All nine ST-4 serogroup A isolates from 1963 to 1990 were fetA F1-5 and nadA negative, while all 17 serogroup A isolates from 1988 to 2007 with ST-5, ST-7, ST-2859 or ST-6035 (all clonal complex (cc) 5) were fetA 3-1, and nadA positive (allele 3). All 19 serogroup X isolates with ST-181, ST-751, or ST-5403 were fetA F3-27 and nadA negative. Of 17 serogroup W135 isolates from 1988 to 2007, 15 cc11 isolates were nadA positive (chimeric 2/3 allele); and 14 of the 15 were fetA F1-1. Among nadA positive serogroup A or W135 isolates, the inferred NadA amino acid sequences were 99% identical. The NadA gene promoter FarR/NadR binding regions were 100% conserved, but TAAA(n) repeats were present in groups of 6, 9, 11 or 12 in different isolates irrespective of ST, cc, or serogroup, which is consistent with previous claims of phase variable nadA expression.

Conclusions. In Africa, the NadA gene is clonally distributed, encodes a highly conserved protein, and is prevalent among recent A or W135 isolates. Although phase-variable, NadA merits further investigation as a vaccine candidate for Africa.

P040

Prevalent sequence types and antigenic subtypes of non-invasive meningococci in Scotland, 1974 – 2004: increased level of genetic diversity compared with invasive disease isolates

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The SHLMPRL has an extensive meningococcal isolate collection dating back to the 1960s representing an invaluable resource. A study was undertaken to characterise 791 non-invasive meningococci isolated from healthy subjects by MLST and PorA VR sequencing to investigate the circulating lineages and subtypes from a 31-year period and to compare with data on 2767 invasive meningococcal disease (IMD) isolates from previous studies. Results:299 STs were identified amongst the non-invasive strain collection of which 70% were not found amongst the IMD strain collection. The most prevalent lineages were ST-41-44/lineage 3 (14.1%); other common lineages included ST-8/Cluster A4, ST-35, ST-22, ST-23 and ST-53 clonal complexes. Approximately 12.5% of non-invasive isolates could not be assigned to a known clonal complex. cc-18, cc-37 and cc-549 were present in the IMD strain collection, but absent from the non-invasive strain collection. Contrastingly, cc-53, cc-175, cc-178 and cc-198 were absent from the IMD strain collection but not from the non-invasive strain collection. The level of diversity was significantly different for both ST (D = 0.981; 95%CI 0.955, 1.01) vs. (D = 0.938; 95% CI 0.930, 0.946) and PorA subtype (D = 0.958; 95% CI 0.948, 0.968) vs. (D = 0.928; 95% CI; 0.927, 0.928) between non-invasive and IMD strain collections, respectively. Conclusions:The prevalence of particular clonal complexes and subtypes varied between the two strain collections and temporal variations were evident. The data presented here will add further to our knowledge on the circulating meningococcal lineages in Scotland over the last three decades and more.

P041

The epidemiology and surveillance of meningococcal disease in England and Wales

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Background: The HPA performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers and characterise strains.

Methods: Clinicians notify suspected cases of meningococcal meningitis/septicaemia to Office for National Statistics. Microbiology laboratories submit isolates for phenotypic characterisation and since, October 2007, by porA sequencing. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacin are determined. Clinical samples are submitted for non-culture detection by PCR.

Results and discussion: Laboratory confirmed cases rose from 1,448 in 1995 to peak at 2,804 in 1999 falling to 1,046 in 2009. The increase in serogroup C cases from 1995-9 resulted in the introduction (November 1999) of serogroup C conjugate (MenC) vaccine into the UK population. In 2009 a total of 1,046 cases of invasive meningococcal disease were laboratory confirmed and of these, 58% were confirmed by PCR alone. For 2009, over 15,000 samples were submitted for meningococcal PCR testing. In 2009, 89% of cases were serogroup B, 6% were serogroup Y (doubling from 28 cases in 2004 to 62 in 2009) whereas only 1% (10 cases) were confirmed as serogroup C and 2% serogroup W135. Phenotypic and genotypic shifts have been observed, specifically the relative contribution of clonal complexes ST-41/44, ST-269, ST-32, ST-213 and ST-11 to meningococcal epidemiology. Surveillance has demonstrated a sustained reduction in serogroup C infections since 1999, a direct consequence of the programme and resultant herd immunity. The apparent small, but detectable increase in serogroup Y cases and the age groups affected is now being closely monitored.

P042

Incorporation of RT-PCR into Routine Public Health Surveillance for Meningococcal Meningitis in São Paulo State, Brazil

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Background: Understanding meningococcal meningitis burden in developing countries is important because of conjugate vaccines. Public health surveillance is hampered by high rate of culture negativity, leading to underestimates of disease burden. Real-time (RT)-PCR has been shown to increase diagnostic yield for bacterial meningitis but incorporation into surveillance in a developing country has not been reported.

Methods: We evaluated multiplex RT-PCR assay for Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae in 12 hospitals in São Paulo and Campinas, Brazil during February 2007-April 2009. Patients with blood or cerebrospinal (CSF) culture positive and/or patients whose CSF profile showed ≥100 leukocytes and ≥60% neutrophils were included. For sensitivity of RT-PCR, culture positive specimens were the gold standard. Specificity was calculated among patients culture positive for another pathogen. CSF specimens were tested for presence of antibiotics using Kocuria rhizophila.

Results: There were 90 persons who were culture-positive for N. meningitidis and additional 328 patients with the specified CSF profile. Sensitivity of RT-PCR for N. meningitidis was 100% (95% CI, 96.0%-100%) and specificity was 100% (93.0%-100%). A total of 83 culture-negative, RT-PCR-positive patients with meningococcal meningitis were identified, for additional yield of 92% of RT-PCR over culture-based results. In multivariable analysis including all three pathogens, the main risk factor for being culture negative but RT-PCR positive was presence of antibiotic in CSF (odds ratio 12.2, 95% CI 5.9-25.0).

Conclusions: RT-PCR was highly sensitive and specific and substantially added to measures of meningococcal meningitis disease burden when incorporated into routine public health surveillance in São Paulo.

P043

Risk factors and epidemiology of *Neisseria meningitidis* carriage among 1-29 year-olds in one urban and two rural districts in Burkina Faso, 2009

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Objectives: Conjugate vaccines can confer herd immunity by interrupting carriage and transmission. We determined carriage prevalence and epidemiology in Burkina Faso before introduction of a novel conjugate serogroup A vaccination program.

Methods: We conducted serial, cross-sectional, multistage cluster sample surveys. For each rural district we used probability proportional to size sampling to select villages, and random sampling of households within villages. In the urban district, we randomly selected city blocks and chose a convenience sample of households. All eligible residents of selected households were invited to participate. Personal digital assistants with GPS capabilities were used to map and navigate to rural households and collect data. Participants reported demographic, health, and household characteristics. An oropharyngeal swab for culture was obtained from participants. Meningococcal identification was performed by Burkina Faso reference labs using standard methods and confirmed by the Norwegian Institute of Public Health.

Results: In 4 pre-vaccination surveys, 20,326 participants were enrolled. Overall, 56.3% were female, 49.6% were aged ≤9 years, and 59.6% reported meningococcal polysaccharide vaccination within 5 years. Prevalence of meningococcal carriage was 4.0%, serogroup A 0.4%. Carriage among the urban and two rural districts was 1.8%, 3.9% and 6.3%, respectively. Males aged 15-19 years had the highest prevalence (7.5%). Prevalence was significantly higher during the dry season and among those with more household residents. Carriage did not differ significantly by vaccination history, travel, or tobacco use.

Conclusion: Variation in carriage by age, gender, season, and geography underscores the need to achieve high coverage to maximize vaccine herd immunity.

P044

Clonal expansion of meningococci in University halls of residence; implications for control of disease

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Objective: To identify distribution patterns of meningococcal clones in students living in university halls of residence; a population known to be at increased risk of invasive disease.

Methods: Pharyngeal swabs were obtained from 190 University of Nottingham students resident in five different halls of residence 1 month after the beginning of term. This was repeated later in the autumn term and again in the spring term. Positive cultures were evaluated for MLST, PorA-type and serogroup.

Further swabbing of over 1000 university students was undertaken in October 2010. Two cases occurred in one hall. Specialised software was used to link cases across this dataset.

Results: The carriage rate was 47% at the first time point and subsequently increased to 51% and 54%. In the three halls which were a single building; the dominant clonal complex in round 1 was also found to be dominant in rounds 2 and 3. The dominant clone was different in each of the five halls. Clones were widespread across individual halls and patterns of spread were linked to the hall architectural layout.

In the outbreak an extensive contact network was identified.

Conclusion: Spread of meningococci appeared to be mainly within halls of residence.

Each hall of residence had a different dominant meningococcal clone and the degree of meningococcal variation was specific to the hall layout. A hall based control strategy should be considered when a case occurs.

P045

Molecular Epidemiology of serogroup W135 and Y meningococcal disease in Germany

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With the introduction of tetravalent polysaccharide conjugate vaccines, the molecular epidemiology of serogroups W135 and Y has gained increasing interest. We present data from the German NRL. Samples were analysed by antigen sequence typing, MLST, and penicillin Etest (243 cases, 210 viable strains, Dec 2001 through May 2010). The incidence rate was 0.035/100000/a for both serogroups taken together (W135, n = 82; Y, n = 162). 50% of W135 and 60% of Y cases were above 18 years of age. 44 of 65 serogroup Y cases with MLST were ST-23, the dominating ST for W135 was ST-11 (15 of 49). The dominant finetypes were Y:P1.5-2,10-1:F4-1 (93 of 162 Y) and W135:P1.18-1,3:F4-1 (28 of 81 W135). Of 29 Y:P1.5-2,10-1:F4-1 strains, for which a serotype was available, 26 were PorB type 3-36, which corresponds to the "late" Y strains associated with increasing incidence in the US (Harrison et al., JID 2006). Interestingly, Y:P1.5-2,10-1:F4-1:porB3-36 was also the dominant finetype among healthy carriers of ST-23 in Germany in 1999/2000 (Claus et al. JID 2005). However, no incidence change was associated with this finetype, and disease was frequently affecting the elderly. The immunodominant PorA antigen P1.4 of the investigational Novartis vaccine was found in only 1% of all cases. 16% of the Y strains showed penicillin MICs of >0.064 µg/ml, in contrast to 51% of the W135 strains. W135 strains therefore represent an outlier with regard to penicillin susceptibility.

P046

Projection of the impact of a Serogroups A and W-135 Meningococcal Conjugate Vaccine in the African Meningitis Belt

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Objective: The meningitis belt of sub-Saharan Africa has the highest global incidence of epidemic meningococcal disease, predominantly caused by serogroups A (MenA) and W-135 (MenW-135). We modelled the impact of a meningococcal AW-135 conjugate vaccine on MenA disease in this region, and assessed the additional benefit of MenW-135 protection.

Methods: A static model estimated morbidity and mortality in the African meningitis belt over a 10-year period due to MenA disease, considering several vaccination schedules in children ≤1 year old with/without prior herd-protection effects. MenW-135 was assumed to contribute 5–10% of overall meningococcal disease burden, with potential to cause epidemics. Age-specific disease incidence rates and case fatality ratios were estimated using endemic population data (1981–1996) from Niamey, Niger, with results extrapolated to an at-risk meningitis belt population of 240 million.

Results: The model projected that 2 doses (14 weeks, 9 months) might prevent 17% of MenA cases and 18–20% of MenA-related deaths; 1 dose (9 months) might prevent 15% of MenA cases and 16% of MenA-related deaths. In a population of 240 million (1 or 2 doses) without (resp. with) prior herd-protection the model predicted ≥170,000 (resp. 40,000) MenA cases averted, and ≥21,000 (resp. 4,000) MenA-related deaths without (resp. with) prior herd-protection. A vaccine including MenW-135 (1 or 2 doses) might avert ≥7,800 additional cases.

Conclusion: A MenAW-135 conjugate vaccine was predicted to reduce MenA cases by ≥15% and MenA-related deaths by ≥16% over a 10-year period. By including a MenW-135 vaccine component, meningococcal disease burden may be further reduced.

P047

Sequence Variation And Degree Of Expression For Five Genome-Derived Vaccine Antigens Included In The MenB Novartis Investigational Vaccine

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Distribution and sequence variation of the genes coding for the five recombinant antigens included in the Novartis investigational vaccine were assessed in isolates from Norway (50 MenB; 16 non-B) collected during a low incidence period (2005-06). The isolates were highly heterogeneous on the basis of multilocus sequence typing and genetic variation for the vaccine antigens. The gene for nadA was present in 30% of all strains (36% MenB); NadA-1 being most frequent. All strains harboured nhba; variants 5 (21%) and 8 (17%) were the most commonly represented. The gene for fHbp was present in all strains. Among MenB strains 64% had fHbp variant 1 (fHbp-1), 28% fHbp-2 and 8% fHbp-3. Sub-variant 1.1 of fHbp was the most abundant in MenB strains (26%). For GNA1030 and GNA2091 substantially less variation was seen. Two strains with a frame-shift mutation in gna1030 and fHbp, respectively, did not express the antigens. A match of 66% for the MenB strains was found between presence of the relevant NadA-/fHbp variants and the vaccine formulation. This vaccine coverage prediction is conservative because NHBA is expected to induce a broad immune response. In addition, GNA1030 and GNA2091 may also contribute. Antigen expression levels were studied using FACS analyses. All variants of NadA and NHBA were expressed on a fairly constant level in the strains possessing these genes. GNA1030 and GNA2091 gave a positive signal in few strains only, suggesting that these two proteins were less accessible to antibody binding. The highest variation in expression was observed for fHbp-1.

P048

Assessment of Spatio-temporal Meningococcal Clusters with Multiple Locus VNTR Analysis (MLVA)

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Multiple Locus Variable-Number-Of-Tandem-Repeats Analysis (MLVA) is a high-throughput typing method, which we recently employed to dissect an outbreak of ST-41/44 complex meningococci (Elias et. al, EID, 2010). Using newly available genomes from carrier (α14, α153, α275, α710) and invasive (NMCC053442) isolates we adapted the scheme by Schouls et al. (JCM, 2006) to a multiplex approach requiring 2 amplification rounds per strain. Further, we developed an open-source tool (http://lazymlva.r-forge.r-project.org) for automated derivation of MLVA-profiles from ABI electropherogram files, which will be included in a web service. In a pilot study we investigated whether 9 previously identified spatio-temporal clusters over 42 months (Elias et al., EID, 2006) with a low probability of alpha-type error ($p \leq 0.005$) differed in diversity or repeat based distance from non-clustered, antigen-sequence-type-(AST)-matched controls. Clustered strains comprised 33 isolates belonging to 8 different ASTs; an equal number of controls from the same time period were randomly selected. MLVA-based Simpson's index of diversity did not differ with 0.985 (CI: 0.971 to 0.999) and 0.970 (CI: 0.944 to 0.995) among clustered and control strains, respectively. Mean distance between repeat profiles, measured with the Manhattan distance metric, was not significantly higher in controls (2.88) compared to clustered strains (2.09, p=0.86). Conclusions may include: 1) selection of clusters was not stringent enough to detect genetical similarity, 2) clock-rate at MLVA-loci is too high to trace possibly linked strains. To clarify these issues, an analysis spanning 96 months, higher stringency of cluster selection, and 2:1 matching will be performed.

P049

Meningococcal Carriage In Army Recruits In Finland, 2004-2005

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Aims: To study meningococcal carriage and diversity of carried isolates among army recruits in Finland during a non-epidemic period in 2004-2005

Methods: 892 military recruits from two intake groups in Kainuu Brigade, Finland, were enrolled in the study in July 2004 and January 2005. Oropharyngeal swabs were collected on entry and leaving the garrison at 6, 9 or 12 months, and during respiratory infection episodes requiring consultation by physician. The swabs were cultured and meningococci identified and pheno- and genotyped by standard procedures. For comparison, 99 invasive disease strains isolated in Finland during the same time period were analyzed.

Results: On arrival, 2.2% of the recruits were culture positive for meningococci compared to 18.5% at the end of service, with a seven-fold higher carrier rate upon entry in January than in July. 74% of the isolates were nongroupable compared to none of the disease isolates. Among groupable strains, serogroup B predominated. The most common phenotype among carriage isolates was NG:NT:P1.5,2.

By MLST, 111 different sequence types (STs) among 215 carrier isolates were identified, of which >60% were new. ST-4146 was most common (29 isolates). 77% of the STs fell into 13 previously defined clonal complexes (cc). The most common was ST-60 complex (61 isolates and 21 different STs). Only 2% of disease isolates belonged to this cc. Among disease isolates, 74 STs were identified among 95 isolates with complete MLST profile of which >60% were new. ST-41 was most common (10 isolates). 37% of disease isolates did not fall into any previously defined cc.

P050

Epidemiology Of Meningococcal Disease In Finland, 1995-2009

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Aims: In Finland, meningococcal vaccination is recommended for high risk groups only including military recruits to whom tetravalent ACYW135 polysaccharide vaccine is given to as part of their vaccination program. The aim of this study was to investigate the epidemiology of invasive meningococcal disease (IMD) in Finland in 1995-2009 with respect to current vaccination policies.

Methods: Reporting of IMD in Finland is obligatory and based on notifications from clinical microbiology laboratories reporting all positive CSF/blood culture, antigen detection and/or PCR findings and clinicians reporting all laboratory-confirmed cases. All case isolates are requested to be sent to THL for confirmation and phenotyping. In 1995-2009, most cases (>90%) were culture proven. Over 90% were sent to THL.

Results: Since a period in 1995 and 1996 with higher incidence caused by serogroup B and serogroup C strains, the incidence of IMD in Finland has fluctuated at low levels between 0.6 to 1.1 per 100 000 population (29-58 notified cases annually). Most cases (73%) have been due to group B. Only 1-9 group C cases have occurred annually (incidence <0.2 per 100,000). The phenotype distribution of the isolates will be presented. Since 2007, >47% of isolates have been non-serosubtypable.

Conclusions: The incidence of IMD in Finland has remained low during the past 13 years. Due to low incidence of group C disease, there are no plans to introduce serogroup C conjugate vaccine into the national vaccination program. High proportion of nontypable isolates necessitates the use of molecular typing methods for epidemiological surveillance, which are planned to be introduced in 2011.

P051

Characterization and antibiotic susceptibility of invasive and carriage meningococci in Burkina Faso in 2006 and 2008

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Objectives: To describe molecular sequence types and antibiotic susceptibility characteristics of invasive and carried meningococci (Nm) isolated in Burkina Faso in 2006 (during a serogroup A epidemic) and 2008 (seasonal hyperendemicity).

Methods: We analyzed 158 carriage and 73 invasive isolates by phenotyping, genotyping and sequence typing. Antibiotic resistance was evaluated by Etest.

Results: Of 148 Nm carriers identified in 2006, (overall Nm carriage prevalence in the general population, 23%) 100 were A:4:P1.9 ST-2859, 47 were Y:14:P1.5,2 ST-4375 and 1 was nonagglutinable NT:NST ST-192. Of 10 Nm isolated from eight carriers in 2008 (overall prevalence 1.5%), four were Y:14:P1.5,2 ST-4375 and one was X:15:P1.6 ST-198. Two polyagglutinable strains were 4:NST ST-4889 and 14:P1.5,2 ST-6920. Three W135 isolates belonged to NT:P1.5,2 ST-2881 (n=1) and NT:NST ST-53 (n=2). NmY, NmA, NmW135 and NmX were respectively distributed in 7, 4, 2 and one pulsed field gel electrophoresis profiles each different from others by one or more bands. During 2006 and 2008, all 73 invasive isolates were A:4:P1.9 with 70 ST-2859 and three new ST-6968. Three isolates showed reduced susceptibility to penicillin (0.125≤MIC≤1); all were susceptible to ceftriaxone and chloramphenicol.

Conclusion: In Burkina Faso during 2006-2008, epidemic NmA belonged to ST-2859 (clonal complex 5), while a new ST-6968 occurred in sporadic cases. Outside epidemics, ST-2859 was found in cases but not in carriers. WHO recommendation, of chloramphenicol or ceftriaxone for the treatment of meningococcal meningitis appears to be valid in terms of resistance.

P052

Understanding epidemic meningococcal meningitis: don't focus on climate, microbiology or immunity alone

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Much effort is put to understand the impact of climate on meningococcal meningitis epidemics in sub-Saharan Africa, while other projects suspect strain variations and immunity. We present an argumentation on which additional research strategies are needed.

We developed an explanatory model for epidemic meningococcal meningitis in the African meningitis belt, based on the following hypotheses:

1. Endemic meningococcal meningitis incidence is similar to that in Europe.
2. The seasonal increase is ubiquitous, observed in all years and concerns all serogroups.
3. Meningococcal meningitis epidemics are highly localized at village level.
4. Epidemic waves consist of many localised epidemics.

To validate the model, we reviewed published and surveillance data on disease incidence (down to the health center level) and carriage. The data indicated that climate is not associated with the occurrence of localised epidemics but with seasonal hyper-endemicity of disease, including that of pneumococcal meningitis. Outbreak strain carriage prevalence is high during localised epidemics, which can be defined by a weekly incidence rate of >75/100,000 over ≥2 weeks. Epidemics due to other serogroups than A usually occur when A-incidence is particularly low.

From this model, several new research questions arise, including

- prevention of climate-associated bacterial hyper-invasiveness (direct invasion from the nasopharynx?),
- the association of viral micro-epidemics with localised meningococcal epidemics,
- the potential of different serogroups to respond to epidemiogenic factors,
- the usefulness of health center level data (counting localised epidemics rather than cases) for risk factor studies and vaccine impact assessment where exhaustive laboratory confirmation is inefficient or not feasible.

P053

The EMERT database 2007 through 2010: results of an initiative of the European Monitoring Group on Meningococci

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The European Meningococcal Epidemiology in Real Time (EMERT) database was designed to connect reference laboratories for exchange of up-to-date molecular typing data. The database serves to identify transnational expansion of clones, and to provide a comprehensive overview of circulating variants in the sense of being a virtual strain collection. The EMERT database structure is provided by the University of Oxford. Datasets contain lab sample id, date received, date sampled, serogroup, PorA VR1/VR2, FetA VR, ST. As of June 14th, 2010, 8782 cases were assembled in the database, and serogroups B and C accounted for 6347 (72%) and 1508 cases (17%). 22 countries contribute to the database, 13 countries reported consistent molecular typing data. Multilocus sequence typing data were provided only for a minority of cases. 21 PorA / FetA combinations made up for 50% of all meningococcal cases. Taken together, EMERT within an astonishingly short period of time compiled a large number of cases and developed to a strong and representative resource for reference laboratories. The EMERT statistics suggest that DNA sequence based typing still needs further development in Europe, a topic currently addressed by the ECDC funded IBD Labnet. MLST, which is part of the TESSy metadataset of the ECDC, appears especially to be hampered by financial constraints.

P054

PorA genotyping of the Irish *N. meningitidis* disease causing population from 1997 – 2009.

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The PorA protein is a major immunogenic component on the outer surface of the N. meningitides cell wall. With the construction of several PorA containing SgB OMP based vaccines, some of which are specific to circulating epidemic clones, knowledge of PorA expression and PorA allelic diversity are of significant epidemiological value. A total of 874 isolates and 1214 clinical samples, sent to the IMMRL between the years 1997 – 2009, were sequenced and the VR1 and VR2 variable regions were determined, yielding 52 unique genotypes and 5 novel variable regions. Nearly 80% of the organisms typed were serogroup B and the most common genotype was B:p1.7,p1.4. To assess expression in the Irish N. meningitidis population isolates received between 2007-2009 were probed for PorA expression, and compression with their genotypes was possible.

P055

Meningococcal disease surveillance in South Africa: added value of PCR identification and serogrouping, 2004-2009

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Introduction: Laboratory-confirmed invasive meningococcal disease (IMD) was defined as isolation of Neisseria meningitidis (NM) from normally sterile site specimens. Culture-negative, but antigen positive for NM or presence of Gram-negative diplococci were excluded due to specificity concerns. Loss of isolate viability during transport resulted in loss of serogrouping data.

Aim: To improve the detection and characterisation of NM using molecular methods.

Methods: PCR for identification (ctrA) and serogrouping (A, B, C, X, Y, W135) was performed on culture-negative clinical specimens (CNCS) (2004-2009); and transport media yielding no growth (NVTM) (2007-2009). Conventional PCR (2004-2007) was replaced by real-time PCR in 2008 (identification) and 2009 (serogrouping).

Results: 3105 cases of IMD were reported; 112 (4%) cases were CNCS, and 95% (106/112) were included due to PCR confirmation. CNCS increased from 3% (11/409) to 6% (26/462) over the 6-year period (p<0.001). CNCS were more likely to be associated with meningitis (94/112, 84%) compared with culture-confirmed cases (2262/2993, 76%) (p=0.04), and less likely to be identified as serogroup B (5/67, 7% vs 384/2047, 19%, p=0.02). NVTM samples decreased from 2007 (124/503, 25%) to 2009 (59/462, 13%) (p<0.001). Serogroup was determined in 97% (62/64), 73% (48/60) and 100% (55/55) of samples for each year. Detection rates differed for serogroups C and Y from NVTM samples compared to cultures (23/165, 14% vs 74/886, 8%, p=0.02; and 5/165, 3% vs 70/886, 8%, p=0.03, respectively).

Conclusions: PCR added significant value in improving data for laboratory-confirmed IMD. Real-time PCR increased the sensitivity of both organism detection and serogroup characterisation.

P056

Typing of Romanian meningococcal isolates from 2008/2009

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To assess the recent epidemiology of meningococci in Romania, a collaboration between the German and the Romanian reference laboratories was initiated.

A subset of meningococci (n=30) isolated in 2008/2009 was serogrouped and antimicrobial resistance was determined. Molecular typing of meningococcal isolates was performed comprising PorA VR1 and VR2, FetA VR and MLST. Furthermore, penA and the vaccine target fHbp were included in the typing scheme.

Serogroups B and C dominated with a total of 24 isolates. Only 8 of 30 isolates were penicillin susceptible with the rest being intermediate or resistant, respectively. Twenty of the respective isolates harbored an altered penA allele. Astonishingly, there was a single dominant penA allele associated with three distinct meningococcal finetypes. In contrast, previous reports and own data from Germany suggest a high genetic diversity of penA alleles in isolates with elevated penicillin MICs. The paradox might be explained by a regional selective sweep of the particular allele.

Twelve Serogroup : PorA : FetA combinations were identified, of which nine were unique to Romania with no matching to those encountered in Germany between 2002 and 2009. This result might be explained by geographic structuring. Only five isolates belonged to known sequence types and finally less than half of the isolates could be assigned to a clonal complex. The study was limited by possible underestimation of the incidence of invasive meningococcal disease in Romania and laboratory surveillance constraints. The development of penicillin susceptibility should be carefully monitored in the future.

P057

Factor H binding protein (fHbp) variability within the ST-41 and ST-44 sub-complexes of cc41/44

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Clonal complex ST-41/44 contains two main sub-complexes: the ST-41 sub-complex is generally associated with invasive disease (in the MLST database the 75% of strains of this sub-complex are from disease), whereas ST-44 shows a relevant proportion of strains associated with carriage (46% of strains of this sub-complex are from carriers). Little is known about the relative genetic diversity of the fHbp in these two sub-complexes.

We analysed the genetic variability of fHbp of strains belonging to the ST-44 sub-complex, and compared it with the diversity of strains belonging to the ST-41 sub-complex. The full fHbp gene sequence was determined for all strains belonging to the cc41/44 included in available strain panels. Thirty-two strains were isolated in the Czech Republic and 14 were selected from the 107 MLST Reference strains database both including carriage/disease cases. Strains collected from disease were 17 isolated in Sweden, 171 in the UK and 21 from a worldwide strain panel.

All strains isolated from carriers belonged to the ST-44 sub-complex, and represented the 36% of this sub-complex isolates. Conversely, all strains belonging to the ST-41 sub-complex originated from disease. We observed different levels of gene variability among carriage and pathogenic strains and among ST-41 and ST-44 sub-complexes. A significant genetic linkage was observed between fHbp variant1 and ST-41, as well as between ST-44 and variants 2 and 3. These findings could support wider understanding of other clonal complexes; similar analysis of ST-269 and the other most "structured" clonal complexes is ongoing.

P058

Global clones and capsule switching identified among invasive *Neisseria meningitidis* isolates, South Africa, 2005

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Introduction: Invasive meningococcal clones belong to a few hypervirulent lineages. For selective advantage meningococci may switch their capsule type by replacement of serogroup-specific genes.

Aim: To characterise invasive meningococcal isolates from January through December 2005.

Methods: Isolates were submitted to a national laboratory-based surveillance system. MLST, PorA and FetA typing were used to characterise all serogroup B (seroB) isolates (n=58), and 20 randomly selected isolates each from seroA, seroC, seroY and seroW135. Capsule switching was identified by isolates of different serogroups sharing a PorA:FetA:ST genotype. Associations between serogroup and clonal complexes (cc) were compared to data on the PubMLST database.

Results: 605 cases were reported, of which 68% (414/605) had viable isolates; 24 (6%), 58 (14%), 21 (5%), 52 (13%) and 257 (62%) were seroA, seroB, seroC, seroY and seroW135, respectively. Most seroB, seroA, seroC, seroY and seroW135 isolates belonged to cc's ST-32/ET-5 (12/58) and ST-41/44/lineage 3 (17/58); ST-1/subgroup/I/II (17/20); ST-865 (16/20); ST-175 (15/20); and ST-11/ET-37 (19/20), respectively. Genotype P1.7-1,1:F1-6:ST865 was found among seroC (n=8) and seroB (n=1) isolates. Genotype P1.5,2:F1-1:ST11 was found among seroW135 (n=19), seroC (n=1) and seroY (n=1) isolates. Capsule switching from seroC to seroB was indicated by seroB isolates of cc's ST-334 (n=2), P1.17,16-4:F4-23:ST334 and P1.5-2,10-1:F1-5:ST6704; and ST-103 (n=1), P1.18-1,3:F3-9:ST1878, which are usually associated with seroC.

Conclusions: Global hyperinvasive lineages associated with specific serogroups were identified in South Africa. cc ST-865 is usually associated with seroB, however we documented the expansion of ST-865 among seroC isolates. Capsule switching was identified in the absence of vaccine pressure.

P059

Descriptive epidemiology of the public health response to cases of meningococcal disease in the East Midlands North Health Protection Unit population

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Objectives: to determine the incidence of invasive meningococcal disease in a region in relation to the socio-economic deprivation at the lower super output level. Focusing on the rural/ urban incidence within the geographical area, ages of mothers in the under 18 years cases and numbers of contacts identified for chemoprophylaxis.

Methods: Retrospective review of meningococcal questionnaires used to manage the control of meningococcal disease, laboratory reports and the enhanced meningococcal database from January 1st 2007 until December 31st 2009. Each case had their deprivation score identified from their postcode. The incidence of meningococcal disease across the region's LSOA's was analysed by dividing these into quintiles of socio economic deprivation.

Setting: East Midlands North HPU population (2,700,000).

Results: 302 cases of probable and confirmed cases were identified. 195 of these cases were laboratory confirmed and the remainder were clinically probable.

The most deprived 20% of the population as compared with the least deprived had double the incidence rate (most deprived: n= 86, least deprived: n=39).

There were 5.45 contacts per case identified for chemoprophylaxis in the most deprived group and 4.94 in the least deprived group.

There were 213 under 18 cases and in the most deprived group the mean age of the mothers was 27.4 with mothers under 21 n= 22 whereas in the least deprived group the mean age was 33.9 and mothers under 21 n= 1.

Conclusion: socioeconomic deprivation influences the incidence and public health management of meningococcal disease cases.

P060

Meningococcal epidemiology in England and Wales: July 2007 to June 2008

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Objective: The meningococcal group C (MenC) glycoconjugate vaccine, introduced in England and Wales in 1999, achieved a 97% reduction in MenC cases by 2006/7. The proportion of MenB cases consequently rose (90% in 2005/6). The proportions of MenW135 and MenY cases were relatively stable between 2003/4 and 2006/7, averaging 2.7% and 2.9%, respectively. Notable MenB trends, between 1999 and 2006, were a decreasing proportion of cc41/44 (~44% to ~28%) and cc32 (~12% to ~6-8%), and increasing proportion of cc269 (~24% to ~46%) and cc213 (~1.6% to ~6.8%) cases. This reversed marginally for cc41/44 and cc269 in 2006/7 (~30% and ~38% of cases, respectively). Presented are epidemiological data for all invasive disease isolates received by the HPA Meningococcal Reference Unit in 2008/9.

Methods: Isolates (n=613) were characterised in terms of sero/genogroup, genosubtype, fetA, and MLST.

Results: Capsular groups present included MenB (87.3%), MenY (4.6%), MenW135 (3.9%) and MenC (2.6%). The cc41/44 vs cc269 balance was further restored (32% vs 33% of cases, respectively) and cc213 increased (~10%). cc11 accounted for 94%, 4% and 1% of MenC, MenW135 and MenB isolates, respectively. Predominant subtypes were: P1.7-2,4 (17.7%), P1.22,9 (14.6%), P1.22,14 (11.8%) and P1.19-1,15-11 (7.5%). Predominant fetA VRs were F1-5 (23%), F5-1 (20%), F5-12 (10%) and F5-5 (9%). Major CCs varied in their porA/fetA diversity, predominant combinations were B:P1.7-2,4:F1-5 (cc41/44) (12.4%), B:P1.22,9:F5-12 (cc269) (7.5%), B:P1.19-1,15-11:F5-1 (cc269) (6.2%) and B:P1.22,14:F5-5 (cc213) (6%).

Discussion: Collectively, the predominant four porA subtypes and four fetA VRs (individually and/or in combination) accounted for 78% of the total isolates.

P061

Carriage of *Neisseria meningitidis* Among Saudi Arabian National Guard Personnel Before and After Vaccination with Meningococcal (Serogroups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid-Conjugate Vaccine

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Background: Decreasing the nasopharyngeal carriage of meningococci interrupts transmission of this pathogen and induces herd immunity. We examined whether diphtheria toxoid-conjugated ACWY vaccine (Menactra®, Sanofi Pasteur, Swiftwater, PA [MCV4]) could reduce meningococcal carriage in Saudi Arabian National Guard personnel stationed in Jeddah, Madinah or Ta'if, and assigned to work on the Hajj premises or otherwise interact with pilgrims.

Methods: In repeated cross-sectional surveys, nasopharyngeal swab specimens were obtained from participants before, 1 month after, and 6 months after MCV4 vaccination. N. meningitidis was identified by standard methods; serogroup was determined by slide agglutination. All isolates are being genetically characterized and compared to invasive clones, such as the W-135 strain that caused the 2000/2001 Hajj outbreaks.

Results: At the Jeddah site, 5 of 1200 participants (prevaccination), 1 of 534 participants (1 month postvaccination), and 0 of 881 participants (6 months postvaccination) were positive for carriage. At Madinah, the analogous data were 0 of 746, 1 of 339, and 0 of 392. At Ta'if, no participants were positive at the first (n=1180), second (n=867), or third (n=739) surveys. From the Jeddah site, 3 of the isolates (2 prevaccination and 1 postvaccination) were serogroup W-135, and the other 3 prevaccination isolates were non-serogroupable. The single isolate from Madinah was of serogroup A.

Conclusion: This large study demonstrates that N. meningitidis continues to circulate in Saudi Arabia. Although low carriage rates were observed at each survey, the data suggest a possible salutary effect of MCV4 on meningococcal carriage.

P062

Capsular switching and persistence of serogroup C ST-32/ET-5 complex *Neisseria meningitidis* as a cause of invasive disease in Brazil

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Background: In 1990, an epidemic of Neisseria meningitidis serogroup B of the ST-32/ET-5 complex peaked in Rio de Janeiro, Brazil. From 2000 onwards, there was an increase of serogroup C:4 strains causing sporadic cases and case clusters, suggesting the possibility of serogroup B→C capsular switching.

Methods: Serological typing was performed at the National Reference Meningitis Centre (Adolfo Lutz Institute, São Paulo). MIC to sulfadiazine was determined by E-test. Molecular characterization was performed by MLST and sequencing of outer membrane protein genes: porA, porB, fetA.

Results: Of randomly selected serogroup B:4 strains, 5 (porB 3-1 allele) were classified as B:P1.19,15:F5-1:ST-33 (cc32) and 10 (80%, porB 3-79 allele) as B:P1.7-1,1:F5-1:ST-639 (cc32). From 1993 to 2009, 34 (83%, 2000 - 2009) N. meningitidis C:4 strains were recovered from patients; 87% showed resistance to sulfadiazine. All isolates had a class 3 porB gene, mostly with 3-79 or 3-1 alleles. By MLST, 27 isolates had STs (81%, ST-639 or ST-33) related to the ST-32/ET-5 complex. According to OMP sequence profiles, 63% were 4:P1.7-1,1:F.5-1 and 30% were 4:P1.19,15:F.5-1. Other observed CCs found were ST-35, ST-376, and ST-41/44. One case cluster (2006) caused by C:P1.7-1,1:F.5-1:ST-7696 (cc32) was identified.

Conclusion: Previous reports of capsular switching (B→C) have resulted in few invasive disease cases caused by the serogroup C strain. In contrast, we report a substantial number of cases caused by ST-32/ET-5 serogroup C strains. The ability of N. meningitidis to cause substantial disease following capsular switching is of concern and has implications for vaccine prevention.

P063

Invasive disease caused by *Neisseria meningitidis* ST-11/ET-37 complex bearing capsule serogroups C or W135 in Brazil

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Background: We previously reported an increase in the proportion of meningococcal disease caused by serogroup W135 after 2003 in Rio de Janeiro, Brazil. We present an analysis of the genetic and immunologic diversity of serogroups C:2a and W135 strains isolated from 1988 onward.

Methods: Serological typing was performed at the National Reference Meningitis Centre (IAL, São Paulo). Molecular characterization was performed by MLST and sequencing of outer membrane proteins genes: porA, porB, fetA.

Results: A total of 29 W135 and 16 C:2a invasive isolates collected from 1988 to 2008 were studied. All C:2a strains had a class 2 porB gene, (81%, 2-2 alleles). In the 1990s, 67% of W135 strains had a class 3 porB gene (86%, 3-35), whilst in the 2000s 94% had a class 2 porB (63%, 2-145). By MLST, C:2a strains and those W135 strains isolated after 1999 had STs (83%, ST-11) belonging to the ST-11/ET-37 complex. Before 1999, 70% of the W135 was related to the ST-174 complex (ST-174 or adk SLVs of ST-174). During the 1990s, W135:P1.21,16:F.5-13 OMP sequence profile predominated, but after 1999 W135:P1.5,2:F.1-1 replaced the early OMP profile. In 2000s, there were 2 outbreaks caused by W135:2a:P1.5,2:F.1-1:ST-11 (cc11) and C:2a:P1.5-1,10-8:F.3-6:ST-11 (cc11).

Conclusion: Recent reports have indicated an increase in serogroup W135 disease. We report sporadic cases and outbreaks caused by ST-11/ET-37complex bearing both serogroups C or W135, which is related to the Hajj clone. Also, we point out the absence of a licensed vaccine in Brazil to protect against W135 disease.

P064

fHBP epidemiology of invasive meningococcal B isolates from Spain and Germany: age based prevalence of fHBP subfamilies

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The N. meningitidis factor H binding proteins (fHBP) are promising vaccine candidates and appear to be critical for survival of meningococci in the human host. Extensive analyses have determined that the gene is present in all invasive meningococcal disease serogroup B (IMDB) isolates and that all fHBP fall within two distinct subfamilies (A and B). Vaccines composed of monovalent fHBP elicit mainly subfamily-specific responses. As a step towards evaluating potential impact of fHBP-based vaccines, the current study examines the age-stratified fHBP epidemiology of strains isolated from IMDB patients.

fHBP characteristics were determined for IMDB isolates systematically selected from national laboratories of Spain (n=346) and Germany (n=205) between 2001 and 2006. Information on patient age, gender, and disease outcome was provided for each isolate where available. Analyses included descriptive statistics and assessment of bi-variate relationship of fHBP characteristics with available clinical parameters.

Isolates from both subfamilies caused disease across the age range, with an increased proportion of subfamily A evident in the youngest age group (children <1 year) as compared to that seen in the population overall; 64% vs 40% in Spain and 45% vs 21% in Germany. Dominant fHBP variants were responsible for the majority of disease in all age groups. No association was seen between subfamily and disease outcome.

Age-stratified data analyses of IMDB isolates from Spain and Germany confirm that a bivalent vaccine containing one member from each fHBP subfamily has the potential to significantly impact disease in all age groups.

P065

Increased virulence of non-encapsulated *Neisseria meningitidis* isolates in the mouse model

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The capsule of N. meningitidis is the major pathogenicity factor that enables these bacteria to overcome host immunity elicited by complement and phagocytes, rendering them capable of surviving in blood. Therefore, capsule-deficient N. meningitidis isolates are generally considered non-pathogenic. Here, we report that non-encapsulated N. meningitidis isolates from patients with meningoccemia were able to cause morbidity and mortality in an intraperitoneal mouse infection model.

The non-encapsulated strains were isolated from blood cultures of two patients in Ontario (strains 2006-178, 2009-044) and one patient in British Columbia (2004-111, see Hoang et al. 2005 Clin. Infect. Dis.). Capsule deficiency was confirmed by serology and PCR for ctrA-D and siaA-C genes as well as serogroups B, C, Y, and W-135 specific siaD genes. For 2006-178 and 2004-111, capsule synthesis genes were replaced by the capsule null locus cnl-2.

Virulence of the non-encapsulated isolates was compared to that of the well-described N. meningitidis strains MC58 and B16B6, and a capsule-deficient mutant of MC58 lacking the siaD locus (MC58ΔSiaD). C57Bl/6 mice were inoculated intraperitoneally with 1E+05-1E+08 bacteria with human transferrin as an iron source. Mortality was observed at a high inoculation dose (1E+08) with two of three non-encapsulated strains: 5/10 mice succumbed to 2006-178 and 2/11 succumbed to 2004-111, whereas no mortality occured with 2009-044 or MC58ΔSiaD. In comparison, 6/8 mice succumbed to MC58 and 7/8 mice succumbed to B16B6 at a dose of 1E+07 bacteria. Our results suggest the acquisition of a new virulent phenotype by these non-encapsulated strains.

P066

Identification of a novel anti-σE factor in *Neisseria meningitidis*

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Background: Gene expression in Neisseria meningitidis needs continuous fine tuning to adapt to changing conditions during different stages in colonization and disease. In many pathogens σE, encoded by rpoE, is essential in mounting responses to environmental challenges. Here we identified genes belonging to the σE regulon of meningococci.

Methods: Knockout and overexpressing mutants of NMB2144 (rpoE) and NMB2145 were constructed and differentially expressed proteins were identified by SDS-PAGE and MALDI-TOF mass spectrometry. Mutations were generated using site-directed mutagenesis and transcription was quantified by RT-PCR.

Results: We showed that σE is part of the polycistronic operon NMB2140-NMB2145. Overexpression of NMB2144 in trans increased transcription of NMB2140-NMB2145, indicating that this operon is autoregulated. σE also controls the expression of methionine sulfoxide reductase (MsrA/MsrB). In the NMB2145 deletion mutant transcription of NMB2140-NMB2145 is also increased. NMB2145 encodes a protein structurally related to anti-sigma domain (ASD) proteins, having a zinc containing anti-σ factor (ZAS) motif in common. Results of site-directed mutagenesis showed that the Cys residues in the ZAS motif and at position 4 of NMB2145 are essential for anti-σE activity. In general, the anti-sigma property of ASD proteins is abrogated by oxidative agents. However, in meningococci oxidative agents had no effect on σE activity.

Conclusions: Meningococci express a functional σE factor, which is transcriptionally autoregulated. Its activity is controlled by a novel meningococcal anti-σ factor related to the ZAS family.

P067

Characterization of the MDA (Meningococcal Disease Associated) bacteriophage

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Comparison of strains from hypervirulent and non-hypervirulent clonal complexes of Neisseria meningitidis by whole genome microarrays revealed the presence of a 8kb island significantly associated with hypervirulent complexes. Further epidemiological investigations have shown that this island appears to be highly present in bacterial strains causing menningococcal disease. This island is composed of 10 ORFs, is integrated in the bacterial chromosome into dRS3 repeat sequences, and constitutes a filamentous bacteriophage since a double stranded replicative form and a single stranded circular form protected by protein have been detected in the cytoplasm of the bacteria and in the extracellular medium, respectively. This is reinforced by structure and sequence homologies of the MDA with other filamentous phages such as M13 or CTXΦ of Vibrio cholerae. In order to confirm these homologies, mutants of each genes were analyzed and the phenotypes observed confirmed the supposed roles of the phagic proteins. Moreover, the production of the phage varies widely between different strains and under stress conditions. Therefore an analysis of the conditions that modulate expression of the MDA phage was realized.

P068

Differential Regulation of Glutamine Biosynthesis Genes *glnA* and *glnE* in *Neisseria gonorrhoeae* by MtrR

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MtrR is a DNA-binding protein that represses transcription of the mtrCDE-encoded efflux pump in Neisseria gonorrhoeae and its production influences levels of gonococcal resistance to certain antimicrobials and impacts in vivo fitness in an experimental murine model of infection. We have shown that MtrR can dampen the expression of nearly fifty genes as well as enhance expression of over twenty other genes. Interestingly, two genes encoding enzymes involved in glutamine biosynthesis were identified as being differentially controlled by MtrR, with glnA (encoding glutamine synthetase [GS]) being subject to MtrR repression and glnE (encoding an adenylase/deadenylase that regulates GS activity) being MtrR-activated. The novelty of a DNA-binding protein, known for controlling efflux gene expression with a capacity to differentially control genes involved in the same biosynthetic pathway, prompted us to study the ability of MtrR to modulate expression of genes outside of the mtr efflux locus. Herein, we report the capacity of MtrR to repress glnA, which is also positively controlled by the product of an MtrR-repressed gene (farR). FarR binding to its target DNA sequence upstream of glnA was reduced in the presence of competing MtrR, suggesting that MtrR-mediated repression of glnA occurs by reducing the gene-activating capacity of FarR. In contrast, expression of glnE was found to be directly activated by MtrR by a FarR-independent mechanism. As glutamine is a scarce amino acid at mucosal surfaces and within phagolysosomes, the ability of MtrR to regulate glnA and glnE likely influences in vivo fitness and survival of gonococci.

P069

Competition between MtrA and MtrR for regulation of the *mtrCDE* efflux pump

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Antibiotic resistance in Neisseria gonorrhoeae is a worldwide public health problem and the MtrCDE efflux pump, which recognises a broad spectrum of host-derived and external antimicrobials, contributes to such resistance. The mtrCDE efflux pump operon is directly regulated by the MtrR repressor and the MtrA activator. We report the first successful purification of MtrA and its DNA-binding properties and show that it competes with MtrR for binding to the mtrCDE promoter; these proteins have different affinities for this site. We found that pre-incubation of MtrA with effectors that induce expression of mtrCDE enhance its binding to the promoter such that under these conditions, MtrR binding is decreased. To determine the nucleotides important for MtrA binding, we used low-fidelity PCR to generate mutations that impacted MtrA binding; these mutations mapped to a region 10-15 base pairs upstream of the MtrR binding site. We verified that these mutations influenced induction of the mtrCDE efflux pump when gonococci were grown on sublethal levels of triton X-100. The results from our study document that regulation of mtrCDE expression is dependent on competition between MtrR and MtrA for sterically close binding sites. Given that an MtrA mutant is less fit compared to its parent strain in a mouse model of vaginal infection (Warner et al., 2008), the inability to displace MtrR and activate expression of the mtrCDE efflux pump operon could, in part, explain this change in in vivo fitness.

P070

Functional domains of FtsA from *Neisseria gonorrhoeae* involved in interaction with divisome proteins from *N. gonorrhoeae*

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Objectives: 1) to investigate the protein-protein interactions of three “early” Neisseria gonorrhoeae (Ng) divisome proteins FtsANg, FtsZNg and ZipANg; 2) to study the interactions between FtsANg and the “late” divisome proteins FtsKNg, FtsQNg, FtsINg, FtsWNg, FtsNNg, FtsENg, and FtsXNg; 3) to identity the functional domains of FtsANg involved in these interactions.

Methods: A model for FtsANg was predicted and its domains were identified based on the resolved crystal structure of FtsA from Thermotoga maritima. Wild-type divisome proteins and FtsANg truncations, which isolated various domains, were cloned into bacterial two-hybrid (B2H) and GST vectors. Protein-protein interactions were tested using B2H assay and confirmed with GST pull-down assay.

Results: FtsANg interacted with FtsZNg, however ZipANg did not interact with either FtsZNg or FtsANg. FtsANg associated with the “late” divisome proteins FtsKNg, FtsQNg, FtsWNg, and FtsNNg. Four domains (1A, 1C, 2B and 2A including 2A1 and 2A2) of FtsANg were identified by structural homology modeling. Domain 2A1 interacted with FtsZNg, FtsKNg, and FtsNNg. Domain 2B interacted with FtsZNg, FtsKNg, FtsQNg, and FtsNNg. Domain 2A2 interacted with FtsZNg, FtsQNg, FtsWNg, and FtsNNg. Divisome formation in N. gonorrhoeae differs from the proposed models for E. coli because the “late” divisome proteins associating with FtsANg differ from those reported in E. coli. Furthermore, the domains of FtsANg interacting with these proteins are also novel.

P071

Gonococcal FetA expression is regulated by MpeR and enables strain FA1090 to utilize enterobactin and its linear derivatives

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Neisseria gonorrhoeae produces no known siderophores but is capable of acquiring iron from xenosiderophores produced by other bacterial species, including Escherichia coli. FetA is a TonB-dependent, outer membrane transporter expressed by all Neisseria species characterized to date. We tested whether N. gonorrhoeae could utilize enterobactin, a cyclic trimer of dihydroxybenzoylserine (DHBS), in addition to the linear dimer, trimer, and monomer derivatives. Plate bioassays and liquid growth assays demonstrated that strain FA1090 utilized enterobactin, in addition to the dimer (D2) and trimer (D3) derivatives in a TonB- and FetA-dependent manner. Use of the monomer of DHBS was largely TonB- and FetA-independent. Genes located downstream of fetA encode a putative periplasmic binding protein and ABC transport system. We determined that these downstream genes are co-transcribed with fetA under iron-deplete conditions. We also established that the iron-regulated, AraC-like regulator, MpeR, is necessary for maximal expression of fetA under iron-deplete conditions in FA1090. MpeR, which is known to regulate the expression of antimicrobial agent efflux pumps, is encoded within a locus that is not contiguous with the fetA operon. Interestingly, mpeR has only been identified in the genomes of pathogenic Neisseria species, in contrast to the fetA operon. Other pathogens utilize substrate-inducible, AraC-like regulators for maximal expression of siderophore utilization genes. We are currently employing qRT-PCR and western blot analysis to test the hypothesis that iron-depleted growth conditions and internalization of enterobactin or the linear derivatives (D2 and D3) results in maximal fetA expression via an MpeR-dependent mechanism.

P072

Synergistic killing of a gonococcal *katA* mutant by hydrogen peroxide and peroxynitrite

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We have previously reported that N. gonorrhoeae is extremely resistant to reactive nitrogen species (RNS) including peroxynitrite (PN). Recent literature suggests that catalase can provide protection against commercial preparations of PN. Though wild type gonococci were shown to be highly resistant to 2mM PN, a gonococcal katA mutant and N. meningitidis were both shown to be highly sensitive to 2mM PN. Analysis of translational fusions of the catalase promoters from N. gonorrhoeae and N. meningitidis to lacZ demonstrated that basal katA expression from gonococci is 80-fold higher than in meningococci, though meningococcal katA retains a greater capacity to be activated by OxyR. This activation capacity was ultimately shown to be due to a single base pair difference in the -10 transcription elements between the two kat promoters. PN resistance was initially shown to be associated with increasing catalase expression, however, commercial preparations of PN were later revealed to contain high levels of contaminating hydrogen peroxide. Removal of hydrogen peroxide from PN preparations with manganese dioxide markedly reduced PN toxicity in a gonococcal katA mutant. There was a synergistic effect of treatment with both hydrogen peroxide and PN simultaneously. Our results suggest that the high toxicity of commercial peroxynitrite preparations is due to contamination with hydrogen peroxide, and that peroxynitrite, on its own, may not be as toxic to bacteria as reported in the literature.

P073

NeMeSys: a biological resource for narrowing the gap between sequence and function in *Neisseria meningitidis*

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Genome sequences, now available for most pathogens, hold promises for the rational design of new therapies. However, biological resources for genome-scale identification of gene function and/or genes essential for cell viability, which are necessary to achieve this goal, are often sorely lacking. This holds true for Neisseria meningitidis, one of the most feared human bacterial pathogens. By determining and annotating manually the complete genome sequence of a serogroup C clinical isolate of N. meningitidis (strain 8013) and assembling a library of defined mutants in up to 60% of its non-essential genes, we have created NeMeSys a biological resource for Neisseria meningitidis systematic functional analysis. To further enhance the versatility of this toolbox, we have manually (re)annotated 8 publicly available Neisseria genome sequences and stored all these data in a publicly accessible online database. The potential of NeMeSys for narrowing the gap between sequence and function was illustrated in several ways, notably by performing an exhaustive functional analysis of type IV pili, one of the most widespread virulence factors in bacteria. By improving our capacity to understand gene function in an important human pathogen, NeMeSys is expected to contribute to the ongoing efforts aimed at understanding a prokaryotic cell comprehensively and eventually to the design of new therapies.

P074

Prevalence and Detailed Mapping of the Gonococcal Genetic Island in *Neisseria meningitidis*

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The gonococcal genetic island (GGI), which is 57 kb and encodes a type IV secretion system (T4SS), is found in 80% of Neisseria gonorrhoeae (gonococcus, GC) and some strains of N. meningitidis (meningococcus, MC). The gonococcal T4SS has been shown to secrete single-stranded chromosomal DNA directly into the culture medium, which is an activity unique among T4SS's. We screened clinical isolates of MC to establish the prevalence of the GGI and ascertain if any strains had a complete GGI. We found 11 of 75 (22%) of isolates we screened have the GGI. In order to identify and characterize the differences between the meningococcal gonococcal GGIs, we performed a PCR walk across the GGI of seven MC strains. We found several differences including a deletion in traD, encoding the putative coupling protein, a longer version of traA, encoding the putative pilin, and a specific allele of traG, encoding a putative inner membrane protein. These differences were not common to all strains, and two strains, 01/241471 and 13102, possess an island nearly identical to that of GC. Both meningococcal and gonococcal GGIs are flanked by direct repeats, called dif, although the sequence differs between species. We are able to detect excision of the GGI, and preliminary results indicate that this occurs at an appreciable frequency in both gonococci and meningococci. The variability in excision frequency may be related to the sequence of dif. We are also investigating possible functions of the T4SS in MC including DNA secretion and roles in infection.

P075

The division and cell wall synthesis clusters of the commensal *Neisseria* spp.

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One of the most highly conserved gene clusters across all bacterial species is the division and cell wall synthesis (dcw) cluster, which encodes proteins essential to these processes. The gene order and sequences are even conserved across the Gram-negatives and Gram-positives as well as between rods and cocci. In some species, additional genes involved in similar processes are also included in the dcw cluster, such as proteins responsible for the division and cell wall synthesis processes associated with sporulation. In the pathogenic Neisseria spp., the dcw cluster has previously been analyzed in detail revealing a Correia Repeat Enclosed Element, a neisserial uptake sequence, and three additional dcw cluster associated genes. One of these genes, dcaC, contains a coding tandem repeat with variable copy number between strains. Another of these genes, dca (pptA), is only present in the pathogenic Neisseria spp., is phase variable in some strains, and is involved in phosphorylcholine addition to pilus. In this study, the dcw clusters of commensal Neisseria spp. were extracted from genome sequencing project data and analyzed for gene content and similarities to the pathogenic Neisseria cluster. This revealed the presence of other additional genes, some of which were conserved between the commensal species. These results show that there are confirmed Minimal Mobile Elements between murE and murF and between murG and murC, with possible others within the cluster. Sequence similarity, gene cluster structure, and the potential phylogenetic relationships revealed between the commensal and pathogenic species will be presented and discussed.

P076

UDP-N-acetylgalactosamine is synthesised by a bifunctional GalE in *Neisseria gonorrhoeae*

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Gonococcal LOS can terminate with endogenously synthesized N-acetylgalactosamine (GalNAc) and this addition increases the rate of invasion of the gonococcus into cells expressing the macrophage galectin-type lectin (MGL). Microbes synthesize GalNAc either via a bifunctional UDP-galactose 4-epimerase (GalE) or a UDP-GalNAc 4-epimerase (GNE). Since N. gonorrhoeae does not contain a GNE homologue, we propose that the GalE of N. gonorrhoeae is a bifunctional epimerase that synthesizes both UDP-galactose (UDP-Gal) and UDP-GalNAc from the substrates UDP-glucose (UDP-Glc) and UDP- N-acetylglucosamine (UDP-GlcNAc), respectively. Bioinformatic analysis of other known bifunctional and monofunctional GalE proteins suggests that a single amino acid in the active site of the enzyme dictates the substrate specificity for UDP-glucose and UDP-GlcNAc. The analogous position in gonococcal GalE is a serine residue which suggests that both UDP-Glc and UDP-GlcNAc will act as substrates for this enzyme. In contrast, meningococcal GalE has a phenylalanine located at the homologous position which we propose will prevent binding of UDP-GlcNAc, hence resulting in a monofunctional epimerase for the production of UDP-Gal alone.

In an effort to gain insight into the functional role that GalE plays in N. gonorrhoeae we cloned galE from FA1090, and recombinantly expressed and purified the gonococcal GalE protein. Incubation of UDP-GlcNAc with the purified enzyme resulted in the production of UDP-GalNAc as confirmed by HPLC analysis.

In conclusion, the species specific synthesis of GalNAc by N. gonorrhoeae is dictated by a conserved single amino acid change in the GalE.

P077

Developing an *in silico* metabolic model of *N. meningitidis* MC58

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N. meningitidis is able to adapt and proliferate in different compartments within the body but very little is known about the substrates it utilises or the metabolic pathways that are active during commensalism and infection. As a first step to developing an understanding of how the pathogen adapts to the host environment we have constructed a genome-scale metabolic network model of the meningococcus, GSMN-Nm. The model was developed from the publicly available metabolic model of E. coli (Feist et al, 2007). N. meningitidis orthologues to E. coli genes were identified and used to replace the E. coli genes in the model. Reactions in which no N. meningitidis orthologues could be identified were removed so long as their removal did not lead to infeasibility of the model. Reactions and pathways unique to N. meningitidis were added and "orphan" reactions that were required to complete essential pathways were also included. The final model consists of 1270 reactions and enzymes encoded by 549 genes.

The model correctly predicts N. meningitidis growth characteristics and substrate utilization under a range of conditions. We have also interrogated the model by comparing gene essentiality predictions of the model with gene essentiality determined by global transposon mutagenesis. The model already provides novel insight into the metabolic capabilities of the pathogen. It will be a useful tool to study the metabolism of N. meningitidis in vitro and during infection.

P078

The sequenced genome of ST-8 isolate *Neisseria meningitidis* strain NMB (B:2B:P1.2,5:L2; CDC8201085)

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The majority of invasive meningococcal disease is caused by a limited number of hypervirulent lineages of which ST-8 is one. ST-8 isolates are characterized by the possession of adhesins such as the four hypervirulent opacity protein alleles, Class II Type IV pili, and lack the opacity-like protein, Opc.

To ascertain if the ST-8 lineage contained novel virulence determinants which could be attributed to increased virulence, we sequenced the genome of Neisseria meningitidis strain NMB (B:2B:P1.2,5:L2: CDC8201085). Although ST-8 strain NMB produces biofilms, it lacks a functional denitrification pathway with deletions in both aniA and norB which are important for anaerobic growth. In addition to containing the genetic islands for the expression of L2 lipooligosaccharide immunotype, it contains the genes for the expression glycosylated proteins with glyceramido-acetamido trideoxyhexose lacking the di-galactose addition. As has been seen in other neisserial genomic sequences, the vast majority of variable changes relate to strain specific patterns of insertion elements, a proportion of which have integrated into operons necessary for membrane biogenesis and metabolism. Very few novel open reading frames were found with the majority having been identified in one or more other sequenced neisserial genomes.

In conclusion, the genome ST-8 strain NMB contains a similar array of genetic features found in other neisserial isolates from different hypervirulent lineages. We conclude that regulatory factors controlling the expression of known virulence determinants must play an important role in determining the virulence of ST-8 isolates.

P079

The role of DprA in meningococcal transformation

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Objectives: DNA processing chain A (DprA) is a protein required for transformation in the constitutively competent meningococcus and has been proposed to be a recombination mediator protein dedicated to natural transformation. It interacts with the single-stranded binding protein SSB and the recombinase RecA in other bacterial species. We are investigating the interplay between DprA and the recombination and transformation machineries in the meningococcus. Curiously, the protein is ubiquitous and widely conserved, and it also occurs in many species that are not known to be naturally competent. Therefore we ask the question: Does the protein have additional functions, for example in recombinational repair?

Materials and methods: Meningococcal null mutant strains were constructed and assessed with regard to transformability, colony morphology and fitness for survival under oxidative and alkylating stress, as well as mitomycin C influence. Recombinant DprA was purified to homogeneity and monitored with regard to DNA binding and substrate specificity with ssDNA/dsDNA and homo- and heterologous DNA by electromobility shift assays. Reverse transcription PCR was employed to assess potential co-transcription of dprA and neighbouring genes.

Results and conclusion: We confirm that the meningococcal ΔdprA strains were not competent for transformation. The sensitivity of the ΔdprA strains to oxidative and alkylating stress and mitomycin C did not differ from the wild-type strain. The dprA gene appears to be a single transcriptional unit. These findings support the hypothesis that DprA exclusively is dedicated to transformation, as of yet no role in recombinational repair could be established.

P080

Genome-wide effects of transformation in *Neisseria meningitidis*

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OBJECTIVES: Neisseria meningitidis readily takes up and incorporates extracellular DNA into its genome through transformation. Transformation contributes to genetic variation and adaptive potential within natural populations of N. meningitidis but many details of the process remain unclear. We have investigated transformation parameters in a number of meningococcal strains and are currently studying the size of recombination events and frequency of concurrent mutation events, using high throughput (HTP) genome sequencing and SNP identification methods.

METHODS AND RESULTS: Transformation frequencies were determined using DNA from donor strains containing either lipopolysaccharide or DNA repair genes interrupted by an antibiotic resistance cassette, or spontaneous point mutation-derived antibiotic resistance, and appropriate unmarked recipient strains. Frequencies differed between the various recipient strains, but the choice of selective marker (antibiotic resistance gene or location) had little effect on the rate of transformation observed. Similar results were obtained in a transformation assay using epithelial cells as a matrix for neisserial culture. The data from these combined experiments identified appropriate strains and conditions to investigate transformation through HTP genome sequencing. For Illumina sequencing, strain MC58 (highly transformable) was used as the recipient strain, while a derivative of strain FAM18 with point mutation-derived rifampicin resistance was used as donor, in a liquid culture transformation assay. Genomic DNA pooled from 200 successful transformants was sequenced and the presence of donor-specific SNPs identified in order to quantify events in the transformation process in N. meningitidis.

P081

Specific necessity of *N. meningitidis* to export manganese through a new metal efflux pump

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Neisseria gonorrhoeae (Ng) and N. meningitidis (Nm) are adapted to different environments within their human host. If the basis of this difference has not yet been understood, previous studies (including our own data) have reported that, unlike Ng, Nm can tolerate high manganese concentration. As transition metals play important roles in regulating host pathogen interactions and are essential nutrients for bacteria, we aimed to identify the genetic reasons leading to Mn2+ tolerance and the pathogenic consequences. To identify novel factors involved in manganese homeostasis we used a model organism, Xanthomonas campestris, which has a 'simple' predicted manganese regulon (mntR, mntH but without perR, mntA, mntE). Bioinformatic screening for genes controlled by the manganese dependent regulator MntR revealed a single candidate, which we identified as a manganese resistance factors as determined by targeted gene deletion. It shows similarities with the SMR transporter family and exports Mn2+ as determined by heterologous expression in E. coli. In the Neisseria genus, this factor is present and functional in all Nm isolates but it is mutated and non functional in the majority of Ng strains and absent in a majority of non pathogenic species. All these data suggest that the presence of a functional Mn2+ exporter is under a strong positive selection in N. meningitidis but not in other neisseria. As anticipated, experimental deletion of this gene in Nm leads to alteration of various phenotypes including stress resistance, expression of virulence factors (e.g. pilin) and virulence, symptomatic of an impaired bacterial homeostasis.

P082

A TonB-dependent heme receptor in *Neisseria meningitidis*

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Neisseria meningitidis employs redundant heme acquisition mechanisms, including TonB-dependent hemoprotein receptors, HmbR and HpuA/B. A putative TonB-dependent receptor, NMB0964, shares significant sequence similarity to HumA, a heme receptor of Moraxella catarrhalis, and contains conserved motifs found in many heme utilization proteins. When expressed in E. coli, NMB0964 allowed heme capture by the E. coli cells and supported heme-dependent growth of an E. coli hemA strain. Excess free heme inhibited binding of NMB0964 in the enriched E. coli membrane fraction to heme agarose. Thus, it was designated as nhuR for Neisseria heme uptake receptor. A meningococcal nhuR mutant created in a strain lacking all known heme uptake systems (hmbR/hpuB/pilQ) displayed no discernible defect in heme-dependent growth, suggesting the presence of additional heme uptake mechanisms in meningococci. Interestingly, distinct from other TonB-dependent iron receptors, nhuR expression was induced by iron in a Fur-dependent manner, and EMSA demonstrated a specific interaction of meningococcal Fur protein with the nhuR promoter. As TonB, but no specific TonB-dependent receptors, has been shown to be required for meningococcal replication within epithelial cells (1), the role of NhuR was examined. We found that the meningococcal nhuR mutant was defective in invasion of and survival within A549 epithelial cells. In correlation, nhuR expression was induced in the presence of epithelial cells when compared to meningococci grown in the cell free culture medium. Thus, the NhuR heme receptor is positively regulated by iron and is important for intracellular replication of N. meningitidis.

P083

Characterization of LtgA, a lytic transglycosylase of *Neisseria gonorrhoeae*

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Peptidoglycan fragments released during the growth of Neisseria gonorrhoeae have been shown to kill ciliated Fallopian tube cells, induce inflammatory cytokines, and cause arthritis. These fragments are produced through the action of lytic transglycosylases, which cleave the glycan backbone at the β-1,4 linkages between N-acetylmuramic acid and N-acetylglucosamine with the formation of a 1,6-anhydro bond on the N-acetylmuramic acid moiety. Previous work in our laboratory identified two lytic transglycosylases, LtgA and LtgD, involved in the production of soluble monomeric peptidoglycan fragments. Mutants in either ltgA or ltgD released monomer at levels that were 62% or 38% of wild-type, and a double mutant lacking ltgA and ltgD failed to release any peptidoglycan monomer. In this work, we heterologously overproduced and purified a soluble form of LtgA and demonstrate using an HPLC-based assay that this enzyme can degrade macromolecular gonococcal peptidoglycan. Treatment of the soluble reaction products with the reducing agent sodium borohydride results in an HPLC elution profile nearly identical to the profile of the unreduced reaction products, consistent with the formation of non-reducible 1,6-anhydro bonds. Furthermore, analysis by LC/MS shows that the major products of the enzymatic reaction are 1,6-anhydro-tripeptide monomer and 1,6-anhydro-tetrapeptide monomer, providing additional biochemical support for the role of LtgA as a lytic transglycosylase. Further characterization of LtgA is aimed at determining substrate specificity, localization, and potential complex formation with LtgD and/or other peptidoglycan-associated proteins.

P084

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

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Due to widespread emergence of antibiotic resistance, only cephalosporins are now recommended for treating gonoccocal infections, a precarious position that demands the development of new antimicrobials directed against Neisseria gonorrhoeae. Cephalosporins (and other β-lactams) disrupt cell wall biosynthesis by inhibiting transpeptidases involved in peptidoglycan cross-linking. The success of these antibiotics suggests that other enzymes of peptidoglycan metabolism could also be viable targets. One enzyme involved in peptidoglycan breakdown is N-acetylmuramyl-L-alanine amidase, known as AmiC. Mutation or deletion of AmiC results in improper cell separation during division and increases susceptibility to a number of antimicrobial agents. The goal of this project is to evaluate AmiC as a prospective antimicrobial target through biochemical and structural studies, and to identify inhibitors of AmiC through screening of chemical compound libraries. We have expressed and purified full-length AmiC, as well as constructs of the catalytic C-terminal domain and the N-terminal domain, whose function is unknown. Zinc-dependent amidase activity of the full-length enzyme and of the C-terminal domain construct was shown by turbidity, zymogen and fluorescent peptidoglycan assays. Surprisingly, the same assays reveal that the N-terminal domain also breaks down peptidoglycan, suggesting that AmiC may be a bifunctional autolysin. We have obtained crystals of the N-terminal domain diffracting to 2 Å and efforts to phase the structure are underway. In tandem, NMR is being employed to solve the structure of this domain. In order to discover inhibitors against AmiC, we have designed a novel fluorescent substrate in readiness for high-throughput screening using a FRET-based assay.

P085

New technology for investigating cell proliferation and cell death during meningococcal infection

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Measuring cell proliferation and cell death during N. meningitidis infection involves performing end-point assays that represent the response at a single time point. A new technology from Roche Applied Science and ACEA Biosciences allows continuous monitoring of cells in real-time using specialized cell culture microplates containing microelectrodes. The xCELLigence system enables continuous measurement and quantification of cell adhesion, proliferation, spreading, cell death and invasion, thus creating a picture of cell function during meningococcal infection. Furthermore, lag and log phases can be determined to estimate optimal times to infect cells.

In this study we used this system to provide valuable insights into cell function in response to several virulence factors, including lipopolysaccharide (LPS), polysaccharide capsule and outer membrane protein (Opc). Furthermore cell function in response to 10 strains of apathogenic Neisseria spp. (N. lactamica and N. mucosa) was analyzed. We observed that prolonged time of infection with pathogenic Neisseria strains led to morphological changes including cell rounding and loss of cell-cell contact as described recently (1), thus resulting in changed electrical impedance as monitored in real-time. In contrast, infection with apathogenic N. lactamica isolates did not change electrical impedance monitored for 45 hours. Together our data show that this system can be used as a rapid monitoring tool for cellular function in response to bacterial infection and combines high data acquisition rates with ease of handling.

1. Schubert-Unkmeir, A., C. Konrad, H. Slanina, F. Czapek, S. Hebling, and M. Frosch. PLoS Pathog 6:e1000874.

P086

Analysis of the effect of PorB antigenic diversity on *Neisseria meningitidis* fitness

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Neisserial PorB is an essential outer membrane protein that is immunoreactive and not phase variable. PorB and other OMPs are antigenically diverse complicating their development and assessment as vaccines. We hypothesize that although PorB is under positive immune pressure, antigenic diversity is restricted by cell physiology and host response. We established a vector-based system for switching the porB gene. We created isogenic tranformants in an MC58 porA(-) background that express: parent MC58 PorB (serotype 15); serotype 4 PorB common to Brazil and New Zealand epidemic strains; prototype serotype 4 PorB; natural hybrid PorB (serotype 4/15); or a laboratory hybrid construct (serotype 15/4). The natural hybrid transformation was difficult and required use of MC58wt with subsequent deletion of porA. To test physiological differences between the porins, we assessed isogenic strain growth under different conditions. Strains differing only in PorB had similar growth in liquid media. Strains lacking porA had 10-fold less CFU/ml than MC58wt following incubation in serum and buffer except the strain expressing Brazilian serotype 4 PorB which had 2.6-fold higher CFU/ml. Differences in antibiotic susceptibility were also detected. PorB serotype 4 Loop1 antiserum exhibited a 4-fold difference in bactericidal activity against two serotype 4 transformants suggesting a conformational affect on the epitope by amino acid differences in non-adjacent regions. The isogenic strain panel expressing PorB with defined differences in surface-exposed loops enabled the initial characterization of affects on strain fitness and will serve as an important tool for investigating immunogenicity, antigenicity and structure or function consequences of PorB diversity.

P087

Functional Analysis of Genes Encompassing the *Neisseria gonorrhoeae* Fur Activated Regulon

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The sexually transmitted disease pathogen Neisseria gonorrhoeae globally regulates gene expression in response to the iron-limited mucosal surface in the human body. Part of this regulation is fulfilled by the ferric-uptake regulator (Fur), a transcriptional regulator that responds to iron availability. Although Fur has been classically defined as a transcriptional repressor, recent studies demonstrated that a subset of genes that are activated under iron-replete growth conditions also contain in silico predicted Fur binding boxes, suggesting that Fur binds to these regulons to directly activate their transcription. However, activation of gene transcription can be both direct (by direct binding to conserved Fur box sequences in the promoters) and indirect via secondary regulatory proteins. Thus in this study we have further characterized the mechanism of Fur mediated direct activation of gonococcal genes. The direct binding of Fur to the putative promoters was determined using EMSA and confirmed that less than 50% of the genes, which contained predicted Fur boxes, bound Fur in vitro. For those promoters to which Fur was demonstrated to bind we observed different binding affinities as well as differences in the positions of Fur boxes indicative of a range of putative regulation. Likewise semi-quantitative RT-PCR analysis of a N. gonorrhoeae fur mutant revealed alterations in gene expression of several of these Fur activated genes compared to that in the wild type. Thus Fur functions as a direct activator of gene transcription via binding to defined promoter regions, which results in a range of transcriptional control.

P088

Variation in TraG and the associated peptidoglycanase affect DNA secretion by *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae secretes chromosomal DNA via a type IV secretion system (T4SS) encoded in the gonococcal genetic island. The secreted DNA is active in the transformation of other gonococci in the population, and this mechanism of DNA secretion may contribute to genetic diversity in this highly variable species. Most genes of the T4SS show a high degree of conservation, but the region downstream of and including traG are variable. There are three different alleles of traG, encoding a putative membrane protein similar to mating-pair stabilization proteins from F-like conjugation systems. Protein fusions were used to study the topology of TraG, and TraG was demonstrated to be an inner membrane protein with either one or two large soluble regions in the periplasm. Downstream of traG is atlA, encoding a peptidoglycan lytic transglycosylase. In some strains a different allele of traG is present and atlA is replaced with the gene for a different peptidoglycanase, an endopeptidase. The putative endopeptidase was demonstrated to bind peptidoglycan, but peptidoglycan cleavage has not been detected. TraG and AtlA are required for DNA secretion. When the traG-atlA region was replaced with the traG-EP region, DNA secretion ability was lost, and DNA secretion could not be restored by expression of AtlA. These studies demonstrate that TraG acts in DNA secretion and that different forms of TraG affect what substrates can be transported by the T4SS.

P089

Prevalence and genetic diversity of candidate vaccine antigens among invasive *Neisseria meningitidis* isolates in the United States

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Objective: Two vaccines are being developed to target Neisseria meningitidis (Nm) serogroup B, but may also protect against other serogroups. We characterized genes encoding factor H binding protein (FHbp), Neisserial adhesin A (NadA), and Neisserial heparin binding antigen (NhbA), in U.S. Nm isolates.

Methods: Isolates were collected through active, population-based surveillance (ABCs)1 from 9 states. 896 Nm isolates (650 Bs, 2000-8; 246 non-Bs, 2006-8) were assayed for fHbp by sequence analysis and for the presence/absence of nadA by PCR. 85 nadA+ isolates and 381 isolates (randomly selected 136 Bs, 2000-8 and 245 nonBs, 2006-8) had nadA and nhbA sequenced, respectively. Proportions are shown weighted for Oregon, which had a NmB outbreak during this time.

Results and Conclusion: The overall fHbp subfamiliy/variant2-3 distribution was 44% B/v1 and 55% A/v2-3, with B/v.1 predominating in NmB (61%) and A/v.2-3 in non-NmB (78%). Eleven NmC isolates contained fHbp with a single base-pair deletion creating a frameshift. nadA and nhbA were present in 38% and 98% of isolates, respectively. NadA were relatively conserved. Pairwise identity of NadA and NhbA were greater than 93% and 78%, respectively. A few FHbp subvariants appeared more frequently in certain clonal complexes than in others. In conclusion, although assessment of vaccine coverage requires understanding of the antigen expression and the ability to induce bactericidal activity, FHbp and NhbA have broader distribution in US isolates than NadA, suggesting that FHbp or NhbA may play role in providing broad protection against multiple Nm serogroups.

P090

Two Partner Secretion Systems Are Associated with Clinical Outcome in Meningococcal Meningitis

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Background: Meningococcal secreted products are considered to be important in the pathogenesis of meningococcal invasive disease. Three Two Partner Secretion (TPS) systems exist among meningococci. Host immune response is elicited by two of the secreted products TpsA2 and TpsA3, but their relevance is unclear. The aim of this study was to evaluate the distribution of the TPS systems among meningococcal isolates from meningitis patients and to correlate this with clinical outcome.

Methods: The Meningitis Cohort Study was a Dutch nationwide prospective observational cohort study of adults with community-acquired bacterial meningitis, confirmed by culture of cerebrospinal fluid (CSF), from October 1998 to April 2002. The study identified 258 episodes of meningococcal meningitis in 258 patients. Prevalent clonal complexes (cc) were cc41/44 (41%), cc11 (24%), and cc32 (16%). We determined the distribution of the TPS systems by PCR and sequencing and correlated the presence of tpsA with clinical data.

Results: Of 254 available meningococcal isolates, 249 (98%) harbour tpsA1, 163 (64%) harbour also tpsA2 and 53 (20%) harbour tpsA1, tpsA2 and tpsA3. Isolates of the hyperinvasive cc11 (n=62) and cc8 (n=10) lacked both tpsA2 and tpsA3. Patients infected with tpsA2/tpsA3 negative meningococci had more often a positive blood culture (53/83 vs. 75/171; p=0.004), CSF white cell count <1000 cells/mm3 (23/76 vs. 24/162; p=0.008), but had more systemic complications (22/83 vs. 23/171; p=0.014) and a worse outcome (Glasgow Outcome Scale <5, 156/171 vs. 68/83 p=0.039).

Conclusion: Meningococci lacking tpsA2 and/or tpsA3 cause more severe disease.

P091

Fructose bisphosphate aldolase is required to establish bacteraemia in a transgenic murine model of meningococcal bacteraemia

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Objectives: Neisseria meningitidis remains an important cause of septicemia and meningitis and is associated with high morbidity and mortality. Recently we have demonstrated that the fructose bisphosphate aldolase (FBA) of N. meningitidis is translocated to the outer membrane and is required for optimal adhesion to host cells. The aim of this study was to characterize the potential role of FBA in the pathogenesis of meningococcal infection using a transgenic mouse model.

Methods: FBA null-mutants were generated in several N. meningitidis strains of different genetic backgrounds. Growth rates of wild-type and mutant strains in BHI broth were assessed by measuring OD600. Groups of five, six-week-old female BALB/c congenic mice expressing human transferrin, were infected by intraperitoneal challenge with 5 × 106 CFU of wild-type or cognate mutant strain, respectively. Bacterial counts in the blood were determined at 2, 6 and 24 h after challenge by plating serial dilutions of blood samples on GCB medium and were expressed in log10 CFU ml-1 of blood.

Results: The FBA-mutant strains grew at the same rate as the parental wild-type strains in vitro. Using the in vivo mouse model, the numbers of bacteria recovered at various time points demonstrated that there were significant differences in the level of bacteraemia established by the mutant strains compared to the wild-type parent strains.

Conclusion: N. meningitidis FBA mutant strains have a reduced capacity to establish bacteraemia in a transgenic murine model suggesting that FBA plays a role in the pathogenesis of meningococcal disease.

P092

Identification of the immunoproteome of the meningococcus by immunoprecipitation and LC-MS/MS

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Most healthy adults are protected from meningococcal disease by the presence of naturally acquired anti-meningococcal antibodies; however, the identity of the target antigens of this protective immunity remains unclear, particularly for protection against group B disease. To identify the proteins targets of natural protective immunity we developed an immunoprecipitation and proteomics approach to define the immunoproteome of the meningococcus. SBA-positive sera (to both a meningococcal C strain L91543 and the B strain MC58) from 10 healthy individuals as well as pooled human sera were used for the analysis. Immunoprecipitations were performed with each serum sample and cells from both meningococcal stains. The immunoprecipitated proteins were captured using Ultralink protein A/G and separated by SDS-PAGE followed by in-gel trypsin digestion. The tryptic peptides were analysed by LC MS/MS to identify the antigens recognised by each antisera.

Analysis of the immunoproteome identified by each individual serum demonstrated common antigens that were recognised by most sera as well as subject-specific protein recognition. Most antigens were found in both meningococcal strains but a few were strain-specific. Most of the immunoprecipitated proteins were previously characterised as surface antigens and were often adhesins and proteases. Several are currently recognised as vaccine candidate antigens. The identification of the surface proteome of the meningococcus provides a basis for understanding the role of each antigen in natural immunity.

P093

The Host Response to Chlamydial Infection Results in Increased Gonococcal Colonization in a Novel Model of Coinfection

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Objective: Neisseria gonorrhoeae and Chlamydia trachomatis cause similar urogenital diseases and up to 70% of individuals with gonorrhea also have chlamydia. Using a novel murine model of gonococcal and chlamydial coinfection we found that significantly more gonococci were recovered from mice coinfected with Chlamydia muridarum, the mouse strain of chlamydia, compared to mice infected with N. gonorrhoeae alone. We hypothesized that the immune response to chlamydial infection makes the genital tract more permissive for gonococcal infection.

Methods: Using an immune-targeted RT-PCR array we screened for alterations in gene expression during chlamydial infection that may account for increased gonococcal colonization. Factors of interest were analyzed by flow cytometry and coinfection studies in wild-type (BALB/c) and TLR4-deficient (C.C3H-TLRLPS-d/J) mice.

Results: Prior to gonococcal challenge, C. muridarum-infected mice had decreased expression of toll-like receptor 4 (TLR4) and antimicrobial peptide (CRAMP, SLPI) genes. Correspondingly, up-regulation of inflammatory markers only occurred in mice infected with N. gonorrhoeae in the absence of chlamydial infection. Flow cytometric analysis suggested that changes in epithelial cell gene expression are responsible for these observations, not differences in the recruitment of immune cells. Importantly, coinfected TLR4-deficient mice did not show increased gonococcal colonization.

Conclusions: These data suggest that the host response to chlamydial infection induces an environment in which protective TLR4-mediated responses and antimicrobial peptides are down-regulated allowing for greater gonococcal colonization in mice. This conclusion is further supported by recent findings that N. gonorrhoeae stimulation of TLR4 leads to a partially protective Th17-type immune response.

P094

Immunoproteomics has revealed potential candidates for inclusion in multi-component serogroup B meningococcal vaccines

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Objective: Neisseria meningitidis outer membrane (OM) vesicles have been utilised as vaccines against outbreaks of serogroup B infection. However, such vaccines offer only strain-specific protection because they contain the antigenic surface components of the donor meningococcal strain. By contrast, meningococcal colonisation is known to stimulate the production of cross-reactive antibodies, but little is known of the nature of the antigens involved. In this study, the objective was to use proteomic methods to investigate the dynamics of immunity in response to meningococcal carriage with the aim of identifying potential vaccine candidates effective against all serogroup B meningococcal strains.

Methods: Matched sera and serogroup B meningococcal carriage strains obtained from first-year undergraduate students during two longitudinal carriage studies were analysed for evidence of a developing immune response to colonisation. Serum bactericidal activity towards homologous colonizing strains and heterologous strain MC58 were determined and the nature of the antigens associated with cross-protective immunity was investigated by Two-Dimensional Gel Electrophoresis of outer membranes combined with immunoblotting and mass spectrometry finger-printing.

Results: Bactericidal antisera induced by colonization recognized 43 proteins on western bolts of 2D gels of heterologous strain MC58. The list of protein immunogens identified included both well-established protein antigens, such as OM porins as well as potential new conserved vaccine candidates.

Conclusions: Data from this study will inform the prospective development of universal serogroup B vaccines based on the inclusion of newly-identified cross-reactive antigens.

P095

Effect of factor H-binding protein (fHbp) sequence variation on factor H (fH) binding and survival of Neisseria meningitidis in human blood

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Binding of human factor H (fH), a down-regulating complement protein, to the surface of Neisseria meningitidis is critical for evasion of innate host defenses. The meningococcal vaccine candidate, fHbp, serves as an fH ligand. To investigate differences in fH binding among fHbp sequence variants, we prepared fourteen recombinant fHbp sequence variants. By ELISA, two proteins, ID 14 (from a group B epidemic New Zealand strain) and ID 15 (from strains causing 23% of endemic group B disease in the UK) showed 5- to 10-fold lower concentration-dependent fH binding than fHbp ID 1 (from a group B epidemic Norwegian strain). To evaluate the effect of lower fH binding on N. meningitidis survival in human blood, we prepared mutants of strain H44/76 that expressed fHbp ID 1, 14, or 15. By flow cytometry, mutants with fHbp ID 14 or 15 had ~5-fold lower fH binding than the mutant with fHbp 1. When incubated for four hours in blood of non-immune donors, all three mutants could grow and showed similar increases in CFU/mL. In contrast, an isogenic fHbp knock-out mutant was rapidly killed. Thus, expression of fHbp sequence variants with lower fH binding had minimal effects on non-immune clearance of meningococci from human blood, and amino acid substitutions that lead to decreased fH binding do not necessarily decrease fitness conferred by fHbp. These results suggest that in human sera, which have high concentrations of fH, binding of fH to fHbp is saturated, even for fHbp sequence variants with decreased affinity for fH.

P096

Differential effect of Neisseria gonorrhoeae infection on Th1, Th2 and Th17 immunity depends on TGF-β

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Our previous studies have demonstrated in a murine model of vaginal gonococcal infection that Th17 axis of immunity played an important role in the innate inflammatory responses elicited by this pathogen. In contrast, N. gonorrhoeae did not strongly induce specific immunity or immune memory. Herein we show in vitro that N. gonorrhoeae could specifically inhibit Th1 and Th2 reactivity of mouse CD4 T cells but enhance Th17 reactivity. Induction of TGF-β by N. gonorrhoeae was critical for the differential effect on Th1, Th2 and Th17 immunity, and different mechanisms and pathways were involved. The inhibitory effect of N. gonorrhoeae on Th1/Th2 response depended on Opa-CEACAM1 interaction as Opa deficiency and CEACAM1 blocking significantly decreased it. While the Th17 response involved gonococcal lipooligosaccharide and TLR-4, TLR-4 deficiency did not compromise the suppressive effect of N. gonorrhoeae on Th1/Th2 responses. Foxp3+ CD4 cells were elevated by culture with gonococci. The natural Th17 immunity to N. gonorrhoeae could be diverted to Th1/Th2 immunity by using anti-TGF-β, but not anti-IL-17. ELISA, microarray and flow cytometry studies indicated that when mice infected with N. gonorrhoeae were treated with anti-TGF-β, the host Th17 immune response was diminished, while Th1/Th2 responses were enhanced. Anti-TGF-β treatment led to faster clearance of infection and induced protection against secondary challenge, which did not occur in control-treated mice. Our results suggest that N. gonorrhoeae suppresses Th1/Th2-mediated adaptive immune response through mechanisms involving TGF-β and Treg cells, and that this effect can be manipulated to enhance specific protective immunity.

P097

S-nitrosothiol depletion by Neisseria meningitidis in a murine model of meningococcal septicaemia

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S-nitrosylation, the covalent addition of nitric oxide (NO) to the thiol group of specific cysteine residues to form S-nitrosothiol (SNO), is a form of post-translational protein modification akin to phosphorylation. Dysregulation of S-nitrosylation has been implicated in a number of chronic conditions, though such dysregulation has thus far been universally attributed to aberrant host cell activities. Recently, we demonstrated that the nitric oxide reductase of Neisseria meningitidis (NorB) is responsible for reducing the concentration of endogenously produced SNO in J774.2 murine macrophage cells (Laver et al., FASEB J, Jan 2010). We hypothesise that during meningococcal infection, NO detoxification tips the balance between de novo SNO synthesis and SNO degradation in favour of the latter, by removing an essential substrate for SNO formation.

To determine whether systemic meningococcal infection affects the concentration of NO-derived biomarkers in vivo, we have developed a murine model of meningococcal septicaemia. Simultaneous intraperitoneal inoculation of E. coli LPS (25,000 EU g-1) and 10^9 viable Neisseria meningitidis (MC58 and ΔnorB) leads to systemic bacteraemia, with sustained blood titres in excess of 10^8 cfu ml-1 after 4 h. Using ozone-based chemiluminescence techniques, in LPS-inoculated mice we have measured significantly increased nM concentrations of SNO in murine liver and plasma. There is a trend toward a lower median SNO concentration in plasma from mice infected with wild type Neisseria meningitidis after 4 h, which is not observed when mice are infected with ΔnorB. We conclude that meningococcal detoxification of NO in vivo may disrupt mammalian NO homeostasis.

P098

Sialylation Protects Intracellular Neisseria gonorrhoeae from Nonoxidative Killing by Polymorphonuclear Leukocytes and Antimicrobial Peptides

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Addition of host-derived sialic acid to the lacto-N-neotetraose species of N. gonorrhoeae (Gc) lipooligosaccharide occurs via the action of sialyltransferase (Lst). We previously reported that an lst mutant of Gc strain MS11 was attenuated during experimental murine infection and that sialylation reduced uptake by polymorphonuclear leukocytes (PMNs). Since Gc are not challenged by phagocyte-derived oxidative defenses, here we tested whether intracellular, sialylated Gc are more resistant to PMN nonoxidative defenses. Wild-type bacteria and the lst mutant were cultured in CMP-NANA and exposed to cathelicidins LL37 (human) and CRAMP (mouse) or the bactericidal/permeability-increasing protein (BPI). Sialylated Gc were significantly more resistant to the APs and also bound more CRAMP, LL37 and BPI than nonsialylated Gc. Complementation of the lst mutation restored resistance to, and binding of APs to wild-type levels. We also tested the susceptibility of intracellular, sialylated versus nonsialylated Gc to PMN killing. Sialylated Gc demonstrated an early survival advantage in both human and mouse PMNs. No survival difference was detected when PMNs from CRAMP-deficient mice were used. Finally, we found the lst mutant was less attenuated in CRAMP-deficient mice compared to normal BALB/c mice during competitive infections with the wild-type strain (competitive index, 10-fold versus >1,000-fold decreased, respectively). We conclude that increased resistance to APs within PMN granules and perhaps epithelial cell-derived APs is another mechanism by which sialylation enhances Gc pathogenesis. The lesser but significant attenuation of the lst mutant in CRAMP-deficient mice suggests nonsialylated Gc also may be challenged by BPI and other factors during infection.

P099

Substitution of Hep2 C3 of Neisseria meningitidis Paraglobosyl LOS prevents Human IgG that binds Lacto-N-neotetraose α Chains from initiating Bacteriolysis; substitution of Hep2 C6 enhances Bacteriolysis

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Objectives: Human IgG that bind LOS with lacto-N-neotetraose (nLc4) α chains are bactericidal for L2-4, & L7 N. meningitides, but it is the IgG that binds the internal trisaccharide (nLc3) that accounts for this activity. We assessed how adornments of Hep2 that determine L-type affect the bactericidal activity of nLc4 and nLc3 LOS IgG.

Methods. We passed human IVIG over 1291 (nLc4) and 1291a (nLc3) LOS coupled to Sepharose and eluted purified IgG. 1291 LOS IgG was passed over 1291a LOS to isolate full-length nLc4 IgG (1291-1291a LOS IgG). We inactivated lpt3 in 7946 (B:L3,7) and lpt3, lpt6 and lgtG in 8024 (B:L2,4), used whole cell ELISA to assess binding of IgG, and human complement to assess bactericidal activity.

Results. The Δlpt3 (7946 & 8024), Δlpt6 and ΔlgtG (8024) mutants, bound IgG as well as the parent strains, but inactivation of these genes affected IgG bactericidal activity. 1291-1291a LOS IgG was not bactericidal for 7946wt, but was for 7946Δlpt3. 1291-1291a LOS IgG killed 8024wt, albeit not as well as 1291a LOS IgG; 8024ΔlgtG was killed by complement alone. In contrast, 8024Δlpt6 resisted killing by both IgG pools.

Conclusions. Substitution of C3 of Hep2 by either PEA (L3,7) or glucose (L2) protects meningococci from the bactericidal effect of human full-length nLc4 LOS IgG, but not that of nLc3 LOS IgG. Substitution of C6 of Hep2 by PEA (L4) enhances killing by both nLc4 and nLc3 LOS IgG. These substitutions do not affect binding of IgG.

P100

Case Report: Recurrent Meningococcal Disease in a Patient with Normal CH50

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A 20 year old male college student presented to clinic with a one day history of fevers, chills, a pounding frontal headache with light sensitivity, and body aches. Initially discharged with the diagnosis of a viral syndrome, the patient returned to clinic nine hours later in extremis and was admitted to an intensive care unit, treated presumptively for meningococcal sepsis. Blood cultures grew an unencapsulated Neisseria meningitidis at 24 hours. He was managed intensively but expired on the fifth day of his illness.

The patient had been hospitalized with meningococcal meningitis at age 11 which resolved without sequeleae with a seven day course of intravenous antibiotics. A CH50 performed one year later in the context of a viral illness was reported as 40 U/mL (normal range 31-66), and presumed to rule out a complement deficiency associated with increased risk of meningococcal disease. After the demise of the patient, evaluation of siblings and parents revealed an inheritable complement deficiency associated with recurrent meningococcal disease.

Inherited conditions that predispose to meningococcal disease continue to be identified. A review of these conditions and the limitations of CH50 as a screening tool will be discussed. Optimal strategies for identifying those at increased risk and mitigating those risks by maintaining a high index of suspicion, vaccination and chemoprophylaxis would substantially decrease the burden of meningococcal disease in adolescents and adults.

P101

The serious sequelae of complement deficiency and repeated meningococcal infections; and a high prevalence of component C6 genetic defects in the Western Cape, South Africa

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A relatively high prevalence of complete deficiency of complement component C6 (C6Q0) presenting with recurrent meningococcal infections was reported from the Western Cape, South Africa in 1987. In 2005 we undertook a case control study to determine long-term consequences of this deficiency and accompanying susceptibility to Neisseria meningitidis. Controls were non-deficient siblings/cousins closest in age. Choosing family members ensured other genetic factors, and living conditions, were similar. 41 matched pairs were investigated. All were categorised as either well, suffering current long term serious illnesses (SI) or died. Some C6Q0 subjects suffered more than one SI; however, classification remained the same. The C6Q0 patients suffered significantly more SI/death than the Controls. What was more even significant was comparing 24 C6Q0 subjects with a history of no or one episode of meningococcal infection with 21 who had had two or more meningococcal infections. They were highly significantly ($p<0.001$) more likely to have died or suffer SI.

Four genetic DNA defects have already been shown responsible for C6Q0 in the Western Cape. Three were first described in Black Americans suffering meningococcal infections. However, the fourth is apparently a Cape defect. These defects are co-dominant and any combination of two leads to C6Q0. 2250 newborn cord bloodspots were screened for these defects. An unexpectedly high frequency of defects was found, predicting about one homozygous or compound heterozygote C6Q0 per 1900 newborns and one heterozygous carrier per 23 newborns. It will require screening of adolescents and adults and/or a newborn screening program with follow-up, to determine the implications of this finding.

P102

Neisseria gonorrhoeae infection induces altered amphiregulin processing and release

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Adhesion of the human pathogen Neisseria gonorrhoeae has established effects on the host cell and evokes a variety of cellular events including growth factor activation. In the present study we report that infection with N. gonorrhoeae causes altered ampiregulin processing and release in human epithelial cells. Amphiregulin is a well-studied growth factor with functions in various cell processes and has been thoroughly investigated for its role in a variety of cancers and other proliferative diseases, but not yet for its part in bacterial infections. The protein is prototypically cleaved on the cell surface in response to external stimuli. Upon infection, a massive up-regulation of amphiregulin mRNA is seen. The protein changes its sub-cellular distribution and is also alternatively cleaved at the plasma membrane, which results in augmented release of an infection-specific 36 kDa amphiregulin product from the surface of human cervical epithelial cells. Further, using antibodies directed against different domains of the protein we could determine the impact of infection on pro-peptide processing. The effects were seen already after 30 minutes, and followed up to 24 hours post infection. In summary, we present data showing that the infection of N. gonorrhoeae causes an alternative amphiregulin processing and sub-cellular distribution and release in human epithelial cervical cells.

P103

Chronic meningococcemia is associated with infection by meningococci expressing underacylated LPS

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Background: Chronic or prolonged meningococcemia (CM) is an uncommon form of meningococcal disease, characterized by prolonged and usually relapsing fever and the presence of meningococci in the blood stream. Underlying conditions of CM are largely unknown, but host predisposing factors are often implicated. Here we present a study among 19 CM patients.

Methods: CM was defined as a case of meningococcal sepsis with a febrile episode of at least 1 week, without meningeal symptoms. Bacterial isolates were characterized by the Netherlands Reference Laboratory for Bacterial Meningitis at the AMC, the Netherlands.

Results: The median age of the 19 patients was 22 yrs (range, 2-62). Median duration of symptoms before diagnosis was 4 weeks (range, 2-5). Skin rash was present in all 15 evaluated patients and arthralgia in 11 of 15 (73%). Meningococci were cultured from the blood of 18 patients and from cerebrospinal fluid in 1 patient. Serogroup B and C were most prevalent: 10 (52%) and 7 (37%), respectively. Five isolates were of clonal complex 41/44, 3 of cc11, 3 of cc461, 2 of cc37, one of each cc32, cc5, cc334, 2 singletons and one unknown. Of 19 isolates, 7 (37%) had a mutation in lpxL1, resulting in penta-acylated lipid A. This frequency is higher than previously found among adult patients with meningitis (9%).

Conclusions: Chronic meningococcemia patients are often infected with meningococci with a mutation in lpxL1 resulting in under-acylated lipid A. Previous research showed that under-acylated LPS results in less inflammatory response and less stimulation of the host immune response, explaining the clinical course in these patients.

P104

Neisseria gonorrhoeae modulation of T-regulatory cells is dependent upon PilC2 expression in vitro

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Gonococcal infection of the genital epithelia is associated with acute inflammation and influx of polymorphonuclear leukocytes (PMNs). Selective gonococcal adherence to adaptive immune cells likely modulates PMN repertoire function and activation. We hypothesized that adherence to immune cells is dependent upon the differential expression of Type IV pilus associated proteins PilC1 or PilC2. This binding would result in the modulation of cytokines produced by these cells and affect the recruitment and activation of PMNs, and adaptive immune cells. To test this hypothesis we used a panel of FA1090 isogenic mutants expressing PilC1 or PilC2 and co-cultured with human peripheral blood mononuclear cells (PBMCs). We demonstrated that expression of PilC2 selectively binds to, but does not invade CD4+ CD25+ T regulatory cells (Tregs). We went on to measure transcriptional activation of immune response genes using RT-PCR. From co-culture of PBMC's with each of the isogenic mutants, we demonstrated that IL-7 was upregulated ($P<0.01$) in response to stimulation with PilC2 expressing gonococci. Taken together, these results suggested that gonococcal expression of PilC2 contributes to the modulatory activity of T-cells and may in part contribute to the poor immune memory response that is characteristic of gonococcal infection.

P105

Neisserial Surface Protein A (NspA) Binds to Factor H and Enhances Meningococcal Resistance to Complement

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Complement forms an important arm of innate immune defenses and one mechanism meningococci employ to limit complement activation on the bacterial surface is binding of the alternative complement pathway inhibitor factor H (fH) to fH-binding protein (fHbp). fHbp is a leading vaccine candidate against group B Neisseria meningitidis and novel mechanisms that enable meningococci to bind fH could undermine the efficacy of fHbp-based vaccines. We observed that fHbp deletion mutants of some meningococcal strains showed residual fH binding and ligand overlay immunoblotting using membrane fractions from one such strain showed that fH bound to a ~17 kD protein, identified by MALDI-TOF analysis as Neisserial surface protein A (NspA). Deleting nspA, in the background of fHbp deletion mutants, abrogated fH binding and mAbs against NspA blocked fH binding, confirming NspA as a fH binding molecule on intact bacteria. NspA expression levels vary among strains and expression correlated with the level of fH binding; over-expressing NspA enhanced fH binding to bacteria. Progressive truncation of the heptose (Hep) I chain of lipooligosaccharide (LOS), or sialylation of lacto-N-neotetraose LOS both increased fH binding to NspA expressing meningococci, while expression of capsule reduced fH binding to the strains tested. Similar to fHbp, binding of NspA to fH was human-specific and occurred through fH domains 6-7. Deleting NspA increased C3 deposition and resulted in increased complement-dependent killing. Collectively, these data identify a key complement evasion mechanism with important implications for ongoing efforts to develop a meningococcal vaccine that employs fHbp as one of its components.

P106

Human antibody responses to the meningococcal factor H binding protein LP2086 during invasive disease

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Objectives: The meningococcal factor H binding protein (fHBP) is a surface-exposed lipoprotein undergoing clinical trails as a vaccine candidate for the prevention of Neisseria meningitidis serogroup B disease. fHBP protein sequences segregate into two subfamilies, A and B. Although extensive functional, biochemical and immunological studies have been undertaken, the human immune response to fHBPs during meningococcal infection is not well understood. We quantitatively and qualitatively characterized the antibody response to subfamily A and B fHBPs in patients with invasive meningococcal disease.

Methods: Sera from eleven patients with invasive disease were obtained upon hospital admission, on subsequent days during hospitalization and after discharge from care. Meningococcal isolates were cultured from these patients and surface levels of fHBP were determined by flow cytometry with a fHBP-specific monoclonal antibody. A multiplex assay was developed on the Luminex platform to measure fHBP-specific immunoglobulin G (IgG) antibodies in human serum. Anti-fHBP IgG titers, expressed in arbitrary units, were determined from a standard curve included on each assay plate. Patient sera were also used for immunoblotting a panel of recombinant fHBPs.

Results & Conclusion: Strong antibody responses were detected in the serum of almost all patients, including those infected with strains that expressed low levels of surface fHBP. Antibodies were detectable upon admission to the hospital and titers generally increased during recovery. These data indicate that fHBPs are expressed in vivo, are immunogenic and elicit a robust antibody response during invasive meningococcal disease.

P107

Binding Of *Neisseria meningitidis* To Galectin-3

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Neisseria meningitidis is a commensal of the human respiratory tract and an important Gram-negative human pathogen, able to cause septicaemia and meningitis. We describe the interaction between N. meningitidis and galectin-3 (gal-3), a host protein produced by/on immune cells during inflammation. Immunohistochemical staining showed that gal-3 is significantly up-regulated in tissues from mice infected with N. meningitidis MC58 (serogroup B) and co-localises with bacterial colonies in human tissues from patients with meningococcal disease. We investigated the possible interaction between N. meningitidis and gal-3 by analysing the binding to bacteria and the consequences in terms of interactions with phagocytic and non phagocytic cells. Binding to N.meningitidis was detected by FACS analysis and was shown to be dependent on lipopolysaccharide (LPS). Inhibition assays with lactose and the use of truncated versions of gal-3 showed that the C-terminal domain (CRD) of gal-3 is responsible for the binding, but the full length of the protein is necessary for this interaction. In order to study the consequences of galectin-3 binding we analysed the adherence of N. meningitidis to phagocytic and non phagocytic cells and the uptake of bacteria by macrophages. We showed that pre-incubation of N. meningitidis with exogenous gal-3 did not affect the adhesion of bacteria to epithelial cells, but increased the attachment of N. meningitidis to phagocytic cells. Further experiments will be performed in order to determine the mechanisms involved and to identify the role of gal-3 in the modulation of the host immune response during infection.

P108

Naturally-acquired immunity against Neisseria meningitidis serogroup X infection and immunogenicity of its capsular polysaccharide in mice

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Neisseria meningitidis is a gram negative encapsulated diplococcus, that can be classified into 13 distinct serogroups on the basis of the antigenicity of the polysaccharide capsule. Among the 13 meningococcal serogroups, serogroups A, B, C, Y and W135 are associated with the majority of cases of meningococcal disease. In the African Meningitis belt, until recently, most large epidemic have been caused by N. meningitidis serogroup A (NmA), but C and W135 epidemics have also been reported. Meningococcal infections due to Neisseria meningitidis serogroup X (Nm X) occurred occasionally in the countries of the meningitis belt during the past 20 years. More recently, during the season 2006, the largest series of laboratory-confirmed NmX meningitis cases occurring in a single season were reported in Niamey (Niger). The incidence of NmX cases in 2006 was exceptionally high and was associated to a very low incidence of serogroup A cases.

The outbreak was clonal and provoked by isolates of the phenotype X: NT: P1.5 and the sequence type ST-181

The threat represented by NmX must be considered seriously because no vaccine exists to protect individuals against this serogroup. We report a case control study (N= 31 matched triplets) which demonstrates a naturally acquired immunity in patients recovered from meningitis due to N. meningitidis serogroup X.

According to serum bactericidal assay, 80.6% of cases could be considered as having a protective immunity to NmX, compared to 51.6% in exposed controls and 9.7% in unexposed controls. The data obtained in mice also confirmed the immunogenicity of purified serogroup X polysaccharide.

Thus, both NmX and purified fraction of polysaccharide X were capable of stimulating immune responses.

P109

The Vaccine Candidacy of Meningococcal Opa Proteins

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Introduction: Meningococcal disease cannot be comprehensively controlled due to the lack of a vaccine against meningococci expressing the serogroup B capsule. The majority of serogroup B and C disease in the past 5 decades was caused by a small number of hyperinvasive lineages. During decades of global spread, these clonal complexes remained stably associated with limited combinations of highly immunogenic outer membrane adhesins called opacity (Opa) proteins. Conflicting studies on CD4+ T cell immunomodulatory effects mediated by Opa-CEACAM1 have been published. The aim of this study was to examine the in vitro immunomodulatory properties of meningococcal Opa using both human PBMCs and enriched CD4+ T cells.

Methods/Results: Opa genes associated with hyperinvasive serogroup B meningococci were cloned, expressed in Escherichia coli BL21 (DE3) and purified using affinity chromatography. Non-CEACAM1 binding Opa proteins (mutants) were generated using site-directed mutagenesis. In combination with various culture conditions (+/- IL-2 pre-stimulation and co-ligation of CD3 and CD28), cellular responses to a panel of Opa antigens including purified Opa proteins and corresponding mutants, Opa containing liposomes, Opa+/Opa- expressing meningococci and outer membrane vesicles (OMVs) were investigated. Whilst anti-CEACAM antibody inhibited CD4+ T cell responses, no significant inhibition of T cell responses by Opa was observed when measured using carboxyfluorescein succinimidyl ester (CFSE) to detect proliferation by flow cytometry.

Conclusion: The reasons for variation in reports of the immunomodulatory effects of Opa-CEACAM1 mediated interactions require further investigation. However, the study presented here supports the consideration of Opa proteins as potential candidates in vaccines for the prevention of meningococcal disease.

P110

Species specificity of factor H interaction with the uniquely human pathogen, Neisseria gonorrhoeae, resides in arginine substitution at position 1203 in domain 20

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Human factor H (HufH) binds selectively to Neisseria gonorrhoeae enabling complement evasion that is not achieved by fH from other primates. The C-terminal domain 20 of HufH harbors an important binding site for gonococci. We exploited differences in amino acid (aa) sequences that exist between human and (non-binding) chimpanzee fH domain 20 to create cross-species aa mutations to define important binding sites to gonococci. Beginning with a chimeric protein containing HufH domains 18-20 (N-terminal) fused to the Fc fragment of mouse IgG2a (C-terminal) (called HufH 18-20/Fc) that binds to N. gonorrhoeae, we replaced 11 disparate aa individually or in pairs; replacement of arginine (R) with asparagine (N) or alanine (A) at position 1203 (R1203N or R1203A) emerged as the single substitution that completely abrogated binding of the modified HufH 18-20/Fc. Modeling of the R1203N human-to-chimpanzee mutation showed a loss of positive charge that protrudes at the C-terminus of domain 20. The functional importance of (human) R1203 for survival of gonococci was shown by serum bactericidal assay. Wild-type HufH18-20/Fc, but not HufH18-20/Fc that contained the R1203A mutation, bound N. gonorrhoeae, inhibited endogenous HufH binding and mediated killing of gonococci by normal human serum (NHS). As a control, a recombinant fH/Fc molecule that contained chimpanzee domain 20, but was humanized only at amino acid 1203 (N1203R), also bound to gonococci, prevented HufH binding and mediated killing by NHS. These findings underscore the importance of HufH in gonococcal serum resistance and provide additional understanding of the molecular pathogenesis and species-specificity of gonococcal infections.

P111

Phosphoryl Moieties of Lipid A from *Neisseria meningitidis* and *N. gonorrhoeae* Lipooligosaccharides Play an Important Role in the Differential Activation of both MyD88- and TRIF-Dependent TLR4/MD-2 Signaling Pathways

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We have previously shown that the lipooligosaccharide (LOS) from Neisseria meningitidis and N. gonorrhoeae engages the innate immune receptor TLR4/MD-2 complex. In this study, we report that LOS from different meningococcal and gonococcal strains differentially activates NF-κB through TLR4/MD-2, and that the relative activation can be correlated with ion abundances in MALDI-TOF mass spectrometry that are indicative of the number of phosphoryl substituents on the lipid A (LA) component of the LOS. The LOS from three of the strains, meningococcal strain 89I and gonococcal strains 1291 and GC56, representing high, intermediate and low potency NF-κB activation potential, respectively, differentially activated cytokine expression through the TLR4/MD-2 pathway in monocytes. In addition to induction of typical inflammatory cytokines such as TNF-α, IL-1β, and IL-6, MIP-1α and MIP-1β also were significantly higher in cells treated with 89I LOS which had the most phosphoryl substitutions on the LA compared to 1291 and GC56. We found that LOS activated both the MyD88-dependent and -independent pathways through NF-κB and IRF-3 transcription factors, respectively. Moreover, LOS induced the expression of costimulatory molecule CD80 but not CD86 on the surface of monocytes via upregulation of transcription factor IRF-1. These results suggest that phosphoryl moieties of LA from N. meningitidis and N. gonorrhoeae LOS play an essential role in the determination of the differential activation of both the MyD88- and TRIF-dependent pathways. These findings are consistent with the concept that bacteria modulate pathogen-associated molecular patterns by expression of phosphoryl moieties on the LA to optimize interactions with the host.

P112

Phosphoethanolamine Modification of Gonococcal Lipid A Alters NFkB Signaling through Toll-like Receptor 4 and Promotes Increased Survival during Competitive Murine Genital Tract Infection

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PEA modification of gonococcal lipid A occurs via the action of the lptA gene and results in increased susceptibility to complement and cationic antimicrobial peptides. Here we investigated the importance of PEA lipid A modification during experimental infection and in inducing signaling through Toll-like receptor 4 (TLR4) based on known differences in the number of acyl chains in lipid A from lptA mutants. In competitive infections of female BALB/c mice, an isogenic lptA::spc mutant was 10- and 100-fold attenuated relative to the wild-type parent bacteria (strain FA19) on days 4 and day 6 of infection, respectively. Complementation restored recovery, and increased fitness >1,000-fold relative to the wild-type strain. The capacity of PEA-modified and unmodified lipid A in protease K-digested extracts to induce signaling through TLR4 was tested using mouse embryonic fibroblast (MEF) cells from C3H/HeN (wild-type TLR4) and C3H/HeJ (TLR4lpsD mutant) mice that carry a reporter gene under the control of the NFkB/AP-1 promoter. Significantly greater induction of NFκB occurred in C3H/HeN MEF cells treated with extracts (~100 endotoxin units) from wild-type versus lptA mutant bacteria. This differential NFκB activation was not observed in C3H/HeJ MEF cells. We conclude that the absence of PEA modification, lipid A confers a survival disadvantage to N. gonorrhoeae during competitive infections with wild type bacteria. The reduced recovery of the lptA mutant may be in part due to the increased presence of TLR4-regulated effectors that occur in response to PEA-modified lipid A of wild-type gonococci.

P113

Activation of the Caspase-1 Inflammasome Complex by *Neisseria* Species

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Interleukin (IL)-1β is a classic proinflammatory cytokine, and is an important mediator of inflammation in a variety of clinical conditions, including both gonococcal and meningococcal disease. IL-1β production is a tightly regulated process that includes regulation at multiple levels and requires two distinct signals for activation and release. A proinflammatory signal first leads to NF-kB activation and synthesis of pro-IL-1β. The second signal involves activation of a cytosolic multiprotein complex known as the inflammasome to generate biologically active caspase-1, which cleaves pro-IL-1β into mature IL-1β.

The recognition of pathogen associated molecular patterns (PAMPs) is key to the induction of an immune response, and the effector proteins that recognize PAMPs are the pattern recognition receptors (PRRs). Toll-like receptors (TLR) are the best studied PRRs, detecting pathogens at the cell surface or in endosomal compartments. The nucleotide-binding domain and leucine-rich repeat containing family (NLR) detects pathogens in the cytosolic compartment, and include the inflammasome components required for the caspase-1 activation and IL-1β processing. Neisseria species activate both TLR4 and TLR2 via recognition of the lipid A component of LOS, and lipoproteins and porins, respectively. However, the role of the NLRs in Neisseria pathogenesis remains largely uncharacterized. We found that live, but not killed, Neisseria gonorrhoeae are capable of inducing caspase-1 activation and processing of IL-1β to its biologically active form in murine bone marrow derived macrophages, suggesting direct activation of the inflammasome. In this presentation we will examine the mechanism by which this occurs and explore potential differences between Neisseria species.

P114

Cellular Responses to Meningococcal Meningitis

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Objective: Meningococcal disease is a leading cause of meningitis worldwide. Among the serious consequences of meningitis are brain edema and disturbed neuronal excitability. These effects are often the direct cause of death or neurological sequelae. However, the molecular basis for these events is poorly understood and virtually nothing is known about the trafficking of membrane proteins governing water homeostasis during the infection process. Other cellular responses to meningococcal meningitis are also poorly characterized; neither the cytokine profile nor the effects on neurotransmitter transporter and receptor activities in glial cells or neurons and consequent changes in excitatory pulse control and neuron excitability during meningitis are characterized to date.

Materials and Results: We have developed a mouse model to determine which components in glial and neuronal cells are affected during the infection process. CD1, BL6 and aquaporin knock-out mice have been subjected to intra-cerebrospinal fluid injection of wildtype meningococci. Mice were clinically monitored by Versa-Max 3D movement registration. Cellular responses in the brain related to inflammation and water homeostasis were monitored at the transcriptional level by real-time qPCR. Protein levels of aquaporin-4 were monitored by western blotting and electron microscopy analysis after immunogold labelling. This mouse model is expected to yield new information on meningococcal meningitis and brain edema, with significant potential for discoveries that can directly influence and inspire new strategies for prevention and treatment of this serious disease.

P115

The Molecular Basis of Human Anti-Capsular Immunity to Group A Meningococcus

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Immunity to group A Neisseria meningitidis is mediated by complement-mediated bactericidal antibodies specific for the capsular polysaccharide (MenA PS), but the molecular basis of this protective repertoire is not known. We investigated the human MenA PS repertoire using a new method for cloning native human monoclonal Fab fragments that employs antigen-specific cell sorting in combination with single B cell polymerase chain. We isolated 105 sequence-unique MenA PS-specific Fab fragments from 5 adults vaccinated with a MenA PS-protein conjugate vaccine. The findings demonstrate a genetically complex repertoire encoded by 15 VH, 14 D and 18 Vκ genes. Diversity is enhanced by substantial CDR-3 heterogeneity and high levels of somatic hypermutation (SHM). Within individuals, the patterns of clonality ranged from preponderance of a single clone and its progeny to the presence of as many as 17 distinct clones. Affinity for Men A PS amongst clonal progeny varied by as much as a hundred-fold because of differential levels of SHM. Bactericidal activity of full-length IgG1 antibodies measured with human complement correlated with affinity for MenA PS. Fab fragments that were reverted to their presumed unmutated (germline) configuration showed very low MenA PS binding affinity. Collectively these findings show that at the population level the MenA repertoire can be encoded by a substantial fraction of genes in the human Ig loci. Therefore, evolution has not selected a canonical combining site but has distributed protective fitness over many genes. Hypermutation appears required for generating protective immunity to group A meningococcus.

P116

Neisseria gonorrhoeae: CEACAM3 synergizes with innate signalling to drive a pro-inflammatory response

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Neisseria gonorrhoeae (Ngo) is the causative agent of the sexually transmitted disease gonorrhea. Despite the availability of antibiotic treatments, this disease remains prevalent in both developed and developing countries. Ngo possesses a number of immunomodulatory mechanisms, rendering adaptive immunity less effective and necessitating the innate immune response to function as the primary defence against infection. However, acute gonorrhoea is characterized by purulent discharge composed of polymorphonuclear lymphocytes (PMNs) and the pathology associated with disease largely stems from this overzealous response. CEACAM3 is a human-restricted receptor found only on neutrophils. It has been shown to act as a decoy receptor to capture human-restricted pathogens that target CEA family receptors, including Ngo. Ngo binding to CEACAM3 drives the efficient opsonin-independent phagocytosis and elimination of bacteria by human PMNs. Herein, we show that neisserial Opa expression also leads to CEACAM3-dependent production of MIP-1α and MIP-2 chemokines, which effectively recruit immune cells, including neutrophils, to the site of infection. While CEACAM3 is essential for the efficient triggering of this proinflammatory chemokine response, it is not sufficient. Instead, a second activating signal triggered by pathogen associated molecular pattern (PAMP) detection synergizes with CEACAM3 to drive NF-κB-dependent transcription in the infected neutrophil. This co-engagement of the ITAM-containing CEACAM3 and more general innate pattern recognition receptors (PRR) thereby combine to initiate the potent CEACAM3-dependent feedback loop that augments the inflammatory response and ultimately leads to the immunopathology of gonorrhea.

P117

The Role Of Sh-2 Domain-Containing 5-Inositol Phosphatase-2 (Ship-2) In Ceacam1-Dependent Epithelial Cell Responses To *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae has a remarkable ability to inhibit the immune response and thereby persist in the host. This potent immunosuppressive effect has been linked to the Carcinoembryonic Antigen-related Cellular Adhesion Molecule-1 (CEACAM1). CEACAM1 is expressed on the surface of epithelial cells, and acts as a receptor for Neisseria gonorrhoeae. The co-inhibitory function of CEACAM1 has been attributed to its cytoplasmic domain-localized immunoreceptor tyrosine-based inhibitory motif (ITIM) recruiting tyrosine phosphatases that dephosphorylate components involved in cellular responses to pathogens. Recently, a bioinformatic screen suggested that CEACAM1 may recruit lipid phosphatases as well as the heretofore described tyrosine phosphatases; however, this function has not yet been investigated. SHIP-2 is an SH2 domain-containing 5-inositol phosphatase considered to be a negative regulator of activating phosphoinositide pathways such as the PI3K pathway. We hypothesize that SHIP-2 associates with CEACAM1 in response to infection by N. gonorrhoeae and negatively regulates phosphoinositide signaling pathways, thereby contributing to neisserial inhibition of epithelial cell immune responses. We assessed the interaction of SHIP-2 with CEACAM1 in an epithelial cell model using immunofluorescence-based imaging. SHIP-2 colocalizes with N. gonorrhoeae-bound CEACAM1 but not with bacteria adhering to other receptors. Consistent with a model that the ITIM of CEACAM1 is phosphorylated during cellular activation and then recruits SHIP-2 via its SH2 domain, the phosphatase inhibitor, pervanadate, caused SHIP-2 colocalization with the gonococci to persist whereas the addition of a Src kinase inhibitor, PP2, decreased SHIP-2 association. These results support a CEACAM-dependent regulation of phosphoinositide pathways contributing to the innate response of epithelial cells to neisserial infection.

P118

DC-SIGN (CD209) recognition of *Neisseria gonorrhoeae* is circumvented by lipooligosaccharide variation

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Our recent studies have demonstrated that the dendritic cell-specific nonintegrin CD209 (DC-SIGN) specifically binds to the core LPS or LOS of several Gram-negative bacteria, including, Escherichia coli, Salmonella Typhimurium, Neisseria gonorrhoeae (Ng or GC), Haemophilus ducreyi, Yersinia pseudotuberculosis and Yersinia pestis. Our results further showed that N-acetylglucosamine (GlcNAc) sugar residues within the core LPS or LOS in these Gram-negative bacteria play an essential role in targeting the DC-SIGN receptor. For example, this DC-SIGN recognition and subsequent phagocytosis of GC by antigen presenting cells (APCs) such as dendritic cells (DCs) are limited, however, to a LOS mutant (lgtB) of GC that exposes its GlcNAc sugar residue. This conclusion is supported by experiments demonstrating that HeLa cells expressing human DC-SIGN (HeLa-DC-SIGN) bind exclusively to and engulf an lgtB mutant of GC, and this interaction is blocked specifically by an anti-DC-SIGN antibody. The results imply that LOS variation may have evolved as a mechanism for GC for avoiding phagocytosis in order to probably escape the immune responses from hosts.

P119

Finding divergent homologues with the Motif Machine

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In this era of exponentially growing sequence data, the identification of homologous sequences has become a very common and fundamental task. The most widely used methods of identifying homologues are sequence-based, like BLAST, which work very well to match up closely related sequences. However, if two species are relatively divergent, they may possess functionally equivalent proteins with sequences that are dissimilar enough so as to be missed by these tools. A more sensitive, and biologically sound, method of sequence comparison is through motif-based searching. Instead of considering the entire sequence of a gene or protein, motifs highlight short segments that are highly conserved, often regions important to structure or function. For example, these methods could match conserved domains in two genes where the rest of the sequences show virtually no similarity at all.

The Motif Machine is a new program that streamlines the process of searching large sequence collections using motifs. It brings together motif extraction, via MEME, and motif searching, via MAST, into one tool designed for high throughput searching by non-expert users. It uses a custom graphical user interface, so the user is not required to master the command line usage. The program is run locally, eliminating any length or other restrictions necessitated by web-based tools, and avoiding possible time or security issues arising from accessing a remote machine. In this study, the Motif Machine software is used to find the previously unannotated siderophore-transport pathway in several species of the Neisseria genus.

P120

The changing genome of *Neisseria gonorrhoeae*

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Sequencing and genomics has revealed that bacterial genomes are not the stable blueprints we might have once believed. Contributing to this instability are such features found in the Neisseria spp. as tandem repeats, expression / silent cassette systems, homologous recombination substrates and orthologous genes, homopolymeric repeat tracts, competence for incorporation and uptake of DNA, 'phage and plasmid integration and excision, and transposons and transposon-like elements such as the Correia Repeat Enclosed Elements. In this study, we have investigated instability in the gonococcal genome. Neisseria gonorrhoeae strain NCCP11495 was grown under standard growth conditions, anaerobic growth conditions, and static liquid growth conditions and changes to the genome were detected by PCR. Differences between the published genome sequence and the PCR products will be presented and their implications discussed.

P121

Regulation of genes involved in the assembly and functionality of type IV pili in *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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Background: The mechanisms controlling the assembly and functionality of type IV pili (tfp) are poorly understood. The gonococcal pilE gene, encoding the major tfp subunit, has been shown to be positively regulated by IHF. Furthermore, transcription of pilE appears to decrease in the stationary phase of growth. Tfp biogenesis and function is likely to require at least 13 additional pil gene products. It is unknown how these pil genes are controlled in Neisseria gonorrhoeae (NG) and Neisseria meningitidis (NM).

Objectives: This study aims at characterizing the regulation of these additional pil genes, and to determine whether expression of these pil genes is growth phase dependant.

Methods: Reverse transcriptase PCR was performed to demonstrate that many pil genes are co-transcribed. Regions containing potential promoters and regulatory elements were fused with a promoterless cat reporter gene. Site-directed and deletion mutagenesis, followed by integration into the gonococcal genome, was used to assess these putative elements. Promoter activity was monitored by measuring CAT levels.

Results and discussion: Correia Repeat Enclosed Elements (CREE), which contain an IHF binding site and may confer promoter activity, were identified upstream of three pil operons. These CREE insertions are not present in both NG and NM. Site-directed and deletion mutagenesis showed that the CREE do not participate in pil gene regulation. This suggests the regulation of these genes is different to that observed with pilE. We have also shown that the pilE promoter and other pil promoters are still highly active in the stationary phase of growth.

P122

*Igor Stojiljkovic Memorial Award Winner**

Host responses to *Neisseria meningitidis* and *Neisseria lactamica* indicate different colonisation strategies at the respiratory tract

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Objectives: Neisseria meningitidis is commonly found at the human nasopharyngeal mucosal surface but occasionally causes invasive disease. In contrast, closely related bacteria such as Neisseria lactamica colonise the nasopharynx but remain commensals. It is hypothesised that early interactions of N. meningitidis and N. lactamica with respiratory epithelial cells are associated with differential host cell responses, and that these alter the outcome of that interaction. Characterising the host responses to these two different neisserial species, and identifying the bacterial mediators involved may uncover how occasionally pathogenic and commensal bacteria modulate the host in different ways.

Methods: The transcriptomes of a human bronchial epithelial cell line (16HBE14) in response to N. meningitidis MC58 and N. lactamica Y92-1009 were determined by whole genome wide microarray technology (Illumina HumanRef-8 V2 BeadChip). The expression of selected genes was verified using quantitative real time RT-PCR and multiplex ELISAs. To determine the contribution of bacterial secreted proteins on differentially expressed genes, crude neisserial secreted protein preparations were harvested and added onto the 16HBE14 cells.

Results and Conclusions: Genes involved in host metabolic and energy production processes were associated with N. meningitidis and N. lactamica, suggesting that they both utilise host resources for energy. In contrast, the data indicated that while N. meningitidis down-regulates host defence genes, N. lactamica initiates a proinflammatory response, suggesting specific colonisation processes that may lead to different clinical outcomes. Neisserial secreted proteins were involved in some of these differential responses, suggesting novel mechanisms for modulation of the host response.

P123

Genotypic and phenotypic modifications of *Neisseria meningitidis* after an accidental *in vivo* passage

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A scientist in our laboratory was accidentally infected while working with Z5463, a Neisseria meningitidis serogroup A strain. The isolate obtained from the blood culture was indistinguishable from Z5463 by pulse-field gel electrophoresis. In order to get insights into genomic modifications that can occur in vivo, we sequenced our original stock of Z5463, the strain derived from Z5463 and suspected to have caused the infection (Z5463PI), and the strain isolated from the blood culture (Z5463BC).

All three strains contained a mutated mutS allele and therefore displayed a hypermutator phenotype, being consistent with the high number of mutations (SNP) detected. We compared the number of non-coding and intergenic SNP between each of the three isolates. Being able to estimate the number of cell divisions separating Z5463 from Z5463PI (from the record of laboratory experiments), we concluded that only few bacterial divisions had occurred in the human body, despite the severe clinical presentation of the patient. As expected the in vivo passage resulted in several modifications of phase variable genes.

This genomic study has been complemented by transcriptomic and phenotypic studies, showing that the blood strain used a different haemoglobin-linked iron receptor (HpuA/B) from the parental strain (HmbR). Different pilin variants have been found after the in vivo passage, which led to different properties of adhesion.

The in vivo passage, despite a limited number of bacterial divisions, permits the selection of numerous genomic modifications that may account for its capacity to cause systemic disease.

P124

Gene chip microarray evaluation of *N. meningitidis* MC58 under different iron growth conditions for genes of low iron up-expressed proteins identified by trypsin-digested native outer membrane vesicle proteomics

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Many iron metabolism involved genes are up-regulated during infection when the bacteria enter low free iron environments such as blood. Due to post-transcriptional events and different stabilities of mRNAs and proteins, mRNA levels may not strictly correlate with expressed proteins. We have therefore examined both DNA microarray analysis and outer membrane vesicle (OMV) proteomics under different iron growth conditions. Identification of surface proteins in N. meningitidis MC58 grown in low and high iron containing media was performed by 2D LC-MS/MS using trypsin-digested native OMVs.

A comparative proteomics analysis of OMVs derived from strain MC58 grown in RPMI (low iron medium) versus RPMI supplemented with ferric nitrate identified 48 proteins that were apparently up-expressed under low iron conditions.

mRNA from MC58 strain grown under the same two media conditions was analyzed by DNA microarray analysis. RNA results showed that mRNA transcription levels for 34 of the 48 proteins identified by proteomics were up-regulated. Annotated and literature described functions of the 34 genes indicated that >50% of these genes are directly involved in iron metabolism. We found the number of genes regulated by iron at stationary phase is more than those at log phase. However, about 80% of log phase transcripts are also found in stationary phase, which may additively contribute to the protein synthesis. Use of iron loaded human transferrin as a medium iron source was also examined by DNA microarray and compared with the ferric nitrate results. Transcriptnomics and proteomics analysis can provide complimentary information.

P125

pilS loci in *Neisseria gonorrhoeae* are transcriptionally active

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In this study, we examined the stability of the pilE message in Neisseria gonorrhoeae using Northern analysis and qRT-PCR. These combined approaches indicated that the primary transcript decreased in abundance over time and that a smaller pil-specifc RNA species could be identified that was not a consequence of pilE mRNA turnover. Furthermore, using a series of pilE deletion mutants we were able to demonstrate that the novel smaller RNA species was not derived from the pilE transcript due to some mRNA processing event. 5' and 3' RACE analysis was then performed on total RNA that was enriched for the smaller RNA species as well as RNA from wild type strains which revealed that the primary pilE transcript is 761 bp in length, with the 3' end terminating within the Sma/Cla repeat located downstream of pilE and that cDNA derived from the smaller RNA species provided a perfect match with a pil gene copy from the pilS1 locus indicating that pilS maybe transcriptionally active. This was confirmed using RNA derived from Escherichia coli that carried either the pilS2 or the pilS6 locus on a plasmid. Further evidence for pilS transcription was obtained using RNA derived from a gonococcal mutant where an RNA pyrophosphohydrolase had been inactivated (this mutation slows down mRNA degradation). In these bacteria we are able to demonstrate a greater abundance of pilE message as well as multiple pil-specific signals of varying size on Northern blots corresponding to transcription from the various pilS loci located in the gonococcal chromosome.

P126

Investigation of the interaction between sRNA NrrF of *Neisseria meningitidis* with mRNA targets *sdhC* and *petA* by site directed mutagenesis

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Objective: Using a GFP-reporter system in E. coli, the interaction of sRNA NrrF with its in silico identified targets, sdhC and petA was demonstrated in vivo. The regions of NrrF predicting to interact with these both targets are two different segments of this sRNA molecule. To elucidate the mechanism by which NrrF represses translation of sdhC and petA, we investigated the interaction between NrrF and its targets by site directed mutagenesis .

Methods: The 5'-UTRs of sdhC and petA were fused in frame to gfp in a low copy vector. NrrF was mutagenized in a high copy vector. Functional interaction of WT and mutagenized NrrF with target mRNA was assessed in vivo by combining both plasmids in E. coli.

Results: NrrF contains two regions which were predicted to interact with region -44 to +6 on sdhC mRNA encompassing the putative SD (-13 to -9) and with region -43 to -30 on petA mRNA upstream from the putative SD (-15 to -11), respectively. Replacement of all guanosines with cytidines and vice versa in NrrF within the region that interacts with sdhC or within the region that interacts with petA completely abolished downregulation of fluorescence of both targets, respectively as compared to wild type control NrrF.

Conclusion: By using site directed mutagenesis combined with an in vivo reporter system, we confirmed that the regions of NrrF that were in silico predicted to interact with the 5'-UTRs of meningococcal sdhC or petA are essential in the translational down regulation of sdhC and petA.

P127

Horizontal gene transfer from humans to *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is an obligate pathogen that has maintained an exclusive and long-term association with humans. N. gonorrhoeae is genetically competent and highly recombinagenic; therefore, it has the means and opportunity to obtain DNA from its host. Publically available genome sequences indicate the presence of DNA with identity to the human long interspersed nuclear element L1 in some N. gonorrhoeae strains. The 685-bp simple insertion contains a truncated portion of L1 ORF1 and is located 14-bp upstream of an irg recombinase family gene of N. gonorrhoeae. The human and N. gonorrhoeae copies share ~99% identity at the nucleotide level, strongly suggesting the acquisition of this DNA through horizontal gene transfer. Southern hybridization, PCR-based screens, and sequencing of L1-containing PCR products all confirmed the presence of the element within the N. gonorrhoeae genome and eliminated the possibility that contamination during genome sequencing yielded these results. Molecular genotyping showed the presence of the L1 fragment in four of 51 tested isolates with no correlation to a particular disease state, but failed to demonstrate the presence of L1 in 11 commensal Neisseria isolates and 47 N. meningitidis strains. Multilocus sequence typing demonstrated that two of the L1-containing strains cluster tightly, suggesting the vertical transmission of this element between strains. Although the functional significance of this insertion is not presently known, L1-containing transcripts were detected by RT-PCR in all four positive isolates. This work presents the first evidence of horizontal gene transfer from human to bacteria and illustrates the genetic flexibility of N. gonorrhoeae.

P128

Transcriptome analysis of *Neisseria meningitidis* during growth in human whole blood

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Neisseria meningitidis (Nm) encounters multiple environments within its host, which makes rapid adaptation a crucial factor for survival. The lack of animal models of meningoccocal disease and the species-specificity of interactions of this pathogen with human factors have limited the study of neisserial pathogenesis in vivo.

To explore Nm adaptations during growth in blood, we performed a whole-genome transcriptome analysis using an ex vivo model of human whole blood infection. We observed complex dynamic changes in the expression of transcriptional regulators, energy metabolism, transport and binding proteins that allowed rapid adaptation. The transcripts of capsule and pili genes were unchanged or down-regulated while several other surface-exposed virulence genes were significantly up-regulated. Interestingly, genes encoding surface-exposed vaccine antigens were also up-regulated.

We selected up-regulated genes coding for surface-exposed proteins involved in nutrient uptake or host-cell interaction and putative lipoproteins. We also selected genes encoding four transcriptional regulators that likely control the complex response used by Nm to adapt to human blood.

Knock-out mutant strains of the selected genes were generated and analyzed with respect to growth and survival in the ex-vivo blood model. Nm mutant strains lacking the genes for the surface-exposed NalP serine protease, the Fur transcriptional regulator, the transferring binding protein Tbp2, the L-lactate permease LctP and the putative lipoprotein NlpD were more sensitive to killing by human whole blood. Screening of the other mutants is ongoing. We suggest that this approach has the potential to identify new virulence factors involved in the survival of Nm in blood.

P129

The expression of NHBA is post-transcriptionally regulated by the Hfq RNA chaperone when the CREN element is present upstream the gene

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The CREN (contact regulatory element of Neisseria) is a 50-150bp repeated sequence, often located upstream of genes, in the genome of pathogenic Neisseria species. It was identified as a cis-regulatory element that coordinates the upregulation of several genes in response to host cell contact, including nhba, which encodes a component of the recombinant MenB vaccine. By sequence analysis on strains belonging to different clonal complexes (cc), we identified the CREN as located upstream of nhba only in strains belonging to ST32cc.

Hfq is an RNA chaperone involved in gene expression shown recently to play a role in virulence in meningococcus. During the investigation of Hfq-dependent effects, we observed that Hfq down-regulates the expression of NHBA in strains where CREN was present in the nhbA locus. Using recombinant strains of MC58 expressing NHBA from the Ptac promoter with or without CREN we confirmed that the expression of the protein is altered by Hfq only when CREN is present. The same Hfq-dependent regulation was observed for crgA, another gene with CREN present in its locus. Our results suggest that when the CREN is present as a 5'UTR, Hfq may down-regulate the expression of the associated gene, measurable in reduced steady state levels of mRNA and correlated protein levels. We demonstrate that Hfq can interact in vitro with a CREN RNA probe supporting this hypothesis. In conclusion, Hfq post-transcriptional down-regulates CREN-associated genes and we propose that this mechanism may contribute to their cell-contact co-regulation including the upregulation of NHBA expression in the ST32cc.

P130

Bioinformatic analysis of lipooligosaccharide biosynthesis in the Neisseriaceae

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Lipooligosaccharide (LOS) is an important virulence determinant in the Neisseriaceae. While the genetic basis of LOS production in the pathogenic Neisseria has been extensively studied, little research has focused on the genetics underlying LOS production in commensal Neisseria. We determined the genomic sequences from a variety of commensal Neisseria, and compared these sequences, along with other available genomic sequences from commensal and pathogenic strains. We determined that all Neisserial strains possess genes with high homology to rfaF and rfaC, genes needed to synthesize the heptose core. However, the flanking genes were variable, being conserved in the gonococcus and meningococcus, and highly divergent in the commensal strains. All commensal strains except N. cinerea and N. lactamica strains possessed a homolog of heptosyltransferase III, suggesting that the commensal strains differ from the pathogenic strains by the presence a third heptose. Likewise, some commensals possessed the genes needed to make the beta and gamma chains. Surprisingly, most commensal strains possess all of the known genes needed to synthesize Lipopolysaccharide (LPS), containing clear homologs to genes like O-antigen ligase, chain length determining enzyme and LPS translocase. However, only Neisseria sicca 4320 was found to produce an LPS. We identified a four gene region in strain 4320 that appears to possess the two glycosyl transferase genes needed to synthesize LPS. The G+C content in this region differs significantly from the rest of the genome, suggesting a horizontal gene transfer event. In all other commensal strains, the two glycosyl transferase genes appear to have been deleted.

P131

Neisseria meningitidis serogroup B (MenB) surface antigens with vaccine potential expressed during growth in blood, identified by transcriptional profiling

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Mining the MenB genome to discover surface-exposed proteins with cross-protective vaccine potential has been frustrated by the antigenic variability of most candidates. We speculate that this is driven by immune-mediated selection in the nasopharynx, the natural environment for the meningococcus, and that surface-exposed proteins of which expression is relatively down-regulated there will show less variability. Any which then are relatively highly expressed in organisms in the bloodstream should be attractive vaccine candidates. We have sought these by a transcriptomic approach, using microarrays to identify genes in MenB strain MC58 which are progressively up-regulated during incubation in non-bactericidal human blood.

Comparative gene expression profiling after incubation up to 4 hours identified 6 genes of interest, which were sequenced in each of 21 strains of established diversity. As anticipated, very limited sequence variation was found, in contrast for example to the situation with PorA, well expressed in the colonising state. Genes were expressed in commensal Neisseria to establish their products' location in the bacterial cell. NMB0390, detectable on the surface by flow cytometry, was investigated further. MC58 recovered from growth in human blood was probed with NMB0390-specific polyclonal antibodies and analysed using flow cytometry to assess surface expression. Exposure at the bacterial surface was found to be variable, a result we currently interpret as reflecting a degree of masking by the polysaccharide capsule or other surface structures with variable expression during bacterial growth. Consistent with this observation, it has not been possible to detect SBA activity on bacteria grown in broth culture.

P132

Meningococcal internalization into human brain microvascular endothelial cells is triggered by the influx of extracellular L-glutamate via GltT L-glutamate ABC transporter in *Neisseria meningitidis*

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It has been believed that meningococcal internalization into human cells is a consequence of meningococcal adhesion to human cells. Here we identified three transposon mutants of Neisseria meningitidis that were primarily defective in the internalization of human brain microvascular endothelial cells (HBMEC), with insertions occurring in the gltT gene or its neighboring gene, NMB1964. GltT has been identified as a sodium-independent L-glutamate transporter, while the function of NMB1964 is still unknown. Since the mammalian cell entry (MCE)-related domain was found in the deduced amino acid sequence of NMB1964, NMB1964 was tentatively named gltM in this study. The internalization deficiency was also confirmed in the null ΔgltT-M N. meningitidis mutant and the defect was suppressed by complementation with wild type gltT+-gltM+ genes in trans in the mutants. The intracellular survival of ΔgltT-M mutant in HBMEC was not largely different from that of the wild type strain under our experimental conditions. Introduction of one bp deletion and amber or ochre mutations in gltT-M genes lost efficient internalization into HBMEC. The defect of meningococcal internalization into HBMEC and L-glutamate uptake in ΔgltT-M mutant was suppressed only in strains expressing both GltT and GltM proteins. The efficiency of meningococcal invasion decreased under L-glutamate-depleted conditions upon meningococcal infection to HBMEC. Furthermore, ezrin, a key membrane-cytoskeleton linker, accumulated beneath colonies of the wild type N. meningitidis strain but not ΔgltT-M mutant. All the results suggested that L-glutamate influx via the GltT-M L-glutamate ABC transporter serves as a cue for N. meningitidis internalization into host cells.

P133

Robust inference of genetic networks for *Neisseria Meningitidis* MC58

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The 'ab initio' inference of a complete regulatory network remains a challenging task for the bioinformatics community and in the case of Neisseria, the challenge is further marked by the organism's natural transformability and dissimilarity to other bacteria.

The present work is a first attempt to infer Neisseria's (MC58 strain) regulatory network from scratch, using minimal to no prior information, with the aim of going beyond significant pairwise interactions and small-scale systems.

At our disposal we have a large single channel gene expression microarray data set comprising of 251 arrays, including different strains, knock-out, double knock-out mutants under different growth and stress conditions.

The method employed is based on a set of heuristics that use information theoretic functionals, namely mutual information and conditional mutual information and a solid statistical framework.

This approach has the advantage that we can account for associations of any parametric form and differentiate between direct and non-direct interactions between variables. With the application of the method on this large dataset, we identified regulatory loops, directed chain-like dependence structures, as well as interesting cooperative, synergistic and antagonistic interactions between groups of genes, awaiting further exploration.

P134

A novel phase variable type III Restriction Modification system in *Neisseria meningitidis* ST41/44

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A class of epigenetic regulators of gene expression has evolved from phase variable methylases of type III restriction modification systems (R-MIII) that control the expression of multiple genes, or 'phasevarions' (phase variable regulons), via differential methylation of the genome. Phase variation, the high frequency reversible switching of gene expression, is a common feature of Neisseria meningitidis (Nm) and two phase variable methylases, ModA and ModB, have been described to date. Here we describe a third novel phase variable R-MIII methylase , ModD, which was identified in the hypervirulent ST41/44 strain NZ05/33. This strain also contains modA and modB genes, indicating the presence of three potentially distinct phasevarions. Analysis of the distribution of modD revealed it is primarily found in ST41/44 strains of Nm. Five separate alleles of this gene exist, based on differences in the encoded central DNA-binding domain. The 5' region of the modD gene contains tandem 5'-ACCGA-3' repeats that vary in number both between strains and within a single strain during growth, leading to altered phases (ON or OFF) of ModD expression. Microarray and Real-Time PCR analysis of the wild type modD ON compared to wild type modD OFF or modD knockout strains revealed that ModD regulates the expression of several genes. The modulation of gene expression through the random on/off switching of a phase variable regulator could have important implications for the pathogenesis of ST41/44 strains. Furthermore the widespread distribution of these 'phasevarions' should be considered during antigen characterisation and vaccine development.

P135

Transcriptional insights into host-pathogen interactive biology and rational vaccine design from a model of meningococcal nasopharyngeal colonization

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To study the meningococcal transcriptome during prolonged epithelial association (modelling colonization: preparatory to comparison with in vitro biofilm- and blood-grown organisms) microarray analysis of gene expression was performed on RNA extracted from meningococci (strain MC58) co-cultivated for up to 21d with the bronchial epithelial cell line 16HBE14. Results have initially been compared to the transcriptome of bacteria grown for 2.5h in tissue-culture medium (DMEM).

Gene expression patterns at 96h and 24h were found to be similar.

Consistent with the well-established reduction in capsulation during colonization, polysaccharide biosynthesis genes synX, siaB and siaC, and export genes ctrA-C, were found to be down-regulated at both time points. By contrast, genes encoding pilus components were upregulated to varying extents, including in particular the major subunit PilE (ten-fold increase in transcription at 24h), PilQ and PilT. Transcription of genes encoding putative non-pilus adhesins was also increased, including in particular the abundant outer membrane protein NMB2095 and the autotransporter adhesin NadA.

Of the five components of the investigational MenB vaccine 5CVMB – NadA, NMB2091, FHBP, NMB1030 and NMB2132 – the first two were upregulated and the last two weakly down. However the additional vaccine candidate PorA was transcribed at high level.

Comparison of 24h (and 96h) transcriptomes to the shorter (3h) co-cultivation experiments reported by Grifantini et al (2002) revealed substantial differences, to be described in detail. In this model system, early stages of “epithelial colonization” are accompanied by significant changes in the meningococcal transcriptome, although by 24h a transcriptional plateau appears to be established.

P136

Whole Genome Sequencing to Identify Genetic Factors Associated with Emergence of Serogroup Y *Neisseria meningitidis*

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Objective: The evolution of novel genotypes plays an important role in Neisseria meningitidis disease epidemiology. An increase in serogroup Y disease in Maryland, USA during the 1990s was accompanied by antigenic shift in outer membrane proteins PorA, PorB, and FetA, as a clone predominating at the beginning of the decade (early clone) decreased in incidence as another clone (late clone) emerged. The objective of this study was to identify other antigenic changes associated with the emergence of serogroup Y disease.

Materials and Methods: Antigenic differences were investigated in a representative early clone and late clone isolate using whole genome sequencing. PCR and Sanger sequencing was used to determine whether the observed differences were found consistently in a population of invasive isolates (10 early and 15 late clone isolates) from the same time period.

Results: Overall early and late clone whole genomes were highly similar. Differences were noted in the gene encoding pilin glycosylation protein B, pglB, and 2 genes encoding iron uptake and utilization proteins (lactoferrin binding protein B, lbpB; hemoglobin-haptoglobin utilization protein A, hpuA), Genes found in only the late clone included zitB, a predicted integral membrane metal ion efflux pump. By PCR and Sanger sequencing, the early and late clone populations consistently contained the same gene variants at pglB, lbpB, hpuA, and zitB as did the early and late clone isolates used for whole genome sequencing.

Conclusion: Sequence diversity at multiple antigenic loci may have contributed to the emergence of serogroup Y disease in Maryland in the 1990s.

P137

Characterization and Genome Sequencing of *Neisseria* species Isolated from Rhesus macaques

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Objectives:

1. Survey for Neisseria species among the bacterial flora of Rhesus macaques.
2. Determine if multiple Neisseria species can be found in the study population.
3. Sequence and annotate the genomes of select isolates.
4. Determine if Neisseria from Rhesus macaques are naturally transformable and have type IV pilin-like fibers.

Methods: Three separately housed groups of Rhesus macaques at the Oregon National Primate Research Center were surveyed for the presence of Neisseria species. Neisseria isolated from pharyngeal sites or the oral cavity were identified by oxidase testing, Gram staining and 16S rDNA sequencing. Multilocus sequence typing (MLST) was used to characterize a collection of Neisseria isolates. Draft genome sequences of two isolates were generated using the Roche 454 platform. The genomes were compared to human Neisseria species. Transformation frequency assays were used to monitor DNA uptake. Electron microscopy was used to search for type IV pilin-like fibers.

Results and Conclusions: Neisseria species are abundant in Rhesus macaques. Single MLST haplotypes can dominate separately housed groups of animals. However, multiple Neisseria species can be found within the population and individual animals. We chose isolates AP206 and AP312 for genome sequencing. AP206 is related to the human Neisseria commensals N. sicca and N. mucosa whereas AP312 has significant differences. Rhesus Neisseria have the genetic capacity to produce type IV pili and are capable of taking up DNA. Type IV pilin-like fibers were detected by electron microscopy.

P138

Phasevarions Mediate Random Switching of Gene Expression in Pathogenic *Neisseria*

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Many host-adapted bacterial pathogens contain DNA methyltransferases (mod genes) that are subject to phase-variation. In Haemophilus influenzae, the random switching of the modA gene controls expression of a phase-variable regulon of genes (a “phasevarion”), via differential methylation of the genome in the modA ON and OFF states. Phase-variable mod genes are also present in Neisseria meningitidis and Neisseria gonorrhoeae, suggesting that phasevarions may occur in these important human pathogens. Phylogenetic studies on phase-variable mod genes associated with type III restriction modification systems reveal that these organisms have two distinct mod genes—modA and modB. There are also distinct alleles of modA and modB, which differ only in their DNA recognition domain. The recognition sites for modA11, modA13 and modB1 have been identified, facilitating studies on the mechanisms underlying gene regulation. Mutant strains lacking the modA11 or modA13 genes were made and their phenotype analyzed in comparison to a corresponding mod ON wild-type strain. Microarray analysis revealed that in both modA alleles multiple genes were either up- or down-regulated, some of which were virulence-associated. In N. meningitidis MC58 (modA11), differentially expressed genes included those encoding the candidate vaccine antigens lactoferrin binding proteins A and B. Functional studies using N. gonorrhoeae FA1090 and the clinical isolate O1G1370 confirmed that modA13 ON and OFF strains have distinct in a primary

human cervical epithelial cell model of infection, and in biofilm formation. This study indicates that phasevarions may be a common strategy used by host-adapted bacterial pathogens to randomly switch between "differentiated" cell types.

P139

Origin of the diversity in DNA recognition domains in phasevarion associated *modA* genes in pathogenic *Neisseria* and *Haemophilus influenzae*

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Phase variable restriction-modification (R-M) systems have been identified in a range of pathogenic bacteria. In some it has been demonstrated that the random switching of the mod (methyltransferase) gene mediates the coordinated expression of multiple genes and constitutes a phasevarion, phase variable regulon. The ModA of Neisseria and H. influenzae can be divided into 3 domains; conserved C- and N- terminal domains and a highly variable, central domain. The variable domain functions in DNA recognition (DRD) and dictates the modA allele. 18 distinct modA alleles have been identified in H. influenzae and Neisseria. The mechanism behind the generation of variability in the DRD is the focus of this study.

To look for the origin of the DRD, the 18 modA DRDs were used to search the available databases for similar sequences. Significant matches were identified between several modA alleles and other non-related bacterial species, such as modA4 with Helicobacter pylori Mod, JHP1296, and modA5 with Moraxella catarrhalis Mod, M. McaRII, indicating one source of the DRD variability was via horizontal gene transfer.

Detailed comparison of the 18 modA alleles revealed significant mosiacism, indicating exchange between the alleles residing in Neisseria and H. influenzae further contributed to the observed diversity in the DRD. Blastn and Blastp identified inter and intra-allele regions of similarity. These regions were mapped onto the corresponding allele and reciprocal exchanges indicated that some modA alleles had undergone recombination more frequently than others. This sequence shuffling has apparently been used to generate further diversity between the modA alleles.

P140

Discovering non-coding small RNA in *Neisseria meningitidis*: from *in silico* prediction to transcriptome sequencing

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Objective: Non-coding small RNA molecules (sRNAs) have been found to play a crucial role in transcriptional regulation in bacteria, including the regulation of virulence factors. A systematic high-throughput approach of identifying and validating the exact transcripts of these sRNAs facilitates further elucidation of their abundance and function.

Methods: Intergenic regions (IGR) of strain MC58 were scanned for: consensus promoter sequences followed by a spacer and a rho-independent terminator (search A), and sequences homologous to known sRNAs of E. coli (search B). The resulting sRNA candidates were validated using preliminary data from deep sequencing Whole Transcriptome Analysis (WTA) of log growth cells using the SOLiD platform (Applied Biosystems), Northern Blotting, and RT-PCR. Fur regulation of sRNA expression was assessed using a Fur knockout.

Results: A total of 34 sRNA candidates, 32 identified by search A and 2 identified by search B were investigated. Of 34 sRNAs, 8 were identified by WTA. Of these, 6 were from search A and 2 from search B. 2 sRNAs were identified in both A and B. 21 sRNAs were positive with RT-PCR and/or Northern Blotting of which 5 were also positive with WTA. The expression of 2 RT-PR and/or Northern Blotting positive sRNAs appeared to be under the control of Fur.

Conclusions: In silico predictions overestimate the total number of sRNAs found by experimental methods. However, the expression of some of the predicted sRNAs may be regulated by specific signals and therefore not detected by WTA, RT-PCR or Northern Blotting.

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Characterization of DsbD in *Neisseria meningitidis*

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Proper periplasmic disulfide bond formation is important for correct folding of many secreted and membrane-associated proteins and is mediated by three oxidoreductases, DsbA1/A2/A3, in Neisseria meningitidis. Incorrect disulfide formation in proteins with multiple cysteines is corrected by the isomerase DsbC that is kept in the active configuration by DsbD, an inner membrane protein that transfers reducing power from the cytoplasmic thioredoxin system to the periplasm. Microarray analysis revealed that the MisR/MisS two-component system positively regulates the expression of dsbD and this regulation is further characterized. qRT-PCR showed significantly reduced dsbD expression in the misR/S mutants, which can be rescued by complementation. Activities of various dsbD::lacZ fusions in the wild type strain and the misS mutant delineated the promoter sequence important for MisR regulation. The dsbD transcriptional start site was determined by primer extension and EMSA demonstrated that MisR indeed directly interacts with the dsbD promoter. The MisR-binding sequence was further mapped by EMSA using various promoter fragments and by DNase I protection assay. A DdsbD::aphA3 mutational construct was generated. Surprisingly, PCR, DNA sequencing and Southern hybridizations revealed that all recovered transformants still carried a wild type copy of dsbD, and the correct dsbD mutation can only be created in the strain carrying a copy of ectopically located dsbD, the dsbA1/A2 double mutant and the dsbA1/A2/A3 triple mutant. Thus, the MisRS system positively regulates the expression of dsbD and, unlike E. coli, meningococcal DsbD is indispensable for the oxidative protein folding catalyzed by DsbAs.

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Genetic analysis of the *Neisseria meningitidis* capsule operon

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Introduction: Thirteen serogroups have been described: A, B, C, D, 29e, H, I, K, L, W-135, X, Y and Z. With the exception of D, capsular polysaccharides have been characterised chemically and structurally with genes involved in capsule synthesis sequenced in serogroups 29e, L, W-135, X, Y and Z. This study explored the genetic relatedness of the meningococal capsular operon among all thirteen serogroups. It has made use of the Bacterial Isolate Genome Sequence Database (BIGSdb).

Methods: The capsule operon was sequenced in a representative N. meningitidis isolate from each serogroup D, 29e, H, I, K, L, W-135 (2 isolates), Y (2 isolates), X and Z. Sequences were deposited in the BIGSdb from which schemes for each capsule region were devised.

Results: All of the serogroups examined had similar organisation of the capsule. Serogroup H contained genes involved in serogroup Z capsule synthesis. Serogroups I and K had identical capsule operons sharing sequence identity with genes involved in capsule synthesis in Pasteurella species.

Conclusion: Sequencing of the capsule operon revealed that serogroup D was identical to C suggesting that this serogroup does not exist. It is proposed that N. meningitidis isolates be classified into 12 serogroups A, B, C, 29e, H, K, I, L, W-135, X, Y and Z. All of the sequenced capsule operons have been deposited in BIGSdb and are being used as a reference set to probe for the capsule operon among N. meningitidis genomic DNA deposited in the database.

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Whole-genome Analysis of 105 Meningococcal Reference Isolates

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Advanced sequencing methods and bioinformatics have begun to redefine the landscape of microbial genomics. Over the last several years there has been a fundamental shift away from Sanger sequencing to whole-genome analysis using advanced parallel sequencing technologies.

Here we present an analysis of the whole-genome coverage of 105 Neisseria meningitidis reference genomes sequenced using the Illumina platform and analyzed using the Bacterial Isolate Genome Sequence Database (BIGSdb). 105 paired-end samples were tagged for multiplex runs producing on average 140-260Mb per sample. Reads were assembled de novo using Velvet assembly software, uploaded to BIGSdb, and queried using curated gene datasets. As of writing, the BIGSdb reference gene datasets represent approximately 3-5Mb of each Neisseria genome. To facilitate analysis we tagged 148 genes, 30 previously sequenced with Sanger sequencing, within the BIGSdb platform. Illumina whole-genome runs captured over 97% of the tagged genes with coverage of 14x or higher, allowing reliable variant calling. All 105 reference genome gene datasets queried showed over 99% concordance with previous Sanger sequencing.

The whole-genome analysis method provides an improvement over Sanger sequencing in both cost and speed. Additionally, recent improvements in cluster generation and sequencing chemistries has increased the total genome yield by approximately 30% cutting the error rate from 1% to 0.5%; and therefore can be reliably used to assemble the difficult G+C bias areas of the genome. The comparison shows the use of parallel sequencing technology is a time saving tool for screening the Neisseria genome, defining novel gene schemes, and constructing whole-genome phylogenies.

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Characterization of a Novel Fur-Activated Regulatory Protein in *Neisseria gonorrhoeae* that Contributes to Invasion of Epithelial Cells

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Gonococcal infections begin at mucosal surfaces, typically the urethra in males and the cervix in females. We have previously demonstrated that gonococcal isolates from men and women with uncomplicated gonococcal infection express a number of genes under the control of the transcriptional regulatory protein Fur (Ferric-uptake repressor). We also reported that in the pathogenic Neisseria Fur functions as a global regulatory protein as both a repressor and activator of gene transcription. EMSA analysis of a subset of Fur regulated genes reveled that Fur could bind to the promoter regions of only a subset of these genes. These results suggested that regulation of gene transcription could be both direct (by direct binding to conserved Fur Box sequences in the promoters) and indirect, via additional regulatory proteins. In this study, we have identified one such indirect mediated mechanism of Fur mediated transcriptional control and subsequently characterized the role of a novel Fur activated regulatory protein (farP). The farP gene was originally annotated as a putative gonococcal phage repressor. RT-PCR analysis demonstrated that farP is activated by Fur and EMSA analysis confirmed direct Fur binding to the farP promoter region. A gonococcal farP deletion mutant exhibited increased adherence and invasion of human endocervical epithelial cells. Further characterization of the farP mutant in vitro identified several regulatory targets of FarP. Collectively these studies have identified a new Fur controlled regulatory cascade that contributes to gonococcal invasion of female epithelial cells.

P145

Proteomic analysis reveals that Lytic Transglycosylase-A Modulates Key Intermediates in Cell Wall Synthesis and the Type IV Secretion system in *Neisseria gonorrhoeae*

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Lytic transglycosylase-A (ltgA) is an enzyme responsible for recycling peptidoglycan monomers and releasing cytotoxic-peptidoglycan fragments that increase virulence via lysis of epithelial cells. To gain a better understanding of the global role of LtgA during exponential and stationary growth phase, a proteomic analysis was performed using a mutant ltgA strain. Proteins were separated by two-dimensional (2-D) gel electrophoresis and the differentially expressed proteins were identified by MALDI-TOF mass spectrometry. There were several proteins that were up-regulated or down-regulated in the mutant ltgA strain compared to wild type. The most notable increase in expression was in an aminosugar (glucosamine-fructose-6-phosphate aminotransferase; glmS), a pyrimidine (uracil phosphoribosyltransferase; upp), and a pilus biosynthesis protein (pilO). Quantitative real-time PCR results correlated with proteomic analysis confirming ltgA modulation of glmS, upp and pilO at the transcriptional level. We also determined that LtgA has the capacity to modulate glmS and upp expression in response to antimicrobial stress and increase resistance to β-lactam antibiotics. A compensatory response may ensure that the cell wall is maintained when there is a reduction peptidoglycan recycling. This is a plausible explanation since glmS and upp serve as key intermediates for cell wall synthesis in generating uridine 5′-diphospho-N-acetyl-D-glucosamine. These results also suggest that there is crosstalk between these systems to ensure cell wall maintenance and temporal positioning of the Type IV pili that transverse the cell wall.

P146

Transferrin binding proteins A and B do not form a ternary complex with transferrin

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Pathogenic Neisseria species rely on an iron acquisition system that acquires iron directly from human transferrin (Tf). The process is mediated by a surface receptor composed of Tf-binding proteins A and B (TbpA and TbpB). TbpA is an integral, Ton-B dependent outer membrane protein that functions as a gated channel for the passage of iron into the periplasm and TbpB is a surface exposed lipoprotein that facilitates the iron uptake process. Results from several studies have implicated an intimate association between TbpA and TbpB during the iron acquisition process, leading to the prediction that they form a ternary complex. This study was initiated to prepare ternary complex for structural studies. Recombinant forms of TbpA were over-expressed in the E. coli outer membrane and purified after selective detergent extraction directly from intact cells. Recombinant TbpAs and TbpBs with a poly-histidine tag were alternately used to affinity capture Tf with the intention of preparing a ternary complex with untagged versions of the other receptor protein. The complex of tagged TbpA and Tf was unable to capture untagged TbpB whereas untagged TbpA removed bound Tf from immobilized TbpB. The inability to isolate a stable ternary complex of Tf with both receptor proteins, and the ability of TbpA to remove Tf from TbpB suggests that the primary role of TbpB is for initial capture of iron-loaded Tf.

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Neisseria meningitidis Lipoologosaccharide expression is modulated through contact with epithelial cells or after growth in human blood

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Neisseria meningitidis expresses a range of lipooligosaccharide (LOS) structures, comprising at least 13 immunotypes (ITs). Meningococcal LOS is subject to phase variation of its terminal structures allowing switching between ITs, which is proposed to have functional significance in disease. The objective of this study was to assess the impact of bacterial environment on IT expression. Three different environments were studied: 1/rich broth medium (BHI) 2/ anti-coagulated fresh non-bactericidal human blood and 3/ human epithelial cells (A549 pulmonary cell line. IT expression was assessed in a set of 10 N. meningitidis strains by flow cytometry or confocal microscopy using anti-L8 and anti-L3 Mabs. After growth in BHI, the bacterial cell population typed either as L3 or L8, but never both.. However after contact with epithelial cells or growth in human blood, both immunotypes could be detected in the population of each strain. By confocal microscopy, we were able to demonstrate that while the bacterial population contained both immunotypes, individual organisms expressed either L3 or L8 immunotype, but not both. N. meningitidis is able to sense different environments and modulate LOS expression as a function of those environments. Regardless of starting immunotype, antigenic variation can lead to L3 and L8 co-existing in N. meningitidis populations after epithelial cells or human blood contact.

P148

Phase variation of meningococcal surface antigens mediates escape of bactericidal antibodies and is frequent during persistent carriage

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Objectives: Multiple surface antigens of Neisseria meningitidis are subject to phase variation (PV) due to mutations in tandem DNA repeat tracts. PV is thought to facilitate adaptation of meningococci to fluctuations in host immune responses. Translational phase variation of lgtG mediates escape of a bactericidal monoclonal antibody1. This study examined whether PV of porA mediated escape of bactericidal antibodies and if PV was frequent during meningococcal carriage.

Methods: A modified serum bactericidal assay was utilised to examine escape of killing by PorA mAbs1. Longitudinal meningococcal carriage isolates and concomitant serum samples were obtained from university students. Repeat tracts were analysed by PCR and/or sequencing and correlated with protein expression levels by ELISA and Western blotting. Antibodies specific for particular proteins were detected with multiplex immunodetection assays.

Results: Meningococcal strain 8047 was shown to escape killing by mAb P1.2 due to alterations in the porA promoter-located repeat tract that correlated with reductions in PorA surface expression. Meningococcal isolates representative of 4-to-24 weeks persistence of a single clone were obtained from multiple carriers. Phase variable changes were observed in multiple genes (porA, nadA, hpu, hmbR). Induction of PorA antigenic variant-specific IgG responses were correlated with gain of carriage but not with phase variable changes in PorA expression.

Conclusion: These results show that meningococcal PV (transcriptional and translational) can mediate escape of bactericidal antibodies and is frequent during carriage suggesting PV facilitates host persistence and could compromise vaccine-induced herd immunity.

References:

1. Bayliss et al. 2008, Infection and Immunity, 76 p5038-5048.

P149

Influence of the Combination and Phase Variation Status of the Haemoglobin Receptors on Meningococcal Virulence

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Objectives: N. meningitidis can utilize haemoglobin and haemoglobin–haptoglobin complexes as sources of iron via two phase variable haemoglobin receptors, HmbR and HpuAB. The study assessed whether the distribution, ON-OFF status and poly-C tract length of both receptors varies between disease and carriage isolates and examined the hpuAB deletion mechanism in hpuAB negative isolates.

Methods: 214 disease and 305 carriage isolates were investigated for the presence of hpuAB and hmbR by PCR amplification. Residues in the poly(C) tracts of the genes were enumerated by gene scanning/sequencing. hpuAB deletion events were examined by PCR with flanking primers followed by chromosomal walking.

Results: No significant difference was observed in the frequency of both genes relative to hmbR only in disease isolates but hpuAB only strains were under-represented in disease isolates. Variation in repeat tract length was evident with a slight over-representation of tract lengths longer than the modal numbers. The receptor was in the ON state in >90% of disease and ~70%carriage isolates with a single receptor. Isolates negative for hpuAB exhibited either complete deletion or replacement by IS1106A3 and events were ST, and/or serogroup associated.

Conclusion: The distribution of both receptors varies among isolates with evidence for selection against an Hpu receptor in invasive strains possibly due to a high level of immunogenicity. Invasive isolates have at least one receptor in an ON state indicating that acquisition of haemoglobin is important during invasion and systemic spread. Deletion of hpuAB is mediated by recombination and mainly occurred in serogroup B isolates.

P150

High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection-based carbotyping of meningococcal lipooligosaccharides

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Meningococcal lipoolysaccharides (LOS) have been classified into 12 immunotypes based on monocolonal antibody recognition. Each LOS immunotype is related to a specific oligosaccharide structure which was established for most of them by a combination of analyses such as wet chemistry, immunoblots, 2D-NMR and mass spectrometry techniques. None of these techniques, which requires for some of them large amounts of purified starting material, can provide complete and unambiguous structural information for these molecules with very high heterogeneity.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been proven to be a rapid and sensitive method for carbohydrates analysis. Position of glycosidic linkages and the overall conformational structures of oligosaccharides are important factors for their separation by HPAEC-PAD. Moreover, the nature, the number and the positions of charged chemical groups, such as phosphoethanolamine (PEA) are critical [1].

To obtain quick and efficient structural information of meningococcal LOS, we have developed an HPAEC-PAD method for structure characterization of oligosaccharides released from meningococcal LOS by mild acid hydrolysis. Identify of LOS immunotype by this method is also possible starting directly from inactivated meningococcal whole cells without any purification step.

This method can determine the number of PEA residues and provide quantitative information and nearly complete structures rapidly.

[1] Swanson, K.V.; Griffiss, J. McL. Carbohydr. Res. 2006, 34, 388-396.

P151

Antigenic variation is key to clonal replacement but infrequent during persistent carriage

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Objectives: Antigenic variation of meningococcal surface antigens is thought to contribute to persistence during nasopharyngeal carriage. Proposed models suggest that the host immune responses are responsible for producing non-overlapping antigenic repertoires and these, subsequently, facilitate sequential carriage. This study aimed at examining the extent of antigenic variation of the capsule, PorA, FetA and other antigens during persistent carriage of a clone or sequential carriage of different clones in healthy individuals.

Methods: A cohort of first year university students were recruited for a 6-month carriage study. Meningococcal isolates, blood and saliva samples were obtained at 4 timepoints: 0; 4; 12 and 24 weeks. Capsular group, PorA, FetA and MLST types were determined by PCR and sequencing-based methodologies.

Results: Sequential carriage of different clones was observed in 18 individuals, 15 of which showed clonal replacement by strains with unrelated antigenic repertoires. Antigenic variation during persistent carriage of a single clone was observed for PorA but not FetA. Isolates from 3 out of 15 persistent carriers had minor changes in the amino acid sequences of the PorA VR2; these were due to substitutions or alterations in the length of a trinucleotide repeat tract within the variable region.

Conclusion: These results suggest that colonization of an individual by a strain with the same antigenic properties as the previous carried strain is unlikely. Conversely, antigenic variation during persistent carriage is infrequent. The ability of a strain with a different antigenic repertoire to replace a carried strain implies a lack of cross-protective immunity against other repertoires.

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RmpM binds to meningococcal porin trimers through the PorB

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Objective: The analysis of porin complexes in OMVs from different Neisseria meningitidis strains revealed at least 9 different combinations of both meningococcal porins and at least other two proteins (RmpM and MIP). The objectives of this study were to determine the associations among the porins and the protein RmpM without the influence of other outer membrane proteins present in OMVs and, to analyse the relevance of the stoichiometry of the two porins in complex formation.

Methods: Three recombinant proteins rPorA, rPorB and rRmpM were purified by IMAC and renatured through incorporation into liposomes prepared by dialysis and extrusion. Protein complexes obtained using different combinations of the three recombinant proteins were analyzed by 1-D hrCNE (high resolution Clear Native Electrophoresis), 2-D hrCN / SDS-PAGE and Western-blotting with anti-serum against rRmpM.

Results: Electrophoretic analysis showed that the three recombinant proteins associated to form, independently of the quantity of each porin, seven complexes with different electrophoretic mobilities, 1 rPorB, 2 rPorA/rPorB, 1 rPorA, 1 rPorB/rRmpM and 2 rPorA /rPorB/rRmpM, agreeing with the trimeric structure of porin complexes and with the pattern of porin complexes in the native OMVs of N. meningitidis. Western-blotting assays confirmed the association of the RmpM only to those complexes where the PorB was present.

Conclusions: Proteoliposomes prepared by dialysis-extrusion allow a complete and an appropriate renaturation of porin complexes giving rise to a pattern similar to that found in the native OMVs of N. meningitidis. The immunoassays using anti-rRmpM serum suggest that the protein RmpM binds only to the rPorB to form the heterocomplexes.

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Porins form associations following similar patterns in different subtype and serosubtype strains of serogroup B *Neisseria meningitidis*

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Objectives: Previous studies in our laboratory, using the Neisseria meningitidis vaccine strain H44/76 and the native electrophoretic technique hrCNE (high resolution Clear Native Electrophoresis, showed that the porins PorA and PorB associate mainly as heteromeric complexes with the participation, in some of them, of the proteins RmpM and MIP. In order to confirm whether these structures are constant among different serotype and serosubtype strains of serogroup B, we analysed the composition of their porin complexes using the same method.
Methods: Electrophoretic separation and characterization of the complexes by 1-D hrCNE, 2-D hrCNE/SDS-PAGE and 2-D hrCNE/hrCNE were carried out as previously described 1. Protein composition of the complexes detected was determined by nano LC/MS-MS and MALDI-TOF.
Results: The results confirmed the presence of the same associations detected previously in strain H44/76 (heteromeric complexes of PorA/PorB, PorA/PorB/RmpM, PorB/RmpM, and PorA/PorB/RmpM/MIP and, homomeric complexes of PorA and PorB). Complexes with identical composition but different electrophoretic mobility were detected in all isolates, possibly due to stoichiometric differences in the proportion of PorA and PorB or due to the presence of minor proteins undetectable by these methods.
Conclusion: We conclude that meningococcal porins always associate mainly as heteromers which can appear together with RmpM or MIP, that the quantitatively most important homomeric associations are those of PorB and unlike the PorB, the PorA does not seem to bind to RmpM.
Reference:
1. Marzoa J. et al. J Proteome Res 2010. 9:611-619.

P154

Mutagenic analysis of PilW, a small lipoprotein affecting the PilQ secretin and Type IV pili biogenesis

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Present in Gram-negative as well as Gram-positive bacteria, type IV pili (Tfp) represent the most widespread class of pili known. Neisseria meningitidis successfully utilises Tfp for various functions including host adhesion, auto-aggregation, DNA competence, and a form of flagella-independent movement called twitching motility. In N. meningitidis and other bacteria, the outer membrane lipoprotein PilW is required for Tfp biogenesis as well as facilitating assembly of stable multimers of the secretin, PilQ. Using the crystal structure of PilW as a guide, we have carried out structure-function studies. By mutating the lipid-anchoring residue C20, we show that proper localisation of PilW to the outer membrane is not required for its function but rather affects its stability and therefore efficiency. PilW possesses a further two cysteine residues that in the crystal structure disulfide bond, but that are absent from the Pseudomonas aeruginosa and Myxococcus xanthus orthologues. Mutating either cysteine renders PilW non-functional. Additionally, we have identified conserved residues that when individually mutated to alanine do not interfere with Tfp biogenesis. These residues cluster to a central cleft of the protein and the mutants specifically disrupt Tfp mediated adhesion without affecting PilQ multimer stability. This result demonstrates that detection of stable PilQ multimers and Tfp biogenesis are not necessarily coupled. The location of the disulfide bond, also within the central cleft, leads us to propose that it provides stability to the tertiary structure, and emphasises the importance of this cleft region for PilW function.

P155

Characterization of putative surface-exposed proteins of the type IV secretion system in *Neisseria gonorrhoeae*

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The 57 kb gonococcal genetic island (GGI) is found in 80% of Neisseria gonorrhoeae strains and some strains of N. meningitidis. The GGI was likely horizontally acquired and encodes a type IV secretion system (T4SS). T4SSs have been identified in a variety of bacterial species and include both conjugation systems and effector systems that translocate proteins or DNA-protein complexes. The gonococcal T4SS secretes single-stranded DNA into the extracellular environment, and this DNA is effective in natural transformation. Little is known about the assembly of T4SS proteins in gonococci. Predicted outer membrane proteins include TraK (a secretin-like protein) as well as TraN and TraB, which may form part of the channel. These proteins are predicted to contain surface-exposed regions and may interact with the host. We have overexpressed TraK and TraN in E. coli and are having specific antibodies raised to these proteins. We have tagged TraB with a FLAG epitope (TraB-FL) in a region that may be surface-exposed. Preliminary data suggests that TraB-FL localizes to the outer membrane of gonococcal cells. Interestingly, localization of TraB-FL to the outer membrane is not altered in the absence of other T4SS proteins. To aid in these studies, we have developed a construct that directs gene expression from an IPTG-inducible promoter at a chromosomal site between the genes trpB and igA. Expression levels of TraB-FL at the trpB-igA site are comparable to expression levels from a previously characterized site between lctP and aspC. These studies should greatly increase our understanding of T4S in gonococci.

P156

Isolating the surface proteome of the meningococcus

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The meningococcus interacts with its environment, including the host immune system, through its surface. Much of the surface is occupied by the capsule and outer membrane but the proteins embedded in the pathogen's outer membrane play a key role in host-pathogen interaction and are key targets for protective immunity. Efforts to develop new vaccines have focussed on identifying outer membrane proteins. Most previous studies have utilised cell fractionation methods, or structural prediction tools to identify surface proteins. We describe here a novel method for identifying surface proteins. The surface of intact meningococcal cells was first labelled with a biotinylating reagent. Cells were then lysed and streptavidin was used to pull down labelled proteins which could then identified by LC-MS-MS. Immunogenic proteins were identified by western blotting with immune sera.

We demonstrated the efficacy of this approach using as well-defined surface protein and demonstrated that the method correctly identifies those epitopes of the protein that are exposed on the outer cell surface. We then use the method to characterise the surface proteome of the meningococcus. Many previously characterised vaccine candidate antigens were captured and identified by the method. We also identified several proteins that were not expected to be on the surface but were confirmed to be surface exposed by FACS analysis. This work is funded by Sanofi Pasteur.

P157

Solution Structure of the C-terminal Domain of NHBA, a Protective Antigen of *Neisseria meningitidis*

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The Reverse Vaccinology approach on the meningococcus B (MenB) genome has led to the identification of a novel protective antigen that binds heparin in vitro through an Arg-rich region. This property correlates with increased survival of the unencapsulated bacterium in human serum. For this reason, the protein has been named Neisserial Heparin Binding Antigen (NHBA).

Two proteases, the meningococcal NalP and human lactoferrin (hLf), cleave the protein upstream and downstream from an Arg-rich region, respectively, generating two different C-terminal fragments.

Here, we present the NMR structure of the C-terminal portion corresponding to the domain released by hLf, which folds in a beta barrel preceded by a highly mobile segment. Possible implications of structure and sequence similarities of NHBA to other bacterial proteins are discussed.

P158

A single residue substitution in the translocator domain of *Neisseria meningitidis* NhhA affects trimerization, surface localization and adhesive capabilities

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NhhA (Neisseria hia/hsf homologue A) is a trimeric autotransporter adhesin which mediates interaction of meningococcus to human epithelial cells and components of the extracellular matrix. In addition, NhhA has been shown to protect bacteria from phagocytosis and against complement-mediated killing, enhancing the ability of the bacteria to survive and multiply in human blood. Finally, NhhA has been considered a potential vaccine antigen.

In this study we analyzed the expression of NhhA in a panel of diverse N. meningitidis B strains. We found that trimeric NhhA is produced at different levels and in some strains, belonging to ST41/44 clonal complex, it is not detectable or present as a monomer. Sequencing of the nhhA gene and generation of complementing strains in different genetic backgrounds, have proved that a single point mutation (Gly to Asp) in the translocator domain affected both trimerization and surface localization of NhhA. In vitro infection assays showed that this mutation impairs meningococcal NhhA-mediated adhesion indicating that the strains naturally expressed a mutated form of NhhA may rely on different strategies or molecules to mediate interaction to host cells. Furthermore we demonstrated that N. meningitidis ST41/44 strains expressing monomeric NhhA are not killed by NhhA-specific bactericical antibodies.

These data may help to elucidate the secretion mechanism of the trimeric autotransporters and to understand the contribution of NhhA in the evolutionary process of host-meningococcus interactions as well as have important implications for the evaluation of NhhA as a vaccine candidate.

P159

Site specificity of phosphorylcholine post-translational modification in *Neisseria meningitidis*

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Pili of pathogenic Neisseria are a major virulence factor. Pili are post-translationally modified by several different modifications including the addition of phosphorylcholine (ChoP) and a glycan. In S. pneumoniae, H. influenzae, and commensal Neisseria, ChoP is important for the colonisation of the nasopharynx. In N. meningitidis, the gene, pptA, encoding the candidate transferase for pili-linked ChoP modification is phase-variable. We have shown that both pili-linked glycan and the ChoP modifications play a role in mediating adherence to host epithelial cells. Structural studies indicate that 2 ChoP modifications are on the C-terminus of the pilin protein in N. meningitidis. Mutagenesis analysis reveals that the charge of the residue in C-terminus transferase target site plays a crucial role in substrate recognition for addition of ChoP. In western analysis of outer membrane preps and cell lysates of N. meningitidis using TEPC-15 (anti-ChoP monoclonal antibody), no other proteins post-translationally modified by ChoP were observed. Our previous studies revealed that AniA is post-translationally modified by the same glycan as pilin. To determine whether ChoP can be added to another target protein, the C-terminal sequence of pilin was added to the C-terminus of AniA. No ChoP modification of this AniA protein was observed indicating factors other than the C-terminal sequence are required for recognition and addition of ChoP.

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Analysis of phase variation (PV) of meningococcal *opa* genes and implications for bacterial pathogenesis and vaccine development

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Background and aims: Opacity-associated (Opa) adhesin proteins are major meningococcal outer membrane proteins involved in infection of the nasopharynx and immune interactions, and are being evaluated as a novel serogroup B meningococcal vaccine candidate. Opa proteins undergo phase variation (PV) due to the presence of a coding repeat (CR) tract containing the pentameric repeat sequence 5'-CTCTT-3'. Modulation of the number of repeats occurs by slipped-strand mis-pairing during DNA replication, resulting in regular frame-shifting, which is a key mechanism of immune evasion. The dynamics of PV of meningococcal Opa proteins is unknown, and has significant implications for bacterial pathogenesis and vaccine development.

Methods: Opa PV was assessed by analysis of genotype and phenotype. For genotype analysis, N. meningitidis was serially passaged and genomic DNA extracted from single colonies. All 4 opa genes were amplified from each DNA sample by polymerase chain reaction and nucleotide sequence analysis of the CR tract undertaken. For phenotype analysis, Opa expression was determined by colony blotting use anti-Opa monoclonal antibodies.

Results: Different opa genes possessed different lengths of CR tracts at the start, and opa genes demonstrated variable rates of PV, in the order of 10-3 to 10-4 per cell per generation. Both increases and decreases in CR tract length were observed

Conclusions: These data provide valuable information for the first time regarding differences in PV between different meningococcal opa genes, and suggest that differences between Opa variants may exist in terms of their function during infection or potential for inclusion in a meningococcal vaccine.

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On the immunoreactive / immunoprotective potential of the PilQ complex

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Background and objectives: The secretin PilQ is a highly expressed outer membrane protein exhibiting limited sequence variation. It is surface exposed and omnipresent in neisserial strains and we are interested in determining its immunoreactive / immunoprotecive potential. We previously isolated the PilQ complex to homogeneity and investigated its 3D structure by electron microscopy and single particle averaging. Also, we showed that the isolated PilQ complex alone can give an immune response similar to an outer membrane vesicle preparation with regard to surface binding and opsonic activity. In addition, a protective effect in a murine meningococcal disease model was demonstrated.

Methods and results: For further evaluation of the immunoreactive and protective potential of PilQ, a mouse model was employed. Triple immunisation with small amounts of PilQ complex induced significant IgG activity against PilQ. Reactive parts of PilQ were identified by the study of partial recombinant proteins by ELISA and immunoblot methods and various sets of sera. Post-vaccination sera from vaccinees in the Norwegian MenBVac program and convalescent sera from patients with meningococcal disease showed an increased IgG activity against the N-terminal part of PilQ.

Conclusions: The outer membrane protein PilQ in its complex form is potentially an immunostimulating antigen that can provide protective activity against meningococcal disease. Specific targeting may be achieved through further definition of the immuno-active determinants of the molecule. As the PilQ complex represents a central component in a neisserial membrane pore complex, a concerted activity with other membrane located proteins can be envisioned.

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Differential expression of *Neisseria gonorrhoeae* PilC results in similar adherence phenotypes to *in vitro* cultured genital epithelial cell lines

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The first step in successful gonococcal colonization is the adherence to epithelial tissues of the female reproductive tract and the male urethra. The initial phases of attachment are initiated by the Type IV pilus of the gonococcus. In the FA1090 strain there are two accessory pilus proteins, PilC1 and PilC2 that are thought to mediate adherence. We hypothesized that expression of PilC1 or PilC2 would result in tissue specific adherence. Interestingly, using a novel whole cell binding immunoassay we demonstrated that expression of either PilC1 or PilC2 results in similar adherence phenotypes. By constructing a panel of FA1090 isogenic mutants expressing either PilC1 or PilC2 and testing adherence phenotypes, we found they were statistically indistinguishable in their binding characteristics to ME180, Hec 1B, HeLa, and two primary transformed cell lines: PURL (penile urethral) and ENA2 (endocervical) cultured cells in vitro. These isogenic mutants had similar expression levels of PilC, were screened to be Opa non-expressing, altered to arrest pilin antigenic variation, phase locked to prevent PilC expression changes and confirmed to be expressing the same pilE sequence. PilC2 from two different serotypes of Neisseria meningitidis expressed in the same gonococcal background had similar binding phenotypes when compared with Neisseria gonorrhoeae FA1090 PilC2. Taken together, these results suggest that PilC1 and PilC2 share redundant binding functions which may enhance the chance of colonization of the host.

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Understanding the Transferrin Binding Proteins interaction with human Transferrin and their Role in Iron Release and Transfer

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Neisseria meningitidis (Nm) causes meningococcal infection in humans. The bacterial transferrin binding protein (Tbp) receptor complex, comprised of TbpA and TbpB, facilitates iron acquisition from its host during infection. The interaction on TbpB and TbpA with hTf remains unknown, and understanding these interactions and iron release process is crucial for vaccine development. Hydrogen-deuterium exchange mass spectrometry (H/DX-MS) can be used to explore the interfacial regions in the Tf-Tbp interactions, and to understand the process of iron release.

hTf was expressed in Pichia pastoris, while Nm B16B6 Tbps were expressed in E. coli. TbpB was processed in its free and hTf-complexed states, using a bottom-up H/DX-MS strategy. Deuterium incorporation was quantitated after an online pepsin digestion and separation by an LC gradient. Peptides were analyzed with a QSTAR Pulsari QqTOF MS, and deuteration calculated with Hydra software.

Methods for analyzing the integral membrane protein TbpA with H/DX-MS are currently being developed, involving a post-digestion chloroform detergent removal step, and disulfide bond reduction in TCEP-quench solution. Complex results will be mapped to pdb structures and homology models.

All H/DX-MS studies mentioned above have been analyzed using 88% sequence coverage of TbpB. Deuteration ratios of the complex vs. free protein are currently being calculated for TbpB.

SUPREX experiments are currently being performed on FbpA after incubation with the Tbp-hTf complexes, to assess the release and transfer of iron.

P164

Binding of human lactoferrin by *N. meningitidis* lactoferrin binding protein B

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The human pathogens Neisseria meningitidis, N. gonorrhoeae, and Moraxella catarrhalis comprise a small group of bacteria that are capable of utilising human lactoferrin (hLf) as a sole source of iron. This is accomplished through the action of the lactoferrin binding complex, a two component system consisting of an integral transmembrane protein (LbpA) and an associated lipoprotein (LbpB). The complex shows significant homology to that of the transferrin binding complex (TbpAB). To date, the role of LbpB is poorly understood and inferred from studies of TbpB. Unlike TbpB however, the presence of two negatively charged regions in the C-lobe of LbpB – the large region I and smaller region II – means binding is possible not just through ligand-specific interactions but electrostatic interaction as well.

In this study, we have expressed intact and subfragments of N. meningitidis LbpB in order to determine those regions important for binding of hLf. Binding was demonstrated with the intact LbpB but reduced using just the N-lobe. The C-lobe fragment was capable of binding hLf but this was lost upon removal of region I. In all instances, these fragments bound both hLf and bovine Lf, indicating that binding was most probably due to the electrostatic interaction. Interestingly, an intact fragment lacking region I was shown to bind specifically to hLf. These preliminary results would suggest that both N- and C-lobes are involved in binding of hLf by LbpB. Studies are underway to further elucidate the roles of these two domains in the binding of hLf.

P165

A Conserved Major Pilin subunit in *Neisseria meningitidis*

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Type four pili (Tfp) are indispensible virulence factors contributing to colonization and disease. Tfp are composed primarily of subunits of the major pilin, PilE, along with minor pilins. Neisseria pilins are categorized into two classes, class I and II, depending upon the pilE allele and reaction with the mAb SM1. Until now Neisseria pilin has been regarded as a paradigm of antigenic variation. Extensive sequence variation of PilE occurs both in vitro and during human infection. Pilin variation has been shown to influence bacterial interactions with cells and is also a proposed mechanism of immune escape.

We have found that the major pilin subunit in certain hyperinvasive clonal complexes (cc) of N. meningitidis is highly conserved. Using PCR analysis to amplify class I or class II pilE genes from clinical isolates of N. meningitidis we have shown that strains belonging to cc8 or cc11 harbour the class II pilE allele and that this encodes a highly conserved pilin subunit. More recently, preliminary studies indicate that serogroup A strains from cc1 and cc5, which are responsible for outbreaks in Africa, may also express pilins that do not vary.

Using murine models we have shown that the conserved pilin in cc11 strains is immunogenic during infection. The immune responses and phenotypes associated with class II pilin expression are currently under investigation.

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Development of a mutant *E.coli* for the expression of heterologous *N. meningitidis* outer membrane associated proteins

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Capsular vaccines against group B meningococci are poor immunogens and may potentially elicit unwanted autoimmune responses; therefore, several candidate group B meningococcal outer membrane vesicle (OMV) vaccines containing surface proteins (i.e. fHBP, NadA, PorA) are being tested for their ability to elicit an immune response and prevent disease. Studying the protective response of these outer membrane proteins as part of complex OMV vaccines involves isolating and purifying each protein and conducting Bactericidal Inhibition assays. Isolation and purification of membrane proteins can be difficult since it involves discreet purification and folding steps to ensure proper native confirmation of the purified protein. To solve this problem, knockout mutant E.coli (lpp-, ompA-) were created with the ability to bleb off outer membrane vesicles (NOMV), like N. meningitidis. Expressing proteins in the outer membrane of this mutant E. coli would presumably ensure them assuming their native confirmation. The N. meningitidis membrane associated protein, fHBP v1, was expressed in the blebbing E. coli under an arabinose promoter. NOMVs containing the meningococcal fHBP v1 in its native conformation were collected, purified and used in a Bactericidal Inhibition assay. Assay results show that fHBP v1 expressed in E.coli NOMV had similar percent inhibition results as purified fHBP v1. Rather than purifying membrane proteins, this method could prove to be an easier and faster approach to generating individual proteins for use in the bactericidal inhibition assay, and consequently studying their roles in generating a protective response in complex OMV vaccines.

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A model of the TbpB-Transferrin recognition based on structural variations of the Transferrin binding site from porcine pathogens TbpB

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Pathogenic bacteria acquire the essential element iron through specialized uptake pathways that are necessary in the iron limiting environments during host infection. In particular the Gram-negative Neisseriaceae and Pasteurellaceae families have adapted to acquire iron from the host iron binding glycoprotein, transferrin, through a receptor complex Tbp protein A and B. As these surface-exposed membrane proteins require a specific interaction with a host protein for function, they are conserved throughout the Neisseria family and are considered prime vaccine targets. The specific interactions between TbpB and transferrin have been sought as this interaction site may serve as the conserved epitope required to create a broad-spectrum vaccine. In this work we focused on the essential mechanism of recognition between the TbpB receptor and the host's transferrin: using the sole available TbpB structures from porcin pathogen as a model for the TbpB receptors, we provide information on the structurally conserved features and key elements involved in the transferrin recognition site, supported by docking simulations and transferrin-binding experiments of both TbpB wild type and mutants.

P168

Evolutionary algorithm approach for investigating the mutation behavior of *Neisseria meningitidis* Transferrin binding protein B

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One of the challenges facing protein based vaccines for Neisseria meningitidis is the wide diversity observed within potential vaccine antigens. This is a result of the naturally transformable capability of Neisseria, which allows the pathogen to rapidly alter the structure of components under selective pressure. The hypothesis of this project holds that in spite of the wide spread recombination and mutation observed in vaccine candidate antigens, certain components must remain conserved such that the antigen's function is maintained. Under this hypothesis it is logical to assume that there are specific rules dictating how an antigen is allowed to mutate.

For this study the nucleic acid sequence for Transferrin binding protein B's (TbpB) from 96 Neisseria meningitidis strains, provided by Dominique Caugant, were sequenced. These sequences were cross-referenced with relevant MLST data and analyzed with e-BURST to identify the most probable ancestor strains. The TbpB sequences from the ancestor strains comprise the initial genes of the evolutionary model. The model then takes the initial genes and randomly recombines them. After recombination the resulting genes are compared against the next generation of TbpB as identified by e-BURST. This first random recombination event is repeated until the resulting sequence meets a predefined threshold of identity. After which the process is repeated for the new generation. As the model runs through a preset number of generations it records the specific series of recombinations. This provides a means for developing rules describing how recombination mutations can occur in N. meningitidis Transferrin binding protein B.

P169

Characterisation of the Effects of Gonococcal Opa Proteins on Neutrophil Biology

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Opacity-associated (Opa) proteins of Neisseria gonorrhoeae (Gc) are phase-variable integral outer membrane proteins which bind to members of the CEACAM superfamily or heparan sulphate proteoglycans on host cells. Although Opa+ Gc are phagocytosed and induce reactive oxygen species (ROS) in neutrophils, our results suggested that Opa-negative Gc also appear to be phagocytosed by adherent primary neutrophils. Interpretation of this finding was complicated by potential presence of 'translucent' Opa proteins, which produce the same phenotype as Opa-negative Gc. These 'translucent' Opa proteins make it difficult to determine the Opa status for a population of Gc over time. In order to study the effect of Opa proteins of Gc and their interactions with host cells, we constructed a derivative of strain FA1090 in which the translucent Opa proteins OpaB, E, G and K were deleted. We then selected for Gc expressing individual phenotypically opaque Opa proteins in this background and identified these proteins by colony phenotype and western blot. In support of previous findings from the Rest group, most of the 'opaque' Opa proteins when expressed in Gc induce ROS in neutrophils, but OpaA expressing Gc behave like Opa-negative bacteria and do not induce ROS. Furthermore, when recombinantly expressed Opa proteins known to induce ROS in Gc are folded into artificial liposomes, they exhibit similar effects on ROS in this context. These complementary systems will be useful to further explore Opa protein structure and biology.

P170

Prevalence and sequence diversity of the Factor H binding protein (FHbp) in invasive isolates of *Neisseria meningitidis* from Rio de Janeiro, Brasil

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Background: FHbp is a novel meningococcal vaccine candidate. The protein appears to be expressed in all Neisseria meningitidis, though level of expression may vary. The aim of this study was to deduce the prevalence and diversity of FHbp in clinical meningococcal isolates from Rio de Janeiro, including nonserogroup B.

Methods: Serogrouping was performed in 166 invasive isolates collected from 1988 to 2008. Molecular characterization was performed by multilocus sequence typing (MLST) and sequencing of outer membrane protein genes: porA, porB, fetA. The fHbp gene was amplified and subsequently sequenced.

Results: The fHbp gene was present in all 166 isolates. All sequences fell into previously named subfamily A (58%) and B(42%). There were 30 unique fhbp alleles (17 previously unreported) encoding 29 unique peptides (17 previously unreported). The majority of FHbp alleles (75%) were 1, 13, 16, 21, 25, and 151. An association between the most prevalent clonal complex and FHbp was observed: ST-32 (86%) with FHbp allele 1; ST-103 (81%) with allele 25; ST-8 (100%) with allele 16; ST-174 (78%) with allele 21; ST-11 with allele 13 and allele 151 (61%). FHbp allele 1 was found in 95% of serogroup B isolates (18/19), in 20% of serogroup C (21/108) and was observed neither in serogroup W135 (0/29) nor in serogroup Y(0/9).

Conclusions: The fHbp gene was universally detected in four different serogroups of meningococcal clinical isolates. Six predominant FHbp alleles identified were associated with the five most prevalent clonal complex in Rio de Janeiro.

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Sequence variation of *N. meningitidis* Lactoferrin binding protein B

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Lactoferrin binding protein B (LbpB) is a lipoprotein associated with the lactoferrin uptake system in various Neisseria and Moraxella species. Its role is poorly understood but its surface-exposed and ubiquitous nature amongst N. meningitidis strains makes it a viable antigen for a broad-range N. meningitidis vaccine. In this study, the lbpB of 106 strains of N. meningitidis was amplified and sequenced, resulting in 65 unique sequences. Sequence variation of lbpB was found to have little correlation with Multi Locus Sequence Typing (MLST) or any other epidemiological marker examined. This suggests a fast rate of evolution through recombination or mutation, a frequent observation in studies involving outer membrane proteins of N. meningitidis.

A model was prepared based on the crystal structure of Actinobacillus pleuropneumoniae Transferrin binding protein B (TbpB). The N-lobe and C-lobe each possess an N-terminal “hand” domain and a C-terminal β-barrel domain. Sequence variation was low within the internal structure of the protein but high in exposed regions. Two negatively charged regions are present in the C-lobe of LbpB. Region I (~ 80 residues) is found within the “hand” domain while region II (~40 residues) is located in loop 7 of the β-barrel. Sequence analysis revealed that one third of the strains possess region II and the remaining two thirds of strains instead have a 12 residue insert in loop 1, next to loop 7 in the β-barrel structure. This alternate loop is not negatively charged, suggesting that the negative residues of region II are not necessary for binding.

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Structural and Functional Features of Lactoferrin Binding Protein B

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Neisseria meningitidis and N. gonorrhoeae are capable of utilizing human lactoferrin (hLf) as a sole source of iron for growth. The acquisition of iron from hLf is mediated by a surface receptor complex comprised of lactoferrin binding proteins A and B (LbpA and LbpB). The role of the transmembrane protein LbpA in iron uptake has been experimentally demonstrated whereas the role played by the lipoprotein LbpB has been inferred by its homology to the well-studied bacterial transferrin binding protein B. LbpB is distinguished from TbpB in that it possesses two unique clusters of anionic residues in the C-terminus lobe. The function of these clusters, termed region I and II, is unknown but has been proposed to be involved in the binding of Lf.

Our studies revolve around the protein structure and function of N. meningitidis LbpB. In particular, we are investigating a second biological role for LbpB. Proteolytic cleavage of hLf leads to the release of the positively charged N-terminal region, a small cationic peptide called lactoferricin which can interact with and disrupt the bacterial cell wall resulting in cell death. The negatively charged LbpB has been suggested to abrogate the microbial activity of lactoferricin by binding the peptide. Functional studies are underway that will evaluate the protection against lactoferricin conferred by a selection of different LbpBs and LbpB derivatives, including ones defective in the anionic clusters.

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Structural Studies of the Lactoferrin Binding Proteins

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Neisseria meningitides, Mycobacterium bovis and Moraxella catarrhalis are host-restricted respiratory pathogens that cause a spectrum of infections including meningitis, pneumonia and ear infections. Currently most vaccines against these pathogens are based on the immunogenicity of the capsular polysaccharides. However, capsular polysaccharides of N. meningitides group B, the most virulent group of these bacteria are poorly immunogenic. An alternative approach is to use protein antigens widely presented on the bacterial cell surface.

Lactoferrin binding proteins (Lbp) are bacterial conserved outer-membrane proteins responsible for the iron uptake directly from host lactoferrin (Lf). This protein is considered an attractive candidate for vaccine development due to its essential role in bacteria survival. Lbp consists of two proteins: LbpA, an integral outer-membrane protein; and LbpB, a bi-lobed peripheral membrane protein which assemble into an oligomeric complex. Protein crystallography studies of both proteins should provide molecular details of the Lf binding determinants on Lbp and the structure of iron-binding site which is essential for vaccine design.

Various LbpB constructs including intact proteins, N-lobes, C-lobes from different strains of N. meningitides, M. catarrhalis, M. bovis were generated. Purification was optimized with general chromatography columns. Crystals of LbpB N-lobes from N. meningitides and M.bovis diffracting to 2.15 Å and 2.8 Å respectively were obtained and the structures of the proteins were solved using TbpB (Transferrin-binding protein) as a molecular replacement search model.

Future experiments include crystallization of the intact LbpB and the iron receptor LbpA.

P174

Phase variation of meningococcal Opa proteins and its impact on anti-Opa serum bactericidal activity

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Objective: Opa proteins from meningococci belonging to the ST-8, ST-11, ST-32 and ST-41/44 clonal complexes induce functional murine immune responses as measured by the serum bactericidal assay (SBA). However Opa expression is phase variable, controlled by stochastic changes in the length of an untranslated pentameric repeat tract within the reading frame of opa genes. The aim of this investigation was to describe variation in the repeat tract length in a diverse collection of hyperinvasive meningococci and to determine the extent to which variation affects the readout of anti-Opa bactericidal assays.

Methods: Genomic DNA from 29 representative meningococcal isolates, targeted in the SBAs, was extracted and locus-specific PCRs performed to separate and amplify the opaA, opaB, opaD and opaJ loci. The number of CTCTT repeats, indicating the inferred on/off expression status, was determined by analysis of the nucleotide sequences.

Results/Discussion: The average length of the repeat tract and the proportion of proteins "switched on" varied between opa loci, with the longest repeats and the highest proportion of expressed proteins observed at the opaD locus. Differences in the number of expressed proteins were also observed between clonal complexes. A positive association between target Opa expression and bactericidal activity was observed. However in some cases Opa proteins were not expressed but bactericidal activity was demonstrated, providing possible evidence of cross-reactive antibodies recognising non-targeted proteins. In conclusion, meningococcal Opa expression tends to correlate with bactericidal activity in vitro; similar data within other isolate collections may aid future meningococcal vaccine development.

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Modification of AniA glycoprotein as a vaccine antigen

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O-Glycosylation is emerging as a common posttranslational modification of surface exposed proteins in bacterial mucosal pathogens. In recent studies we have identified an additional outer membrane glycoprotein in pathogenic Neisseria, the nitrite reductase AniA, that is glycosylated in its C-terminal repeat region by the pilin glycosylation pathway. We have performed detailed characterization of the characteristics of the protein acceptor that allow efficient glycosylation of AniA.

Antigenicity studies of animals with various glycoforms of the AniA protein showed that the antibody response was directed predominantly towards the glycosyl moiety of the protein. When animals were immunised with a truncated form of AniA, completely lacking the glycosylated C-terminal region, a strong antibody response was observed against AniA regardless of the glycosylation state of the protein. This apparent immune evasion strategy, conferred by the phase variable immunodominant O-linked glycan attached to AniA, was investigated further using AniA purified from wild-type and glycosylation pathway mutant Neisseria strains. In addition, recombinant AniA proteins with various truncations expressed in an E. coli host were used as antigens to define the ideal antigen for generation of a cross reactive response. A strong, non-native immune response against AniA can be generated by immunisation with truncated forms of this protein.

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Development and Characterization of Monoclonal antibodies that recognize *Neisseria gonorrhoeae* PilC on the bacterial cell surface

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The pilin accessory protein, PilC, has long been recognized as being involved in Neisserial adhesion. Our goal was to define the parts of the 110kDa PilC protein that directly interact with the eukaryotic cell surface by developing monoclonal antibodies (Mabs) that block bacterial adhesion. “Refolded” recombiniant PilC1 was used to immunize mice and draining lymph nodes were harvested to make hybridoma lines. These lines were screened for antibodies that recognized PilC1 by ELISA and on the surface of N. gonorrhoeae. After mapping their epitopes using a PilC1 peptide array, we realized that several of the lines produced antibodies that recognized identical peptides. This redundancy could be due to immuno-dominance or development of sibling lines. Three of the Mabs recognized linier epitopes at the N-terminus, C-terminus, mid-region of PilC. None of the Mabs tested had adherence inhibition activity even when used at high concentrations. The epitope of the Mab recognizing the C-terminus was conserved between PilCs and across multiple gonococcal and meningococcal strains. None of the Mabs exhibited complement mediated bactericidal activity even when used at high concentrations on a serum-sensitive, cross-reacting strain of N. gonorrhoeae. Interestingly, the Mab recognizing the N-terminus only saw PilC on the surface of gonococci when it was also expressing a pilus while the other Mabs confirmed the presence of PilC regardless of a pilus. This phenotype could indicate that PilC is oriented with it N-terminus buried in the membrane prior to incorporation into an intact pilus. This model was partially confirmed by immuno-electron microscopy.

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Effect of Increased CRM197 Carrier Protein Dose on Meningococcal C Bactericidal Antibody Response

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Objectives: Increasing numbers of pediatric multivalent CRM197-based meningococcal and pneumococcal conjugate vaccines are available or in development. When vaccines with the same carrier protein are administered simultaneously, reduced antibody responses to the vaccine antigen(s) might occur. We reviewed studies of meningococcal C-CRM197 (MenC-CRM) vaccines to assess the effect of CRM197 carrier protein dose on bactericidal antibody response.

Methods: Cochrane Database, PubMed, Embase and clinical trial registries were reviewed for studies among healthy children <2 years old who were given MenC-CRM vaccine co-administered separately with pneumococcal or (PCV) Hib CRM197-based vaccines. Evaluated meningococcal outcomes included serum bactericidal geometric mean antibody titer (SBA GMT) and seroresponse rates >=1:8 and >=1:128.

Results: A trend towards lower MenC SBA GMTs occurred when CRM197 carrier protein amounts up to ~50ug/per dose were given concomitantly with DTaP-containing vaccines. At least 95% of children achieved a MenC rSBA or hSBA titer >=1:8 after the last infant or toddler dose. MenC antibody response was not affected when similar total CRM197 dose was given with concomitant DTwP-containing vaccines. Similar MenC SBA GMTs were observed following 2-dose vs. 3-dose MenC-CRM vaccine regimens, each co-administered with a PCV.

The effects of co-administered CRM197-based conjugate vaccines have implications for infant immunization strategies, which could include 15-64ug/dose and 20-32ug/dose of CRM197 in meningococcal and pneumococcal conjugate vaccines, respectively. Cumulative CRM197 effect on meningococcal antibody responses are also important to evaluate in children given three or four PCV7 (cumulative 60-80ug CRM197) infant immunizations, and meningococcal CRM197 or diphtheria conjugate vaccination in adolescence.

P178

Immunogenicity and Safety of a Recombinant Meningococcal Serogroup B Vaccine and a Quadrivalent Conjugate Vaccine in Laboratory Workers

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Background: Laboratory staff exposed to meningococci are at increased risk for invasive disease.

Methods: This was the first study in laboratory workers who received both a conjugate vaccine against meningococcal serogroups A, C, W-135 and Y (Men ACWY-CRM, Menveo®) and an investigational multicomponent vaccine against serogroup B containing factor H binding protein, Neisserial Adhesin A, Neisseria Heparin Binding Antigen and New Zealand strain outer membrane vesicles (4CMenB). Healthy adults (18-50 years of age) received 3 doses serogroup B vaccine at baseline, 2 and 6 months followed by a single dose of MenACWY-CRM 1 month later. Immunogenicity was assessed via serum bactericidal assay using human complement (hSBA) 1 month postvaccination; solicited reactogenicity and adverse events were monitored.

Results: Fifty-four participants enrolled. Bactericidal immune responses were evident after each dose of 4CMenB, as assessed by geometric mean titers and percentages of subjects with hSBA titers ≥4 or ≥8 or a four-fold rise over baseline. One month postvaccination, most MenACWY-CRM recipients had hSBA titers ≥8 against serogroups A, C, W-135, and Y. Both vaccines were well-tolerated, although rates of solicited reactions were lower after MenACWY-CRM.

Conclusions: Laboratory workers who received 4CMenB and MenACWY-CRM showed evidence of bactericidal immune responses to all 5 serogroups. Both vaccines were well-tolerated in these participants.

P179

Clinical Immunogenicity and Safety Profile of Two Quadrivalent Meningococcal Conjugate Vaccines in 2907 2-to-10-year-old Children

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Background: Current guidelines in the US and Canada advocate the routine administration of conjugate quadrivalent meningococcal vaccine for adolescents and certain high risk groups. We compared the I\immunogenicity and safety profiles of Menveo® (MenACWY-CRM) versus Menactra® (MCV4) in 2- to 10- year-olds.

Methods: Eligible 2-to-5-year-olds were randomized 1:2:2 to receive 2 doses of MenACWY-CRM197 or 1 dose of MenACWY-CRM or MCV4; 6-10 year olds were randomized (1:1) to receive a single dose of MenACWY-CRM or MCV4. The primary immunogenicity assessment was seroresponse in the two age cohorts 30 days following a single dose of MenACWY-CRM or MCV4. Noninferiority and superiority criteria were predefined. Solicited injection-site and systemic reactions were evaluated for 7 days postvaccination.

Results: 2907 children were vaccinated and underwent assessments(March 2008–October 2009). For seroresponse rates, MenACWY-CRM was statistically superior versus MCV4 for serogroups W and Y and noninferior for serogroup C in both age strata. For serogroup A, the seroresponse rates for MenACWY-CRM and MCV4, respectively were 72% and 77% in 2-5 year olds and 77% and 83% in 6- to 10- year-olds. Across all ages combined (2-10 years), MenACWY-CRM was non-inferior to MCV4 for all four serogroups, and statistically superior for serogroups C, W and Y. Safety parameters were overall similar across age cohorts and vaccines groups.

Conclusions: Both MenACWY-CRM and MCV4 were immunogenic and well tolerated in this study population. In children aged 2-10 years, MenACWY-CRM was statistically noninferior to MCV4 for all serogroups, and statistically superior for serogroups C, W, and Y.

P180

Immunogenicity of an Investigational Multicomponent Meningococcal Serogroup B Vaccine in Healthy Infants at 2, 4 and 6 Months of Age

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Background: Meningococcal serogroup B disease is the most widespread cause of bacterial meningitis and septicemia in infants for which no licensed, broad-spectrum vaccine exists. Whole-genome sequencing was employed to develop a multicomponent meningococcal serogroup B vaccine containing three recombinant protein antigens: Factor H binding protein(fHbp), Neisserial Adhesin A (NadA), Neisseria Heparin Binding Antigen(NHBA), which were combined with New Zealand Strain 98/254 outer membrane vesicles. Phase 2 studies showed immune responses in healthy infants.

Methods: 3630 healthy infants were randomized to receive one of three lots of investigational vaccine with routine infant vaccines (a diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Haemophilus influenzae type b, and hepatitis B combination vaccine plus a 7-valent pneumococcal conjugate vaccine), routine vaccines alone, or meningococcal C conjugate vaccine plus routine vaccines at ages 2, 4, and 6 months. Primary immunogenicity assessment was based on a serum bactericidal assay using human complement (hSBA) against three serogroup B strains (5/99, NZ98/254 and H44/76) 30 days after final study vaccination.

Results: Evidence of immune responses to the investigational vaccine, as assessed by hSBA titers, was observed. In pooled data across all lots, 100% of infants who received three doses of study vaccine had a seroprotective response to 5/99 and H44/76 as did 84% of infants to NZ98/254. Lot consistency, GMTs, and immune responses to the routine vaccines will be presented.

Conclusions: Three doses of an investigational multicomponent serogroup B vaccine in healthy infants, who also received routine vaccinations, induced robust bactericidal responses.

P181

Antibody persistence of group A meningococcal conjugate vaccine (MenAfriVac TM) in 2-29 years old subjects from Africa and India

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The Meningitis Vaccine Project (MVP) was funded in 2001 as a partnership between WHO and PATH to eliminate meningococcal epidemics in Sub-Saharan Africa through accelerated development and introduction of meningococcal group A conjugate vaccine. The new MenA conjugate vaccine (PsA-TT) (SIIL MenAfriVac ™) has been licensed in India and will be used in the African meningitis belt countries as a single dose in 1-29 years old population. We report one year antibody persistence in subjects vaccinated between 2 and 29 years of age in Africa and India.

Methods: Subjects vaccinated in the clinical studies in Africa (900 subjects aged 2-29 years) and India (340 subjects aged 2-10 years) were followed up for antibody persistence one year after immunization with one dose of either PsA-TT or polysaccharide vaccine (PsACWY-GSK Mencevax ACWY®)

Results: One year after immunization African subjects in PsA-TT group had higher serum bactericidal antibody rSBA GMTs than those in PsACWY group (2889 95% CI 2643-3159vs 922 95% CI 743-1143) as well as a higher proportion of subjects with rSBA ≥ 1:128 (99% 95% CI 98-100) vs those in the PsACWY group (93% 95% CI 90-96). Similar trends were observed for anti-PsA IgG GMCs and proportion ≥ 2 µg/ml. Results similar to those in African subjects were obtained in 2-10 years old Indian children.

Conclusions: One year after immunization with one dose of MenAfrivac 2 -29 years old African subjects and 2-10 years old Indian subjects had sustained antibody titres and these titres persisted better than those vaccinated with PsACWY vaccine.

P182

Tolerability of a Three-dose Schedule of an Investigational, Multicomponent Meningococcal Serogroup B Vaccine and Routine Infant Vaccines in a Lot Consistency Trial

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Background: A multicomponent meningococcal serogroup B vaccine comprised of recombinant protein antigens and outer membrane vesicles was developed via genomic technology. Infants are a key age group affected by serogroup B disease.

Methods: Healthy infants (n=3630) were randomized to receive one of three lots of the multicomponent serogroup B vaccine with routine infant vaccines (a diphtheria, tetanus, acellular pertussis, inactivated poliovirus, haemophilus influenzae type b, and hepatitis B combination vaccine as well as a 7-valent pneumococcal conjugate vaccine), the routine vaccines alone, or monovalent C conjugate vaccine plus routine vaccines at ages 2, 4, and 6 months. Solicited injection site and systemic reactions were recorded for 7 days postvaccination; adverse events were evaluated throughout the study.

Results: The serogroup B vaccine was considered generally well-tolerated when administered with routine immunizations; few infants discontinued the study due to reactogenicity following any study vaccination. In preliminary data, safety outcomes were generally similar for the three lots of investigational vaccine. The most commonly-reported solicited reactions in all groups were sleepiness, irritability, unusual crying, as well as injection site tenderness, erythema, and induration. Updated data will be presented. The most frequently reported adverse events were otitis media, upper respiratory tract infections, bronchitis, and nasopharyngitis.

Conclusions: The multicomponent meningococcal serogroup B vaccine had an acceptable safety profile when administered with routine immunizations. Few vaccinees discontinued the study due to reactogenicity.

P183

Robustness of the Serum Bactericidal Activity (SBA) Assay for *Neisseria meningitidis* serogroup B

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Objective: Human factor H binding protein (fHBP), a Neisseria meningitidis outer membrane lipoprotein, is under development as a vaccine antigen for the prevention of N meningitidis serogroup B (MnB) disease. The serum bactericidal assay (SBA) using human complement is the surrogate of protection used for in vitro evaluation of meningococcal vaccine. MnB SBAs used in late phase clinical studies supporting vaccine licensure must be robust. The effect of several assay parameters, such as shaker speed during reaction incubation, incubation time and human complement source, were investigated.

Methods: A design of experiment (DOE) approach was used to investigate the effect of shaking and incubation time on the SBA for four MnB strains. Several human complement sources were assessed for their effect in the SBA using a panel of immune sera from adult vaccines in bias analyses.

Results and Conclusions: For all strains tested, robustness of shaking speed and incubation time was demonstrated around the conditions used for SBAs supporting clinical studies. Human complement lots used in SBAs must be rigorously qualified prior to use to identify lots that will perform consistently over the course of a clinical study.

P184

Phase I study of a meningococcal C strain 2135 conjugate vaccine

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

Methods: Phase I randomized, double-blinded study, in 60 healthy male and female adult volunteers (18-50 years): 30 received 0.5 mL of the candidate vaccine, and 30 received 0.5 mL of a reference vaccine, Neisvac-C®. Only volunteers with < 5 IU/mL of tetanus antibodies were included. Candidate vaccine: meningococcal C polysaccharide 10 µg (strain 2135), conjugated to tetanus toxoid 10-30 µg, aluminium hydroxide 0.35 mg (Al+3)/ dose.

Clinical biochemical tests before vaccination and 48 hours later. Serology for meningococcal C polysaccharide and tetanus toxoid before vaccination and 1 month later. Clinical examinations before and 2, 7 and 30 days after vaccination. Adverse events within 30 days of vaccination were recorded.

Results: Safety: both vaccines were well tolerated, and adverse events were mild or moderate.

Immunogenicity: bactericidal (geometric mean) titers for 2135 strain (95% CI), BM vaccine, pre: 8.6 (5.4; 13.7); post: 280.8 (156.6; 503.3); Neisvac®, pre: 7.6 (6.2; 9.5); post: 294.1(156.3; 553.1). For C11 strain, BM vaccine (95%CI), pre: 5.7 (3.3; 9.7); post: 406.4 (210; 786.4). Neisvac®, pre: 4.8 (3.3; 7.0); post: 630.3 (351.1; 1131.8). Seroconversion (4-fold increase in titer): 2135 strain, 30/30 for BM vaccine, 27/30 for Neisvac®. C11 strain, 28/30 for both BM vaccine and Neisvac®.

Conclusion: BM vaccine showed safety and immunogenicity comparable to a licensed vaccine.

P185

A Meningococcal Antigen Typing system, (MATS) for estimating the potential effectiveness of protein-based vaccines against *Neisseria meningitidis*

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The serum bactericidal assay using human complement (hSBA), is a surrogate marker of protection used in evaluations of meningococcal vaccines. For vaccines based on novel proteins, existing methods may not reflect the variations in sequence and expression levels of the antigens. Testing large panels of strains in the hSBA against immune sera is impractical.

We developed a method, the Meningococcal Antigen Typing System (MATS), to define the potential of diverse MenB strains to be killed by vaccine-induced bactericidal antibodies. MATS is a modular system that may combine both genotypic and phenotypic information depending on the composition of the vaccine to which it is linked. To measure the contributions of fHBP, NadA, and NHBA, this method uses a novel sandwich ELISA performed on detergent extracts of bacteria. The measurement is sensitive to variations in both the immunologic recognition of each antigen and its expression level. Because the vaccine under study also contains OMV, the contribution of PorA is assessed by conventional subtyping methods.

We correlated MATS results from 124 MenB strains with hSBA data on pooled immune serum from different age groups, and found that a positive MATS result indicates strains that are highly likely to be killed in the hSBA. The method can be performed on large collections of strains, making it possible to survey regional populations of MenB bacteria to determine the potential for strain coverage by a candidate MenB vaccine.

P186

Use of immunoglobulin depletion to develop a universal complement source for use in the meningococcal serum bactericidal assay

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Goldschneider et al (1969) established the serum bactericidal assay (SBA) using human complement (hC') as a correlate for protection against meningococcal disease. Extensive efforts are required to match hC' donations without endogenous killing to strains of interest as donors commonly have acquired natural immunity. Therefore, we are attempting to develop a "universal" complement source by depleting immunoglobulin (Ig) from human plasma while preserving the endogenous complement for use in the SBA. Plasma collected by phresis from adult volunteers was depleted of Igs with Protein A/G+ resin at 4C. Plasma was immediately tested using the CH50 hemolytic complement test. IgG, IgM and complement components C1q, C3, and C9 were measured by immunodiffusion. Plasma was frozen at -80C for later use as complement. The SBA was performed with calcium and magnesium supplementation to reverse chelation during phresis. Ig-depleted plasma supportive of bactericidal killing was obtained for some strains. Endogenous killing activity could be removed while retaining CH50 activity similar to untreated plasma. When using a plasma-to-resin ratio of 2:1, depletion of IgG and IgM was >90% and 15-35%, respectively. Reductions in C1q, C3, and C9 were also observed up to 37%. IgG depletion was less effective at 5:1. Bactericidal titers obtained with Ig-depleted plasma previously frozen at -80C were comparable to untreated. Titers varied by resin and plasma lot tested. Removal of endogenous bactericidal activity following Ig-depletion was possible for many plasma samples tested. Complement function in the SBA was retained in Ig-depleted plasma following a freeze-thaw cycle.

P187

Importance of IgG subclass on complement-mediated bactericidal activity of antibodies to factor H binding protein (fHbp)

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The role of IgG subclasses on anti-fHbp functional activity is unknown. The serum IgG anti-fHbp GMT of ten adults immunized with a meningococcal vaccine containing recombinant fHbp increased from 1:48 before vaccination to 1:535 after 3 doses of vaccine. For 9 of 10 subjects IgG1 predominated (mean, 93% of IgG), with lesser amounts of IgG2 (5.3%) or IgG3 (≤1%). To investigate IgG subclass anti-fHbp functional activity, we constructed expression vectors encoding either human IgG1, IgG2, or IgG3 constant regions, which were each paired with one of two murine fHbp-specific binding domains ("JAR3" or "JAR5") that recognized overlapping epitopes in the fHbp N-domain. Constructs were expressed in CHO cells. The concentrations of the JAR3 mAbs required for human complement-mediated bactericidal activity against group B strain H44/76 were 12, 40 and 0.8 µg/ml, respectively, for IgG1, IgG2 and IgG3. The corresponding concentrations of the JAR5 mAbs were 18, 32 and 3.3 µg/ml. By flow cytometry, the JAR5 IgG3 chimeric mAb elicited 5-fold greater C4 deposition on the bacteria than the IgG1 mAb, and 25-fold greater activity than the IgG2 mAb. Activation of C4 deposition required C1q (a marker of classical complement pathway activation). Thus, IgG3 anti-fHbp mAbs, a subclass infrequent in serum, had greater bactericidal activity than IgG1 or IgG2. When a mAb targets a sparsely distributed antigen such as fHbp, the elongated Fc hinge region of IgG3 may permit more efficient engagement of C1q and classical pathway activation than IgG1 or IgG2 Fc regions with identical binding domains.

P188

Evaluation of the contribution of protein antigen NHBA to bactericidal antibody responses in sera from human vaccinees enrolled in clinical trials

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NHBA (Neisserial Heparin Binding Antigen) is present in all known serogroup B (MenB) meningococcal strains and exhibits limited polymorphism. NHBA, (fused with GNA1030) Neisserial Adhesin A (NadA) and factor H Binding Protein (fHBP) (fused with GNA 2091) comprise a novel MenB vaccine being evaluated in clinical trials alone and combined with MenB strain NZ98/254 Outer Membrane Vesicles (OMV).

We took a subtractive approach to investigate the contribution of NHBA to killing in the serum bactericidal assay. In strains mismatched to the vaccines for fHBP and PorA and lacking NadA, we screened for the quantity of NHBA using a sandwich ELISA (Meningococcal Antigen Typing system or MATS) with polyclonal antibodies against the individual vaccine components. Strains that expressed different levels of NHBA were analyzed in a competition serum bactericidal assay; in which the different vaccine antigens were added as competitors. A naturally occurring MenB strain, in which addition of NHBA inhibited killing by pediatric immune sera, was used to demonstrate that the vaccine induced bactericidal anti-NHBA antibodies even in young children.

In addition, a recombinant strain that was not killed directly by antibodies to any other vaccine component was complemented with the nhba gene.

Combining NHBA and OMV increased the inhibitory effect of NHBA even if the target strain was not matched to the OMV PorA component. Thus antibodies to NHBA can provide direct bactericidal activity and also synergize with antibodies against other protein targets.

P189

In vitro levels of NadA expression may underestimate the potential effectiveness of immune responses against *nadA in vivo*

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NadA is a component of a Multicomponent Meningococcal Serogroup B Vaccine being evaluated in Phase III clinical trials. We used a novel meningococcal antigen typing system (MATS) that correlates with killing in the serum bactericidal assay using human complement (hSBA) to define the quantity of the NadA expression that allows strains to be killed in hSBA. Under the in vitro conditions of growth of the bacteria for hSBA and for typing, NadA antigen is repressed by a regulatory protein NadR/NMB1843.

To understand the importance of NadR for the diversity in expression levels of the NadA antigen observed in MenB strains, a strain panel covering a range of NadA levels was selected and the nadR gene was knocked out in each. The resultant nadR- strains all expressed comparably high levels of the NadA antigen. Sera from mice immunized with NadA, alone or combined with other antigens, showed increased NadA-specific bactericidal activity on nadR- strains compared with wild type strains. Sera from clinical trial subjects of different age groups immunized with the rMenB vaccine were able to kill all of the nadR- strains in a bactericidal assay with human complement, confirming that NadA is a powerful immunogen.

In vivo, we expect NadR repression to be alleviated due to niche-specific signals, such as the small molecule inducer 4HPA that is present in saliva, resulting in high levels of NadA expression from any nadA+ strain and therefore efficient killing by the anti-NadA antibodies.

P190

An experimental detoxified L3-derived lipooligosaccharide meningococcal vaccine offer good safety but low immunogenicity in healthy young adults

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This open, randomized phase I study (#105779) evaluated the safety and reactogenicity of an experimental meningococcal serogroup B (MenB) vaccine obtained from outer membrane vesicle detoxified L3-derived lipooligosaccharide (LOS). The vaccine showed good safety and immunogenicity in preclinical and toxicological studies. Healthy adults (n=150) were randomized to receive either experimental vaccine (provided under five formulations, n=25 per group) or VA-Mengoc-BC® (control, n=25). Serum bactericidal assays (SBA), performed against 3 MenB wild-type strains, assessed immune response defined as a 4-fold increase between pre- and post-vaccination.

No serious adverse events related to vaccination were reported. Solicited adverse events graded 3 (i.e. preventing daily activity) were: pain (up to 17% of subjects, vs. 32% in control), fatigue (up to 12%, vs. 8% in control), headache (up to 4% in any group). Swelling greater than 50 mm occurred in up to 4%, vs. 8% in control.

The immune responses ranged from 5% to 36% across experimental vaccines for H44-76 (L3 type) strain (vs. 27% for the control), 0% to 11% for NZ98/124 (L3 type) strain (vs. 23% for the control), and 0% to 13% for 760676 (L2 type) strain (vs. 59% for the control). All geometric mean titers were below those measured with the control vaccine.

In conclusion, the experimental formulations tended to be less immunogenic than the control. In contrast, the safety profile was acceptable, with less grade 3 symptoms (especially pain) than the control, without signal of toxicity supporting the benefit of detoxification operated on the LOS for the experimental formulations.

P191

Antibody persistence of group A meningococcal conjugate vaccine - MenAfriVac- in African children

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A new MenA conjugate vaccine (SIIL MenAfriVac ™), has been licensed in India and will be administered to subjects 1-29 years old in the African meningitis belt countries.

Methods: Children aged 12-23 months received randomly a dose of MenA conjugate vaccine (PsA-TT) or meningococcal polysaccharide vaccine (PsACWY) (GSK Mencevax ACWY®) or Haemophilus influenzae type b vaccine (Hib) (GSK Hiberix®). Ten months later, children randomly received a booster of PsA-TT, or 1/5 dose of PsACWY or Hib in each group. Antibody persistence 2 years after primary immunization (14 months after booster) is reported here.

Results: 589 children received two vaccinations. Two years after primary vaccination, children vaccinated with PsA-TT followed by Hib had higher serum bactericidal antibody rSBA GMT (1314 95% CI 875-1972 vs 545 95% CI 311-958) and showed a higher proportion of subjects with rSBA ≥ 1:128 (97% 95% CI 89-100 vs 88% 95% CI 78-95) than those who received ACWY polysaccharide followed by Hib. Fourteen months after booster subjects who had received 2 doses of PsA-TT had rSBA GMT of 2721 (95% CI 1960-3777) vs 1314 (95% CI 875-1972) of those who had PsA-TT followed by Hib. The proportion of subjects with rSBA ≥ 1:128 was the same in both groups (98% 95% CI 91-100).

Conclusion: Children vaccinated two years earlier with MenAfriVac had an appreciable antibody decay. However, almost all subjects had rSBA ≥ 1:128. Two doses of PsA-TT had no significant advantage over one dose in the first year of life.

P192

Defining a *Neisseria meningitidis* group B (MenB) strain pool for estimating vaccine effectiveness

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Background: The evaluation of vaccines intended to provide broad protection against meningococcal serogroup B (MenB) strains requires a strain pool that is relevant to current epidemiology to determine the coverage potential of the vaccines. In collaboration with the ECDC, we sought to establish a representative strain pool based on strains collected in EU member states that together represent much of the overall MenB disease burden.

Methods: We determined a representative subset of strains based on year of isolation, distribution, serogroup and diversity of isolates. To account for the seasonal peak of incidence during winter, we used a recent epidemiological year from which one full season collection was available: July 2007 to June 2008.

Results and Discussion: Together, England & Wales, France, Germany, Norway and Italy accounted for 2,037 of 3,408 reported confirmed MenB cases in the EU in 2006. Each of the most common 33 MenB PorA types listed by EU-IBIS were represented among cases from these five countries (EU-IBIS Annual Report, 2006). The 5 most abundant clonal complexes identified by multilocus sequence typing (MLST) (ST-32, ST-41/44, ST-11, ST-8, ST-269 complexes) are represented in these countries, as well as the ST-162, ST-213 complexes, and other minor STs. Therefore, the strain distribution among these countries includes the major meningococcal clonal complexes. Based on these data, these 5 countries, taken together, constitute a minimum representative fraction of both the total MenB caseload and strain diversity in the EU. This basic list may be supplemented with additional countries and epidemic years.

P193

Kinetics of serum antibody responses one year post-meningococcal serogroup-C conjugate vaccination and correlation with B- and T-cell responses

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Some individuals who receive conjugated meningococcal vaccine exhibit waning of serum bactericidal antibody (SBA) titres to non protective levels by 12 months. We sought to measure persistence of humoral immunity and memory responses in young adults, 12 months after receiving serogroup-C meningococcal conjugate vaccine (MCC).

A group of 94 volunteers were vaccinated with MCC; SBA and specific IgG were measured at day 28. Twelve months post-vaccination serological response was again measured and volunteers were rechallenged with nasal administration of 25μg serogroup-C meningococcal polysaccharide (to mimic meningococcal colonization). In addition, activation of volunteers' peripheral B-and T-cells in response to polyclonal stimuli was assayed to determine if low SBA levels correlated to a defect in cellular activation.

While 100% of donors were protected (SBA ≥8) 28 days after vaccination, 11.7% had dropped below protective SBA levels after 12 months.

Following rechallenge, and measurement of antibody responses after 4, 7 and 10 days, rises in SBA and IgG levels by 10 days were only observed in donors with low (<128) or non-protective (<8) SBA levels at the point of inoculation (12 months post-vaccination). Only one donor did not attain protective levels 10 days after rechallenge. Throughout we found that SBA and serum IgG levels were positively correlated. No correlation of SBA with cellular activation was observed.

These data reveal detailed information on the kinetics of memory responses one year post-vaccination and suggest that exposure to intra-nasal antigen only induces serum responses in individuals with sub-threshold antibody concentrations at the time of new challenge.

P194

The effect of human serum on expression of neuraminic acid-containing polysialic acid (NeuPSA) antigens in *Neisseria meningitidis* group B (NmB) and functional activity of anti-NeuPSA against NmB

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Objectives: Humans are obligate hosts for NmB yet culture media and functional assays typically contain supplements derived from bovine blood products (BBP). We investigated the effects of BBP compared to human serum (HuS) on binding and functional activity of two mAbs that recognize different NeuPSA epitopes.

Methods: Three NmB strains were cultured in Müller-Hinton (MH), BBP-free chemically defined medium (CDM), or CDM supplemented with 5% HuS (CDM HuS). Anticapsular and anti-NeuPSA mAb binding was measured by flow cytometry. Bactericidal activity (BCA) against NmB in buffers that did or did not contain BBPs was evaluated using human complement without or with PMNs and in whole blood.

Results: The anti-NeuPSA mAbs SEAM 3 and 2 bind to NeuPSA epitopes with degree of polymerization (Dp) <4 containing a non-reducing end Neu residue or Dp>10 containing ~50% Neu, respectively. The presence of BBP in the culture medium did not affect anticapsular mAb or SEAM 3 binding to cells or BCA of the mAbs with the NmB stains tested. In contrast, the expression of longer, more highly de-N-acetylated PSA epitopes recognized by SEAM 2 increased considerably for NmB cultured in CDM or CDM HuS compared to MH. Also, the concentration of SEAM 2 required for BCA increased up to 100-fold in the presence of BPP regardless of culture medium. Both anti-NeuPSA mAbs had the highest BCA in human plasma with PMNs or whole blood.

Conclusion: The results raise questions about the clinical relevance of NmB grown and BCA measured in the presence of BBP.

P195

Serological Antibody Kinetics and BT-cell responses after Primary Immunization with Meningococcal Serogroup C Conjugate Vaccine or Secondary Immunization with either Conjugate or Polysaccharide Vaccine in Adults

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Objective: We investigated the characteristics and development of antibody (iso)types and cellular immune responses after primary, secondary MenCC or PS vaccination.

Methods: Volunteers, 18-55 years, were immunized with MenCC (primo-group) or received secondary immunization with MenCC (MenCCgroup) or MenC-PS (PSgroup). Blood samples were obtained pre- and seven time-points post-immunization. IgGsubclasses, IgA, IgM and avidity were assessed by a multiplex immunoassay, functional antibodies by SBA, plasma cells memory B-cells and T-cell cytokine responses were detected after activation of PBMCs by ELISpot assay or multiplex immunoassay.

Results: High levels of antibodies were still present 5 years in primo-group. Secondary immunization resulted in increased IgG and SBA titers after 5-7 days. In primed individuals, IgM was still present, and only increased in PSgroup. Immunization with PS induced higher IgG2 responses compared to MenCC. MenC PS-specific memory B-cells were detectable from day 7-10 onwards in primo-group and MenCCgroup and were highest on day 17 in the MenCCgroup persisting up till 25 days. The main cytokines induced upon conjugate vaccination are of Th1 type but also Th2 cytokines were induced, albeit at lower levels.

Discussion: Secondary immune responses are quite slow. The Ig (iso)type distribution is different between MenCC and PS maybe influencing functional titers. MenCC immunization results in a clear induction of MenC PS-specific memory B-cells possibly related to an activation of carrier protein-specific T-cell immunity. Although immunological memory was previously induced by a single MenCC vaccination, the study highlights the importance of sustaining protective antibody levels against N. meningitidis infection.

P196

Interlaboratory standardization of the MATS ELISA: a protein antigen phenotyping method for *N. meningitidis* serogroup B

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The Meningococcal Antigen Typing System (MATS) combines phenotypic and genotypic information to assess whether a given MenB strain may be covered by a vaccine. It uses a sandwich ELISA (MATS ELISA) to measure the fHbp, NadA, and NHBA content of tested isolates relative to a reference strain (relative antigen potency). The relative antigen potency is sensitive to variations in both the immunologic recognition of each antigen and its expression level. In preliminary studies, MATS accurately predicted killing in the serum bactericidal assay using human complement, an accepted correlate of protection for meningococcal vaccines.

We conducted an inter-laboratory standardization study at 7 independent laboratories to assess the robustness of MATS ELISA relative potencies for fHbp, NadA and NHBA, and to validate the potential for strain coverage in defined geographical regions. Each laboratory performed MATS assays on a set of 18 shared reference strains; each of which was assayed multiple times for the three antigens. 'Consensus' relative potencies were derived for each specimen/antigen combination using a mixed-model analysis of variance from the present data. These values were used to quantify accuracy, reproducibility, and precision among the seven laboratories. We used scatter plots and plots of accuracy, precision, bias and variance components. We calculated Pearson correlation coefficients (r), coefficients of accuracy (Ca), and concordance correlation coefficients (rc), to assess agreement. We evaluated the

ability of the seven laboratories to reproduce relative potencies among themselves and against the consensus relative potencies using the estimated values from the ANOVA models.

P197

Diversity of the Novartis investigational vaccine in German invasive meningococcal isolates 2007/2008

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The recombinant meningococcus B (rMenB) - OMV vaccine containing fHbp, NadA, P1.4, and NHBA targets the antigenetically variable population of MenB. Pre-licensure prediction of vaccine effects relies on DNA sequence data, antigen expression analysis, and serum bactericidal assays. Antigen sequence variants were analysed in 316 invasive isolates from Germany (07/2007 and 06/2008; MenB: 223, 70.3%). At the time of writing, 140 ST and 15 cc were assigned to 274 strains. 86, 51, 46, and 12 strains belonged to ST-41/44 cc, ST-32 cc, ST-11 cc, and ST-269 cc, respectively. For the subgroup of 187 MenB with ST assignment, ST-41/44 cc accounted for 84 isolates (45%), ST-32 cc for 44 (24%), ST-269 cc for 10 (5%). 176 of 276 strains with fHbp family assignment were variant 1 (64%), which is used in the vaccine. For MenB this value was 153 of 193 (79%). 231 of all 316 strains lacked nadA (73.1%; 76% for MenB). As expected, most ST-269 cc and ST-41/44 B strains were nadA negative. Of 315 strains with PorA VR2 assignment, 47 harboured P1.4, the immunodominant PorA antigen of OMV, all of them were MenB. 202 of the 276 isolates fully analysed at the time of writing, either harboured P1.4, nadA, or variant 1 fHbp (73%). This number was 158 of 193 fully analysed MenB (82%). In conclusion, even when excluding contribution from NHBA, rMenB-OMV is predicted to have a satisfying coverage in the MenB population circulating in Germany. Further data on NHBA will be presented.

P198

Human Complement Bactericidal Activity in a Phase 2 Safety and Immunogenicity Study Following Vaccination with a New Meningococcal A Conjugate Vaccine in Healthy Africans Living in Sub-Saharan Africa

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Background: Epidemic group A meningococcal meningitis plagues the region of Africa known as the meningitis belt during the dry months December through June. The Meningitis Vaccine Project (MVP) partnership is working to develop an affordable group A conjugate vaccine, PsA-TT, for widespread use in Africa.

Methods: Serum bactericidal activity was determined using pooled human complement (hSBA) for sera from an observer-blind, randomized (2:1), controlled safety/immunogenicity study conducted in 900 subjects 2 to 29 year of age in Mali, Senegal and The Gambia. Study volunteers received one dose of PsA-TT conjugate or PsACYW polysaccharide vaccine. A subset of 380 participants stratified by age was randomly selected for hSBA testing of sera obtained prior to and 28 days post-immunization.

Results: The hSBA geometric mean titers [GMT (95% CI)] of PsA-TT post-immunization sera for 2-5, 6-10, 11-17, 18-29 year olds were 31.1(20.6,46.8), 38.4(24.6,59.8), 43.7(26.5,72.0) and 54.4(36.3,81.7) compared to 3.4(3.0,3.9), 4.4(3.6,5.4), 5.2(4.1,6.7) and 9.6(6.9,13.4) pre-immunization. PsACWY post-immunization sera GMTs were 4.8(3.2,7.1), 3.9(2.8,5.6), 16.0(9.1,28.1) and 21.3(11.7,38.8) for the same age groups respectively. The proportion [%,(95% CI)] of PsA-TT and PsACWY subjects with hSBA titers ≥1:8 post-immunization were 78.6%(67.1%,87.5%) and 32.3%(16.7%,51.4%) in 2-5 year olds, 83.6%(71.9%,91.8%) and 22.2%(10.1%,39.2%) in 6-10 year olds, 80%(67.7%,89.2%) and 71%(52.0%,85.8%) in 11-17 year olds and 90%(79.5%,96.2%) and 75.9%(56.5%,89.7%) in 18 to 29 year olds.

Conclusions: PsA-TT induced strong hSBA responses in all age groups studied consistent with previously reported A PS IgG and rabbit complement SBA results. hSBA was more discriminatory between vaccines than rSBA especially in young age groups.

P199

Comparison of Two Assays Used for the Measurement of Anti-Capsular Antibody Avidity in Relation to Antibody Bactericidal Activity

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Antibody avidity is an important determinant of antibody functional and protective activity. Solid phase enzyme-linked immunoabsorbent assays (ELISA) using chaotropic agents such as thiocyanate to disrupt binding have been used for measuring antibody avidity, but reports attempting to validate these assays using monoclonal antibodies (mAbs) have been conflicting. The objective of this study was to compare avidity values obtained using two different methods, thiocyanate elution and liquid phase radioantigen binding assay (RABA), and to correlate these values with complement-dependent antibody bactericidal activity. Seven human IgG mAbs were evaluated: 3 specific for the capsular polysaccharide (PS) of group A Neisseria meningitidis and 4 specific for the capsular PS of Haemophilus influenzae type B. RABA avidity indices showed a significant correlation with bactericidal activity ($p = .028$). Thiocyanate avidity indices gave a significant correlation with bactericidal activity for 6 of the 7 mAbs ($p = 0.007$). The one exceptional mAb required a high concentration of thiocyanate to disrupt binding despite having only moderate bactericidal activity and RABA avidity. These data indicate that in most cases both RABA and thiocyanate elution methods provide avidity values that predict anti-capsular antibody bactericidal activity. However, there appear to be exceptional antibody-antigen reactions for which the thiocyanate avidity index may not accurately reflect antibody functional activity. The mechanism of this phenomenon is not known, but we speculate that chaotropic disruption of antigen binding may in some cases reflect unique combining site chemistries that are not directly related to avidity determination.

P200

Vaccinology (Clinical)

Serological Evaluation of Meningococcal C Conjugate Vaccine Programs in Canadian Children

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Invasive meningococcal disease (IMD) is endemic in Canada, serogroup C strains causing nearly half of cases and deaths. Canada is one of the first countries to establish infant MenC immunization programs, with three variants: Alberta's 3-dose program (2, 4, 12 months) using meningococcal C-tetanus toxoid conjugate (MenC-TT), 2-dose program in BC (2, 12 months) using MenC-TT and single dose programs (12 months) in other provinces, using MenC-TT or licensed meningococcal C-diphtheria toxin conjugate (MenC-CRM) vaccines. An optimal vaccination program would provide a high level of protection throughout peak years of risk (0 to <5 years), yet the most effective, efficient schedule is not yet determined. To this end, 165 subjects recruited in each of AB, BC and Halifax will provide patient records and specimens for analysis.

C IMD immunity is primarily antibody-mediated, serum concentration of which is measured by bactericidal assay (SBA), while polysaccharide-specific antibodies are measured by enzyme-linked immunosorbent assay (ELISA).

Immunity will be assessed in both manners at the University of Calgary, one of only two public sector serum bactericidal assays in Canada. All sera will be assayed for serogroup C bactericidal activity using standardized procedures and inocula

The functional approach the Translational Research lab at the University of Calgary has chosen will assess various provincial MenC immunization programs in a real-world context. Results obtained will aid in refining domestic programs by providing policy makers with much needed information regarding efficacy and efficiency.

P201

Improved OMV vaccine against *Neisseria meningitidis* using genetically engineered strains and a detergent-free purification process

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The use of detergent-extracted outer membrane vesicles (OMV's) is an established approach for development of a vaccine against N. meningitidis serogroup B. Selective removal of lipopolysaccharide (LPS) decreases toxicity, but promotes aggregation and narrows the immune response. Detergent-free OMV vaccines retain all LPS, which preserves the native vesicle structure, but results in high toxicity and lower yield. The present study assessed the effects of gene mutations that attenuated LPS toxicity (lpxL1) or improved OMV yield (rmpM) in combination with the available OMV purification processes. The results substantiate that OMV's from a strain with both mutations, produced with a detergent-free process provide better vaccine characteristics than the traditional detergent-based approach. With comparable toxicity and yield, no aggregation and cross-protection against other PorA subtypes, these OMV vaccines are potentially safe and effective for parenteral use in humans.

P202

Re-evaluating FetA as a meningococcal vaccine antigen

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The search for a vaccine providing comprehensive protection against meningococcal disease has largely focused on outer membrane protein antigens. One such protein, FetA, has often been dismissed due to the limited cross-reactivity between variants and the unpredictable iron-regulated expression.

FetA is known to be expressed by the meningococcus in vivo as specific antibodies have been found in convalescent sera. Antibodies against FetA are also bactericidal in vitro and only a few FetA variants are associated with hyperinvasive lineages, suggesting that this protein could be a suitable vaccine antigen. In this study, meningococcal strains have been constructed which constitutively express different FetA variants at increased levels. Outer membrane vesicles from these strains have been shown to induce FetA-specific antibodies in a murine model and, as such, could be used to develop a vaccine targeting FetA. A probe-based Reverse Transcription-PCR assay was also developed to investigate the variability of fetA expression in a range of isolates. When compared with genomic DNA sequences, the variation seen in fetA expression cannot simply be attributed to iron availability (via Fur) and slipped-strand mispairing in the -10 to -35 region. However, despite this variation, results suggest that a significant proportion of strains are likely to express sufficient amounts of FetA to be killed by specific antibodies. FetA might prove to be an effective meningococcal vaccine component, particularly if combined with other outer membrane protein antigens to improve potential coverage.

P203

The Impact of Immunization with Transferrin-Binding Proteins TbpA and TbpB on Vaginal Colonization in a Mouse Model of Gonococcal Infection

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The transferrin binding proteins TbpA and TbpB of Neisseria gonorrhoeae comprise a bipartite system for acquisition of iron from transferrin. Conservation of these proteins among strains combined with their role in pathogenesis has made these proteins attractive vaccine candidates. The goal of this study was to determine the effects of mucosal immunization with TbpA and TbpB on colonization rates in a murine model of gonococcal infection. Animals were immunized intranasally with TbpA, TbpB, or a combination of TbpA and TbpB, with cholera toxin B subunit (Ctb) as a mucosal adjuvant. A control group received Ctb alone. Three weeks after the final immunization, mice were challenged intravaginally with 3E6 colony forming units of the homologous strain of N. gonorrhoeae. Vaginal colonization was monitored for 8 days post-inoculation. Reduced colonization rates occurred in immunized mice compared to control mice beginning 5 days post-inoculation. At 8 days post-inoculation, 53.8% of the control group remained colonized. In contrast, only 30% of animals receiving either TbpA or TbpB remained colonized, and only 23% of mice immunized with both antigens maintained detectable levels of bacteria. These data suggest that immunization with TbpA and TbpB is potentially protective in this gonococcal infection model. Current studies are aimed at examining antibody titers and anti-gonococcal activity of serum and vaginal fluid from immunized animals to evaluate potential correlates of protection. These results represent an important initial examination of the potential of transferrin binding proteins as protective antigens in an in vivo model.

P204

Role of TLRs in host response during outer membrane vesicle immunization and experimental meningococcal disease

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Outer membrane vesicle (OMV) vaccines from Neisseria meningitidis contain a variety of TLR agonists, such as lipopolysaccharide (TLR4) and lipoproteins (TLR2) which may work as internal adjuvants and thereby determine the adaptive immune response. To investigate this, we immunized mice deficient in TLR2, TLR4, or TRIF with OMV vaccines. Readouts were total IgG titers, IgG subclass distribution, serum bactericidal titers, and PorA-specific T cell responses. The overall immune responses were not lower in TLR2-/- mice but tended to be even higher. In contrast, TRIF-deficient and TLR4-deficient mice showed clearly impaired immunity. Together our data demonstrate that TLR4 activation contributes to immunogenicity of the N. meningitidis OMV vaccine, but that TLR2 activation is not required, at least not when TLR4 is also activated.

We also investigated the role of TLR4 activation during meningococcal infection, using a mouse model of meningococcal sepsis and N.meningitidis mutant strains deficient in TLR4 activation due to underacylated lipid A. Strains with mutations in the lpxL1 and lpxL2 genes have penta-acyl lipid A, lacking the secondary C12 chain at the 2' and 2 position, respectively. We showed that the lpxL2 mutant activates murine TLR4 less efficiently than lpxL1, which was again less active than the wild type. Interestingly, we found that the lpxL2 mutant is more virulent in mice than the wild type strain, whereas the lpxL1 mutant is actually much less virulent than the wild type strain. These results demonstrate the crucial role of TLR4 activation in determining the outcome of infection with N. meningitidis.

P205

Evaluation of the cross-protective capabilities of the *Neisseria meningitidis* fHbp antigen sub-variant 1.1 through the generation of engineered meningococcal strains

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fHbp is a Neisseria meningitidis (Nm) surface-exposed lipoprotein, identified by reverse vaccinology, that induces high levels of bactericidal antibodies. fHbp is part of the Multicomponent Meningococcal Serogroup B Vaccine currently being evaluated in clinical trials.

Although fHbp variants (1, 2 and 3) display very low levels of cross-protective bactericidal response, recent analysis of sub-variants identified according to allelic variations raised questions about cross-reactivity within variants.

We evaluated whether antibodies raised against the fHbp vaccine variant (1.1) cover other common sub-variants 1. A genetic approach was used to overcome the variability caused by the different susceptibility of Nm strains, the limitations of compatible complement sources and the variable expression of the fHbp sub-variants among different strains. We engineered a well characterized Nm strain (5/99) to express different sub-variants 1 from a constitutive heterologous promoter.

The expression of the sub-variants was evaluated by Western blot and FACS analysis. Strains were also characterized by bactericidal assays using sera from animals immunized with the fHbp recombinant protein and from humans in varying age groups immunized with Multicomponent Meningococcal Serogroup B Vaccine in clinical trials. We observed cross protection against all sub-variants in adults and adolescents vaccinated with Multicomponent Meningococcal Serogroup B Vaccine. Analysis of infant sera suggested that fHbp antigen alone may not be sufficient to efficiently protect this age group.

P206

Comparison of different carrier proteins for meningococcal serogroup A, C, W-135, and Y oligosaccharides

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Meningococcal disease receives prominent public health attention because of its extremely rapid onset and progression; the disease can be fatal in a matter of hours.

Surface polysaccharides have long been key antigens for vaccines against major disease-causing meningococcal serogroups A, C, W-135 and Y (Men A, MenC, MenW-135, MenY). Conjugation of polysaccharides to a protein carrier has conferred important public health benefits; little research examines the differences between carriers.

We compared different protein carriers that are currently used in licensed vaccines. We prepared and characterized menigococcal serogroup A, C, W-135 and Y glycoconjugates with CRM197, Diphtheria Toxoid (DT), Tetanus Toxoid (TT). These carriers are used in licensed vaccines.

The different conjugates were tested in a mouse animal model: Balb/C mice were each injected subcutaneously with 2µg of MenA, and 1µg of MenC, MenW-135, and MenY with AlumPhosphate as adjuvant. Control animals were injected with adjuvant only. Additional doses were administered after 14 and 28 days. Vaccinated and control animals were bled 27 and 42 days after the second and third conjugate injection.

Post immunization sera were analyzed by ELISA for specific anti-polysaccharide and anti-carrier IgG. Functionality of the antibodies elicited against the capsular polysaccharide was assessed in a serum bactericidal assay using rabbit complement (rSBA).

Preliminary data showed comparable results for CRM197, TT and DT conjugates.

P207

Minor pilins as novel vaccine candidates against *Neisseria meningitidis*

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There is an urgent need for development of vaccines against N. meningitidis serogroup B. Among the major surface-exposed attributes of N. meningitidis are type IV pili (Tfp), which contribute to its virulence. Tfp are composed of subunits of PilE, the major pilin, and less abundant minor pilins PilX, PilV and ComP, which have important roles in Tfp biology. Tfp have been attractive candidates for vaccine development, however development of a pilus-based vaccine has been halted due to extensive antigenic variation of PilE. We therefore decided to evaluate the possibility that minor pilins could be better vaccine candidates against N. meningitidis. We first investigated the prevalence and sequence variation of genes encoding minor pilins in 56 clinical isolates of N. meningitidis. The genes encoding minor pilins were present in all clinical isolates and were found to be highly conserved in contrast to pilE. While ComP is almost identical in all isolates, phylogenetic analysis of PilX and PilV proteins revealed 3 major protein variants for each protein. We next tested the possibility that antibodies directed against these proteins might interfere with Tfp-linked functions and/or mediate complement-mediated bactericidal activity. Although sera directed against minor pilins did not have high bactericidal effects, they interfered with Tfp-mediated functions to various extents which does not rule them out as constituents of a broad vaccine against N. meningitidis.

P208

N. menigitidis PorB Adjuvant Activity is Mediated Through TLR2 and Induces Isotype-Specific Subclasses of IgG

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The Neisseria meningitidis porin PorB has been shown to act as an adjuvant, and immune stimulant in a TLR2-dependent manner. To determine whether antibody generation to a test antigen would also be TLR2 dependent, the following experiments were performed. WT C57Bl/6 and TLR2 -/- (KO) mice were immunized with Ovalbumin (Ova) alone and Ova + PorB. TLR2 -/- mice generated lower Ova-specific IgG levels, overall, when compared to WT mice. Although Ova-specific antibody levels were lower in KO mice that received Ova + PorB, they were still higher than in KO mice that received Ova alone. The antibody responses in mice vaccinated with Ova + PorB was predominantly of the IgG1 and IgG2b subclasses, the latter not seen in mice vaccinated with Ova alone. The levels of both of these IgG subclasses were significantly reduced int eh TLR2 KO mice. These results confirm that the adjuvant activity of PorB depends on a TLR2-mediated pathway, and demonstrate that the porin enhances induction of antigen specific subclasses of IgG.

P209

An *in vitro* potency test for conjugate vaccines based on bactericidal antibodies against capsular polysaccharides

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Immunogenicity evaluation, a widely used approach for measuring the antigenic potency of vaccines, depends on in vivo responses, which are variable, linked to the immunization regimen and provide little information about antibody function. Immunogenicity testing for product release requires repeated studies involving laboratory animals which should be reduced whenever possible.

We sought to measure the antigenic potency of a meningococcal polysaccharide-protein conjugate vaccine with an in vitro relative potency assay based on the ability of the antigens to bind to bactericidal, serogroup-specific monoclonals directed against N. meningitidis. As polysaccharide conjugate vaccines are very well characterized by chemico-physical and spectroscopic analyses, it is possible to confirm whether the in vitro test is an appropriate way of defining the antigenicity of vaccine components.

We developed an antibody binding competition assay based on the interaction of specific monoclonal antibodies and capsular polysaccharides of serogroups A, C, W-135 and Y meningocci. The assay is a competitive ELISA in which the binding of a monoclonal antibody to the native polysaccharide is specifically inhibited by the quadrivalent ACWY conjugate vaccine (Menveo®). The read-out of the assay is a relative potency determination, calculated by comparing the inhibition curve of a reference vaccine with the curve of an unknown vaccine sample.

We show the validation of the assay as applied to the analysis of a quadrivalent polysaccharide–protein conjugate vaccine. Our results demonstrate the sensitivity and reproducibility of the method and highlight the feasibility and usefulness of its application to other vaccine formulations.

P210

Meningococcal 5CVMB vaccine antigens – prevalence and polymorphisms of the encoding genes in *Neisseria gonorrhoeae*

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A serogroup universal Neisseria meningitidis vaccine has been designed. This non-capsular 5 Component Vaccine against Meningoccocus B (5CVMB) includes five genome-derived Neisseria antigens (GNAs): fHbp, NHBA, GNA1030, GNA2091 and NadA. Preclinical studies of 5CVMB have shown promising results. An effective N. gonorrhoeae vaccine has never been designed.

The aim was to investigate the prevalence and polymorphisms of the 5CVMB genes in Neisseria gonorrhoeae.

The WHO 2008 N. gonorrhoeae reference strains (n=8) and divergent gonococcal isolates from 28 different countries worldwide (n=103), were examined using serovar determination, full-length porB sequencing, N. gonorrhoeae multiantigen sequence typing (NG-MAST), and sequencing of fHbp, nhba, gna1030, and gna2091. nadA presence was screened by using in silico examination.

With exception of nadA (lacking in all isolates), all the 5CVMB genes were present in all gonococcal isolates. However, a total of 25 isolates (22.5%) were not able to express complete fHbp (n=9) and/or NHBA (n=18). Among the N. gonorrhoeae isolates, the GNA genes were highly conserved; 92.8-99.5% in their respective nucleotide sequence and 89.5-99.5% in the amino acid sequence. However, compared to the N. meningitidis reference strain MC58 the sequences were far more diverse; 69.7-95.4% in the nucleotide sequence and 61.1-94.9% in the amino acid sequence.

In conclusion, four of the five antigens included in the 5CVMB are expressed in most N. gonorrhoeae strains worldwide. The potential effects of the 5CVMB vaccine on gonococci as well as the commensal Neisseria species remain unknown. Some level of cross-immunity can not be excluded and should be further examined.

P211

Mucosal and systemic immune response of mice to capsular polysaccharide of N. menigitidis serogroup C coadministered intranasally with AFCo1

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Simple administration of antigens at mucosal sites have demonstrated be inefficient to stimulate the immune system by the weak immunogenicity inherent of these antigens. The use of mucosal adjuvants is capable to change this problem, increasing the mucosal and systemic immune responses. AFCo1, a microparticle, had demonstrated be a potent mucosal adjuvant with thyme-dependent antigens. In this work we demonstrated that intranasal immunisation (i.n) of 3 doses in Balb/c mice of capsular polysaccharide (PSC) of Neisseria meningitidis serogroup C and AFCo1, mixed together, promote higher statistics specific IgA anti-PSC responses ($p<0.001$) in saliva and vaginal wash than the i.n immunization of PSC alone and VAMENGOC-BCTM by the intramuscular route (i.m). Also, were induced a statistics significant specific IgG response anti-PSC ($p<0.05$) superior to i.n plain PSC, but not different to i.m VAMENGOC-BCTM. The subclasses pattern and the IgM response were analyzed. The long-time mucosal and systemic memory responses after a booster immunisation with plain PSC 70 days later of the last immunisation were evaluated. These results suggest that AFCo1 is a potent mucosal adjuvant not only for thyme-dependent antigens, also to thyme-independent antigens type 2 without the need of conjugation and is capable to induce memory responses.

P212

Natural *Neisseria* Derive Proteoliposome And Cochleate As Potent Vaccine Adjuvants

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Proteoliposome (PL) and its derivated- Cochleate (AFCo1) contain immunopotentiating properties and delivery system capacities required for a good adjuvant. Consequently, we hypothesized that PL and AFCo1 will function as good vaccine adjuvants. PL is a detergent-extracted outer membrane vesicle from live Neisseria meningitidis B and can be transforming into AFCo1 in calcium environment. Both are produced at industrial scale and control at Finlay Institute under GMP conditions.PL and its AFCo1 have exceptional characteristics because they: combine in the same structure the potentiator activity, the polarizing agents, and the delivery system capacities; exhibit multimeric copies (repetition of porins and other proteins); contain multiprotein composition (Hmbr, FrpB, PorA, PorB, rmpM, etc.); contain multi PAMP components (LPS, porins, phospholipids, DNA, etc); contain synergistic PAMPs-TLRs interactions (TLR4 and TLR9); act co-administrated but also allow the inclusion of other PAMPs, proteins, peptides, etc; induce both type I IFN and IL-12 cytokines which suggest the stimulation in human of plasmocytoid precursor and conventional dendritic cells, respectively; induce a preferential Th1 pattern of response; induce T CD4+, T CD8+, and cross presentation; has polyclonal B cell activity, and function by parenteral and mucosal routes. The constitution of Proteo-Cochleate permits per se their function as a vaccine. The inclusion of heterologous PAMPs and the formation of Cochleates using PL obtained from other microorganisms is a real fact. Preliminary results against Malaria and sexual transmitted disease are very promising. PL and Cochleate acting directly as vaccines or used as adjuvants are very promising.

P213

Production and characterisation of a multi-protein meningococcal vaccine enriched in heat shock proteins

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We have developed a meningococcal disease vaccine containing a mixture of protein antigens which is enriched in the highly immunogenic heat shock proteins (Hsps). Sera from mice immunised with this vaccine show promising bactericidal and opsonophagocytic responses against a panel of N. meningitidis strains. The vaccine also provides protection against lethal challenge in a mouse model of meningococcal septicaemia. Our objective is to develop a consistent process and the required characterisation tools for this vaccine to demonstrate process robustness, vaccine stability and batch consistency.

The vaccine is prepared from a heat shocked culture of the commensal N. lactamica which has a majority of antigens in common with N. meningitidis and offers advantages for large scale production, particularly that biocontainment conditions are not required. The bacterial paste is recovered by centrifugation, the bacteria are broken by homogenisation and clarified supernatant is applied to an anion exchange column. Fractions enriched in Hsp65 are pooled to produce the vaccine. We have used a number of assays to characterise the Hsp-enriched vaccine including protein content by SDS-PAGE and LPS by LAL. We have also used western blotting and ELISA to quantify the important antigens such as Hsp65. The effect of key production parameters, such as fermentation conditions, process and formulation buffers, on product consistency and immune responses are currently being evaluated and will be described.

P214

Pilot scale production of AFCo1 as Vaccine Adjuvant

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The use of new adjuvants in vaccine formulations is a subject of current research. Only few parenteral adjuvants have been licensed. We develop a mucosal and parenteral adjuvant entitled AFCo1 (Adjuvant Finlay Cochleate 1) from proteoliposome of Neisseria meningitidis B, using dialysis procedures at lab scale. In order to increase the production process, we used a crossflow system (CFS) that allows easy scale up to obtain large batches in an aseptic environment. The aim of this work is to produce AFCo1 at pilot scale conserving adjuvanticity. Results. AFCo1 was successful produced by tangential filtration to scale pilot. It passes preclinical toxicity studies and preliminary stability. The nasal immunogenicity evaluated in Balb/c mice induced specific saliva IgA and serum IgG. The induction of Th-1 response was demonstrated by the induction of IgG2a, IFNg and not IL-5. The self adjuvant action as well as against co-administered antigens were similar to Lab scale lots. The AFCo1 obtained at pilot scale conserve the mean physic-chemical and immunological characteristics for 2 years with no preclinical toxicity

P215

A meningococcal native outer membrane vesicle (NOMV) vaccine with attenuated endotoxin and over-expressed factor H binding protein (fHbp) elicits broad serum bactericidal antibody (SBA) responses in infant rhesus monkeys

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Objective: To develop an improved group B vaccine, we prepared NOMV vaccines from LpxL1KO mutants expressing attenuated penta-acylated LPS and engineered to over-express fHbp. Immunized mice developed broad SBA responses. However, mouse TLR4 is activated by both hexa- and penta-acylated meningococcal LPS, whereas human TLR4 is mainly stimulated by wildtype hexa-acylated LPS. The mouse immunogenicity therefore may have resulted from an adjuvant effect of stimulation of mouseTLR4 by the mutant LPS, which would be much less in humans. In the present study we evaluated the immunogenicity of NOMV vaccines in infant rhesus primates.

Methods: Animals, ages 2–3 months old, received a mixture of two NOMVs (25µg, N=4) from LpxL1KO mutants of strains H44/76 and NZ98/254 with over-expressed fHbp v.1 or 2, respectively, or received aluminum hydroxide alone (negative control, N=6). Two doses were administered separated by one month with a booster dose given four months later. Bactericidal titers (human complement) were measured in sera obtained two weeks after dose-3.

Results: With human PBMCs, the NOMV elicited similar cyokines as elicited by conventional detergent-treated OMV. In monkeys, the NOMV vaccine elicited titers ≥1:4 against all 10 strains tested, including 9 with heterologous PorA to the NOMVs. GMTs were 67-121 for fHbp variant 1 strains, 15-188 for variant 2 strains, and 16-128 for variant 3 strains. All negative control animals had titers <1:4.

Conclusion: The NOMV vaccine from mutants elicited broad protective SBA responses in a non-human infant primate model likely to be more relevant than mice for predicting human responses.

P216

AFPL1 as adjuvant to an allergy vaccine

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Background and Aims: Allergic diseases are caused by altered Th2 immune response, where IgE is the main antibody involved. Consequently, vaccine strategies are developing to circumvent this Th2 response inducing a non-pathological Th1 or Tr1 responses. The AFPL1 adjuvant (Finlay Institute) containing the Proteoliposome (PL) from Neisseria meningitidis serogroup B exerts a Th1 effect. Dermatophagoides siboney (Ds) house dust mite is a common cause of respiratory allergy in Cuba. Therefore, we sought to determine the influence of AFPL1 over Ds allergen-specific response in mice. Methods. Mice immunization experiments using AFPL1 and Ds adsorbed onto Aluminum Hydroxide (Alum) or Ds onto Alum were performed. Total and specific IgE, specific IgG and IgG subclasses, and cytokines after immunization and before and after allergen challenge were determined. Results. Significant reduction in total and anti Ds IgE, induction of specific IgG2a prominent response, IFNg and not IL-4/5 after AFPL1+Ds in comparison to Ds+Alum, were detected. The Ds challenge induced neither pulmonary inflammatory signs in lung tissues nor eosinophilia in AFPL1+Ds immunized mice, without affecting the IL-10 local production in broncho-alveolar lavage. The candidate vaccine was well tolerated without severe toxicity symptoms. Conclusion. AFPL1 is a promising adjuvant for developing therapeutic or prophylactic allergen-based vaccines.

P217

Imunization With Afco1 And Afpl1 As Adjuvants Enhanced Antibody Immune Response Againt Protein Antigens In Neonatal Mice

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Neonates have a poorly developed immune system. Hence safe and powerful adjuvants may be used to enhance the immune response in early life. Neisseria meningitidis B proteoliposome (AFPL1 when it is used as adjuvant) and its derivative-Cochleate (AFCo1) contain immunopotentiating and immunomodulating properties and delivery system capacities required for a good adjuvant. In the current study, the ability of AFPL1 and AFCo1 adjuvants to enhance the immune response against OVA and MSP1 from P. falciparum in neonatal mice was tested. One week old mice were immunized with the incorporated antigen into de adjuvants (AFPL1-OVA and AFCo1-OVA) or co-administrated with those adjuvants (AFPL1+OVA, AFCo1+OVA and AFCo1+MSP1) by intranasal route (in), 3 times, 7 days apart and anti-specific antigens systemic and mucosal antibody responses were measured by ELISA. AFCo1+MSP1 was also administered intramuscularly (im) with two doses, 14 days apart. AFPL1 and AFCo1 induced significant levels of anti OVA IgG antibodies in comparison with controls. It was higher when OVA were delivered incorporated into the adjuvant. Anti OVA secretory IgA in saliva were only found in AFPL1-OVA and AFCo1-OVA groups. The addition of AFCo1 as adjuvant enhanced MSP1-specific IgG responses following either in or im MSP1. Although it was not achieved the levels obtained in of adults mice with similar immunization. These results suggest that the use of AFCo1 and AFPL1 as adjuvant may be able to circumvent some of the limitations of neonatal antibody response in early life.

P218

Mucosal And Systemic Immune Response Against *Neisseria meningitidis* B Induced By Single Time Vaccination Strategy

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Immunization have been helping to reduce child mortality, improving maternal health and combating infectious diseases. In spite of its undisputed past success and promising future, however, immunization remains an unfinished agenda because of them inadequate coverage. Several factors have been largely responsible of a difficulty to attain immunization coverage and have been recognized as a problem of current vaccines. To bear in mind these principals problems of current vaccines, a novel protocol for vaccination named Single Time Vaccination Strategy (SinTimVaS) was developed. Using female BALB/c mice, we induce systemic and mucosal immune responses against N. meningitidis with only one parenteral and one mucosal dose at the same time, employing the Finlay Adjuvants derivate from N. meningitidis (AFPL1 and AFCo1) respectively.

P219

A novel OM antigen as a potential vaccine candidate against serogroup B meningococci

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Objective: Using a proteomics approach, we recently identified the presence of a novel protein in high abundance in the outer membrane (OM) of the meningococcus. Our objective was to investigate the potential of this protein as a vaccine candidate by preparing a recombinant protein and testing its ability to induce functional bactericidal antibodies.

Methods: The gene encoding the OM protein from our meningococcal B strain was cloned into the pRSETA system and propagated in E.coli. Soluble protein was expressed and purified by nickel column chromatography under native conditions. Recombinant protein was refolded into i) liposomes and ii) Zwittergent 3-14 micelles, with and without the adjuvant monophosphoryl lipid A (MPLA), iii) adsorbed onto aluminium hydroxide gel, and used to immunise mice. Raised antisera were tested in ELISA and western blotting for specific anti-protein responses and reactivity against our strain; immuno-fluoresence and serum bactericidal activity (SBA) assays.

Results: The novel OM protein was successfully expressed as a recombinant protein in E.coli and purified to homogeneity with high yield (74 mg/L bacterial culture). Antisera reacted with both the homologous recombinant antigen and the protein present in isolated OM in ELISA and recognised the antigen in western blot. The highest levels of SBA (50% end point titres of 1/1024) were induced by immunisation with antigen in liposomes or Zwittergent micelles, in the presence of MPLA.

Conclusions: A novel OM protein induces serum bactericidal antibodies and deserves consideration as a potential candidate for inclusion in new serogroup B meningococcal vaccines.

P220

Construction of Opa-deficient mutant strains of *Neisseria meningitidis* to evaluate a potential serogroup B meningococcal vaccine

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Background and aims: Opacity-associated adhesin (Opa) proteins are major meningococcal outer membrane proteins. A limited number of Opa variants have been associated with hyperinvasive serogroup B meningococci globally over the last 60 years, suggesting their use as a potential novel vaccine. Immunisation of mice with Opa elicited high levels of meningococcal-specific bactericidal antibodies, demonstrating proof in principle. Opa proteins are critical in meningococcal pathogenesis, mediating bacterial adherence to host cells, and modulating human immunity via interactions with T cells, B cells and neutrophils, although there are conflicting data regarding their effects. We have constructed Opa-deficient meningococci to enable these interactions to be more fully understood before Opa can be exploited as a vaccine component.

Methods: All 4 opa genes from Neisseria meningitidis strain H44/76 were amplified by PCR, cloned into the plasmid vector pBluescript, and disrupted using the antibiotic resistance genes ermC, aph, or tetA (conferring resistance to erythromycin, kanamycin or tetracycline respectively). The resultant plasmids were used to transform H44/76. Mutant bacteria were identified by growth on erythromycin, kanamycin or tetracycline-containing media, and confirmed by PCR.

Results: All possible combinations of N. meningitidis strains deficient in one, two, three, or all four opa genes were constructed by sequential transformation and homologous recombination of different disrupted opa genes.

Conclusions: These mutant strains will allow precise evaluation of bactericidal antibody responses to Opa and will be used to assess the effects of Opa on T cells, B cells and neutrophils, assisting vaccine formulation and increasing understanding of meningococcal pathogenesis.

P221

Development and assessment of pre-clinical immune responses to a heat shock protein-enriched meningococcal disease vaccine

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Objective: To develop a meningococcal disease vaccine enriched in heat shock proteins (Hsps) and to characterise the immune responses obtained in mice against a representative panel of meningococcal strains to determine if this has potential to induce cross strain protection.

Hsps are molecular chaperones that bind polypeptides, prevent aggregation and support protein folding. Hsps can also induce innate immune responses, activate dendritic cells and deliver peptides leading to antigen presentation and development of adaptive immunity. We have developed a vaccine containing a mixture of protein antigens, enriched in the highly immunogenic Hsps. Vaccines have been prepared from heat-shocked cultures of Neisseria meningitidis and N. lactamica. The bacteria are broken by homogenisation and a Hsp-enriched fraction is prepared from clarified supernatant using anion exchange chromatography.

Protection provided by meningococcal and N. lactamica Hsp-enriched vaccines was compared in a mouse model of meningococcal septicaemia with the N. lactamica vaccine providing the greater protection. Sera from mice immunised with these vaccines have been analysed for opsonophagocytosis (OPA), antibody-mediated complement component C3 and C5-9 binding and serum bactericidal activity (SBA), against a panel of meningococcal strains. Mouse serum raised against outer membrane vesicles from the homologous strain was used as a positive control. Cross strain OPA responses against the meningococcal panel were obtained with the Hsp-enriched vaccines and these were greater than the positive control for 5/7 of the strains, with the N. lactamica vaccine producing the greatest responses. Greater antibody-mediated complement binding and SBA were also seen with the N. lactamica vaccine.

P222

High throughput flow cytometric opsonophagocytosis and antibody-mediated membrane attack complex assays for the assessment of meningococcal vaccines

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Objective: To develop high throughput assays to characterise antibody-mediated immunity provided by meningococcal vaccines and to correlate these with the functional killing assays.

Serum bactericidal activity (SBA) has been established as a correlate of protection for meningococcal vaccines. However, there is increasing evidence from vaccine trials, seroepidemiology studies, animal infection models and in vitro experiments, that SBA is not the only mechanism of protection. We have developed a high throughput opsonophagocytosis (OPA) and a duplexed antibody-mediated complement deposition assay that determines both C3c and C5b-C9 deposition. This study examines the correlation of the responses from these high throughput assays with more labour-intensive viable count SBA and OPA assays. Complement source is key to these assays. Thus we have developed a functional IgG-depleted human complement source, which can be used in all immunological assays against any meningococcal strain.

Using a panel of human and laboratory animal sera, good correlations were observed ($R=0.78$, $p<0.01$) between a high-throughput flow cytometry-based OPA performed using fixed N. meningitidis, IgG-depleted human plasma and DMF-differentiated HL60 granulocytic cells , and an opsonic killing assay using C7-depleted complement. Results obtained in a high throughput antibody-mediated complement deposition assay using fixed meningococci, IgG-depleted human plasma, and fluorescent anti-C3c and C5-C9 antibodies demonstrated good correlations with both a standard SBA ($R=0.87$, $p<0.01$) and the high-throughput OPA ($R=0.92$, $p<0.01$). These results show that these high-throughput assays, performed using azide-fixed meningococci, using small serum volumes are suitable for large-scale clinical serology testing against an extended strain panel.

P223

Development and validation of an ELISA assay specific for human antibodies against serogroup X meningococcal polysaccharide

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Serogroup X N. meningitidis has recently emerged as a cause of localized disease outbreaks in sub-Saharan Africa. In order to prepare for vaccine development we aimed to purify and characterize the serogroup X polysaccharide (MenX PS) and develop a specific ELISA assay. MenX PS was purified from MenX isolates by Cetavlon precipitation, phenol/ethanol extraction and size exclusion chromatography (SEC). Structure and purity of the MenX PS was analysed by silver staining of gel electrophoresis separated sample, and by 1H, 31P, total correlation spectroscopy (TOCSY) and 1H-13C heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectroscopy analyses. Molecular size distribution was assessed using HPLC-SEC. NMR analyses verified the structure as identical to that reported previously, and no O-acetylation was observed. The resultant MenX PS was of comparable purity and of the same high molecular weight as standard preparations of meningococcal polysaccharides. Poor specificity was found when adapting the standard methylated human serum albumin-based meningococcal polysaccharide ELISA to MenX PS. A novel ELISA assay for quantification of human anti-MenX PS antibodies based on covalent linkage of the MenX PS to the microtiter plates was therefore developed and validated. The repeatability and intermediate precision were well within the pass criterion of <20% for coefficients of variation (CV), and the failure rate was <4% for inter- and intradilution variation. The ELISA assay was also shown to be specific, as demonstrated by inhibition with 100µg/ml MenX PS,. This novel ELISA assay may prove useful for assessing polysaccharide based MenX PS vaccines.

P224

Preclinical immunogenicity of polysaccharide and outer membrane vesicles derived from serogroup X *N. meningitidis*

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Serogroup X Neisseria meningitidis is an emerging cause of meningitis in Sub-Saharan Africa, and there is no vaccine against this organism. We studied the immunogenicity of polysaccharide and outer membrane vesicles (OMVs) from serogroup X (MenX) ST-181 isolates from Burkina Faso. MenX polysaccharide was purified by established methods using detergent precipitation, phenol extraction and size exclusion chromatography. Detergent extracted OMVs (dOMVs) were prepared from the wild-type MenX isolate BF7/07 and serogroup B isolate 44/76, whereas native OMVs (nOMVs) were prepared from isolate BF12/03 modified to express an LPS with a penta-acylated lipid A. The OMVs were characterised by gel separation and electron microscopy. NIH mice were immunised with 2 doses of polysaccharide or a mixture of polysaccharide and OMVs, using aluminium hydroxide as adjuvant in all groups. Evaluation of immune responses in ELISA showed that nOMVs alone and polysaccharide mixed with nOMVs both elicited a significant polysaccharide-specific IgG response, indicating that the nOMVs also contained polysaccharide. The polysaccharide did not induce detectable levels of IgG when administered alone, with aluminium hydroxide or when combined with MenX or serogroup B dOMVs. In an nOMV ELISA, the nOMV group induced high levels of IgG, in contrast to the dOMVs from strain BF7/07 which were poorly immunogenic. Surprisingly, the antibodies were not directed against PorA P1.5-1, 10-1 by immunoblotting. A polysaccharide-nOMV based vaccine was immunogenic in mice and has the potential to prevent MenX disease.

P225

Characterization of factor H binding protein (fHbp) subvariants for surface exposure and ability to bind factor H (fH), mediate serum resistance and induce bactericidal antibodies

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Commonly found fHbp subvariants (using the 3-variant classification scheme) from a worldwide strain collection were characterised for their level of surface exposure and ability to bind fH, mediate serum resistance and induce bactericidal antibodies. For serum bactericidal assays, sera from mice immunised with each subvariant were tested against strains carrying homologous or heterologous subvariants. Cross-reactive bactericidal activity was seen within each variant group, although the degree of activity varied suggesting that amino acid differences within each variant have an influence on bactericidal activity. For each subvariant, expression, surface exposure, and ability to bind fH was evaluated by flow cytometry using live bacteria, as well as by far-western and biacore analysis with purified proteins. In an analysis of natural strains, all subvariants could bind factor H; differences in binding capability were not correlated with surface expression level, but may correlate with affinity for fH. To overcome the issue of variable fHbp expression between strains, we genetically engineered strain MC58 to express different subvariants from a constitutive heterologous promoter, and characterized these recombinant strains for fH binding and survival in human sera. This data confirmed that the subvariants bind different levels of fH and that difference in residues within a variant group can influence fH binding. However, survival in human sera does not directly correlate with the level of fH binding, suggesting that the amount of fH bound is not the only factor influencing serum survival. Further analysis is ongoing to clarify these mechanisms and identify other residues important for fH binding.

P226

Diversity of Neisserial Heparin Binding Antigen in US Carriage Isolates

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Background: The neisserial heparin binding antigen (NhbA) is a component of a multivalent vaccine undergoing Phase III clinical trials for prevention of serogroup B meningococcal disease. The effect of NhbA vaccination on meningococcal carriage is unknown.

Objective: To define the diversity of NhbA among 214 US carriage isolates.

Methods: The NhbA amino acid sequence was determined by genetic sequence analysis of 214 carriage isolates obtained from high school students in Georgia and Maryland in 2006-2007. Meningococcal genetic lineage was determined by multi-locus sequence typing.

Results: A total of 40 amino acid sequence alleles encoding 36 NhbA proteins were identified among 214 US carriage isolates. Proteins ranged in size from 419 – 499 amino acids. The majority of the carriage isolates (148/214, 69.2%) harbored NhbA sequences previously described. The most prevalent NhbA sequence (54/214, 25%) corresponded to NhbA from the E26 serogroup X reference strain and was primarily associated with isolates belonging to clonal complex 198 (42/54, 77.7%). There were 22 previously unreported NhbA sequences identified. Insertion sequences were detected downstream of nhbA in 8/214, (3.7%) carriage isolates. Sequences showing similarity to IS1655 were found in the majority of IS containing isolates (7/8, 88%). Genetic lineage was associated with the NhbA protein.

Conclusions: The distribution of NhbA sequences among carriage isolates from GA and MD in 2006-2007 was diverse. No single protein sequence predominated. The NhbA sequence was associated with meningococcal genetic lineage. Further investigation is required to determine the potential effect of NhbA vaccination on meningococcal carriage.

P227

Scale-up of process for GMP production of a serogroup A and W135 meningococcal outer membrane vesicle vaccine for Africa

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Epidemics of meningococcal disease in the African meningitis belt are mainly caused by serogroup A and occasionally by serogroup W135 strains. Affordable vaccines providing long-term protection in all age groups are highly needed. Antibodies against the non-capsular antigens are able to exert bactericidal activity. Previous vaccines based on serogroup B outer membrane vesicles (OMV) have demonstrated to be safe and efficacious. In order to establish methods for GMP production and formulation of a vaccine based on OMVs from serogroup A and W135 meningococci, two candidate wild-type serogroup A (ST-7) and one W135 (ST-11) strain were cultivated in modified Frantz growth medium. The production process was scaled up to 100 L cultivation volume in a fermentor. OMVs were extracted by use of deoxycholate and purified by ultracentifugation and gel filtration. At the end of the purification process, OMVs were either precipitated with alcohol, or diafiltrated against sucrose solution. The OMVs from the A and W135 strains were adsorbed to aluminum hydroxide, mixed (1:1) and and used for characterization, quality control and immunization of mice. The resulting OMVs contained the major relevant proteins (e.g. PorA, NadA, Opc, FetA). The LOS content and endotoxin level were both within the accepted ranges for OMV vaccines. The vaccine elicited prominent immune responses in mice. The scale-up of the production of the serogroup A+W135 OMV vaccine was successful. These results showed that a combined OMV vaccine could probably be an affordable alternative or supplement to the conjugate approach in countries with mixed A and W135 epidemics.

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Sequence Diversity of Factor-H Binding Protein in US Carriage Isolates

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Background: Neisseria meningitidis serogroup B vaccines in development target factor H-binding protein (FHbp), an immunogenic, surface protein. Three FHbp variants (1, 2 or 3) or 2 subfamilies (A or B) have been described. The effect of FHbp vaccination on meningococcal carriage is unknown.

Objective: To define the diversity of FHbp among 408 US carriage isolates.

Methods: Carriage isolates (n=408) obtained from high school students in Georgia and Maryland from 2 time periods (1998 and 2006-2007) were subjected to fHbp sequence analysis and multi-locus sequence typing. FHbp alleles were determined by querying the database at pubmlst.org/neisseria/. Data were compared with ST and FHbp of invasive isolates. Association of FHbp with genetic lineage was determined using the asymmetric Goodman-Kruskal Lambda statistic.

Results: Of 30 FHbp alleles identified, 14 were unique to carriage. The FHbp variant 2/subfamily A was predominant (270/408, 66.2%) due to increased ST23 carriage in Georgia in 1998 and the association of allele 25 with ST23. Genetic lineage was associated with FHbp alleles. Prediction of FHbp alleles given the ST was 91%, although evidence of fHbp recombination was also observed. FHbp allele 94 was unique to carriage and comprised the majority of variant 3 FHbp (22/25, 88.0%). The proportion of FHbp allele 1 (vaccine candidate) was <1% among carriage isolates.

Conclusions: The FHbp alleles in carriage isolates from Georgia and Maryland were predominantly variant 2/subfamily A. Further investigations are necessary to determine the potential effect of FHbp-based vaccines on meningococcal carriage.

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Characterization and immunogenicity evaluation of serogroup A and W135 outer membrane vesicles derived from growth in modified Frantz and Catlin medium

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Serogroup A and W135 meningococcal disease causes a high burden of disease in many African countries. Plain polysaccharide vaccines are available, but they are poorly effective in young children, and polyvalent conjugate vaccines are considered to be too expensive. Outer membrane vesicle (OMV) vaccines from serogroup B meningocci have proven to be safe and efficacious in various epidemic situations. Two "wild–type" A:4/21:P1.20,9 (ST-7) and one W135:2a:P1.5,2 (ST-11) strain from recent epidemics in Africa were cultured in either Frantz or Catlin synthetic media, and the yield, protein expression and immunogenicity of produced OMVs were compared. The OMVs were characterized by SDS-PAGE and by immununoblots with monoclonal antibodies. Balb/C mice were immunized with either two doses of 0.5, 2 or 10 µg of OMV proteins, or with one dose of 50 µg. Immune responses were tested by ELISA and in serum bactericidal assay (SBA). There were no significant differences in the protein profile of OMVs from the two culture media and all relevant proteins were present in the vesicles. One of the two A strains showed higher levels of NadA than the other one, and was preferred. High levels of IgG antibodies against OMVs from all three strains were detected in ELISA. OMVs derived from both serogroup A strains produced in Frantz medium induced similar high SBA titers. Culture medium was not of major importance for protein content or immunogenicity. An A+W-135 OMV combination seems to be a feasible way forward for production of an affordable meningococcal vaccine for Africa

P230

Single aminoacidic mutations in meningococcal antigen fHbp have sizeable and predictable impact on its immunogenicity

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Serum bactericidal assay using rabbit complement (rSBA) was used to evaluate 83 meningococcal strains carrying 30 different fHbp peptides in sera from mice immunized with GNA2091-fHbp fusion protein (variant 1 peptide 1, 1.1) present in the 4CMenB vaccine. Strains with variant 2 or 3 fHbp resisted rSBA killing; variant 1 strains were killed in rSBA, with a wide range of titers. Weak correlations were observed for non-1.1 variant 1 strains between number of amino acid (aa) mismatches vs. 1.1 fHbp and reduction in SBA titers (R^2=0.12, p-val=0.03) vs. H44/76 strain (reference for 1.1).

To identify aa associated with killing, and to develop a sequence-based predictor of fHbp-mediated killing, we correlated sequence variation and rSBA titers, disregarding the influence on killing of fHbp expression levels. Multiple sequence alignment of fHbp protein sequences showed 50 distinct aa groups. Minimization of the linear correlation system converged to statistically supported solutions, and identified 15 aa groups whose variation is significant for the cross-protection when immunizing with the fHbp-1.1.

Validation by sequentially removing fHbp peptides showed that, within the genetic variability investigated, significant sequence variation predicts rSBA with the average accuracy of ±1.2 dilutions, comparable to the experimental accuracy of the assay (R^2=0.92, p-val<10^-4). However, validation by testing of mutated forms of fHbp in the H44/76 genetic background supported the results of the model only in part.

The approach successfully identified aa associated with killing, and indicated that in order to accurately predict fHbp-mediated killing, variability of expression levels should also be considered.

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Structure based epitope design as a means of engineering novel vaccine immunogens

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We employed a novel, structure-based epitope design to engineer a possible vaccine immunogen. As factor H binding protein (fHbp), a protein antigen under study in vaccines against meningococcal serogroup B disease, can be divided into 3 different and non-cross reactive antigenic variants, it was determined to be a good candidate for examination. Structural analysis showed that epitopes of variants 1 and 2 do not overlap, suggesting that variant 2 epitopes could be grafted onto a variant 1 backbone to provide additive immunogenic effects. We divided the surface of fHbp into 10 partially overlapping areas corresponding to the surface usually covered by an antibody. Then, 54 different antigenic variants were designed and expressed, in which the non-contiguous amino acids residing in one of the ten overlapping areas were systematically mutated to introduce variant 2 and 3 specificities.

Seventeen of 54 engineered molecules induced bactericidal antibodies against the prototypic strains of all 3 variants. Of these, 8 promising candidates were purified on large scale, their folding was characterized by Nuclear Magnetic Resonance, and their ability to induce protective antibodies was tested against a panel of 15 meningococcal serogroup B strains representative of the main antigenic variants and sub-variants. One molecule induced high levels of protective antibodies against all strains tested. Our structure-based epitope design can likely be used to engineer novel immunogens and may represent a significant advance in vaccinology.

P232

Nasal immunization with AFCo1 induces immune response to *N. gonorrhoea* in mice

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Neisseria gonorrhoeae infections are common sexually transmitted diseases. Increased antibiotic-resistant of N. gonorrhoeae strains were reported. N. meningitides is another human restricted bacterium transmitted through mucosa. However, the induction of systemic specific IgG antibody against some proteins between the two species is known, but the mucosal immune response to these pathogens is not clear. We hypothesized that N. meningitides could induce immune response against N. gonorrhoeae. Therefore, serogroup B Proteoliposome (PL) was transformed into AFCo1 (Adjuvant Finlay Cochleate 1) and used for nasal immunization of C57Bl/6 mice. In parallel, PL absorbed into Al(OH)3 was used by intramuscular route. The specific IgG and IgG subclasses against both antigens in sera and vaginal extraction were measured by ELISA. Specific proliferation (3H incorporation) of spleen cells recall in vitro with PL or N. gonorrhoeae total antigens was measured. The anti PL human saliva IgA and serum IgG antibody response were evaluated against N. gonorrhoeae antigens by ELISA. Serum and vaginal anti N. meningitidis and N. gonorrhoeae IgG as well as the induction of IgG subclasses were detected. N. gonorrhoeae induces specific proliferation of spleen cells from immunized mice. The recognition of N. gonorrhoeae antigens by specific saliva IgA and serum IgG in human was also detected. In conclusion, PL and AFCo1 induce anti N. meningitidis and anti N. gonorrheae immune responses in mice.

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Passive Transfer of Monoclonal Antibody Directed to an *N. gonorrhoeae* Conserved LOS-derived Carbohydrate Protects Mice Against Gonococcal Challenge

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A lipooligosaccharide (LOS)–derived carbohydrate epitope present on ~95% of clinical isolates of N. gonorrhoeae is recognized by monoclonal antibody (mAb) 2C7. mAb2C7 is bactericidal, opsonic and is elicited in response to human infection. mAb2C7 overcomes the complement-inhibitory effects of human factor H (fH) and C4b-binding protein (C4BP) that bind to most serum-resistant N. gonorrhoeae. Sialylation of gonococci diminishes but does not eliminate killing by mAb2C7. We immunized estrogen treated Balb/c mice with 10 µg of mAb 2C7 (controls received normal saline) intraperitoneally (ip) daily for 4 days. Serum and vaginal washings contained 0.490 +/- 0.011 µg/ml and 0.008 +/- 0.001 µg/ml of mAb 2C7, respectively. Each group (n=4) was inoculated intravaginally with N. gonorrhoeae strain FA1090 (105 CFU) or 15253 (106 CFU). The average duration of gonococcal vaginal colonization of 2C7-immunized mice was 3 days (range 0-5 days) with strain FA1090 and 4.75 days (range 0-11 days) with strain 15253. Control mice harbored each of the gonococcal strains respectively for 6.5 days (range 1-11 days) and 8.75 days (0-12) days. Trends in mean colonization over time were significantly different between immunized and control groups (p=0.04) and “area under the curve analysis" showed a significant difference between the passively immunized groups combined, compared to controls (p=0.04). These results indicate that systemically (ip) administered antibodies reach the lower genital tract and that 2C7 antibody protects mice against experimental challenge with N. gonorrhoeae.

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Phenotyping a subset of US invasive *Neisseria meningitidis* group B (MenB) isolates for antigens included in a novel MenB vaccine

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A candidate MenB vaccine (4CMenB, Novartis) contains 4 major components: an outer membrane vesicle (OMV) and 3 proteins selected by genome screening; factor H binding protein (fhbp), Neisserial adhesin A (NadA) and Neisserial heparin binding antigen (Nhba). An ELISA based meningococcal antigen typing system (MATS ELISA) was used to quantify surface-exposed protein (phenotype) of 112 US clinical isolates selected from Active Bacterial Core surveillance (ABCs, CDC, Atlanta) MenB culture collection. The amount of each protein expressed on standard strains, as measured by MATS, was used to determine the relative potency or positivity for test isolates. Isolates with relative potency of over 2%, 3% and 30% for fhbp, NadA and Nhba respectively were considered positive. Among the MenB test isolates, 69 % (77/112) had a positive phenotype for one or more of the 4CMenB vaccine proteins: 29.5% (33/112) were fhbp positive, 18% (20/112) Nhba positive and 21% (24/112) were positive for both fhbp and NHBA. Nad A was found in low levels on 14% (16/112) of the MenB isolates. Approximately 5% of strains expressed PorA 1.4 (OMV) protein. MATS phenotyping of >700 US isolates for 4CMenB vaccine proteins is underway. While in vitro bactericidal activity assesses the vaccine-induced protection, MATS phenotype analysis may be used to explore the strain coverage for this vaccine.

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Elicit Broadly Cross-Reactive, Bactericidal Antibodies against *Neisseria gonorrhoeae*

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Gonococcal porin is expressed from either the porB1A or porB1B allele and is a promising vaccine target due to its stable expression and surface abundance. The development of a porin vaccine is challenged by sequence differences in surface-exposed loops. We previously reported that long cyclic peptides corresponding to the surface-exposed P1A loops of the Neisseria gonorrhoeae (Gc) strain FA19 induced surface-binding antibodies. Here we report that mouse antisera against cyclic peptides P1A1, P1A3, P1A6, P1A7 and P1A8 bound strains of several different P1A variable region (VR) types. P1A2- and P1A5-specific antisera recognized the homologous porin and some heterologous porins. Antisera against P1A1, P1A3, P1A6, P1A7 and P1A8 were bactericidal against a serum sensitive P1A strain. Interestingly, P1A2- and P1A5-specific antisera did not bind the Gc surface, but were bactericidal against the serum resistant homologous strain. Of four different P1B loop-specific antisera tested, only anti-P1B5 was broadly reactive against strains of different P1B VR types. P1B5 antiserum was bactericidal against one of four P1B strains tested. Anti-P1A8 antisera bound both strain FA1090 (P1B) and FA19 (P1A), and therefore, targeting the P1A8 loop may be effective against P1B strains. Our results support the use of cyclic peptides to preserve the native conformation of porin loops. Cyclic peptides against P1A loops 1, 3, 5, 8 and P1B loop 5 are the most promising in terms of broad reactivity and bactericidal activity. Studies to measure the capacity of porin loop-specific antibodies to inhibit porin-mediated functions and prevent experimental gonococcal infection are underway.

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Humanized Mouse Models of Gonococcal Infections

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Neisseria gonorrhoeae, the causative agent of gonorrhea, is strictly a human pathogen and their association with host cells in vitro are largely human-specific. While a mouse model of gonococcal infection has been established, it cannot consider the contribution of virulence mechanisms involving the specific engagement of proteins of human origin. Herein, we compare infection of wild type and transgenic mice expressing various combinations of human CEACAM1, CEACAM3, CEACAM5 and/or CEACAM6, each of which can function as receptors for different neisserial Opa protein variants. The human CEACAMs are each expressed with a distribution pattern reflecting that in humans, and their capacity to bind and engulf Opa-expressing gonococci is retained in mouse cells. A significant increase in gonococci colonizing the vagina was apparent with the transgenic animals, with bacteria effectively penetrating into the subepithelial spaces. Consistent with the fact that the neutrophil-restricted CEACAM3 functions as a decoy receptor that allows the effective capture and killing of Opa-expressing gonococci, neutrophil depletion caused a substantial increase in gonococci colonizing the urogenital tract of transgenic animals. CEACAM3 expression also correlated with a substantial increase in the number of neutrophils recruited to the site of gonococcal inoculation. Combined, these results establish that epithelial cell-expressed CEACAMs facilitate N. gonorrhoeae colonization of the urogenital tract, while the decoy receptor CEACAM3 promotes both bacterial clearance and amplification of the neutrophil's pro-inflammatory response.

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Effectiveness of a bivalent factor H binding protein vaccine across *Neisseria meningitidis* serogroups

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Objective: A bivalent recombinant lipidated N. meningtidis serogroup B (MnB) factor H binding potein (fHBP) vaccine candidate (rLP2086) is under clinical evaluation for prevention of MnB disease. Preclinical and clinical studies of the candidate vaccine demonstrated bactericidal antibodies against diverse invasive MnB isolates following immunization. In this study we assessed the potential effectiveness of this vaccine against N. meningitidis serogroups C, Y and W135.

Methods: fhbp genes were sequenced from 274 meningococcal invasive strains collected in 2000-2001 through Active Bacterial Core Surveillance program. The surface expression level of fHBP and capsule were analyzed by flow cytometry. Immune sera generated after immunization with the rLP2086 vaccine candidate were tested for bactericidal activity against diverse N. meningitidis Mn C, Y and W135 isolates.

Results and Conclusions: All meningococcal isolates tested contained fhbp genes irrespective of serogroup. fhbp with a single nucleotide deletion were observed in nine serogroup C isolates. All fHBP sequence variants segregated into two subfamilies, non-MnB serogroup isolates showed a higher proportion of subfamiliy A variants (50% and 98% for MnC and MnY, respectively) compared to MnB (30%). No correlation between the levels of capsule and fHBP expression was observed. In general, fewer non-MnB isolates expressed fHBP in vitro (69%) than was observed for MnB, (98%). rLP2086 vaccine immune sera killed strains in a serum bactericidal assay (SBA) regardless of serogroup or fHBP variant. These findings suggest the value of testing fHBP containing vaccines against multiple N. meningitidis serogroups in addition to MnB.

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The Global Meningococcal Initiative. A new worldwide expert group to raise awareness and help prevent invasive meningococcal disease

Ray Borrow, Stanley Plotkin

Global Meningococcal Initiative

The Global Meningococcal Initiative (GMI) is a new international expert group of scientists and clinicians, chaired by Stanley Plotkin. Collectively, these individuals have expertise in meningococcal clinical practice, epidemiology, immunology, public health, vaccinology, manufacturing, microbiology, and heath economics. The GMI has been formed with the intention to help prevent invasive meningococcal disease worldwide through education, research, and cooperation. Given the geographic variability seen with this disease, the GMI will address these issues at both regional and global levels. The GMI held their first summit meeting in Barcelona, Spain, on June 18-19, 2010, attended by 25 experts in the field. The scope of the meeting was global, discussing all geographic regions and meningococcal serogroups. Topics of discussion included: worldwide epidemiology, including the African meningitis belt, Asia, and the Hajj; introduction of novel vaccines, including MenAfriVac; diagnostic and case confirmation best practice and advice to low-resource regions; variations in regional vaccination strategies across the world. The meeting concluded with discussion on novel prevention strategies where the importance of serogroup C conjugate vaccine catch-up campaigns, in developed countries, was highlighted in controlling disease through herd immunity. It became apparent that uncertainty existed concerning the choice between monovalent serogroup and polyvalent serogroup conjugate vaccines, and that data on meningococcal incidence in Asia are sparse. The GMI is supported by an unrestricted grant from sanofi pasteur.

sparse. The GMI is supported by an unrestricted grant from sanofi pasteur.

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