Poster Abstracts

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P1

A preliminary molecular assay for detection of antimicrobial resistant *Neisseria gonorrhoeae*

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Background: The incidence of antimicrobial resistant *Neisseria gonorrhoeae* continues to rise in Canada, however, there is no antimicrobial resistance data for the approximately 70% of gonorrhea infections that are diagnosed directly from clinical specimens by Nucleic Acid Amplification Testing (NAAT) in Canada each year. We aimed to develop a molecular method that can be applied to both cultures and NAAT specimens to monitor antimicrobial resistance in *N. gonorrhoeae*.

Method: Real-time qPCR assays were developed to detect single nucleotide polymorphisms (SNPs) in seven loci that are associated with cephalosporin, azithromycin or ciprofloxacin resistance (*ponA, mtrR, penA, gyrA S91, gyrA* D95 *parC* and *porB*) and one gene that is specific for *N. gonorrhoeae* (*porA*). The DNA sequence was used as the gold standard to evaluate the performance of the assay in detection of SNPs. We tested the RT-PCR assay with cultures of 254 strains of *N. gonorrhoeae* in triplicate and tested cross-reactivity against 47 non-gonococcal species of bacteria. The qPCR assay was then applied to ten clinical specimens that were previously used for diagnosis by NAAT.

Results: For 254 strains of *N. gonorrhoeae* tested in triplicate, the agreement between the DNA sequence information and qPCR was 100% for *porA, ponA, gyrA* S91, *parC* and *penA*; 99.6% for *mtrR* and *gyrA* D95; and 95.3% for *porB*. The specificity for *N. gonorrhoeae*, compared to 47 non-gonococcal strains, was 100% for *porA*, 93.6% for *ponA*, 97.9% for ***mtrR***, 95.7% for *porB*, 91.5% for *gyrA* S91 and *gyrA* D95, 63.8% for *parC* and 76.6% for *penA*. For 8 of the 10 NAAT specimens, we successfully detected the SNP and determined the *N. gonorrhoeae* multi antigen sequence type (NG-MAST) of each strain.

Conclusions: We demonstrated the utility of a qPCR assay for sensitive detection of known antimicrobial resistance markers. Preliminary results with NAAT specimens were encouraging. Our goal is to use this assay with NAAT specimens in our national surveillance program of antimicrobial resistant *N. gonorrhoeae*.

P2

Distribution of antibiotic resistance genes in a global *Neisseria* genome collection

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Although antibiotic resistance in the meningococcus is not as widespread a problem as it is for *N. gonorrhoeae*, the possibility of increased resistance is always a concern and is therefore worthy of close monitoring. Specific mutations in four genes have been shown to be associated with increased resistance to specific antibiotics: *rpoB* (rifampicin), *penA* (penicillin G), *gyrA* (ciprofloxacin)and *folP* (sulphonamide). These genes were analysed in a collection of publically accessible *Neisseria* species genomes on pubMLST.org. There were 1505 isolates in the collection analysed and comprised 22 species of which 73.1% were *N. meningitidis*, 13.8% *N. gonorrhoeae* and 7.4% *N. lactamica*. The time period spanned by the collection was 1937-2013 and included isolates from six continents and 50 countries. Analyses were carried out using the embedded database tools. The temporal and geographic distribution of resistance associated alleles was analysed along with their presence amongst other *Neisseria* species.

P3

Fluoroquinolone resistance-conferring gyrA91/95 mutations provide enhanced in vivo fitness to *Neisseria gonorrhoeae* strain MS11 by increasing resistance to cationic antimicrobial peptides

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The impact of antibiotic resistance mutations on microbial fitness is an important aspect of microbial pathogenesis and may predict the spread of resistance. We recently reported that fluoroquinolone-resistance conferring gyrA91/95 mutations increase the fitness of *Neisseria gonorrhoeae* (Gc) strain FA19 during experimental genital tract infection of female BALB/c mice. We have since demonstrated that a *gyrA*91/95-mediated fitness advantage occurs in two other Gc strains, FA1090 and MS11, and at a 10-fold higher level than that which occurs in strain FA19. Here we tested the *gyrA*91/95 mutants of each strain for differences in sensitivity to H2O2 and cationic antimicrobial peptides (CAMPs) compared to the corresponding wild-type parent strains. Only JD4, the *gyrA*91/95 mutant of strain MS11, showed increased resistance to these antimicrobial substances in vitro. Exposure of JD4 to 3% H2O2 in a disc diffusion assay resulted in a significantly greater zone of inhibition compared to MS11 (7.2 mm versus 5 mm, respectively, $p < 0.04$). JD4 was also signficantly more resistant to the polymyxin B, and the murine cathelicidin-related antimicrobial peptide (CRAMP), the latter of which exhibited a > 2-fold higher ED50 against JD4 compared to strain MS11. To test whether the increased in vivo fitness of JD4 is due to better evasion of host innate effectors, we performed competitive infections with wild-type MS11 versus JD4 bacteria in normal and CRAMP-deficient (cnlp-/-) BALB/c mice. As expected, JD4 out-competed wild-type Gc in normal BALB/c mice. Importantly, however, this fitness advantage was not observed in cnlp-/- BALB/c mice. The basis of the increased CAMP resistance in JD4 is not yet known and is interesting in that strain MS11, which is more infectious in humans and mice, is already inherently more resistant to CAMPs due to over-expression of the MtrC-MtrD-MtrE active efflux pump. We conclude that the mechanism(s) by which clinically relevant *gyrA*91/95 mutations confer increased fitness to Gc are strain-specific, and in some strains, may be due to increased resistance to host cathelicidins. These findings also demonstrate that resistance mutations can further increase the in vivo survival of an already fitness-enhanced strain, and thus underscore the importance of research in this area.

P4

Structural effect of the Asp345a insertion in penicillin-binding protein 2 from penicillin-resistant strains of *N. gonorrhoeae*

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Sexually transmitted infections caused by *Neisseria gonorrhoeae* are becoming increasingly difficult to treat due to dissemination of strains exhibiting resistance to most antibiotics used clinically. A major mechanism of resistance to β-lactam antibiotics in *N. gonorrhoeae* is mutations in penicillin-binding protein 2 (PBP2). PBP2 is an essential transpeptidase that catalyzes the formation of peptide cross-links during peptidoglycan synthesis. A hallmark of PBP2 from penicillin-resistant strains of *N. gonorrhoeae* is insertion of an aspartate after position 345. Although the kinetic consequences of this mutation are well established, how it impacts the structure of PBP2 is unknown.

We have solved the crystal structure of a truncated construct of PBP2 containing all five mutations present in PBP2 from the penicillin-resistant strain 6140 to a resolution of 2.2Å. The Asp insertion resides on a loop near the active site and is immediately adjacent to an existing aspartate (Asp346) that forms a crucial hydrogen bond with Ser363 of the conserved SxN active-site motif. Because the Asp insertion is adjacent to Asp346, there is ambiguity as to whether the insertion is after position 345 or 346. Our previous studies have shown that only Asp can be inserted either before or after Asp346 without abrogation of transpeptidase function and, in keeping with this strict specificity, the insertion does not cause disordering of the structure, but rather induces localized flexibility. The crystal structure resolves the ambiguity as to whether the insertion is Asp345a or Asp346a, because the hydrogen bond between Asp346 and Ser363 is preserved and the insertion is therefore Asp346a. The side chain of Asp346a projects toward the active site, where it hydrogen bonds to Asn364 of the SxN active site motif and likely impedes binding of antibiotic or breakage of the β–lactam ring during acylation.

P5

Genomics of antibiotic resistance emergence within *Nesseria gonorrhoeae*

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The goal of this project is to understand the evolutionary mechanisms driving antibiotic resistance in *Neisseria gonorrhoeae*, using whole genome sequencing (WGS) analysis. *Neisseria gonorrhoeae* is the causative pathogen of the global sexually transmitted disease gonorrhea. This infection has emerged as a public health threat because of increasing resistance of the pathogen to known antibiotics and a dearth of new antibiotics to treat the condition.

There have also been a number of studies that have helped to elucidate the underlying molecular mechanisms driving the resistance of this pathogen to the major classes of antibiotic drugs used to treat the condition. While past studies on this pathogen population in specific geographic regions have focused more on a number of representative genes or regions of the genome of *N. gonorrhoeae*, no extensive studies has been done on a collection of strains from different geographical locations across the globe. Our approach of genome wide analysis of multiple strains of the pathogen across the globe will provide both the depth in terms of more genomic regions covered and the breadth of all the possible sub-populations impacting the evolution of antibiotic resistance within the species.

P6

Establishment of a *Neisseria gonorrhoeae* reference lab & repository for understanding the spread of antimicrobial resistant gonorrhea in the U.S. military

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Sexually transmitted infections (STIs) rank high in incidence in the U.S. military. Gonorrhea is particularly concerning due to its impact on military readiness, reproductive health, the health and well-being of neonates, and its role as a cofactor in the spread of human immunodeficiency virus. Moreover, control measures are complicated by the rapid emergence of antibiotic resistant strains and the increasing resistance of *Neisseria gonorrhoeae* to the extended-spectrum cephalosporins has led to the potential threat of untreatable gonorrhea. Gonococcal resistance usually first emerges in Asia, but levels of resistance in other parts of the world where the military are deployed are not well documented. The Infectious Disease Clinical Research Program (IDCRP) has established a network of military sites in the continental United States (CONUS) to conduct research and surveillance of resistant *N. gonorrhoeae* and has partnered with overseas (OCONUS) labs conducting similar studies. Here we describe the establishment of a DoD-specific reference laboratory and GC repository at the Uniformed Services University (USU) in Bethesda, Maryland. Isolates sent from CONUS and OCUNUS sites will be processed for identification and/or confirmation of *N. gonorrhoeae* based on growth of typical appearing colonies on non-selective (Chocolate agar) and selective (Thayer-Martin) media and standard biochemical, enzymatic, and serological tests. All isolates will undergo susceptibility testing using E-test strips to determine the minimal inhibitory concentrations of selected antibiotics and the nitrocefin test to detect β-lactamase production. High resistance isolates will be genotypically characterized by *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) and by sequencing of resistance determinants. GC strains from within this repository will be made available to researchers within and outside of the DoD for translational research in the areas of improved diagnostics, therapeutics and vaccine development. This repository will also be a valuable resource for future studies on STI transmission networks and of the spread of antimicrobial resistant gonorrhea.

P7

Antimicrobial susceptibilities of *Neisseria gonorrhoeae* in Nanjing, China, 2013

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Background: *Neisseria gonorrhoeae* has developed widespread resistance to antimicrobials including extended-spectrum cephalosporins (ESCs), the most widely used agents for treatment of gonorrhea. Monitoring antimicrobial resistance (AMR) of *N. gonorrhoeae* is crucial for guiding effective treatment. We report the result of amtimicrobial susceptibility testing of 187 gonococcal strains isolated in 2013.

Methods: 187 *N. gonorrhoeae* isolates were isolated from male adults with symptoms/signs of urethritis attending an STD clinic in Nanjing, China in 2013. The MICs for penicillin, tetracycline, ciprofloxacin, azithromycin, spectinomycin, cefixime and ceftriaxone were determined by the agar dilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI). Penicillinase-producing *N. gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG) were examined and typed for β-lactamase and tetM encoding plasmids respectively.

Results: All 187 *N. gonorrhoeae* isolates were resistant to ciprofloxacin; 37%(69/187) to azithromycin. All isolates were susceptible to ceftriaxone (MIC ≤ 0.125 mg/L), cefixime (MIC ≤ 0.25 mg/L) and spectinomycin (MIC ≤ 32 mg/L). 24 (12.8%) isolates had an MIC of 0.125 mg/L for ceftriaxone and 5 (2.7%) isolates had an MIC of 0.125-0.25mg/L for cefixime. The prevalence of PPNG and TRNG was 46.5% (87/187) and 36.4% (68/187), respectively. 71 % (62/187) of PPNG isolates carried the Asia type β-lactamase encoding plasmid and 90% (61/68) of TRNG isolates carried the Dutch type tetM containing plasmid.

Conclusion: Among *N. gonorrhoeae* strains isolated in Nanjing, all were resistant to ciprofloxacin. The prevalence of azithromycin resistance has increased rapidly: from 9.3% in 2009 to 37% in 2013). Ceftriaxone and spectinomycin remain effective empiric first-line therapy for gonorrhoea in Nanjing. However, the frequency of gonococcal isolates with ceftriaxone and cefixime MICs of 0.125–0.25 mg/L is rising.

P8

In vitro activity of the novel DNA gyrase inhibitor AZD0914 against 187 clinical *Neisseria gonorrhoeae* isolates with multi-resistance to other antimicrobials

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Background: The global spread of multi-drug resistant *Neisseria gonorrhoeae* has increased, and recently, gonococcal strains resistant to extended spectrum cephalosporins have emerged. This increases the need for novel agents, with no cross-resistance to marketed agents which can be used to treat gonococcal infections. AZD0914 is a novel spiropyrimidinetrione molecule that inhibits DNA synthesis and is active against *N. gonorrhoeae*, in vitro, including isolates resistant to other antimicrobials.. Here we investigated the in vitro activity of AZD0914 against clinical gonococcal isolates that included multi-resistant strains.

Methods: 187 *N. gonorrhoeae* isolates were isolated from male adults with symptoms/signs of urethritis attending an STD clinic in Nanjing, China in 2013. All isolates were resistant to ciprofloxacin (MIC ≥1 mg/L), 36% (69/187) were resistant to azithromycin(≥1 mg/L). 46.5% were PPNG, 36% TRNG, and 24 isolates had an MIC of 0.125 mg/L for ceftriaxone. The MICs of AZD0914 were determined by the agar dilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI).

Results: The MIC50, MIC90, and MIC ranges of AZD0914 were 0.03mg/L, 0.06mg/L, and ≤0.008 to 0.125mg/L, respectively. 2.1%(4/187) of the isolates had an MIC of 0.125mg/L and 97.9% (183/187) had an MICs of ≤0.06 mg/L for AZD0914.

Conclusion: This study showed that AZD0914 has excellent in vitro activity against clinical gonococcal isolates and might be an effective treatment option for gonorrhea.

P9

Population genomic analysis identifies a strong association of the gonococcal genetic island with third generation cephalosporin resistance in gonococci

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The gonococcal genetic island (GGI) enhances natural transformation between *Neisseria gonorrhoeae* via a type IV secretion system (T4SS) which has the potential to facilitate the spread of antibiotic resistance determinants. The prevalence and diversity of the GGI was assessed using whole genome sequences (WGS) of 186 *N. gonorrhoeae* for which antibiotic resistance profiles had been determined. WGS data were assembled de novo using VELVET and the resultant assemblies were deposited and annotated in the *Neisseria* PubMLST Database (pubmlst.org/*neisseria*). The GGI was then curated along with the conjugative plasmid and additional loci of interest. The GGI was found in 79% of isolates (147/186) and was consistently located between the genes encoding uracil-DNA glycosylase and a putative periplasmic protein (NEIS1116 and NEIS1125 respectively). A total of 65 loci were identified including three hypothetical proteins that had not been previously described and all loci were highly conserved. Six isolates possessed a GGI variant previously described in *Neisseria meningitidis*. Isolates that contained the GGI were statistically more likely to exhibit decreased susceptibility to antibiotics ($p < 0.0001$) including ciprofloxacin (125/147, 85%), tetracyclin (112/147, 76.2%), penicillin (106/147, 72.1%), cefixime (90/147, 61.2%), cefpodoxime (76 /147, 51.7%) or ceftriaxone (23/147, 15.6%, $p < 0.01$). However, the opposite was apparent for azithromycin (4/147 2.72%, $p < 0.0001$). None of the isolates lacking the GGI were resistant to ceftriaxone and cefpodoxime. These results show a direct link between antibiotic resistance and possession of the GGI in *N. gonorrhoeae*.

P10

Recent increase in reduced susceptibility to ceftriaxone in *Neisseria gonorrhoeae* after a decrease of the frequency of the presence of the mosaic *penA* gene

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Introduction: Resistance of *Neisseria gonorrhoeae* against third generation cephalosporins is a threat to public health. A major determinant is the presence of a mosaic *penA* gene in *N.gonorrhoeae*, partially derived from commensal *Neisseria* spp. We report resistance figures of *N.gonorrhoeae* against third generation cephalosporins and looked for the frequency of *penA* mosaic strains covering the years 2007-2013.

Methods: MICs for cefotaxim were determined by E-test on 6857 clinical isolates obtained from 2007 to 2013 from patients visiting the STI outpatient clinic. MICs for cefixim and ceftriaxone were analysed from 2010-13 (4191 strains). A real time PCR identifying strains with a mosaic *penA* gene was performed on 180 randomly chosen proctal isolates from males and on 346 cervical isolates from females evenly distributed over the years 2010-12.

Results: The frequency of strains resistant to cefotaxim (MIC > 0.125) was 2.1% in 2007, peaked to 7.8 – 8.4 % in 2008-10 and decreased to 3.0 % in 2011 and 2.3 % in 2013. The peak was most obvious in proctal isolates from male patients, up to 12.3 % in 2008, and followed by a decrease to 2.5% in 2013. In urogenital strains from females, resistance was never over 3.1%. Resistance against cefixime or ceftriaxone (MIC > 0.125) was not found. The frequency of strains with an increased MIC against cefixime (> 0.032) decreased from 14.0% in 2010 to 5.5% in 2013. The frequency of strains with an increased MIC (> 0.032) to ceftriaxone was 5.2% in 2010, dropped to 2.0 and 3.1% in subsequent years, but increased again to 7.8% in 2013. The frequency of the mosaic penA gene decreased from 35% to 3% in proctal isolates from males between 2010 and 2012, and from 5% to 3% in isolates from females.

Conclusion: The decrease in the frequency of the mosaic *penA* gen in *N.gonorrhoeae* in the period 2010-12, especially in proctal isolates from males, correlated with a decrease in occurrence of strains resistant to cefotaxim and reduced susceptibile to cefixime and ceftriaxone. In 2013, strains with an increased MIC to ceftriaxone, but not cefixime, were again more prevalent.

P11

Meningococcal serogroup A, C, Y and W serum bactericidal antibody profiles in Hajj pilgrims

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Background: The religious seasons of Hajj and Umra in the Kingdom of Saudi Arabia have historically been associated with epidemics of meningococcal disease. Due to effective preventive measures including vaccination, in recent years, no meningococcal outbreaks have been reported during Hajj or were Hajj-associated. However, little is known about the immunological profile of pilgrims. The aim of this study was to assess the immunological profile of pilgrims on arrival to KSA against the four meningococcal serogroups, A, C, W and Y, contained within the quadrivalent vaccine.

Methods: Following consent, socio-demographic factors and health related information was collected from pilgrims arriving at King Abdul Aziz International Airport followed by a blood sample. Antibodies were quantified by serum bactericidal antibody assay using baby rabbit complement (rSBA) against the four meningococcal serogroups, A, C, W and Y.

Results: Of the 796 pilgrims serum samples collected, rSBA results were obtained for all four serogroups for 741 samples. For pilgrims who had their age recorded, this ranged from 18 to 92 years (median 52 years). A total of 48 (6.5%) Hajjis had previously attended Hajj, ranging from 1 to 14 times (median 2 times) and 98.2% had received meningococcal quadrivalent vaccine in the last 3 years. Of the 13 who had not, all originated from Bangladesh with 4 reporting no previous meningococcal vaccination and 9 received vaccination over 3 years ago. For serogroup A only one pilgrim from Indonesia had a rSBA titre <8. For serogroup C, W and Y, the percentage of pilgrims with rSBA titres <8 were 9.9%, 17.4% and 9.4%, respectively. Of note was the high prevalence of non-complement-mediated lysis in pilgrims originating from Nigeria (28/47; 59.6%) and Afghanistan (21/47; 44.7%) but not the other countries. This may be a reflection of the type and pattern of antibiotic usage among these communities.

Conclusion: The vast majority of pilgrims are vaccinated and protected against meningococcal serogroups A, C, Y and W.

P12

Effect of the Eculizumab (Soliris (R)), on the meningococcal serogroup B (MenB) serum bactericidal antibody (SBA) assay

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Background: Eculizumab (Soliris(R)), a monoclonal antibody, targets complement protein C5 and inhibits terminal complement-mediated haemolysis associated with Paroxysmal Nocturnal Haemoglobinuria (PNH) and atypical Haemolytic Uremic Syndrome (aHUS). Following the licensure of Bexsero(R) in the UK, patients with PNH and aHUS may be vaccinated with Bexsero. Therefore it is important to know whether these patients have protective serogroup B (MenB) serum bactericidal antibody (SBA) titres during Eculizumab therapy. The MenB SBA assay is a functional measure of the ability of antibodies in conjunction with human complement to kill the meningococcus. The aim of the study is to exam the effect of Eculizumab on the MenB SBA titres and if the effect of the Eculizumab could be blocked by the complement protein C5.

Methods: Two samples with known high SBA titres against the target strain NZ 98/254 were assayed in the MenB SBA assay in the presence of Eculizumab, at varying concentrations from 1250 µg/mL to 0.0003 µg/mL. One sample's SBA titre was measured in the presence of Eculizumab (20 µg/mL) with different concentrations of C5 from 100 µg/mL to 3.125 µg/mL.

Results: Both test samples gave titres of less than 4 in the presence of Eculizumab at concentrations greater than 5 µg/mL. One of samples when assayed with 20µg/mL of Eculizumab and 100 µg/mL of C5 gained titres within assay variation to those obtained when the neat sample was assayed. Reduction of the C5 to a concentration of 50 µg/mL resulted in reduced titres as compared to the neat sample, and further dilution of C5 resulted in negative titres.

Conclusion: These data suggest that Eculizumab inhibits the human complement used in the MenB SBA assay. Complement protein C5 can be used in the SBA assay to block the Eculizumab inhibition.

P13

Comparison of different serogroup A immunoassays following a single dose of either MenAfriVac or quadrivalent polysaccharide vaccine in healthy Africans 2- to 29- years of age

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Introduction: Immune responses to new meningococcal vaccines are used to infer vaccine effectiveness. Complement-mediated antibody-dependent killing is the primary mechanism of human protection against invasive meningococcal disease and bactericidal antibody can be quantified in assays using exogenous human (hSBA) or rabbit (rSBA) complement.

Methods: We compared assays using sera from a random, age-distributed subset of 360 subjects from a meningococcal group A conjugate vaccine (MenAfriVac®, PsA-TT) study in which 900 subjects between 2 and 29 years of age were randomized to receive PsA-TT or quadrivalent polysaccharide vaccine (PsACWY). Sera collected before immunization and 28 days and 1 year after vaccination were tested; geometric mean responses, fold-rise and threshold analyses within vaccine groups and age groups were assessed. Agreement among the assays was determined overall and by vaccine, age group and time-point.

Results: All three assays showed that immune responses to PsA-TT were substantially higher than those to PsACWY at both 28 days and 1 year following immunization and were similar among the 2-10, 11-17 and 18-29 year age groups at 28 days. Differences between assay results were also observed. Pre-existing antibody and the responses to PsACWY increased with age by IgG and hSBA but not rSBA. rSBA and IgG, but not hSBA data indicated that antibody persisted at 1 year in a high proportion of subjects who received PsA-TT regardless of age, although hSBA and IgG data both showed a greater decline in antibody persistence following PsA-TT in the 2-10 year age group. Agreement between assay results for individual sera was poor regardless of age, vaccine, or time-point.

Conclusion: While serologic assays are critical for the evaluation of meningococcal vaccines, comparison of results generated in different serologic assays remains difficult. The high level of protection against both invasive disease and carriage resulting from MenAfriVac® immunization campaigns is consistent with the strong immune responses observed with each assay but future implementation decisions will likely depend on immunologic data and their long-term correlation with disease and carriage prevention. Expanded immunologic studies and epidemiologic surveillance may improve our interpretation of differences between these immunoassays.

P14

Simultaneous quantitation of PorA and PorB in outer membrane vesicles of Bexsero vaccine using isotope dilution mass spectrometry

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Introduction: Bexsero vaccine for serogroup B meningococcal disease consists of four antigenic components; three recombinant proteins and outer membrane vesicles (OMV) from a New Zealand epidemic strain. Consistency between OMV preparations is currently demonstrated by determination of the proportion of protein bands to total protein by SDS-PAGE. These seven bands include the immunodominant protein PorA. The complex nature of OMVs and the inherent inaccuracy of SDS-PAGE limit the ability to accurately quantify antigens which could be indicators of vaccine potency and consistency of production. We present an approach for absolute quantitation of PorA and PorB proteins in OMV using isotope dilution mass spectrometry (IDMS).

Method: The workflow involves tryptic digestion followed by liquid chromatography and multiple reaction monitoring mass spectrometry (MRM-MS). Putative target peptides were selected using an in silico trypsin digest of PorA and PorB. Optimal peptides were identified on the basis of signal intensities of parent and fragment ion transitions. Three distally separated peptides were chosen from each protein to ensure complete digestion. These were synthesised as both native species and labelled analogues. Leucine, isoleucine or valine was replaced with its 13C15N isotope to increase the peptide mass of the analogues by 6-7 Da. Labelled peptide as an internal standard was added to a tryptic digest of OMV and native peptides.

Results: Absolute quantitation of PorA and PorB in OMVs was based on their corresponding three peptides. These peptides were quantified against a native peptide standard curve where a ratio of peak area from native and labelled peptide was used. The detection was linear from 15 to 250 fmole/µL and quantitation of as little as 1 µg/ml of each protein was achievable. All six pairs of PorA and PorB peptides were separated and analysed within a sample injection under a 60-min gradient.

Conclusions: Our results demonstrate that IDMS is a robust and accurate method for the simultaneous quantitation of PorA and PorB in OMVs. The sensitivity allows quantitation of PorA and PorB protein in the range expected in bulk OMV samples.

P15

The Global Meningococcal Initiative, report from the second summit meeting, Cape Town, South Africa

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Background: The Global Meningococcal Initiative (GMI) is an international expert group, composed of 50 scientists and clinicians, which seeks to prevent invasive meningococcal disease globally through education, research, and cooperation. Collectively, the GMI group has expertise in meningococcal immunology, epidemiology, public health and vaccinology.

Methods: On 17–18 November 2013, the GMI convened its second Summit Meeting in Cape Town, South Africa. The objectives of this meeting were to discuss the lessons learned from existing meningococcal immunisation programmes, define key issues and address remaining challenges in the control of meningococcal disease, including improvement of surveillance systems, set up of outbreak preparedness plans and development of local immunisation strategies.

Results: Although much is known about meningococcal disease and the impact of vaccination, many research/knowledge gaps still exist. The true burden of disease is probably underestimated in several countries and there is a need to improve the diagnosis and establish more uniform quality surveillance. Indeed, the group felt that more studies and data were needed on aspects such as immune memory, carriage, correlates of protection and how to evaluate the impact of the recently licensed serogroup B vaccine. Experience from both Brazil (where routine MenC vaccination for infants was implemented) and Chile (where a reactive vaccination with the MenACWY vaccine for children aged 9 months to 5 years was implemented), demonstrated a dramatic decrease on the incidence rates in the targeted age groups, without early impact on age groups not vaccinated. In the African meningitis belt, the implementation of MenA vaccine, including persons 1 to 29 years, provided impact in all age groups, including those non-vaccinated. However, there is concern regarding the increasing incidence of serogroups W and X disease and thoughts may now move to implementing multivalent vaccines targeting other serogroups in Africa.

Conclusions: There were many lessons to be learned from existing immunisation strategies, such as the importance of catch-up programmes as well as an integrated surveillance system and transparent dissemination of data to the public. As the epidemiology of meningococcal disease is constantly changing, dissemination of such lessons will be of great importance globally. *On behalf of the GMI.

P16

Structural insights reveal a novel trimeric autotransporter adhesin fold in the meningococcal vaccine antigen NadA and the mechanism of its ligand-dependent transcriptional regulation by NadR

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Background: Serogroup B *Neisseria meningitidis* (MenB) is a major cause of severe sepsis and invasive meningococcal disease, which is associated with 5–15% mortality, and devastating long term sequelae. *Neisseria*l adhesin A (NadA), a meningococcal trimeric autotransporter adhesin (TAA) that acts in adhesion to, and invasion of, host epithelial cells, is one of the three protein antigens in Bexsero®: the first genome-derived vaccine against MenB, which was recently approved in Europe, Australia and Canada. We sought detailed insights into the structure of NadA and into the molecular mechanisms governing its transcriptional regulation by NadR.

Methods: A comprehensive set of biochemical, biophysical and structural techniques were applied to investigate several recombinant forms of NadA and NadR, allowing detailed characterizations of their key molecular features.

Results: Here we present the first high-resolution structures of NadA and NadR. The crystal structure of NadA variant 5 revealed a novel TAA fold predominantly made of trimeric coiled-coil with three protruding wing-like structures that create an unusual N-terminal head domain. Transmission electron microscopy confirmed a similar overall topology of NadA variant 3, the vaccine variant. The fine mapping of the binding site of a bactericidal antibody revealed a protective epitope on the head of NadA - a functional region important for binding to human Chang epithelial cells. The crystal structures of NadR in ligand-free and ligand-bound states showed that apo-NadR is structurally preconfigured for DNA binding, while in the presence of its ligand, 4-HPA (4-hydroxyphenylacetic acid), there is a large allosteric conformational change apparently incompatible with DNA binding.

Conclusions: The structure of NadA provides new insights into the understanding of its biological and immunological functions. Moreover, the proposed molecular mechanism by which 4-HPA controls the DNA-binding activity of NadR will enable a deeper understanding of the regulation of NadA expression in vitro and in vivo and its role in pathogenesis. Most importantly, this will open new insights in the effective contribution of NadA to vaccine coverage, which is known to be underestimated.

P17

Sequence, structural and functional differences between different isotypes of transferrin binding proteins A and B from *Neisseria meningitidis*

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N. meningitidis is highly adapted to its human host, and acquires necessary iron in the host environment via specialized receptor systems that sequesters it from circulating host glycoproteins; notably transferrin (Tf). The transferrin receptor system consists of two proteins: the first is an integral membrane protein, transferrin binding protein A (TbpA), that functions as a TonB dependent conduit allowing iron transport across the outer membrane, and the second is a surface-exposed outer membrane anchored lipoprotein, transferrin binding protein B (TbpB), that selectively binds to iron loaded Tf. Previous studies have identified two isotypes of TbpB based on biochemical properties and demonstrated that the isotype I TbpB in strain B16B6 is required for in vitro growth dependent upon exogenous iron-loaded Tf. This study was initiated to address the hypothesis that there is a companion isotype of TbpA that would require an isotype I TbpB to acquire iron under in vitro growth conditions. The *tbpA* and *tbpB* genes from a collection of 200 strains were sequenced and bioinformatics analyses revealed the presence of an isotype I lineage of TbpA associated with the isotype I TbpB. The major structural difference between the crystal structures of isotype I and isotype II TbpBs is the absence of large flexible loop regions localized to the TbpB C-lobe. Similarly structural models of isotype I TbpAs revealed that they may lack critical regions for interacting with Tf thus lowering their affinity. These observations prompted us to initiate functional studies to examine the synergistics effects of TbpA and B partnerships in iron utilization from Tf. We have begun introducing various combinations of tbpB and tbpA genes into a common strain so that we can explore their impact on Tf-dependent growth. These studies should enable us to address our primary hypothesis and provide insights into the mechanism of iron acquisition.

P18

Development and characterization of a low cost synthetic oligomer based meningococcal serogroup C conjugate vaccine

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Background and Aims: *Neisseria meningitidis* is among the leading causes of bacterial meningitis and pneumonia around the globe especially in the sub-saharan meningitis belt. Capsular polysaccharide (PS) of the meningococci has been a target for vaccine development against meningococcal infections. The conjugate vaccines having PS conjugated to the carrier protein are effective in widest possible age groups but are costly and out of reach of most of the needy people in developing world. We have attempted to explore the development of synthetic oligomer based conjugate vaccines against meningococci as a cost effective alternative.

Methods: Tetramers of *N. meningitidis* serogroup C capsular polysaccharide (MenCTM) were synthesized by organic synthesis. The tetramer synthesis scheme was designed to have a linker with free amine group on its reducing end. The tetramers were conjugated to tetanus toxoid (TT) as a carrier protein utilizing thio-ester linkages. The purified oligomers and conjugates were characterized by extensive physico-chemical characterization. Inhibition ELISA was used to evaluate antigenicity of the oligomers and conjugates. Immunogenic potential of well-defined conjugates was investigated in mouse immunogenicity studies in comparison to vehicle control and a licensed conjugate vaccine comparator. The animal sera were titrated for serum bactericidal and total IgG titers.

Results: The MenCTM compiled with expected physico-chemical parameters having >95% purity by high-performance liquid chromatography. The MenCTM could be successfully conjugated to TT to achieve an oligosaccharide:protein ratio of 0.3±0.05. The conjugates were successfully characterized as per WHO specifications. In vitro, the MenCTM as well as MenCTM-TT inhibited antibodies raised against licensed conjugate vaccine. The MenCTM-TT conjugates were highly immunogenic in mice after subcutaneous injections. Significant bactericidal and total IgG titers were obtained which were comparable or better as compared to licensed comparator in all 3 immunogenicity studies including different conjugate lots.

Conclusions: Our MenCTM-TT conjugate vaccine can be efficiently and effectively prepared using synthetic tetramers. The possibility of synthesizing the oligomers in an organic synthesis laboratory, the increased conjugation yields due to in-built linker and least possibility of modification of the oligosaccharide chain together with high stability makes this approach a low cost solution towards reducing the meningitis disease burden worldwide.

P19

Variation in Factor H-Binding Protein distribution among culture and non-culture meningococcal disease cases in England and Wales in 2011

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Background: Immune cross-protection provided by individual Factor H-Binding Protein (fHbp) variants is largely subfamily-specific. Consequently, understanding the distribution of fHbp among circulating invasive strains is essential to predict the coverage of group B vaccines containing this antigen. Approximately 50% of English and Welsh disease cases do not yield a positive culture. Protocols for typing fHbp from these 'non-culture' specimens have not been previously available, resulting in an incomplete picture of fHbp distribution.

Methods: A novel PCR-based sequencing assay was used to type fHbp from 81.8% (n=404) of English and Welsh non-culture cases during the 2011 calendar year. These data were compared to those derived from whole genome analyses of cultured isolates from the same period (n=482).

Results: The ten most common fHbp variants collectively represented 78.9% of all cases. A significantly greater proportion of group B non-culture specimens possessed B44 (pubMLST variant 15) than group B isolates (19.7% vs. 10.4%, respectively) suggesting that strains bearing this variant may be less likely to be successfully cultured. Age analysis revealed a greater proportion of B44/15 among non-culture cases than culture cases across all age groups, with greater disparity observed among children and adolescents.

Analysis of almost 2000 isolates from four previous epidemiological years revealed a strong association between variant B44/15 and the ST-269 cluster of ST-269 complex. ST-269 cluster isolates exhibited a greater susceptibility to Penicillin than those of other group B lineages. Furthermore, insertion sequences disrupting/flanking genes putatively involved in virulence and/or viability were found exclusively within isolates of this population. Finally, the absence of NalP, encoding a serine protease autotransporter of potential importance to the survival of meningococci in vivo, was confirmed for 122/124 (98.4%) ST-269 cluster isolates.

Conclusions: Whilst the ten most prevalent fHbp variants accounted for approximately 80% of both cultured and non-culture cases, significant differences were observed in the distribution of B44/15, a variant strongly associated with the ST-269 cluster. Features specific to this population were identified that may explain a supposed lower viability and could provide a basis for future work to conclusively identify the most significant determinants of culturability.

P20

Structural characterization of endotoxin from *Neisseria Neisseria meningitidis* B and subsequent evaluation of its pro-inflamatory activity

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Lipooligosaccharide (LOS) is a major factor responsible for the induction of pro-inflammatory processes in meningoccocical disease; it´s formed by lipid A moiety which is covalently linked to a oligosaccharide core and is responsible for inflammatory process. The heterogeneity of LOS makes its characterization an important step to understand the innate immune responses. The aim of this study is to characterize native and deacylated LOS (dLOS), from *Neisseria meningitidis* Serogroup B, in order to establish the structure-activity relationship in the induction of inflammatory activities. The LOS was hydrolyzed in acetate buffer. Lipid A was recovered by centrifugation. The oligosaccharide was fractionated by chromatography, generating fractions labeled as OS-1 and OS-2, in a 4:1 ratio. Oligosaccharide composition analysis were performed by methanolysis, derivatizated with BSTFA, analyzed by GCMS. The major oligosaccharide connectivity information was based on NMR studies (1H,13C,31P,2D-COSY, TOCSY, NOESY, ROESY, HMQC, HMBC). These data allowed the determination of the oligosaccharide core as L7 immunotype. Mass spectroscopy was used as an effective technique for analyzing lipid A. MALDI-TOF/MS analysis showed an ion [M+H]+ at m/z 1960,98 attributed to hexa-acylated 1,4'- diphosphoryl-ethanolamine units. The phosphorylation pattern was also determined by NMR-31P, and the data are in conformity with that ones obtained by ESI-MS. The fatty acids obtained by hydrolysis were esterified and analyzed by GC-MS. The results were in accordance with the studies of members of the family *Neisseria*ceae. The kinetic detoxification of LOS was carried out with NaOH at 30 , 60, 90, 120 and 150 minutes, monitored by ESI-MSn. It was possible to verify a large substrate conversion after the 30th minute of reaction, producing a di-acylated-1',4-monophosphorylated-dLOS as a major product; however, there was a residual quantity of di-acylated-1',4-diphosphoryl-dLOS, that gradually decreased during the 150th minute of reaction. The pyrogen test showed the inflammatory effect decreases when detoxification reaction time extends. It suggests a strait relationship between the presence of diphosphoryl group and inflammatory process. Further perspective is to develop mass spectrometry protocols to measure di-acylated 1,4'-diphosphate-dLOS to diminish the accomplishment of in vivo tests, establishing a save protocol for vaccinal component production.

P21

A recombinant protein truncation strategy induces bactericidal antibodies to the Macrophage Infectivity Potentiator protein of *Neisseria meningitidis* and circumvents potential cross-reactivity with human FKBP proteins.

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Background: *Neisseria meningitidis* produces a Macrophage Infectivity Potentiator (MIP) protein that is found in the outer membrane of the organism. Previously, we reported that a recombinant (r)MIP delivered in saline alone and liposomes induces significant serum bactericidal antibody production in mice and is a candidate meningococcal vaccine antigen [1]. However, bioinformatics analysis of MIP shows some amino acid sequence similarity with human FK506-binding proteins (FKBPs) in residues 166-252 located in the globular domain of the protein, which also includes PPIase active site. To circumvent the potential concern over generating antibodies that could recognize human proteins, we therefore developed and tested a recombinant protein truncation strategy.

Methods: Using a structural prediction model based on the L.pneumophila MIP protein, we constructed recombinant MIP proteins that were truncated after the extended α-helix, thereby removing the globular domain. Mice were immunised with saline or liposomes containing recombinant Truncated Type I rMIP proteins that lacked the signal leader peptide (LP) sequence (amino acids 1-22) and with the HIS purification tag at either the N- or C-terminus. The immunogenicity of Truncated rMIP proteins was compared to Full rMIP proteins (containing the globular domain) with either a N- or C-terminal HIS tag and with or without the LP sequence.

Results: By comparing the functional murine antibody responses to these various constructs, we identified that C-term HIS Truncated rMIP (-LP) delivered in liposomes, but not saline, induced high levels of antibodies that bound to the surface of wild-type but not ΔMIP mutant meningococci and showed bactericidal activity against homologous Type I MIP (median titres of 128-256) and heterologous Type II and III (median titres of 256-512) strains, thereby providing >90% strain coverage. Significantly, this bactericidal activity was identical to that induced by the Full rMIP (LP) construct reported previously. In addition, antisera to C-term HIS Truncated rMIP (-LP) did not cross-react human FKBP2 protein in western blot.

Conclusions: This vaccine strategy obviates any potential concerns regarding cross-reactivity with human FKBP proteins and the C-term HIS Truncated rMIP deserves consideration for inclusion in a defined meningococcal antigen vaccine. [1] Hung et al., Infect Immun 2011, 79: 3784–3791 (2011).

P22

The *Neisseria meningitidis* amino acid ABC transporter substrate-binding protein, NMB1612, induces functional cross-protective bactericidal antibodies

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Background: in a previous immuno-proteomics study, we identified that individuals colonized by meningococci showed increases in both serum bactericidal activity (SBA) and antibody reactivity with the amino acid ATP-binding cassette (ABC) transporter, periplasmic substrate binding protein (NMB1612). In the current study, we investigated the conservation and expression of NMB1612 and the vaccine potential of recombinant (r)NMB1612 protein.

Methods: the gene encoding NMB1612 (strain MC58) was cloned into the pRSETA system and rNMB1612 protein expressed in *E. coli* and purified by NI-NTA chromatography. BALB/c mice were immunized with rNMB1612-Al(OH)3, rNMB1612-liposomes, or rNMB1612-MPLA-liposomes and rabbits with rNMB1612-Freund's adjuvant. The biological and functional properties of antibodies to rNMB1612 were characterized by ELISA, western immunoblotting, FACS, immunofluorescence (IF) and SBA assays. Conservation of NMB1612 was examined in the BIGS database of sequenced *Neisseria* genomes (n>2500) and in our selected meningococcal strains (n=13) sequenced commercially.

Results: rNMB1612 is expressed as a monomer-dimer (Mr ~34-68kDa) soluble protein and high titres of murine antibodies were raised against the protein using different adjuvant-delivery systems. Specificity of the immune response was demonstrated by western blot reactivity of anti-rNMB1612 sera with a single protein band in meningococcal lysates of Mr ~29kDa, consistent with native NMB1612 protein. Native protein was expressed similarly by different meningococcal strains in our collection, as judged by western blotting. FACS and IF assays, using both bactericidal murine and rabbit antisera demonstrated the presence of the protein on the meningococcal surface. Examination of our sequenced strains and the BIGS database identified 25 different non-redundant alleles in serogroup B meningococcal strains (n=839): ~80% of these isolates expressed protein belonging to either allele 1 (29%), 62 (14%), 64 (15%), 68 (14%) or 99 (8%). The different types of NMB1612 protein represented by the 25 alleles shared 90% amino acid sequence similarity. Murine antisera to rNMB1612-Al(OH)3, -liposomes or -liposomes-MPLA induced similar SBA (titre 128-256) against both homologous (MC58, allele 62) and heterologous (MC173, allele 64; MC90, allele 1) strains.

Conclusion: the attributes of amino acid sequence conservation, surface presentation, stable expression and ability to induce cross-strain bactericidal activity suggest that NMB1612 is a serious candidate for a universal meningococcal vaccine.

P23

The biology and function of Adhesin Complex Proteins of pathogenic *Neisseria*

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Background: the Adhesin Complex Protein (ACP) of *Neisseria meningitidis* (Nm-ACP) is an adhesin present in the outer membrane (OM) that induces cross-strain bactericidal activity and merits consideration for incorporation into new meningococcal vaccines. *Neisseria* gonorrhoea also expresses an ACP protein (Ng-ACP) and herein we report on our current studies on the biology, function and vaccine potential of ACP proteins from both species.

Methods: ACP diversity was examined in the BIGS database of sequenced *Neisseria* strains and our collection of meningococcal (n=13) and P9 gonococcal strains; recombinant Ng- and Nm-ACP proteins were prepared in pRSET and pET systems and used for immunisation studies; the biological and functional properties of anti-ACP antibodies were characterized by ELISA, western immunoblotting, FACS, immuno-fluorescence (IF) and bactericidal assays.

Results: Analysis of the ACP amino acid sequences from our meningococcal strains and the expanded BIGS database (~2400 isolates) showed that meningococci express 8 different ACP proteins. However, the majority of meningococci express a type II (92% of isolates) or type I (7.7% of isolates) ACP with the remaining proteins expressed by single isolates only; the type I and II proteins show high similarity (>98%) with only one amino acid difference (Asp25 → Asn25). Gonococcal ACP is also highly conserved: 92.4% of isolates express Ng-ACP that shows >98% sequence similarity with a second Ng-ACP expressed by the remaining 7.6% of isolates, with only two observed amino-acid changes (Ala20 → Ala20del and Asn25→Asp25). Ng-ACP proteins and Nm-ACP proteins share ~95% similarity and rabbit anti-Nm-ACP sera cross-react with gonococcal P9 ACP. Data are presented on trial immunisations of mice with Ng-ACP delivered in saline alone, liposomes or adsorbed to AL(OH)3; these data include antibody reactivity against Ng-ACP and isolated OM (ELISA, western blot), demonstration of gonococcal surface expression (FACS, IF), examination of anti-Ng-ACP serum inhibition of binding of recombinant protein and Ng-ACP -expressing bacteria to human cell cultures in vitro, and bactericidal responses.

Conclusions: ACP proteins represent a class of small OM-located *Neisseria* adhesins, capable of inducing functional antibody responses and studies are on-going to further characterize the structural and biological role of these proteins.

P24

Pre-clinical evaluation of the vaccine potential of the highly conserved, expressed and surface-exposed Cell Binding Factor (CBF, NMB0345) protein of *Neisseria meningitidis*

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Background: in a previous immuno-proteomics study, we identified that human sera with positive serum bactericidal activity generated by meningococcal colonisation, showed increased reaction with the putative Cell Binding Factor (CBF, NMB0345) protein. In the current study, we investigated the conservation and expression of CBF and the vaccine potential of a recombinant (r)CBF protein.

Methods: The gene encoding NMB0345 (strain MC58) was cloned into the pRSETA system and rCBF protein expressed in *E. coli* and purified by NI-NTA chromatography. BALB/c mice were immunized with rCBF-Al(OH)3, rCBF-saline, rCBF-liposomes, rCBF-Zwittergent (ZW)3-14, rCBF- MPLA-ZW3-14 or rCBF-MPLA-liposomes. The biological and functional properties of antibodies to rNMB1612 were characterized by ELISA, western immunoblotting, FACS, immunofluorescence (IF) and SBA assays. Conservation of CBF was examined in the BIGS database of sequenced *Neisseria* genomes and in our selected meningococcal strains (n=12) sequenced commercially.

Results: rCBF is expressed as an insoluble monomer (Mr ~36kDa) protein. High titres of murine antibodies were raised against solubilised protein using different adjuvant-delivery systems. Specificity of the immune response was demonstrated by western blot reactivity of anti-rCBF sera with a single protein band in meningococcal lysates of Mr ~31.5kDa, consistent with native CBF protein. Native protein was expressed similarly by different meningococcal strains as judged by western blotting and FACS and IF assays demonstrated the presence of CBF protein on the meningococcal surface. Examination of our sequenced strains and the BIGS database identified nine different non-redundant alleles in serogroup B meningococcal isolates (n=847): ~93.5% of these isolates expressed protein belonging to either allele 1 (71.2%) or 18 (22.3%). The nine different types of CBF protein have only one amino acid difference between each other (99.7% similarity). Murine antisera to rCBF- induced SBA (titre 256) against both homologous (MC58, allele 1) and heterologous strain (L2470, allele 18), but this required both MPLA and introduction into Zwittergent micelles.

Conclusion: the attributes of amino acid sequence conservation, surface location, and ability to induce cross-strain bactericidal activity suggest that CBF is a candidate for a universal meningococcal B vaccine. However, further optimisation of the adjuvant-delivery system is needed.

P25

Potential public health impact of having included *N. meningitidis* serogroup B in the 2005 recommendation for adolescent meningococcal serogroup A, C, Y, and W-135 vaccination

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Background: Routine conjugate vaccination against IMD caused by *N. meningitidis* serogroups A, C, W-135 and Y (MenACWY) began in the United States in 2005, with a single dose of MnACWY vaccine given at age 11 years, and later augmented to include a booster dose at age 16 years. At that time, there was no licensed, broadly protective vaccine against serogroup B (MenB), which represented half of the IMD cases reported in children aged 5-17 in the US. We estimated the potential historical public health impact, measured in cases of invasive meningococcal disease (IMD) avoided, if an adolescent MenB vaccine had been available and included in the 2005 recommendation.

Methods: A transmission dynamic model was developed to simulate prevalence of meningococcal carriage and incidence of IMD within the US population over the past 10 years following initiation of the five serogroup adolescent vaccination program. Carriage prevalence and vaccine efficacy against IMD and carriage were obtained from the published literature and expert opinion. Sensitivity analyses were performed with recent IMD incidence estimates from the national surveillance system in 2005 and 2012. Cases and deaths averted were calculated.

Results: The model estimated that 784 serogroup B IMD cases and 73 deaths would have been prevented through 2014, if a MnB vaccine had been similarly implemented in 2005. At 2012 epidemiology, the cases and deaths avoided would have been 77 and 7, respectively.

Conclusions: IMD incidence, including that of serogroup B, has declined in the US over the past 15 years. IMD is severe and unpredictable, and it is unknown whether incidence will remain low. While the impact of a MnB vaccine at current epidemiology will be limited, had a MenB vaccine been available and recommended alongside MenACWY in the US, substantial disease avoidance could have been realized over the past 10 years.

P26

Potential impact of vaccination of college-age adolescents against *N. meningitidis* serogroup B: Results of a transmission dynamic model

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Background: Studies in the 1990s establishing an increased risk of invasive meningococcal disease (IMD) in first year college students living in residence halls were the foundation for US ACIP recommendations for routine meningococcal serogroup A, C, W-135 and Y (MenACWY) vaccination in this group. Recent college campus outbreaks of meningococcal serogroup B (MenB), such as that at Princeton University where the attack rate was 134 per 100,000, highlight the unpredictability of IMD and the need in the US for a licensed, broadly protective MenB vaccine. We estimated the potential impact of introducing routine MenB vaccination of students preparing for the 1st year of college.

Methods: A transmission dynamic model was adapted to simulate MenB carriage prevalence and IMD incidence within the college-attending US young adult population (17-22 year olds). Carriage prevalence and vaccine efficacy against IMD and carriage were obtained from published literature and expert opinion. Incidence of IMD was estimated from the US national surveillance system utilizing the 2008 to 2011 average. College attendance data were obtained from US Bureau of Labor and Statistics. IMD cases and deaths avoided over a 30 year period were calculated for the entire US population, and separately for a closed (i.e., no transmission into or out of the population) college-attending cohort.

Results: In a closed-cohort of college-attending young adults aged 17-22 years, routine vaccination of 90% of 17-yr-olds entering college would prevent an estimated 293 cases and 35 deaths. If college attendees had limited interaction with other members of general population, an estimated 2,278 cases and 224 deaths could be prevented in the US. An estimated 2,788 cases and 266 deaths could be prevented with routine vaccination of 11 year olds receiving a booster dose at 16 years of age.

Conclusions: A strategy of vaccinating first year college entrants could have a sizable impact on MenB disease and help control college campus outbreaks. However, the overall cases of disease and deaths prevented utilizing a strategy of vaccinating college entrants are fewer than if an age-based vaccination strategy was pursued. A program addressing disease in college students and adolescents would have the greatest impact.

P27

Are transferrin receptor-mediated iron acquisition systems primarily limited to pathogenic bacteria that inhabit the upper respiratory tract?

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A number of *Neisseria* species that exclusively reside in the upper respiratory tract of the host rely on an iron acquisition system that extracts iron directly from the host iron binding glycoprotein, transferrin. As a consequence, the surface receptor proteins that mediate this process have been identified as ideal targets for vaccines. The impact of vaccination on the microbiome has recently arisen as an important issue, due to the demonstration that systemically administered conjugate capsular vaccines not only prevent infection, but also eliminate colonization by the targeted bacteria. Recent experiments with a humanized transgenic mouse model demonstrated that immunization with TbpB eliminates colonization, thus it will be important to determine the impact that TbpB-based vaccines have on the microbial community of the upper respiratory tract. For this study genetic and immunological approaches are being used to evaluate the prevalence of potential targets for TbpB-based vaccines in the upper respiratory tract. Upper respiratory tract isolates obtained from young children and adults through microbiome studies, are being screened for genes involved in iron acquisition, to identify those carrying tbpB genes. Preliminary screens were performed targeting the conserved periplasmic *fbpA* (ferric binding protein) genes or the *feoAB* genes, as the former is invariably present in receptor containing bacteria. The results demonstrate that the receptor proteins are only present in a subset of the colonizing *Neisseria* strains. The genes encoding TbpB from microbiome isolates will be PCR amplified, sequenced and cloned into a series of expression vectors and strains that we use to evaluate the cross-reactive and cross-protective properties of the immune response against TbpB-based vaccines. The directed bioinformatics-based approach will identify anticipated immunological cross-reactivity due to known targets. A parallel approach will be implemented using FACS sorting to identify microbiome isolates that react with antisera directed against TbpB-derived antigens, to potentially identify unanticipated cross-reactivity of the antisera.

P28

Antigen engineering of transferrin binding protein B as a vaccine antigen against infection by *Neisseria meningitidis*

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Background: Transferrin binding protein B (TbpB) is an outer membrane lipoprotein of *Neisseria meningitidis* that is required for iron acquisition from host transferrin. To increase TbpB immunogenicity and induced cross-protection, we have initiated a structure-based protein engineering program for developing improved TbpB-based antigens from different pathogens and have adopted a strategy of evaluating the immune response in different host species to enhance our ability to validate the results.

Methods: Informed antigen engineering has been undertaken with knowledge of both sequence variability and structure of TbpB to produce the most efficacious and cross-reactive vaccine antigen. Animal immunizations have been carried out with various TbpB-derived antigens adjuvanted with emulsigen-D in mice, rabbits and pigs. Collected sera have been used to evaluate cross-reactivity and immunogenicity through ELISA.

Results: Multiple TbpB variants have been produced through structurally informed antigen engineering to produce stable domains, loop deletions, hybrid antigens and multimers as a strategy to optimize the immunogenicity and cross-reactivity. Our results to date indicate that the presence of flexible, highly variable loops on the same polypeptide reduces the induction of a cross-reactive response and that stable derivatives with loops reduced or eliminated retain immunogenicity. The production of stable multimers and hybrid antigens suggest that TbpB derivatives may serve as an effective epitope display scaffold. Immunization experiments in pigs with antigens derived from TbpB of the pig pathogen *Actinobacillus pleuropneumoniae* suggest that binding of host transferrin may interfere with induction of an effective immune response. Aside from this effect, the immune response against TbpB-derived antigens has similar effects in different host species. Since prevention of colonization has become recognized as an important attribute for forthcoming vaccines we initiated experiments in a humanized mouse model. The results demonstrate that colonization of the nasal passage by *N. meningitidis* is preventable through immunization of TbpB in CEACAM1-expressing C57BL/6 mice.

Conclusions: Antigen engineering has been carried out in a sequence and structure-informed manner in order to provide an array of TbpB variants that remain immunogenic and improve cross-reactivity. Further work is being pursued to produce the most protective antigen with focus put on the ability to elicit sterilizing immunity.

P29

Interchangeability of meningococcal group C conjugate vaccines with different carrier proteins in the United Kingdom infant immunisation schedule

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Introduction: In the United Kingdom, a 13-valent pneumococcal conjugate vaccine replaced the 7-valent pneumococcal conjugate vaccine in the infant immunisation schedule in April 2010. During 2011-12, a study was undertaken to evaluate vaccine antibody responses in infants after completion of the routine primary infant immunisation schedule, which included two doses of meningococcal group C (MenC) conjugate (MCC) vaccine at 3 and 4 months, with no restriction on which of the three licensed MCC vaccines was used for each dose.

Methods: Healthy term infants registered at participating general practices (GPs) in Hertfordshire and Gloucestershire, UK, were recruited prospectively to provide a single blood sample four weeks after primary immunisation, which was administered by the GP surgery. Vaccination history was obtained at blood sampling. MenC serum bactericidal antibody (SBA) and IgG antibodies against *Haemophilus influenzae* b (Hib), pertussis toxin (PT), diphtheria toxoid (DT), tetanus toxoid (TT) and thirteen pneumococcal serotypes were analysed according to MCC vaccines received.

Results: Hib IgG geometric mean concentrations (GMC) were significantly lower in infants receiving a diphtheria cross-reacting material-conjugated MCC (MCC-CRM) vaccine followed by TT-conjugated MCC (MCC-TT) vaccine (n=15; GMC, 0.60 µg/mL; 95% CI, 0.27-1.34) compared to those receiving two MCC-CRM (n=83; GMC, 1.85 µg/mL; 95% CI, 1.23-2.78), two MCC-TT (n=83; GMC, 2.86 µg/mL; 95% CI, 2.02-4.05), or MCC-TT followed by MCC-CRM (n=18; GMC 4.26 µg/mL; 95% CI, 1.94-9.36). The same group also had the lowest MenC SBA geometric mean titres (GMT; 82.0; 95% CI, 39-173) compared to 418 (325-537), 277 (223-344) and 553 (322-949), respectively. Our results indicate that two MCC-TT or MCC-CRM doses, or MCC-TT followed by MCC-CRM together with other routine vaccinations provided adequate protection against MenC in almost all infants. However, in infants receiving MCC-CRM followed by MCC-TT, MenC responses were significantly reduced. In infants receiving mixed MCC vaccine schedules, TT-specific and DT-specific antibody responses also appear to be influenced by which MCC vaccine was administered first.

Conclusion: When several MCC vaccines are available, children requiring more than one dose should receive MCC vaccines with the same carrier protein or, alternatively, receive MCC-TT first wherever possible.

P30

Potential coverage of the BEXSERO® MenB vaccine on non-B meningococci

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Background and Aim: A novel meningococcal serogroup B vaccine, Bexsero®, has been recently licensed in Europe, Australia and Canada. The genes encoding for the main antigens, fHbp, NHBA and NadA are also harbored by meningococci belonging to other serogroups, suggesting that immunisation with Bexsero® could provide protection also against non-B strains. However, unlike serogroup B, a quantitative assay able to measure the relationship between expression level/antigen diversity and bactericidal killing for the non-B strains has yet to be defined. Therefore to evaluate the impact that Bexsero® vaccination may have on non-serogroup B disease in different countries, we tested the serum bactericidal antibody (SBA) activity of sera from vaccinated subjects to kill meningococcal strains belonging to serogroups C, Y and W isolated in Europe and Brazil.

Method: A collection of non-B disease isolates was provided by the reference laboratories in UK (n=78), Germany (n=93), France (n=56) and Brazil (n=41). All strains were isolated in Europe in the years 2007/2008 or in 2012 in the case of Brazil. A subpanel of 147 strains selected to be representative of the genetic diversity for each country, including 20 strains from Brazil, 44 from UK, 52 from Germany and 31 from France was tested in human SBA (hSBA) assay using pooled sera from adolescents and infants.

Results: Sera from subjects vaccinated with the MenB vaccine showed SBA activity against most of the strains tested, corroborating our previous published finding on serogroup X strains. The overall proportion of MenC, Y and W strains killed at hSBA titres ≥8 ranged from about 45% to 90%.

Conclusion: These data clearly demonstrate the impact of Bexsero® on meningococcal disease caused by serogroups other than B.

P31

Expression of *Neisseria meningitidis* antigens of the 4CMenB vaccine; comparison between MATS and FACS for prediction of hSBA

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Background: 4CMenB, a multi-component vaccine against serogroup B meningococcal disease, was recently approved for use in Europe, Canada and Australia. The Meningococcal Antigen Typing System (MATS) has been used to predict 4CMenB strain-coverage worldwide. Little is known about the stability of MATS predictions over time. Also, since MATS is based on bacterial lysates, surface exposure of antigens detected by MATS may be questioned.

Materials and methods: Forty-eight serogroup B meningococcal disease isolates, from a low incidence period in Norway (2005-06), were analyzed using MATS and a Fluorescence-activated cell sorting (FACS) assay based on the same rabbit polyclonal antibodies employed in the MATS sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA). Nucleotide sequences for 4CMenB protein antigens (fHbp, NHBA and NadA) had been previously determined on this panel. A subset of 18 isolates was also tested in a serum bactericidal antibody assay with human complement (hSBA) using pooled infant sera before and after four 4CMenB doses.

Results: MATS predicted 72% (CI 64-94%) 4CMenB strain-coverage for 2005-06, not significantly different from 2007-08 (85%, CI 76–98%, p-value = 0.22, measured in a previous study). No significant difference was observed in strain-coverage predicted for individual vaccine components either (p-values >0.17). FACS detected measurable levels of surface-exposed vaccine antigens on 96% of the strains predicted covered by MATS. However, FACS mean fluorescence intensities (MFIs) were poorly or not correlated to MATS relative potencies (RPs). MATS RP differentiated antigen genotypes of fHbp and NHBA antigens (p-values <0.001), while FACS MFI did not (p-values >0.33). Both assays confirmed low expression of NadA as already observed in other studies. Coverage predicted by MATS and bactericidal killing were significantly associated for fHbp and NHBA (p-values <0.05). Conversely, no threshold on FACS MFI was significantly associated with killing in hSBA (p-values >0.42) for either antigen.

Conclusions: MATS predictions of 4CMenB strain-coverage were stable in Norway over four epidemiological years. Strains predicted to be covered by MATS expressed vaccine antigens detectable by FACS on the bacterial surface. MATS was more sensitive to antigenic sequence variation than FACS performed with the same antisera, and was a better predictor of killing in hSBA.

P32

Immunogenicity and safety of a single dose of CRM-conjugated (Novartis) or TT-conjugated (GSK) meningococcal quadrivalent vaccine in adolescents who were primed with Meningitec™, Menjugate™ or NeisVac-C™ at preschool age

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Background: Protection from childhood meningococcal C (MenC) conjugate (MCC) vaccination is short-lived, thus a booster is recommended in adolescence. Using a quadrivalent meningococcal vaccine as booster should protect adolescents against the additional serogroups A, W135 and Y, but could risk possible interference with the vital MenC response. The primary vaccine given is a key determinant of response to Hib-MenC booster given at age 12 months, but the relationship between primary vaccine and booster vaccines given at adolescence remains unclear. Therefore this study compared responses to two MenACWY vaccines in adolescents who were primed at preschool age with different MCC vaccines.

Methods: 93 adolescents (16-19 years), who at 3-5 years of age had been randomised to receive a single dose of MCC-CRM (Meningitec™ or Menjugate™) or MCC-TT (NeisVac-C™); were now randomised for booster vaccination with either MenACWY-CRM (Menveo™, Novartis) or MenACWY-TT (GlaxoSmithKline). Serotype-specific rabbit-complement serum bactericidal antibodies (rSBA) were measured before and 28 days after vaccination, with titre ≥8 considered protective.

Results: Both MenACWY-CRM and MenACWY-TT induced protective SBA against all four serogroups in ≥98% of adolescents. In an interaction effect, MenACWY-TT generated higher MenC titres in MCC-TT-primed than MCC-CRM-primed subjects ($p = 0.031$). For MenA, MenACWY-CRM induced higher titres ($p = 0.017$). Pre-boosting (≥12 years after primary vaccination), 53% of NeisVac-C (MCC-TT)-primed adolescents still had protective MenC SBA, higher than those primed with MCC-CRM vaccines (Meningitec 42%, Menjugate 24%) ($p = 0.03$).

Conclusion: MenACWY-CRM and MenACWY-TT vaccines induced comparable levels of protective antibodies against all four serotypes in MCC-primed adolescents. Men-ACWY-TT appeared to elicit greater antibody levels in those primed with a TT-conjugated MCC.

P33

Bivalent rLP2086 elicits antibodies in individuals that provide broad coverage against MnB strains expressing prevalent and outbreak-associated fHBP variants

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Background: Pfizer's investigational vaccine, bivalent rLP2086, is composed of two lipidated factor H binding proteins (fHBPs), one representative of subfamily A and one representative of subfamily B fHBPs. Bactericidal antibodies measured in serum bactericidal assays using human complement (hSBAs) have been correlated with protection from meningococcal disease and hSBA responses have been used routinely as surrogates of vaccine efficacy. Global epidemiological studies of fHBP diversity revealed that ~80% of meningococcal disease is caused by strains that express one of 10 prevalent fHBP variants.

Objective: To demonstrate potential breadth of coverage by evaluation of hSBA responses as a surrogate of efficacy in subjects immunized with bivalent rLP2086.

Methods: hSBA responses to *Neisseria meningitidis* serogroup B (MnB) strains expressing the 10 most prevalent fHBP variants in the US and Europe (B24, B16, B44, A22, B03, B09, A12, A19, A05 and A07) in individual human subjects immunized with bivalent rLP2086 were evaluated. Twenty-three MnB test strains were obtained from Pfizer's MnB strain collection, including strains systematically collected from the US and Europe between the years 2000 and 2006. In addition, isolates from recent MnB disease outbreaks were included in the analysis. Matched prevaccination and postvaccination sera were obtained from adolescents and young adults enrolled in clinical studies B1971005, B1971012 or B1971003.

Results: All 23 MnB strains were susceptible in hSBA with sera from individual subjects immunized with bivalent rLP2086. While baseline hSBA seroprotection rates (proportions of subjects achieving hSBA titers ^{3}1:4) were low, robust seroprotection rates were observed in adolescents and young adults with postvaccination sera. Postvaccination seroprotection rates for strains expressing the most prevalent subfamily A and B fHBP variants, B24 and A22, ranged from 81.0% to 100%, and 77.8% to 100% for recent outbreak strains expressing fHBP variants B24 and B153.

Conclusions: Bivalent rLP2086 elicits robust seroprotective hSBA responses in individuals to diverse invasive MnB strains expressing prevalent fHBPs in the US and Europe, as well as newly emerging variants (B153). The data support that bivalent rLP2086 has the potential to provide broad protection of adolescents and young adults from invasive meningococcal serogroup B disease, including disease from recent outbreaks.

P34

A prototype GMMA based vaccine against meningococcal meningitis caused by multiple serogroups in sub-Saharan Africa

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Background: *Neisseria meningitidis* is a major cause of invasive bacterial meningitis in sub-Saharan Africa. Introduction of the group A polysaccharide conjugate vaccine resulted in a remarkable decrease of serogroup A disease but W and X are an ongoing problem. An affordable vaccine covering multiple serogroups in sub-Saharan Africa is still need. Our vaccine approach is based on generalized modules for membrane antigens (GMMA), outer membrane particles released by strains, which have been genetically engineered to have increased blebbing and contain modifications that decrease their reactogenicity and increase their immunogenicity.

Methods: We prepared GMMA from an African serogroup W strain (PorA 1.5,2) engineered to have stable over-expression of fHbp variant 1, ID5, deleted native fHbp v.2, lpxL1, capsule biosynthesis and gna33. We also prepared native OMV from an isogenic mutant with intact gna33. Reactogenicity was analyzed by stimulation of IL-6 released from human PBMCs and the ability to stimulate human Toll-like receptor (TLR) 4. Mice were immunized with two doses of 2.5 ug GMMA based on protein. Control mice were immunized with a group ACWY polysaccharide conjugate vaccine. All vaccines were adsorbed on aluminum hydroxide. Sera were analyzed by anti-fHbp v.1 ELISA and serum bactericidal assays using human complement against invasive serogroup W, X, C and A strains.

Results: Two doses of the GMMA induced high anti-fHbp v.1 antibody responses as measured by ELISA. Bactericidal antibody responses were similar to those induced by the native OMV from the isogenic strain without gna33 KO when measured against genetically diverse serogroup W, X, C and A strains expressing five different fHbp IDs. Bactericidal antibody responses induced by GMMA against the A and W strains were comparable to those induced by the polysaccharide conjugate vaccine. The GMMA with the *lpxL1* KO mutation stimulated the release of 50 to 100-fold less IL-6 from PBMC and stimulated TLR-4 1000- fold less compared with GMMA from a control strain expressing wildtype hexa-acylated lipooligosaccharide.

Conclusion: GMMA from a *N. meningitidis* mutant with over-expressed fHbp and gna33 KO can provide the basis for an affordable, highly immunogenic and broadly protective vaccine against invasive meningococcal meningitis in sub-Saharan Africa.

P35

Characterization of the human antibody repertoire to type B meningoccoccus vaccine

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The first vaccine against type B meningococcus (Bexsero®, Novartis Vaccines) has been recently licensed in Europe, Australia and Canada. Meningococcal factor H-binding protein (fHbp), *Neisseria* heparin-binding antigen (NHBA) and *Neisseria* Adhesin A (NadA) are the key antigens able induce bactericidal antibodies in humans. To gain a comprehensive picture of MenB vaccination-induced antibodies we have sorted single plasma cells circulating 8 days after vaccination in the blood of 3 vaccinees. Based on their variable region repertoire, 41 sequence-unique monoclonal antibodies have been identified, expressed as Fab fragments in ad-hoc *E. coli* expression system and purified. The antigen binding affinity of single Fabs has been determined to each cognate antigen. Twenty-six Fabs were NadA-specific, 14 fHbp-specific and 7 NHBA-specific. We demonstrated that most of the Fabs were able to recognize the native proteins expressed on the surface of different type B meningococcus strains. A deeper characterization of the recognized antigenic regions has been performed by protein chips carrying protein fragments previously identified or predicted as immunoreactive. Based on antigenic fingerprinting we have defined clusters of Fabs that recognize different regions of the proteins. Fabs representative of different clusters have been used to map the epitopes by Hydrogen-Deuterium Exchange analysis assisted by Mass Spectrometry (HDX-MS). Finally the most interesting were selected for full length mAb expression, and their functionality tested in serum bactericidal activity (SBA) assay against a panel of meningococcal strains. These studies represent the first unique approach to map the human immune response to Bexsero® antigens, providing new insights in the dissection of the protective immunity following MenB vaccination.

P36

Using monoclonal antibodies to understand the molecular basis for the cross bactericidal activity of NHBA antigen

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The *Neisseria*l Heparin Binding Antigen (NHBA) is a protective antigen of *N. meningitidis* and one of the components of the recently licensed vaccine Bexsero. NHBA was demonstrated to be an important virulence factor and to be able to elicit a robust immune response in humans against meningococcal strains expressing homologous and heterologous NHBA peptides. In order to better understand the cross protective nature of NHBA we performed a screening with the purpose to obtain monoclonal antibodies (mAbs) to be used as tools in the process of characterization. Mice were immunized with different forms of recombinant NHBA peptide 2. The recombinant proteins sequences are derived from the *N. meningitidis* NZ98/254 strain that is the same peptide present in the Bexsero formulation. Two of the new mAbs displayed bactericidal activity and other two mAbs were bacteriostatic when tested in rSBA against meningococcal strains expressing the homologous protein. Antibody binding and antibody affinity were measured on the recombinant protein by enzyme-linked immunosorbent assay (ELISA) and Surface Plasmon Resonance (SPR) experiments, respectively, whereas Fluorescence-activated cell sorter (FACS) analysis was used to detect the native protein on bacterial surface. Cross-bactericidal activity was assayed by testing the selected mAbs on a panel of MenB strains expressing different NHBA peptides. Finally, a plethora of epitope mapping techniques, including protein chip analysis on NHBA fragments and Hydrogen-Deuterium Exchange (HDX-MS) were adopted in order to precisely map the position of the protective epitopes. By this approach we have mapped protective epitopes on NHBA and studied their possible role on NHBA cross bactericidal activity.

P37

Expediting development of and access to new vaccines

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Background: Recent outbreaks of meningococcal serogroup B (MenB) disease on two US college campuses have heightened public interest about regulatory processes to expedite availability of new vaccines.

Methods: Information about regulatory options available for expediting development of and access to biological products was obtained from search of the Code of Federal Regulations and guidance documents. A summary of key elements and qualifying criteria for processes that have been used for vaccines is presented here.

Results: The FDA expedited programs for serious medical conditions specifically recognize that patients and physicians are generally willing to accept greater risk and potential benefit for investigational treatment for a serious condition where there is an unmet medical need. Statutes enable the FDA to legally grant use of a vaccine for treatment (prevention) of serious conditions before the vaccine is licensed in the US (e.g. expanded access [EA] treatment IND) or before clinical benefit has been verified (e.g. accelerated approval [AA]). Breakthrough therapy (BT) is a new designation for promising products in development which may benefit from additional FDA review resources and intensive regulatory guidance to facilitate planning of clinical trials and timelines for manufacturing processes to fulfill statutory requirements for licensure. With regard to effectiveness, qualifying criteria include:

- Preliminary clinical data showing substantial improvement on a clinically significant endpoint, such as an effect on an established surrogate endpoint (BT).
- Data to support that a surrogate endpoint is reasonably likely to predict clinical benefit (AA), with additional confirmatory study(ies) in the post-marketing period.

For an EA IND, the risk benefit analysis takes into consideration potential risks of the intended vaccine use overall and the assessment that the risks are not unreasonable in the context of the serious condition. IND requirements for IRB-approved protocols and adverse event reporting are the same.

Conclusions: Regulatory mechanisms to expedite development and access to new vaccines are based on a common principle of addressing the treatment of a serious condition and that the potential benefits outweigh their uncertain risks.

P38

Variation in B sequence promotes generation of non-PorB-specific bactericidal antibody

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Despite the introduction of conjugate meningococcal vaccines, infection with *Neisseria meningitidis* remains a leading global health problem. Partly, this is a result of the wide distribution of serogroup B strains which are not included in current formulations due to poor immunogenicity. Accordingly, interest has turned to subunit vaccines for targeting of serogroup B strains. These are composed of outer membrane proteins (OMPs) which are accessible to the immune system and can promote generation of bactericidal antibodies that aid in complement-mediated killing. The porins PorA and PorB are the most abundant OMPs on the meningococcal surface and have been proposed as vaccine candidates. Yet, *porA* is subject to phase variation and strains exhibiting no PorA expression are virulent. In contrast, no isolate has been found that lacks PorB, highlighting its importance in meningococcal survival. We hypothesized that in the absence of PorA, PorB may function as an immunodominant antigen and target of bactericidal activity. To test this, we generated outer membrane vesicles (OMVs) from isogenic strains expressing serotype 4 (Cu385, BB1350) and 15 (MC58) PorB, as well as a naturally occurring (Ch501) and lab-constructed (OCh) serotype 4/15 hybrid, all deleted for porA expression. Rabbits were immunized and sera tested for bactericidal activity. All sera were bactericidal against *porA*- and wild type parental strains, regardless of OMV preparation, with titers to *porA*- strains 4-fold higher compared to *porA*+ strains for 8 of 10 sera. Sera obtained from rabbits immunized with the OCh PorB hybrid strain exhibited an enhanced ability to kill heterologous strains relative to the other four. This activity was independent of anti-PorB antibody, as OMV antisera adsorbed against the corresponding *porB* knockout strain was not bactericidal. These data suggest that the influence of PorB sequence in PorA-independent bactericidal activity is specific to effects on outer membrane composition and/or OMP complex formation. To examine this more closely, we have generated a panel of six MC58-Cu385 hybrid PorB (*porA*+ and *porA*-) strains which we are currently using to examine the effects on OMP expression and cross-protective antibody generation.

P39

Examination of the role of dendritic cells, macrophages and b cells in the function of vaccine adjuvants, including meningococcal porb

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Vaccines are vital in the fight against infectious diseases. Most vaccines are successful because of adjuvants added to their formulations or in the case of live attenuated vaccines because the adjuvants are inherent and are components of the pathogen being recognized by pattern recognition receptors. These adjuvants have been used with little understanding of their mechanisms. PorB, a TLR2 agonist, is the major outer membrane protein of *Neisseria meningitidis* and has potent adjuvant activity with a wide variety of antigens. In this study we investigated the role of individual antigen-presenting cell (APC) types, e.g. B cells, macrophages and dendritic cells (DC) in the adjuvant activity of PorB with comparisons to other vaccine adjuvants in vivo. Current adjuvanted vaccines induce mainly a Th2 type response, which is a less diverse response and does not induce robust cellular immunity. We also examined PorB's ability to induce both Th2 and Th1 type responses. Wild type and mice where MyD88 is either deleted in B cells or DC or macrophages using the Cre/lox system were immunized three times at two week intervals with ovalbumin (OVA) alone or OVA mixed with either PorB, Alum, MF-59, MPL or CpG. Analysis of sera by ELISA indicated that MyD88 is required in B cells, macrophages and dendritic cells for the ability of PorB and other TLR dependent adjuvants to induce high levels of OVA specific-IgG. However, the humoral response was affected to the greatest extent with the deletion of MyD88 in macrophages, as compared to other APC types, when TLR dependent adjuvants were used. PorB was also able to induce both Th1 and Th2 type responses, as determined by serum cytokines measurements and IgG subclasses characterization. Interestingly, the adjuvant activity of MF-59, a MyD88 dependent adjuvant, was not affected by deletion of MyD88 in any of the APC types; however the humoral response was completely abrogated in complete MyD88 knockout mice, suggesting that its adjuvant activity is dependent on MyD88 in other cell types (i.e. stromal cells). These insights will aid in vaccine development by allowing more intelligent and judicious use of these and other vaccine adjuvants.

P40

Examination of the role of meningococcal PorB adjuvant in the induction of vaccine induced immune responses using a system biology approach.

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Vaccines play an important role in the fight against infectious diseases. Most vaccines are successful because of adjuvants added to their formulations or in the case of live attenuated vaccines because the adjuvants are inherent and are components of the pathogen being recognized by pattern recognition receptors. However, these adjuvanted vaccines have been developed empirically, with little understanding of both their mechanism of action as well as the number of immunizations necessary to achieve long lasting protection. There is a great need for better understanding of how vaccine adjuvants and multi-injection schedules. These types of insights would greatly contribute to the rational design and intelligent use of vaccines. In this study, we took a system biology approach where we integrated immunogenetics, immunogenomics, immune profiling and functional studies in order to understand and predict vaccine-induced immune responses. We immunized C57BL6J mice with ovalbumin (OVA) or *Neisseria meningitidis* Porin B (PorB) or OVA were mixed with PorB (antigen+adjuvant) or mock vaccinated with PBS. We observed that they're more robust differential gene expressions with OVA/PorB verses OVA vaccinations. Using the Gene Set Enrichment Analysis (GSEA), we showed that upregulated genes clustered in cell cycle and proliferative processes sets, expression of these genes increased after each immunization. Interestingly, genes that belong TLR signaling pathways, endocytosis, lysosomal degradation, inflammation and cytokines signaling increased in expression after the second immunization and decreased after the first and third immunizations. We also used Ingenuity Pathway Analysis software to analyze the data, the cell cycle and inflammatory response network was the highest scored when comparing OVA/PorB to OVA after the second immunizations. Some of the genes in these pathways are very important in vaccine induce immune responses. We also observed multiple miRNA that were differentially expressed for e.g miR15a was increased after the second immunization with OVA/PorB. This shows that PorB as an adjuvant changes specific genes expression that are related to the immune system as well as modulate the adjuvant induced immune responses.

P41

Pooled hSBA titers predict seroresponse rates of infants vaccinated with 4CMenB

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Background: 4CMenB is a four-component vaccine against serogroup B meningococcal (MenB) disease recently approved for use in Europe, Canada and Australia. The Meningococcal Antigen Typing System (MATS) estimates 4CMenB strain coverage by predicting killing in the Serum Bactericidal Antibody assay with human complement (hSBA) using pooled infant sera. As pooled sera may not reflect individual immune response to vaccination, we studied the diversity of hSBA titers across individual subjects and the relationship with pooled hSBA titers, against a diverse panel of MenB invasive isolates.

Materials and methods: We analyzed: i) individual hSBA titers from 8133 infants vaccinated with 4CMenB in phase III clinical studies against 7 MenB strains, at different vaccination timepoints ii) hSBA titers from 30 infants immunized with 4CMenB, both individual and pooled sera, against 11 diverse MenB strains, and iii) pooled hSBA and MATS data from an epidemiologically representative panel of 40 invasive MenB strains isolated in England & Wales.

Results: Against each strain/timepoint, different proportions of vaccinees mounted a protective immune response (seroresponse rate, SR, 7%-100%). When few subjects responded they had low bactericidal titers. When many subjects responded they had high titers. By strain/timepoint, the titers of seroresponders had compact distributions (average standard deviation = 1.1 base-2 logarithms). Overall, the arithmetic mean of individual hSBA titers predicted well the SR (Pearson's $\rho = 0.81$, p-value < 10^-3). Pooled hSBA titers were linearly correlated with the arithmetic mean of the titers from the individuals composing the pool ($\rho = 0.97$, p-value < 10^-3), and the pooled hSBA titer accurately predicted the SR ($\rho = 0.94$, p-value < 10^-3). Using this predictive relationship on the England & Wales panel, average predicted SR was 77% (interquartile range, IQR: 66-100%) against strains classified as covered by MATS, 39% (IQR: 18-46%) against strains classified as non-covered.

Conclusions: Individual responses to the 4CMenB vaccine are homogeneously distributed and can be predicted from the hSBA titer of the pooled infants serum. 4CMenB strain coverage predicted by MATS is associated with high seroresponse rates, but non-negligible proportions of vaccinees are predicted to mount protective titers also against strains not predicted to be covered by MATS.

P42

Antibody responses in humans after vaccination with a novel serogroup A and W outer membrane vesicle (OMV) vaccine targeted for the African meningitis belt – results from a phase I study in Cuba

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Background: Serogroups A (MenA) and W (MenW) are the main causes of meningococcal disease in sub-Saharan Africa. An affordable conjugate vaccine against MenA disease has been extensively used in the meningitis belt, but MenW continue to cause disease. Outer membrane vesicle (OMV) vaccines have proven to be safe and efficacious to combat serogroup B epidemics, and an OMV vaccine based on outer membrane proteins against MenA and MenW could therefore be an alternative approach for this region. In collaboration between NIPH, Norway and FI, Cuba an affordable AW-OMV vaccine was developed for African countries. The vaccine was tested in a phase I trial in Cuba in 2013 and found to be safe, and we here present the specific antibody responses in sera from vaccinees.

Methods: The vaccine was produced from African MenA and MenW meningococcal strains isolated in Africa by detergent (deoxycholate) extraction, purification of OMVs by gel filtration and adsorption to Al(OH)3. 30 healthy individuals aged 18-50 years were recruited in a controlled, randomized, double-blind phase I study. One group (N=15) received the AW-OMV vaccine (25µg A-OMV + 25µg W-OMV) whereas a control group (N=15) received the B-OMV vaccine VA-MENGOC-BC® (50µg B-OMV). Two doses of vaccine were given 6 weeks apart; serum samples were collected before vaccination and 6 weeks after each dose. IgG responses against live bacteria were analyzed by flow cytometry and by immunoblotting using the individual vaccine OMVs as antigen. Protective antibody responses were measured as serum bactericidal activity using human complement (hSBA) and as opsonophagocytic activity (OPA), both using live bacteria of the vaccine strains as target.

Results: Preliminary results showed increased IgG responses against MenA in all individuals vaccinated with AW vaccine (GMC=19AU/mL before vaccination, GMC=69AU/mL after 2 doses). All AW vaccinees except one showed an IgG response against MenW (GMC=1AU/mL and 16AU/mL before and after vaccination, respectively). The AW vaccine induced a substantial amount of opsonizing antibodies in the majority of vaccinees: MenA: GMT=3 before vaccination, GMT=23 after 2 doses. MenW: GMT= 11 before vaccination, GMT=69 after 2 doses. This indicates that the vaccine induced protective antibodies. Immunoblotting and hSBA analysis are ongoing.

P43

An OMV vaccine derived from a capsular group B meningococcus with constitutive FetA expression: preclinical evaluation of immunogenicity and toxicity

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Introduction: Following the introduction of effective protein-polysaccharide conjugate vaccines against capsular group C meningococcal disease in Europe, meningococci of capsular group B remain a major cause of deaths and sequelae among children under five years of age. The outer membrane proteins (OMPs) PorA and FetA have previously been shown to induce bactericidal antibodies in humans. Despite considerable antigenic variation among PorA and FetA OMPs in meningococci, systematic molecular epidemiological studies revealed a stratified variation of these antigens that is co-evolving with a few dominant hyperinvasive clones causing most of meningococcal disease in Europe over a period of decades.

Methods: We developed a prototype vaccine against capsular group B meningococcal disease based on a *Neisseria meningitidis* isolate genetically engineered to have high and constitutive expression of FetA. The vaccine strain was found to be genetically stable and a small scale GMP process to produce an outer membrane vesicle (OMV) vaccine was developed. Deoxycholate OMVs (dOMVs) extracted from cells cultivated in modified Frantz medium contained 21.8% PorA protein, 7.7 % FetA protein and 0.03 µg lipopolysaccharide per µg protein (3%). The dOMV based vaccine was tested in three mouse species to study the immunological response, and further tested for toxicological profile in New Zealand white rabbits.

Results: Administration of the vaccine, MenPF-1, when given by intramuscular injection on four occasions over a nine week period, was well tolerated in rabbits up to 50 µg/dose; with no evidence of systemic toxicity. Antibodies against FetA and PorA were induced in both mice and rabbits, and were shown to be bactericidal against a panel of strains engineered to elucidate the individual contributions of PorA and FetA specific antibodies.

Conclusion: These results provide the necessary data to support a phase I clinical trial, and a promising alternative for the development of a multicomponent OMV based vaccine able to prevent capsular group B meningococcal disease.

P44

Elimination of meningococcal A epidemics in Africa is within reach

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On behalf of the Meningitis Vaccine Project (MVP) and partners.

The Meningitis Vaccine Project (MVP) was established in 2001 as a partnership between the World Health Organization (WHO) and PATH, funded by the Bill & Melinda Gates Foundation, with the mission to eliminate epidemic meningitis as a public health problem in sub-Saharan Africa through the development, testing, licensure, introduction, and widespread use of affordable meningococcal conjugate vaccines.

Following global international standards, an affordable monovalent group A conjugate vaccine, MenAfriVac® (Serum Institute of India, Ltd), was developed through an innovative public-private partnership. After obtaining marketing authorization from India (December 2009) and WHO prequalification (June 2010), MenAfriVac was introduced at public-health scale in countrywide vaccination campaigns with a single dose among 1- to 29-year-olds.

Since mass introduction campaigns started in 2010, over 153 million persons have received the vaccine in 12 countries of the African meningitis belt: Benin, Burkina Faso, Cameroon, Chad, Ethiopia, Ghana, Mali, Niger, Nigeria, Senegal, Sudan, and The Gambia. Evaluation of vaccine safety and effectiveness consistently demonstrated that MenAfriVac had a good safety profile and a dramatic reduction of carriage and invasive disease in vaccinated districts among both vaccinees and non-vaccinees, thereby suggesting a robust herd protection effect. Mass immunization campaigns will continue until 2016 to cover at-risk populations in the 26 countries where disease burden is greatest. Following the initial mass vaccination campaigns, countries should have the option of protecting new birth cohorts through routine immunization, catch-up campaigns, or a mixed approach so that population protection is maintained in the long term. Thus, in parallel to the large-scale vaccination campaigns, clinical studies have been conducted in infants and toddlers providing comprehensive information on the safety, immunogenicity, immune persistence, and optimal dosage of MenAfriVac when coadministered with other EPI vaccines. One or two vaccine doses administered in infancy could provide sustained and long-lasting protection.

Considering the impressive immediate impact of the MenAfriVac wide-scale vaccination programme in Africa and the promising results from the infant trials conducted in Ghana and Mali, the deadly and devastating meningitis A epidemics in sub-Saharan Africa could soon belong to the past.

P45

Vaccine development using genetic fusions of surface-exposed loops from *Neisseria meningitidis* PorB and TbpA conjugated to the cholera toxin B subunit

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The development of subunit vaccines targeting transmembrane proteins (TMPs) is hampered by their inherent hydrophobicity. Large-scale recombinant production of TMPs often results in inclusion body formation followed by denaturation using high concentrations of chaotropic salts like urea. Once purified, protein refolding into a native state can be a complex process requiring detergents or membranes to keep the TMPs in solution. Native TMP structure is critical if protein-specific conformational antibodies are to be elicited. For vaccine development, a full-length TMP with multiple transmembrane spanning domains may not be an ideal vaccine antigen, as immunodominant epitopes may elicit epitope-specific antibodies that are non-protective, thereby subverting antibody responses to potentially protective and more conserved but immunorecessive epitopes. The study of individual TMP loops or peptides may be advantageous; however they are generally small in size and therefore poorly immunogenic. Conjugation of surface-exposed loops or peptides to carrier proteins, either via chemical conjugation or genetic fusions, has been suggested as a way to overcome the limitations of poor loop/peptide immunogenicity. The non-toxic cholera toxin B subunit (CTB) has been widely used as an adjuvant in animal studies and has been demonstrated to enhance antibody responses to conjugated antigens relative to mixed antigens. CTB is a ring-like structure composed of five identical B subunits that bind together in a non-covalent fashion. Thus CTB constitutes an ideal carrier protein for genetic fusions, as each formed CTB-fusion molecule would ideally contain five attached antigen epitopes. Using *Neisseria*l TbpA we designed expression vectors encoding various loops in frame and downstream of the ctb gene. Upon expression in *Escherichia coli* we were able to purify soluble and stable CTB-Loop chimeric antigens that retained the ability to bind GM1 ganglioside; a characteristic necessary for CTB induced adjuvanticity. In addition, using the crystal structures from *N. meningitidis* PorB, we are developing PorB-specific CTB-loop chimeras. Importantly this approach can be easily adapted to study small loops or peptide antigens from a variety of TMPs for evaluation in immunogenicity and protective efficacy studies against pathogenic *Neisseria*.

P46

Safety and immunogenicity of a serogroup A and W meningococcal outer membrane vesicle vaccine: Results from a Phase I clinical study in Cuban volunteers

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Introduction: Serogroup A and W meningococcal disease causes the highest burden of disease in many African countries. Plain polysaccharide vaccines are available, but they are poorly effective in young children, the highest risk group. Polyvalent conjugate vaccines are considered to be too expensive for Africa. An affordable conjugate polysaccharide vaccine against serogroup A has been extensively used in the meningitis belt, but it is possible other serogroups can emerge as a result of this massive application. Outer membrane vesicle (OMV) vaccines from serogroup B strains have proven to be safe and immunogenic in various epidemic situations. Therefore a bivalent OMV vaccine against serogroups A and W could be an attractive alternative for this region.

Materials and Methods: A phase I clinical trial, was conducted in Cuba with a A+W serogroup OMV vaccine produced at Finlay Institute, Cuba, in collaboration with NIPH. Thirty 18-50 years old, male and female Cuban volunteers, were given the vaccine in a two doses scheme; 15 of them with 25 µg of each OMVs in each dose. Other 15 volunteers received same schedule but VAMENGOC-BC as a control vaccine. Adverse events were monitored one week after each inoculation and immune response were measured by ELISA and serum bactericidal assays (SBA) at the beginning, the day of the second dose and 6 weeks after second dose.

Results: No serious adverse events or other damages to the volunteers due to the vaccine under study were reported during the clinical follow-up period. High pre-vaccination titers against the A strain were observed in most of the Cuban vaccines with 15% >4 fold increase in SBA titers after immunization with 2 doses of the A+W OMV vaccine. The pre-vaccination titers were lower against the W than against the A strain and more than 80 % showed seroconversion by ELISA and bactericidal antibodies determination against serogroup W with the investigational vaccine.

Conclusions: The A+W OMV candidate vaccine have been shown to be safe and highly immunogenic in a phase I clinical trial in Cuban volunteers and may be an alternative, affordable, efficient vaccine against meningococcal disease for Africa.

P47

Epidemic meningococcal meningitis in Africa: success using a Group A conjugate vaccine and a development update on a new pentavalent vaccine (A/C/Y/W/X)

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Background: Group A meningococcal meningitis has long been a vexing public health problem in Sub Saharan Africa. In 2010 MenAfriVac™, a WHO prequalified PsA-TT conjugate vaccine, was successfully introduced and has had a dramatic impact. The vaccine when used in well conducted campaigns aimed at 1-29 year olds has generated herd protection and Group A meningococcal disease has rapidly disappeared. Over 158 million Africans in 14 countries have received a dose of MenAfriVac™.and no major safety issues have been reported. Campaigns will continue through 2016 and birth cohorts will be protected through EPI based routine immunizations and/or catch up campaigns that will begin in 2015. In short, the prospect of eliminating Group A meningococcal infections in Africa is a distinct possibility. Nonetheless, meningitis outbreaks caused by non A meningococcal strains remain a threat for this region and the availability of an affordable, heat stable polyvalent meningococcal conjugate vaccine that includes Group X would be an important product for Africa.

Methods: Meningococcal polysaccharide (PS) manufacturing processes for A, C, Y, W and X were developed and optimized at Serum Institute (SIIL). Purified and sized PS were individually conjugated to either TT or CRM using cyanylation chemistry. Immunogenicity of monovalent and polyvalent meningococcal conjugate vaccines was evaluated in mice and rabbits using a bead based immunoassay for IgG titers and a serum bactericidal assay (rSBA). Conjugate vaccines were thermally stressed for extended periods at 40 C.

Results: Purified PS complied with WHO specifications and NMR analyses confirmed structural identity of the individual PS. Purified cyanylate conjugate vaccines gave yields ≥ 20% and murine immunogenicity studies demonstrated that all conjugates, including Men X, generated strong bactericidal activity. The rabbit SBA and IgG studies consistently showed that a candidate polyvalent vaccine was equivalent or better than a licensed comparator for Groups A, C, Y and W. The PsX-TT conjugate also generated robust SBA titers. An alum adjuvant improved the immunogenicity of the polyvalent vaccine. The freeze dried vaccine formulation was stable at 40 C and retained its ability to elicit robust immune responses.

Conclusions: MenAfriVac™ has had a profound effect on Group A meningococcal infections and a new thermostable polyvalent meningococcal conjugate vaccine that includes Group X will begin clinical trials in 2015.

P48

Immune response against proteoliposomes incorporating the recombinant meningococcal macrophage infectivity potentiator protein (rMIP)

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Currently, the most promising approach for an anti-serogroup B meningococcal vaccine is based on a multicomponent design using several outer membrane proteins and OMVs. However, concerns about the immunogenicity, variability and even toxicity of this approach led us to the search for new immunogenic proteins withless variability that are able to elicit strong responses by themselves. In recent studies, we found that the macrophage infectivity potentiator protein (MIP) could be good candidate vaccine antigen. The MIP gene was cloned from strain H44/76, expressed in *Escherichia coli* and purified using Ni-NTA affinity chromatography. The recombinant protein was incorporated into phosphatidylcholine/cholesterol membranes and treated to form unilamellar proteoliposomes, which were then used to obtain anti-MIP immune sera in mice. The sera were characterized in Western blotting assays against OMV proteins separated by SDS-PAGE and high resolution clear native electrophoresis (hrCNE), and showed reaction with only the MIP protein, either in the homologous or several heterologous strains. The rMIP was highly immunogenic, giving endpoint titres of 1:10.000 in Westen blotting.

Anti-rMIP sera were tested using a serum bactericidal assay (SBA) and flow cytometry to analyse opsonophagocytosis and antibody-mediated deposition of C3b and the membrane attack complex (MAC).

Our results showthat the rMIP induces antibodies that effectively kill the homologous and one of the heterologous strains with SBA titers higher than 1: 256, and show a good bactericidal activity against other two strains. Only one of the strains tested was not killed.

Anti-rMIP sera obtained from proteoliposomes mediated opsonophagocytosis of 4/6 meningococcal strains. Deposition of C3b and C5b-9 was also mediated by rMIP antibodies to 4/6 strains. Results in the flow cytometry assays demonstrated that inclusion of the protein into liposomes increased the immune response obtained when compared with sera obtained using only the rMIP as antigen.

In conclusion, we propose that the meningococcal MIP protein is a good candidate for multicomponent antiserogoup B vaccine.

P49

Meningococcal antigen typing system (MATS) based coverage for Bexsero on invasive MenB strains isolated from infants aged less than one year in Germany 2007-2013

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In 2013, Bexsero® was approved by the European Medicines Agency for immunisation from two months of age upwards to prevent invasive meningococcal serogroup B (MenB) disease (IMD). Before licensure, >1000 MenB isolates collected from 01.07.2007-30.06.2008 in five European countries were analysed by the Meningococcal antigen typing system (MATS) to estimate the proportion of isolates potentially covered by Bexsero® (Vogel et al., 2013), including 222 German MenB strains. MATS combines PorA genotyping with expression of fHbp, NadA, and NHBA as determined by ELISA. Strains are considered covered when the level of expression is above the positive bactericidal threshold (PBT), which is predictive of killing by vaccination-induced bactericidal antibodies (Donnelly et al., 2010). Predicted coverage in all German strains was 82% (95% coverage interval [CI]: 69-92%). It was significantly lower in infants than 1-24 year-olds (64% vs. 90%, p chi224 years (77%, p=0.16). To better characterize predicted coverage of invasive MenB isolates in infants, we analysed a further 148 MenB strains isolated from infants in Germany from 01.07.2008-30.06.2013 by MATS. Predicted coverage was 68% (95% CI: 57-79%), similar to the earlier estimate of 64% (p=0.83). Coverage increased from 59% (95%CI: 45%-78%) in infants aged 0.5) In summary, coverage estimates in infants were stable over time, corroborating the previously observed lower strain coverage by Bexsero® of invasive MenB strains isolated from infants versus older age groups in Germany. While differences between age groups are attenuated if PBT values at the lower range of their 95% confidence intervals (as defined by Plitaykis et al. 2012 based on observed intralaboratory variation for MATS) are used in the calculations, lower coverage estimates are in keeping with an increased genetic finetype diversity observed in infants compared to older children and adolescents (results not shown). Further monitoring of vaccine antigens in meningococci from both unvaccinated and vaccinated IMD cases will be vital to better characterize true vaccine coverage if Bexsero® is widely used.

P50

Engineering antigens derived from transferrin receptors – importance of surrogate host-pathogen systems and an integrated vaccine design and evaluation pipeline

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Due to their surface accessibility and critical role in vivo, transferrin receptors have long been considered important targets for vaccine development. Prior efforts at developing a vaccine against group B meningococci with the surface lipoprotein component, transferrin-binding protein B (TbpB), were initially very encouraging. However, results from a Phase I trial in humans ultimately led to abandonment of the vaccine development program. Although the reasons for no longer pursuing a TbpB-based vaccine were not clearly identified, it is apparent that a blunted response against TbpB in the native host and the degree of cross-protection were important issues. We adopted a structure-based protein engineering approach to design antigens with an enhanced ability to induce an enhanced cross-protective immune response in the native host and, preferably, to eliminate colonization by the targeted bacteria. The need to develop rational strategies for engineering antigens and rapidly assess their impact prompted us to design robust, scalable and integrated methods of evaluation – a vaccine evaluation pipeline – to optimize the antigen engineering process. A robust ELISA assay was developed that involves capture of recombinant TbpBs with a biotinylation tag so that structural genomics methods can be used to generate libraries of antigen variants to screen for cross-reactivity. Methods for generating libraries of strains have been designed so that cross-protection can readily be assessed in in vitro killing assays (SBAs) and animal infection of colonization models. The ability to prevent colonization in a humanized mouse model will be a particularly important feature to optimize. The parallel pursuit of engineered antigens against *N. meningitidis* and porcine pathogens that also possess transferrin receptors enabled us to probe why native TbpB vaccines were less effective in their natural host. A series of site-directed mutants of TbpBs were prepared, evaluated for binding activity by affinity capture methods and biophysical assays and their structures compared to the native protein. The demonstration that a selected site-directed mutant provided superior protection against infection than a commercial vaccine directed against the challenge strain or native TbpB, provides strong evidence that interaction with host Tf interferes with development of an optimal immune response.

P51

Duration of immunity and immunological memory induced by a Brazilian meningococcal C conjugate vaccine

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Introduction: A meningococcal C conjugate vaccine candidate produced by Bio-Manguinhos/Fiocruz (BM), Brazil, by reductive amination using hydrazide-activated tetanus toxoid as carrier protein has proved be safe and immunogenic in phase 1 and phase 2 studies. To follow up on vaccine development, the duration of immunity and immunological memory induced by the candidate vaccine were assessed.

Objectives: To assess the antibody levels 1 year after a single dose and after a booster dose of a Brazilian meningococcal C conjugate vaccine.

Methods: Phase II randomized, single-blinded study, in 258 healthy male and female children from 1 to 9 years of age, vaccinated with one dose in a primary schedule received a booster dose 1 year later: 171 received 0.5 mL of the candidate vaccine and 87 received a reference vaccine, Neisvac-C®. BM candidate vaccine: meningococcal C polysaccharide 10 µg (strain 2135), conjugated to tetanus toxoid 10-30 µg, aluminium hydroxide 0.35 mg (Al+3)/ dose. Serum samples were obtained before and 30 days after revaccination for SBA assay using rabbit complement.

Results: Adhered to protocol 240 children (156 BM vaccine; 84 Neisvac-C®).

Duration of immunity: Seroprotection (rSBA titer ≥ 1:8) dropped from 94.2% after immunization one year before to 19.9% (31/156; 95% CI 13.9; 27.0) for BM vaccine and from 98.3% to 53.6% (45/84; 95% CI 42.4; 64.5) for Neisvac-C® in the same period. Bactericidal geometric mean titers (GMT) fell from 295.1 after immunization to 3.9 one year later for BM vaccine and from 1248.3 to 16.0 for Neisvac-C®.

Immunological memory: After booster dose allchildren were seroprotected in both groups, and bactericidal geometric mean titers (GMT) for C polysaccharide (95% CI) were: BM vaccine, 849.7 (95% CI 742.5; 972.4); Neisvac-C®, 2802.3 (95% CI 2386.1; 3291.0). GMT ratio vaccine in test/reference vaccine: 0.30 (95% CI 0.25; 0.37).

Conclusion: The marked reduction in vaccine-induced immunity after one year and the rapid and intense response to a booster dose suggest that future trials should consider a two-dose immunization schedule. Adjustments in antigen composition of the BM candidate vaccine aiming higher immunogenicity are in progress.

P52

Decline of protective antibodies after serogroup C meningococcal conjugate vaccine in patients with sickle cell disease

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Background: A decline of protective antibody titers after the serogroup C meningococcal conjugate (MCC) vaccine has been demonstrated primarily in healthy children. This should be an issue of concern to risk groups. There are no studies evaluating the persistence of protective antibodies in risk groups, such as sickle cell disease (SCD).

Methods: We conducted a study with 141 SCD patients 2 to 8 years after MCC vaccination. They were distributed according to age at priming in two groups: group I (2 months to < 2 years) and group II (2 to 13 years) and evaluated by years since vaccination (2-3, 4-5 and 6-8). Serum bactericidal antibody titre (rSBA) and specific IgG concentration (ELISA) were measured. The correlate of protection was an rSBA titre of ≥ 8.

Results: In SCD children primed under 2 years with MCC vaccine, only 53,3%, 21,7% and 35,0% had at least SBA ≥ 8 after 2-3 years, 4-5 years and 6-8 years respectively. Seroprotection rates were higher in those primed at ages 2-13 years, being respectively 70, 0%, 45,0% and 53,5% after the same interval since vaccination. Individuals from the group vaccinated 6-8 years ago had received MCC vaccines from three different manufactures and higher seroprotective rates (78,5%) were observed in patients that received MCC-TT vaccine [Baxter] than in those vaccinated with MCC-CRM197 (33, 3% - Novartis, 35, 7%- Pfizer) (p =0.033).

Conclusion: SCD Children aged 2-13 years at MCC vaccination had higher concentrations of bactericidal antibody over time compared to children primed under 2. Protection rates start to fall 2-3 years after vaccination, especially in those primed at younger ages. Seroprotective rates were significantly higher in individuals that received MCC-TT (Baxter) vaccine than in those immunized with MCC-CRM197 (Pfizer or Novartis) vaccines. A booster dose after 3 years is suggested for SCD children.

P53

Timing of adolescent booster after single primary MenCC immunization at young age and the role of saliva in evaluating the effect of vaccination

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Background: Due to waning antibody levels after primary vaccination, several countries are considering the implementation of a Meningococcal serogroup C conjugate (MenCC) booster vaccination in adolescents.

Aim: To establish an appropriate age for an adolescent MenCC booster vaccination.

Methods: Three age-groups were recruited with healthy 10-year-olds (n=91), 12-year-olds (n=91) and 15-year-olds (n=86). All participants were primed with the MenC-PS tetanus toxoid conjugated vaccine 9 years earlier, and received the same MenCC vaccination at the beginning of the study. Blood and saliva samples were collected prior to (T0) and 1 month (T1) and 1 year (T2) after vaccination. Functional antibody levels were measured using the serum bactericidal antibody assay (SBA). MenC-PS specific IgG, IgG subclass, avidity, IgA and IgA subclass levels were measured using a fluorescent-bead-based multiplex immunoassay (MIA).

Results: At T0, 19% of the 10-year-olds still had an SBA titer≥8, compared to 34% of the 12-year-olds (P=0.057) and 45% of the 15-year-olds (P<0.001). All participants developed high MenC-specific serum antibody levels at T1. The high IgG levels after vaccination were mostly caused by a rise in IgG1, although the role of IgG2 seems to increase with age. The booster did not induce an increase in avidity of MenC-specific IgG. At T2, 100% of participants still had an SBA titer≥128, but the 15-year-olds showed the highest protective antibody levels and the lowest decay. MenCC vaccination also induced salivary MenC-specific IgG and IgA responses, mainly of the IgG1 and IgA1 subclass. These levels correlated well with MenC-specific antibody levels in serum.

Conclusion: Nine years after primary MenCC vaccination adolescents develop high protective antibody levels after a booster with good persistence up to 1 year. Our results suggest that persistence of protection increases with the age at which an adolescent booster is administered and that saliva might be used to monitor antibody levels after vaccination.

P54

Predicting serum bactericidal activity with a high-throughput flow-cytometric complement deposition assay using an expanded serum panel

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Serum bactericidal activity (SBA) has long been established as a correlate of protection for capsular polysaccharide and outer membrane vesicle-based meningococcal vaccines, with SBA titres of ≥1:4 measured using human complement established as providing protection. Thus potential vaccine efficacy is widely assessed by the measurement of serum bactericidal antibodies and this method is currently required for vaccine licensure. However, the serum bactericidal assay requires large volumes of sera and can be laborious to perform. For new serogroup B vaccines it is vitally important to determine whether protection will extend to all strains that can cause disease. The limited volume of serum available, particularly from paediatric clinical trials, limits the number of strains that can be assessed. The non-functional MATS assay was introduced to try to answer this problem and while somewhat successful, there are issues surrounding antibody synergy and the comparisons of adolescent and paediatric samples. Thus the development of high-throughput functional assays, which require very low volumes of serum, is important to determine the potential effectiveness of a vaccine in a particular setting.

We have developed a flow cytometric assay measuring antibody-mediated complement deposition. The assay uses fixed meningococci, IgG-depleted human plasma as the complement source and fluorescent-conjugated antibodies to measure deposition of C3b/iC3b and C5b-9 (membrane attack complex) on the surface of *Neisseria meningitidis*. The assay uses a single dilution containing 5µl serum per assay, which is considerably less than that required for a standard SBA. We have analysed antibody-mediated complement deposition and SBA with a panel of 134 human sera and 6 diverse serogroup B meningococcal strains (H44/76, NZ98/254, M01-240364, M01-240355, M01-240101, M00-242922). The correlations between antibody-mediated C5b-9 deposition and SBA titre were significant ($p = <0.01$) for all 6 strains using the Pearson Correlation Coefficient. We have established a cut-off value for C5b-9 deposition that will predict a positive SBA response. Using this value we have demonstrated that this flow cytometric assay has Positive Predictive Value of 96% with a Sensitivity of 82%. This assay has the potential to be even further miniaturised, allowing true high-throughput functional analysis using very small volumes of serum.

P55

Randomized controlled trial comparing the immunogenicity of 3- and 4-dose schedules of a meningococcal MenACWY conjugate vaccine in healthy infants

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Background: In the US, the quadrivalent meningococcal CRM197 glycoconjugate vaccine, MenACWY (Menveo®, Novartis Vaccines), is currently licensed for use in persons 2 months through 55 years of age. In this study, we assessed the immunogenicity of a 4-dose MenACWY vaccination schedule in young infants, compared with that of a 3-dose schedule (NCT01214837).

Methods: In total, 750 healthy full-term infants aged 55–89 days were enrolled and randomized into the following groups: the ACWY3 group (n=250) received MenACWY at 2, 4 and 12 months of age; the ACWY4 group (n=250) received MenACWY at 2, 4, 6 and 12 months of age; and a control group (n=250) received no MenACWY vaccination. All subjects received routine infant vaccinations concomitantly throughout the study. A serum bactericidal assay with human complement (hSBA) was used to measure antibodies against MenACWY antigens, with titers ≥1:8 considered to be seroprotective. Seroprotection per antigen at 13 months of age was considered sufficient if the lower limit of the two-sided 95% confidence interval (CI) for the percentage of subjects with seroprotective titers were >80% against serogroup A and >85% against each of serogroups C, W and Y. Non-inferiority of the 3-dose versus the 4-dose schedule was established if the lower limit of the 95% CI around the difference in percentages of subjects with seroprotective titers against serogroups C, W and Y (ACWY3 minus ACWY4) at 13 months was greater than -10%.

Results: At 13 months of age, the percentages of subjects with seroprotective titers for the 3-dose, 4-dose and control groups respectively, were 88% (95% CI: 82%–93%), 96% (91%–98%) and 1% (0%–5%) for serogroup A, 95% (90%–98%), 99% (95%–100%) and 1% (0%–5%) for serogroup C, 99% (96%–100%), 99% (96%–100%) and 3% (1%–8%) for serogroup W, and 100% (98%–100%), 99% (96%–100%) and 1% (0%–5%) for serogroup Y. Pre-specified sufficiency and non-inferiority criteria were met.

Conclusions: Both schedules led to high percentages of subjects achieving seroprotective titers against each vaccine serogroup following toddler vaccination, with the 3-dose schedule being non-inferior to the 4-dose schedule for serogroups C, W and Y.

P56

Identification of an optimal formulation of MenABCWY vaccine in adolescents using desirability analysis

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Background: Novartis has licensed meningococcal vaccines against serogroups A, C, W and Y (MenACWY-CRM) and serogroup B (4CMenB). We evaluated four investigational formulations of MenABCWY vaccines with intent to select an optimal choice using a desirability index (DI) based on reactogenicity and immunogenicity characteristics (Clinicaltrials.gov NCT01272180).

Methods: Healthy adolescents were randomized into equal groups to receive two doses of different formulations of MenACWY (Menveo®, Novartis Vaccines) and rMenB, with or without Outer Membrane Vesicles (OMV):MenABCWY vaccine (50 µg each of NHBA, NadA and fHbp recombinant protein (RP)), or MenABx2CWY (100 µg of RPs), or MenABCWY+OMV (50 µg RPs and 25 µg OMV), or MenABCWY+¼OMV (50 µg RPs and 6.25 µg OMV), or control vaccines rMenB (50 µg RPs), or one dose of MenACWY followed by placebo.Antibodies against MenABCWY antigens were measured by serum bactericidal assay with human complement (hSBA) at baseline and one month after the second vaccination. The ratios of geometric mean titers (GMT) in subjects receiving MenABCWY versus rMenB against serogroup B test strains, and versus MenACWY against serogroups ACWY were calculated. The GMT ratios and the rates of severe local or severe systemic reactions following vaccination were transformed into desirability indices (DI, value between 0 and 1) based on pre-specified functions.Overall DIs were computed for each of the four MenABCWY formulations based on different component weightings.

Results: Responses against serogroups A,C,W and Y were higher for all MenABCWY formulations compared with MenACWY; DIs for NHBA and fHbp responses were highest in the OMV-containing groups, for NadA response in the MenABx2CWY group, and for PorA in the MenABCWY+OMV group. For the reactogenicity parameters, the highest DIs were observed in the groups who received MenABCWY and MenABx2CWY.Modelling using different weighting scenarios (equal weights, epidemiology-driven weights) indicated a potential advantage of OMV-containing formulations. For the primary weighting schedule the overall DIs were 0.58 (MenABCWY+OMV), 0.56 (MenABCWY+¼OMV), 0.46 (MenABx2CWY) and 0.27 (MenABCWY).

Conclusions: The two investigational MenABCWY formulations containing OMV were selected as candidates for further vaccine development as the model demonstrated they had the most appropriate balance between immunogenicity and reactogenicity, without any identified safety concerns.

P57

Evaluation of meningococcal C conjugate vaccine programs in Canadian children

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Background: The diversity of universal infant meningococcal C conjugate (MenC) immunization programs in Canada is unique among countries providing MenC vaccines and offers a rare opportunity to determine the optimal immunization program. Alberta offers a 3-dose program (2, 4 and 12 months); British Columbia provides 2 doses (2 and 12 months) and Nova Scotia offers 1 dose at 12 months. This analysis of 2 years of follow up data from a 4-year cohort study presents data to assess differences in protection in provinces providing early priming doses in infancy.

Methods: In this prospective comparative cohort study, three similar cohorts of healthy children from 1, 2 and 3 dose programs were enrolled prior to the 12 month MenC dose and immunized with MenC-Tetanus conjugate. All sera were assayed for serogroup C bactericidal activity using standardized procedures with rabbit as the exogenous complement source. SBA was measured at baseline (12 months of age) and 1 month after the 12 month MenC dose (13 months of age) and 2 years later (36 months of age). SBA titers >= 1:8 were considered protective.

Results: Intent to treat results at 36 months of age were available for 384 subjects (1 dose = 119; 2 dose = 119; 3 dose = 146). 57 (13%) were lost to follow up or missing a blood sample at 36 months of age. Gender and age of all subjects were similar at each center and remained similar at the 36 month follow up visit. Protective titers at 36 months were significantly different between the 1 dose and 2 or 3 dose programs: 1 dose 92% (95% CI 86% - 96%) vs. 99% (95%-100%) with 2 doses and 100% (97% – 100%) with 3 doses. GMT were 12.1 (10.8-13.5), 32.4 (28.9-36.2) and 50.6 (45.7-55.9) in the 1, 2 and 3 dose programs respectively.

Conclusion: At 36 months of age, there was little difference in the proportions with protective titers between the 1, 2 and 3 dose groups. The majority of children retained protected titers.

P58

Fibroblast Growth Factor1 is required for optimal meningococcal invasion into human brain microvascular endothelial cells

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Neisseria meningitidis (meningococcus) is an obligate human commensal bacterium that can cause meningitis and sepsis. Crossing the Blood-Brain Barrier (BBB) is a crucial step in the development of meningitis, but the mechanisms used by the meningococcus to achieve this are not fully understood. The aim of this study was to investigate the role of the Fibroblast Growth Factor1-IIIc isoform (FGFR1-IIIc) in the attachment to, and invasion of, Human Brain Microvascular endothelial cells (HBMECs) by *N. meningitidis*. Confocal microscopy showed that micro-colonies of adhered *N. meningitidis* recruit activated FGFR1. Direct interaction between meningococci and the extracellular domain of FGFR1-IIIc was demonstrated by ELISA, confirming the ability of this bacterium to bind FGFR1-IIIc. Other bacterial meningeal pathogens, including *Streptococcus pneumoniae* and *Haemophilus influenzae*, did not bind this receptor confirming the specificity of the interaction. siRNA knock-down of FGFR1 resulted in a significant reduction in adherence and a dramatic reduction in invasion of meningococci into HBMECs, demonstrating that the receptor plays a crucial role in invasion of meningococci into this cell type. This study has identified a host receptor molecule, FGFR1, which plays an important role in the attachment to, and invasion of, human endothelial cells and thus improves our understanding of the pathogenesis of this important human pathogen.

P59

Establishment of the gonorrhea mouse model for pre-clinical testing of antimicrobial agents against gonorrhea

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The recent emergence of resistance to the extended-spectrum cephalosporins in *Neisseria gonorrhoeae* (Gc) has led to the call for new antibiotics against gonorrhea. The 17β-estradiol-treated mouse model of Gc genital tract infection is well-characterized with respect to kinetics of infection, host responses, and the importance of several virulence factors in Gc adaptation in vivo. This model has also been extensively used to test gonorrhea vaccines and topically applied vaginal microbicides, and more recently, novel therapeutic compounds that show efficacy against Gc in vitro. Here we describe the protocol that we have established for testing new antibiotics in this model and demonstrate that strain H041, which is a ceftriaxone-resistant pharyngeal isolate isolated in 2009, is infectious in mice and can be used as a challenge strain for product testing. The basic protocol designed to test the in vivo efficacy of compounds against Gc is as follows. Groups of estradiol-treated female BALB/c mice are inoculated vaginally with the dose of Gc that infects 80-100% of mice. Mice are cultured on days 1 and 2 post-inoculation to confirm infection, and test compounds are administered on the afternoon of the second day of infection. Control groups receive a single dose of ceftriaxone (15 mg/kg) via i.p. injection, the compound vehicle, or are left untreated. The number of colony forming units (CFU) per ml vaginal swab suspension and duration of colonization is then determined over an eight day period. With this protocol, 100% of mice given ceftriaxone clear infection within 24-48 hrs, and at least 80% of untreated mice remain colonized for an average of 10 days. Thus far, all strains used in the mouse model are susceptible to fluoroquinolones and extended-spectrum cephalosporins, and do not represent the strains that are of concern today. To expand the choice of challenge strains, mice were inoculated with strain H041. The duration of colonization and the number of CFU recovered was similar to that observed with other strains. Infectious dose studies on strain H041 and dose response studies with ceftriaxone and cefixime against sensitive and resistant Gc strains are underway to better define the model for antibiotic testing.

P60

Development of a broth microdilution assay for determination of in vitro susceptibility of *Neisseria gonorrhoeae*

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Background: The ability to address drug resistant *Neisseria gonorrhoeae*, which the CDC has identified as an immediate public health threat, is hindered by a lack of efficient tools to support drug discovery. The Clinical Laboratory Standards Institute (CLSI) recommends an agar dilution method for determining susceptibility. However, this method is not amenable to a high throughput approach severely limiting drug discovery efforts. To address this problem a broth microdilution assay was developed to enable rapid screening for growth inhibitors.

Methods: The broth microdilution method was based upon the standard CLSI broth microdilution method. The media was changed from MHB II to a GC broth base supplemented with metals, vitamins, and carbon sources. While this change allowed for growth of N. gonorrhoeae it was not sufficient to allow for standard minimum inhibitory concentration (MIC) endpoint analysis. To overcome the inability to determine endpoint by eye, Alamar Blue™ (AB) was added 1 hour prior to determining MIC. The analysis of % AB reduction allowed for efficient and reproducible MIC endpoint analysis.

Results: MIC values for multiple classes of antibiotics against *N. gonorrhoeae* ATCC 49226, such as ceftriaxone, penicillin, erythromycin, tetracycline, and ciprofloxacin, matched the ranges established for the agar dilution assay by the CLSI. Spectinomycin was an exception with MIC values typically elevated two-fold over the CLSI QC range. 100% growth signal was >20 fold over background. Isolates such as wild type FA19 and efflux over-expressing MS11 also performed consistently in this assay.

Conclusions: A 96 well MIC assay in broth for *N. gonorrhoeae* that is amenable to automated handling and higher throughput was developed. The reduction in time required per sample, the amount of compound needed per assay, and the increase in number strains able to be tested will allow for significant increase in the ability to process new therapeutics against *N. gonorrhoeae*. Ultimately, this approach may accelerate efforts to discover new anti-gonococcal therapeutics.

P61

In vitro activities of the novel bicyclolides modithromycin (EDP-420, EP-013420, S-013420) and EDP-322 against multidrug resistant clinical *Neisseria gonorrhoeae* isolates and international reference strains

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Objectives: New antimicrobials are essential to prevent gonorrhoea becoming an untreatable infection. Herein, the in vitro activities of the novel bicyclolides, belonging to the macrolide class, modithromycin (EDP-420, EP-013420, S-013420) and EDP-322 against *Neisseria gonorrhoeae* strains were investigated and compared to antimicrobials currently or previously recommended for treatment of gonorrhoea.

Methods: MICs (mg/L) were determined using agar dilution method (modithromycin and EDP-322) or Etest (seven antimicrobials) for a large geographically, temporally and genetically diverse collection of clinical *N. gonorrhoeae* isolates (n=225) and international reference strains (n=29), including diverse multidrug and extensively-drug resistant isolates.

Results: The MIC range, modal MIC, MIC50, and MIC90 of modithromycin and EDP-322 were 0.004-256 mg/L, 0.25 mg/L, 0.25 mg/L, 1 mg/L and 0.008-16 mg/L, 0.5 mg/L, 0.5 mg/L, 1 mg/L, respectively. The activities of modithromycin and EDP-322 were mainly superior to those of azithromycin and additional antimicrobials investigated. There was no cross-resistance with cefixime or ceftriaxone.

Conclusions: Modithromycin and EDP-322 exhibited high levels of in vitro activity against *N. gonorrhoeae*, including isolates resistant to azithromycin, cefixime, ceftriaxone, spectinomycin, ampicillin, tetracycline, and ciprofloxacin. However, some cross-resistance with high-level azithromycin resistant strains (MIC=4096 mg/L) was observed. Modithromycin and EDP-322 could be effective options for treatment of gonorrhoea, particularly for cases resistant to extended-spectrum cephalosporins and potentially as a part of a combination antimicrobial regimen. It is important to evaluate further the in vitro selection, in vivo emergence and mechanisms of resistance, pharmacokinetics/pharmacodynamics in humans, optimal dosing, and perform appropriate randomized controlled clinical trials.

P62

High in vitro activity of the novel spiropyrimidinetrione AZD0914, a DNA gyrase inhibitor, against multidrug resistant *Neisseria gonorrhoeae* isolates suggests a new effective option for oral treatment of gonorrhea

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Neisseria gonorrhoeae resistance to most antimicrobials has spread globally, and recently, the first gonococcal strains highly resistant to extended-spectrum cephalosporins (ESCs) were described. Untreatable gonorrhea might arise since ESCs are the last options for first-line antimicrobial monotherapy, and new treatment options are essential. The in vitro activity of the novel spiropyrimidinetrione AZD0914 (DNA gyrase inhibitor) against gonococci was investigated and compared to antimicrobials currently or previously recommended for treatment.
MICs were determined using agar dilution (AZD0914) or Etest (seven antimicrobials) against clinical *N. gonorrhoeae* isolates and international reference strains (n=250), including strains with diverse multidrug and extensive-drug resistance. Regions of the *gyrA* and *gyrB* genes were sequenced.
The MIC range, MIC50, and MIC90 of AZD0914 was 0.004 to 0.25 µg/ml, 0.125 µg/ml, and 0.25 µg/ml, respectively. The MICs of AZD0914 were substantially lower than those of the fluoroquinolone ciprofloxacin and most other antimicrobials examined. The isolates with resistance to ESCs showed low MICs of AZD0914 (0.06-0.125 µg/ml). Sequence analysis confirmed that fluoroquinolone resistance mutations in the *gyrA* gene did not affect the MICs of AZD0914 and no AZD0914 resistance mutations were found in the *gyrB* gene. Overall, the in vitro activity of AZD0914 was high against both antimicrobial susceptible and resistant gonococcal isolates, indicating a lack of cross-resistance to other antimicrobials. These in vitro results suggest that AZD0914 should be evaluated in additional studies including: in vitro selection, in vivo emergence and mechanisms of resistance, pharmacokinetics/pharmacodynamics in humans, optimal dosing, and performance in appropriate randomized and controlled clinical trials.

P63

The neisserial LOS phosphoethanolamine transferase: crystal structure, catalytic function and progress on drug design

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In recent times, *Neisseria gonorrhoeae*, the causative agent of gonohorrea has become increasingly resistant to antibiotic treatment. Due to the community burden of disease, estimated to be around 62 million new cases per annum, there is an increasing urgency to examine new treatment strategies. Endotoxins from *Neisseria gonorrhoeae* are composed of a Lipid A moiety, which is modified by the addition of phosphoethanolamine (PEA) by lipopolysaccharide phosphoethanolamine transferase (LptA). Various studies have provided evidence that the PEA decoration of lipid A plays a vital role in determining the interaction of the pathogen with the host innate immune system and disease progression.

We hypothesise that inhibition of LptA can extend the effectiveness of currently existing antibiotics by improving clearance of *N. gonorrhoeae* by the human innate immune response. Therefore, the structure of LptA has been solved with the aim of pursuing a structure guided drug design approach to develop inhibitors. Full length LptA has been crystallized and a model resolved to 2.75Å. The crystal structure provides insights into the mechanism of substrate binding and suggests possible conformational changes during catalysis. The protein consists of an N-terminal transmembrane (TM) domain and a periplasmic globular domain. The electron density map reveals eight alpha-helices in the TM domain. Five of these helices are oriented parallel/antiparallel to each other and are the expected length for TM helices. A sixth helix, which bridges the TM domain and the soluble domain is oriented approximately 90° to the membrane surface. The position of this helix relative to the 5-TM helices suggests it may lie buried within the membrane. The active site of the soluble domain, containing the bound Zn2+ ion, is oriented towards the TM helices with the bridging helix positioned a short distance away. It is bounded on two sides by two smaller helices which are localized in a loop between two TM helices. A pipeline for the discovery of inhibitors using fragment based drug design is underway.

P64

Identification of putative drug targets and functional annotation of hypothetical proteins of *Neisseria gonorrhoeae* using bioinformatics tools

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As 'a paradigm of classical Venereology' [1] for many decades, the second most common sexually transmitted infection of bacterial origin is Gonorrhea caused by *N. gonorrhoeae*. Hitherto, most important challenge is the emergence of multidrug resistant *N. gonorrhoeae*, which is the major reason for public concern. In order to have an improved treatment regime, it is therefore very crucial to identify the factors which are involved in the virulence and resistance of the bacteria and could be employed as potent drug and vaccine candidates.

In the present study, we used 204 hypothetical proteins (HPs) of *Neisseria gonorrhoeae* FA1090 (whole genome sequence retrieved from NCBI) to identify the sub cellular localization and virulent factors using bioinformatics tools available in public domain. We found 140 HPs in cytoplasm using four different tools (PSORTB, PSLpred, CELLO, cell-ploc). Out of which 6HPs (NGO0883, NGO1163, NGO1186, NGO1593, NGO1604, NGO1723) were found to be virulent by using VCIMpred and virulentpred.

In future, we will use these six HPs to identify binding pockets of drugs using docking studies. Knowledge of docking analysis will facilitate identification of potential therapeutic drug targets.

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P65

Novel antimicrobial agents against *Neisseria gonorrhoeae* from extracts of natural products.

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Neisseria gonorrhoeae is the aetiologic agent of the sexual transmitted disease gonorrhoea. *N. gonorrhoeae* is already resistant to many antimicrobial agents and isolation of strains that have resistance to first-line treatment antimicrobials is becoming more common. Novel treatment options have to be identified in a post-antimicrobial era to either treat or prevent *N. gonorrhoeae* infection. Selected oils (flax seed, hemp, and coconut oil) and essential oils (Black pepper, citronella, lemongrass, marjoram, and spearmint) were tested for their antimicrobial properties. The oils were also treated with a purified lipase. To assay antimicrobial activity, the disc inhibition test (DIT), a widely used method used for sensitivity testing, was used with modifications to accommodate these natural products. In addition, log reduction assays were conducted, which were used to measure the bactericidal activity of the natural products. The untreated flax seed oil, hemp oil, and coconut oil were not bactericidal nor did they prevent growth of the bacteria. Treatment of these three oils with a lipase produced large DIT zones of up to 35 mm when tested undiluted. However, only coconut oil was bactericidal. Addition of a 1/100 dilution of the lipase-treated coconut oil was enough to kill 107 gonococcal cells within two minutes. The essential oils, diluted 1/100, produced DIT zones of up to 20 mm in diameter. All inhibited growth and the order of strength of inhibition was: lemongrass > black pepper > citronella > spearmint > marjoram. All essential oils were bactericidal, with lemongrass essential oil still being effective down to a concentration of 1/4000. The order of bactericidal strength was lemongrass > citronella > spearmint > marjoram > black pepper. The lipase-treated coconut oil shows the most promise out of the oils as a possible antimicrobial agent. From of the essential oils, extracts from the plant genus *Cymbogon* (citronella and lemongrass) have the greatest anti-gonococcal activity.

P66

Carbohydrate transport and metabolism in *Neisseria meningitidis*

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Background: The exclusively human pathogen *Neisseria meningitidis* is a β-proteobacterium that possesses an incomplete PEP: carbohydrate phosphotransferase system (PTS) composed of the proteins PtsP, NPr and two EIIAs and a homologue of HPr kinase/phosphorylase. The corresponding genes are organized on two different loci (ptsN-hprK and npr-ptsP-ptsM). Lacking EIIB and EIIC components, this PTS cannot transport carbon source and was therefore proposed to carry out only regulatory functions. *N. meningitidis* contains potential permeases for lactate, glucose, maltose and gluconate. However, it lacks the glycolytic enzyme phosphofructokinase and the two saccharides as well as gluconate are probably metabolized via the Entner Doudoroff or pentose phosphate pathways.

Methods and Results: We constructed mutants of the permeases GlcP (glucose), MalU (maltose) and GntP (gluconate). The mutants had lost the capacity to transport the corresponding carbon source. In addition, expression of the *N. meningitidis glcP* or *malU* genes in *E. coli* strains lacking the proteins for glucose or maltose transport, respectively, restored the corresponding uptake function. Similarly, expression of *N. meningitidis gntP* in a *Bacillus subtilis gntP* mutant restored growth on gluconate. Surprisingly, the *glcP* mutant exhibited highly elevated synthesis of the capsule for reasons not yet understood. We also cloned the genes for eight metabolic enzymes, including glucose kinase, gluconate kinase, β-phosphoglucomutase, gluconate-6-P dehydrogenase, phosphogluconate dehydratase, ribulose-5-P 3-epimerase, ribulose-5-P isomerase and fructose-1,6-bisphosphate aldolase into a His-tag expression vector, purified the proteins and determined their activities by carrying out appropriate spectrophotometric assays.

Conclusions: We could identify the *N. meningitidis* uptake systems for the glucose, maltose and gluconate. Our results suggest that in *N. meningitidis* these carbon sources are mainly metabolized via the Entner Doudoroff and pentose phosphate pathways, the role of the uptake in meningococcal virulence will be explored according to the availability of these carbon sources in different sites during the infection.

P67

Pyrophosphate-mediated iron acquisition from transferrin in *Neisseria meningitidis* does not require TonB activity

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The ability to acquire iron from various sources has been demonstrated to be a major determinant in the pathogenesis of *Neisseria meningitidis*. Outside the cells, iron is bound to transferrin in serum, or to lactoferrin in mucosal secretions. Meningococci can extract iron from iron-loaded human transferrin by the TbpA/TbpB outer membrane complex. Moreover, *N. meningitidis* expresses the LbpA/LbpB outer membrane complex, which can extract iron from iron-loaded human lactoferrin. Iron transport through the outer membrane requires energy provided by the ExbB-ExbD-TonB complex. After transportation through the outer membrane, iron is bound by periplasmic protein FbpA and is addressed to the FbpBC inner membrane transporter. Iron-complexing compounds like citrate and pyrophosphate have been shown to support meningococcal growth ex vivo. The use of iron pyrophosphate as an iron source by *N. meningitidis* was previously described, but has not been investigated. Pyrophosphate was shown to participate in iron transfer from transferrin to ferritin. In this report, we investigated the use of ferric pyrophosphate as an iron source by *N. meningitidis* both ex vivo and in a mouse model. We showed that pyrophosphate was able to sustain *N. meningitidis* growth when desferal was used as an iron chelator. Addition of a pyrophosphate analogue to bacterial suspension at millimolar concentrations supported *N. meningitidis* survival in the mouse model. Finally, we show that pyrophosphate enabled TonB-independent ex vivo use of iron-loaded human or bovine transferrin as an iron source by *N. meningitidis*. Our data suggest that, in addition to acquiring iron through sophisticated systems, *N. meningitidis* is able to use simple strategies to acquire iron from a wide range of sources so as to sustain bacterial survival.

P68

Differences in AmpG sequence increase pro-inflammatory peptidoglycan fragment release in *Neisseria gonorrhoeae* compared to *Neisseria meningitidis*

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Neisseria gonorrhoeae (GC) is an obligate human pathogen and the causative agent of the sexually transmitted disease gonorrhea. *Neisseria meningitidis* (MC) is most well known for causing bacterial meningitis, although it can and does maintain asymptomatic colonization in the nasopharynx of healthy humans. Both GC and MC release pro-inflammatory peptidoglycan (PG) fragments, although MC releases less PG monomers compared to GC. Limiting the release of PG monomers may dampen the inflammatory response, allowing *N. meningitidis* to persist in the nasopharynx as an asymptomatic colonizer instead of being cleared by the immune system. This difference in PG monomer release is in part mediated by AmpG, a permease that transports PG fragments into the cytoplasm for recycling. It is possible that MC expresses more AmpG protein, which enhances PG fragment recycling and reduces PG fragment release. However, we found that *N. meningitidis* expresses AmpG at lower levels compared to *N. gonorrhoeae*. Expression of the meningococcal AmpG protein in GC reduces PG monomer release by GC, although not to the levels of wild type MC. We performed chimeric analysis and found that differences in three residues near the C-terminus of AmpG (M391, R398 and I402 in GC; L391, Q398 and A402 in MC) impact PG recycling efficiency. Mutation of residue 391 or residue 402 is sufficient to reduce PG fragment release in GC; however, mutation of all three residues is required to reduce PG monomer release to a similar level to when the entire gene was replaced. These residues may work together to directly bind PG fragments, or affect efficiency of PG transport through the transmembrane channel, or interact with periplasmic peptidoglycanases. More work is needed to elucidate the role of these residues in modulating AmpG function.

P69

Common cell shape evolution of nasopharyngeal pathogens

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Respiratory infectious diseases are the third cause of worldwide death. The nasopharynx is the portal of entry and the ecological niche of many microorganisms of which, some are pathogenic to humans, such as *Neisseria meningitidis* (Nm) and *Moraxella catarrhalis*. These microbes possess several surface structures that interact with the actors of the innate immune system. In our attempt to understand the past evolution of these bacteria and their adaption to the nasopharynx, we first studied differences in cell wall structure, one of the strongest immune-modulator. We were able to show a modification of peptidoglycan composition (increased proportion of pentapeptides) and a cell shape change from rod to cocci selected along the past evolution of Nm. Using genomic comparison across species, we correlated the emergence of the new cell shape (cocci) with the deletion, from the genome of Nm ancestor, of only one gene: yacF. Moreover, the reconstruction of this genetic deletion in a bacterium harboring the ancestral version of the locus together with the analysis of the peptidoglycan structure, suggest that this gene is coordinating the transition from cell elongation to cell division. Accompanying the loss of *yacF,* the elongation machinery was also lost by some of the descendants leading to the change in the peptidoglycan structure observed in Nm. Finally, the same evolution was observed for the ancestor of *M. catarrhalis*. This suggests a strong selection of these genetics events during the colonization of the nasopharynx that may have been driven by the interaction with the immune system by reducing the cellular surface exposed to immune attacks without reducing the intracellular storage capacity.

P70

Generation of a finished Swedish serogroup Y genome belonging to the ST-23 clonal complex

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In the last ten years, there has been an increase in serogroup Y meningococcal disease in Europe and the United States. Scandinavian countries, in particular, have reported some of the highest rates of disease with, for example, the relative proportion of serogroup Y cases reported to be 49% in Sweden in 2012. Characterisation by MLST and sequencing of the *fetA, fHbp, penA, porA* and *porB* genes revealed that three serogroup Y clones were responsible for disease in Sweden with isolates containing the distinct genotype: Y:P1.5-2, 10-1: F4-1: ST-23 (cc23) along with *porB* allele 3-36, *fHbp* allele 25 and *penA* allele 22 the most predominant, known as clone YI.

In this study, a finished YI genome is presented. It was sequenced using a combination of next generation sequencing technologies including PacBio RS, Illumina GAIIx and MiSeq platforms which can generate up to 10kb, 150bp and 250bp reads respectively. An assortment of contigs were produced following assembly with PacBio RS generating a single 2,187,871bp contig, GAIIx producing 163 contigs with a mean size of 12,964bp and MiSeq generating 302 contigs with a mean size of 6,981bp. The PacBio assembly was annotated using the rapid bacterial annotation software Prokka and further curated with Artemis. This was then used as a reference genome for comparison with GAIIx and MiSeq assemblies to check for inconsistencies.

Preliminary results indicate that this genome contains 2, 063 coding sequences and demonstrates the potential of using PacBio RS to generate high quality finished bacterial genomes.

P71

Persistent meningococcal carriage is associated with low levels of genetic variation

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Neisseria meningitidis is frequently found on the nasopharyngeal tissues of humans where it persists in an asymptomatic state known as carriage. Meningococcal strains can persist in individuals for several months resulting in elicitation of antigen-specific immune responses. It is hypothesised that adaptation to fluctuations in the host environment is mediated in part by genetic variation. We have recently shown that eight outer membrane proteins undergo frequent phase-variable changes in gene expression, due to mutation in simple sequence repeats, during host persistence but the extent of other types of genetic variation is unclear. Multiple isolates of *Neisseria meningitidis* were collected from individuals subject to persistence colonisation for up to six months. Whole genome sequences were generated by Illumina methodologies for pairs of isolates from 25 carriers. Sequences were assembled using Velvet and analysed using programs in the BIGSdb database. Allelic variation in coding sequences occurred in an average of seven genes per pair of isolates. PCR-based experimental analyses of multiple isolates per time point demonstrated that some mutations were infrequent whilst others exhibited sweeps in later time points. Genome sequences were also obtained from ten isolates per time point at four isolation times from one carrier. Allelic variation in these isolates is also limited and often involves indels rather than point mutations. Intragenomic recombination in the opa gene is also infrequent. We conclude that allelic variation is not a major factor contributing to persistent carriage of meningococci in the upper respiratory tract of humans.

P72

Comparative analysis of Canadian *Neisseria meningitidis* serogroup B isolates

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Background: The Canadian Immunization Monitoring Program Active (IMPACT) surveillance network is population-based and includes over 50% of adults and children in Canada. Meningococcal serogroup B (MenB) is endemic in Canada, accounting for 40%-80% of invasive meningococcal disease depending upon age and region. A MenB vaccine (bivalent rLP2086) containing two factor H-binding proteins (fHBP) representative of subfamilies A and B is under review for licensure in the US. We examined MenB strains circulating in Canada to determine the distribution of fHBP variants and compared this to MenB strains from other geographical regions.

Methods: The study included a total of 226 invasive MenB strains collected through the Canadian IMPACT surveillance network between 2006-2011. Isolates were typed for fHBP and other genetic markers at the National Microbiology Laboratory. Comparative analysis to a MenB reference collection of contemporary isolates from the US and regions in Europe was conducted.

Results: All Canadian MenB strains express fHBP. Overall 37% of MenB strains were from fHBP subfamily A and, like the US MenB isolates, the percent of fHBP subfamily A was greater in Canada than in Europe. Similar to other geographic regions, Canadian children <1 year and adults >65 years had the highest proportion of subfamily A strains. About 80% of the 2010-2011 Canadian MenB isolates expressed fHBP variants that were among the ten most prevalent variants in the MenB reference collection. B44 and A22 were the most prevalent fHBP variants. Each year over half of the Canadian MenB strains were classified as either cc269 or cc41/44. fHBP variant B44 and cc269 were also prevalent in Europe, but were not prominent in US MenB isolates.

Conclusions: Surveillance of circulating invasive MenB isolates is critical to predict and then monitor the efficacy of vaccines. Previous studies demonstrated that the candidate bivalent fHBP vaccine induces antibodies that kill MenB isolates that express diverse subfamily A or B fHBP variants in the MenB reference collection. Analysis of MenB epidemiology and fHBP variant distribution in Canada predicts isolates will be susceptible to bivalent rLP2086 immune sera and suggests that the vaccine will provide broad coverage against serogroup B disease.

P73

Whole genome comparison of *Neisseria meningitidis* isolates from patients and their close family contacts using gene-by-gene analysis

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Whole genome sequencing (WGS) is increasingly being applied in meningococcal epidemiology, for example in the Meningitis Research Foundation Meningococcus Genome Library (MRF MGL) (http://www.meningitis.org/research/genome). Important applications will include very precise contact tracing and an improved understanding of the molecular changes between carriage and invasion. For both of these goals, it is necessary to understand meningococcal variation among and within patients at WGS resolution using a databank of previously analysed isolates. We have contributed to this endeavour this by a reanalysis of a unique set of meningococci isolated from cases of disease and their familial contacts in Greece previously studied by MLST.

The WGSs of 47 isolates from 15 patients and 32 of their close family contacts were compared using a gene-by-gene approach, including analysis of the meningococcal core genome (cgMLST), ribosomal protein sequence type (rST) and whole genome (wgMLST) analysis. Visualization of the data with the NeighborNet algorithm, executed in SplitsTree 4 in the PubMLT.org/*Neisseria* website, established the relationships of the samples at WGS resolution to each other and to other genomes in the database, including the MRF MGL. This analysis precisely and exhaustively identified the numbers, identity, and mechanisms of variation of the genes that differed among these very closely related isolates and demonstrates that multiple closely related but distinct strains were simultaneously present.

P74

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

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The PubMLST *Neisseria* database has hosted allelic diversity data for multilocus sequence typing (MLST) and major antigens for the past decade and currently has records for approximately 28,000 isolates sampled from over 100 countries. In anticipation of the increased availability of whole genome sequence data, the PubMLST database began hosting genomic data in 2009.

The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates using the BIGSdb platform. It now has whole genome data for over 3150 isolates, a large proportion belonging to the Meningitis Research Foundation Genome Library. Loci have been defined within the database for most of the core genome and parts of the accessory genome in a manner analogous to MLST so that sequence diversity is now indexed at >1800 loci with each unique gene sequence assigned an allele number.

The platform facilitates many applications including:

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

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

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

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

P75

A new *Neisseria* species?

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Background: Among the *Neisseria* isolates collected during a carriage study in Burkina in 2009-2012, we identified one *Neisseria lactamica* isolate with a new sequence type (ST): ST-9100. Phylogenetic analysis based on multilocus sequence typing data showed that ST-9100 was distant from all *N. lactamica* and *N. meningitidis* STs, except for a small cluster of four STs, represented by the genotypes of isolates assigned to *N. lactamica*. We further explored the taxonomical position of this ST-9100 isolate and related isolates using whole genome sequencing.

Methods: Three isolates belonging to this small cluster were available to us: one from Burkina Faso (ST-9100) and two from Oman (ST-3783 and ST-3784). The isolates had been collected in carriage studies and classified as *N. lactamica*. Standard microbiological and enzymatic tests were repeated and whole genome sequencing was performed using a GS junior apparatus. We compared their genome sequences with those from a pool of 44 isolates from 7 different *Neisseria* species, the same pool as in Bennet et al, J Clin Microbiol 2014, using the Bacterial Isolate Genome Sequence Database (BIGSdb).

Results: Phenotypic properties of the three isolates were consistent with *N. lactamica* (β- galactosidase-positive, γ-glutamyl transferase-negative, oxidase-positive, Gram-negative diplococci), with the exception of the carbohydrate utilization test where none of the isolates produced acid on agar containing lactose, glucose or maltose.

Splits trees based on genome sequences confirmed that the three studied isolates were clustered, ST-3783 and ST-3784 being closer to one another than to ST-9100. This cluster was visibly distant from all other *Neisseria* species, the closest being *N. lactamica*.

The lacY (putative lactose permease) in the three isolates had identical polymorphism pattern: 10 point mutations were found at the same positions. This could negatively impact the efficacy of lactose permease and explain the lack of acid formation from lactose despite having functional β-galactosidase.

Conclusions: The phenotypic and genotypic resemblance between these three carriage isolates and their significant genetic distance to other known *Neisseria* species support the hypothesis that isolates assigned to ST-9100, ST-3783 and ST-3784 might belong to a new *Neisseria* species.

P76

A genetic characterization of the 4CmenB vaccine antigen genes in serogroup B isolates from invasive meningococcal disease (IMD) cases in the four Western Canadian provinces of British Colombia, Alberta , Saskatchewan and Manitoba from 2009 to 2013

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Background: Invasive meningococcal disease (IMD) due to serogroup B isolates remains a significant threat to the health of canadians since the introduction of effective conjugate vaccines against other IMD serogroups (A,C,Y.W135). The capsule of serogroup B (MenB) isolates is not immunogenic due to its cross reactivity with self- component which makes MenB capsule not a suitable candidate for vaccine production. Reverse vaccinology has led to the discovery of newer vaccine candidates such as factor H binding protein (fHbp), *Neisseria* heparin binding antigen (NHBA) and *Neisseria* adhesion A (NadA). These are formulated together with an Outer Membrane Vesicle (OMV) vaccine containing the PorA antigen P1.4 in a new MenB vaccine Bexsero or 4CMenB. Objective: To characterize the 4CMenB vaccine antigen genes in all invasive MenB isolates in western Canada from 2009 to 2013, and to review the distribution of the different predicted vaccine peptide types among the MenB isolates from each of the 4 provinces in western Canada according to their MLST types.

Methods: The 4CMenB vaccine antigen genes (fHbp, nhba, nadA, and porA) were sequenced and identified according to the information described in the *Neisseria*.org website.

Results: 69 invasive MenB isolates from western Canada were grouped by MLST into 40 sequence types (STs), with 31 of them classified into 8 clonal complexes (ccs), with the remaining 9 STs were not associated with any known cc. There were 31 porA genotypes , 26 fHbp peptide types and 23 NHBA peptide types while nadA gene was absent in most strains except in 5 isolates which were predicted to have 2 different NadA peptide types .

Conclusions: Genetic characterization of the 4CMenB vaccine antigen genes MenB isolates from Western Canada showed the diversity of the vaccine antigen peptide types. Of the 69 MenB isolates studied, 2 isolates with genes that predicted fHbp peptide 1 while genes from 26 isolates predicted fHbp variant 1 peptides. 9 isolates encoded NHBA peptide 2 and 5 isolates encoded for NadA-1 peptide but no isolate encoded for NadA-2 and NadA-3 peptides.

P77

Genetic analysis and quantitation of factor H binding protein expression in US invasive meningococcal serogroup B isolates from population-based active bacterial core surveillance (2010-2012)

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Background: Bivalent rLP2086 is a vaccine under development for the prevention of invasive meningococcal serogroup B (MnB) disease. The vaccine is composed of two lipidated factor H binding proteins (fHBPs), one each from subfamily A and subfamily B. Bivalent rLP2086 recently obtained "Breakthrough Therapy" designation from the FDA and a BLA filing under Accelerated Approval regulations is underway. Comprehensive studies of MnB isolates collected from 2000 to 2008 to assess fHBP diversity (Murphy, Wang) were used to estimate the potential broad coverage of bivalent rLP2086 against MnB invasive disease strains. Similar analyses of contemporary US isolates have now been conducted to confirm earlier findings.

Objective: To evaluate the prevalence, genetic diversity, molecular epidemiological features and expression of the fHBP vaccine antigen in US isolates collected through Active Bacterial Core surveillance (ABCs) sites, from 2010-2012 in comparison to MnB strains collected prior to 2010 from the US and European countries.

Methods: Invasive *Neisseria meningitidis* isolates (all serogroups, n=232) from the ABCs were collected during 2010-2012. The genotype of fHBP and additional epidemiological markers in the 72 MnB strain subset was determined. In addition, the surface expression level of fHBP was determined using the validated MEASURE assay.

Results: Meningococcal serogroups Y (38%), B (31%) and C (22%) account for >90% of the isolate collection. All MnB isolates code for a full length fhbp gene and, consistent with strains collected prior to 2010, the majority are fHBP subfamily B variants. Using a flow cytometry based assay (MEASURE), surface expression of fHBP was detected for over 97% of the MnB isolates. Common molecular epidemiological markers (e.g., CC and PorA type) were not predictive of the fHBP variant expressed, and there were no notable differences in CC or PorA type profiles compared to the analysis of MnB isolates in strain collections that predate 2010.

Conclusions: The epidemiology of fHBP variant distribution, fHBP surface expression level and associated molecular epidemiological characteristics has remained essentially unchanged in the contemporary US MnB strains compared to strains collected prior to 2010. These data further support that bivalent rLP2086 has the potential for broad coverage against serogroup B disease.

P78

Exploring the evolution of three pandemic waves of serogroup A meningococci using whole-genome analysis and mathematical modelling: how important is immune escape?

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Background: Serogroup A meningococci of the ST-5 clonal complex have given rise to three pandemics over the past 50 years, and represented a major cause of meningococcal disease in sub-Saharan Africa. Despite a succession of different sequence types (STs), the diversification of this lineage between the three pandemics has not yet been studied at a whole-genome scale.

Methods: We analysed 153 meningococcal genomes sampled between 1963 and 2011, using the Genome Comparator Tool (pubmlst.org), to identify genetic changes specific to each pandemic wave and delineate the evolution of this global lineage over time. We also developed a deterministic mathematical model, in which strains comprised antigenic as well as metabolic and virulence genomic elements, to explore the changing properties of prevailing pandemic strains.

Results: A large number of genetic differences between isolates from the three pandemics were identified, with alleles at 112, 69 and 94 loci unique to the first, second and third pandemic waves, respectively. Of these, several previously unidentified antigenic differences were found. However, the proportion of allelic differences among antigenic genes was noticeably low (< 3%), while metabolic (45%) and genetic information-processing (15%) differences were most frequent (35% of the differences were either uncharacterised/associated with other functions). In line with this, simulations of our model showed successive replacement by strains with increasing metabolic fitness but similar antigenic properties. Of particular note, preliminary tests of selection suggested neutral changes at 60% of loci and strong purifying selection at 40% (mean DN/DS < 0.12). Recombination analyses suggest that many of the observed allelic changes result from genetic exchange from both within and outside the ST-5 complex, including *N. lactamica* and *N. subflava*.

Conclusions: The observed differences in antigenic alleles between pandemic waves supports previous work that immune escape represents a primary mechanism underlying the emergence of epidemic clones. However, here we use whole-genome data and theoretical methods to show that metabolic genes may also play an important role in this pattern of emergence. Furthermore, the large number of allelic changes between the genomes, coupled with the strong purifying selection observed, suggests that these pandemic strains could predate the earliest isolate (1963).

P79

Structural insight into the translocation mechanism of the zinc-uptake receptor ZnuD, a vaccine candidate against *Neisseria meningitidis*

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Transition metals are nutrients of critical importance for all living organisms due to the essential role they play in many crucial biological processes. The essential trace element zinc is the second most abundant transition metal in mammals. Approximately 9% and 5% of the entire eukaryotic and prokaryotic proteome are bound to zinc, respectively. Exploiting the strict requirement of pathogenic bacteria for transition metals, the mammalian hosts sequester these elements to prevent colonization of potential bacterial or fungal invaders, a process called nutritional immunity. To overcome zinc restriction, Neisseriaceae express high-affinity zinc transporters, including Calprotectin-binding protein A (CbpA) and the Zinc uptake components ZnuABCD[1]. To better understand zinc acquisition within *Neisseria meningitidis*, we have used a combination of X-ray crystallography and molecular dynamic simulation to gain molecular mechanistic details of the 82 kDa outer membrane gated pore ZnuD, a transporter belonging to the TonB-dependent receptor family. By solving structures of ZnuD in three different states, we reveal translocation intermediates identifying multiple zinc-binding sites on the external and periplasmic sides connected by an obstructed channel. Our data reveal the extensive dynamic flexibility of the external loops of ZnuD, which undergo a drastic surface modification of the receptor upon substrate binding with the remodeling of alpha helical sequence into beta strand motifs. Since immunization with ZnuD elicits anti-bactericidal antibodies against N. meningitis[2], we have mapped and characterized the previously identified antigenic regions on the surface of the ZnuD structure. This has allowed us to use structure-based design of ZnuD derivatives to examine their protective effects on *N. meningitidis* infection in the CEACAM-humanized mouse model.

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P80

Temperature reduction stimulates proteomic changes enhancing meningococcal biofilm formation

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Introduction: *Neisseria meningitidis* (N.m., meningococci) frequently colonizes the human nasopharynx. Biofilm formation is thought to support colonization. Within the nasopharynx, meningococci encounter temperatures below 37°C. The adaptation of Nm to reduced temperatures is unexplored.

Methods: Using stable isotope labeling of Nm with 15N and highly sensitive and highly accurate mass spectrometry, we compared the proteomes of Nm grown at 37°C and 32°C.

Results: We found the highest rate of deregulated proteins between 37°C and 32°C among outer membrane (OM) and periplasmic proteins, whereas inner membrane and cytosolic proteins were rarely affected. Nm grown at 32°C showed markedly elevated levels of biofilm formation and autoaggregation. By testing knockout mutants we found that the three highly upregulated OM proteins all contributed to biofilm formation and autoaggregation. Furthermore, an autotransporter protease, which suppresses Nm biofilm formation by the cleavage of adhesins, was less expressed at 32°C. Finally, we showed that increase of biofilm formation at slightly decreased temperatures was a general phenomenon of Nm and the related species *Neisseria gonorrhoeae* and *Neisseria lactamica*.

Conclusion: Temperature reduction from 37°C to 32°C influences the OM proteome, e.g. the vaccine antigen NHBA. The changes consecutively elevate biofilm formation and autoaggregation in Nm. Temperatures in the human nasopharynx might therefore serve Nm as a signal for the recognition and colonization of its desired niche.

P81

Contribution of the TbpA loop 3 helix to transferrin-iron acquisition by *Neisseria gonorrhoeae*

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While evolving to exclusively infect humans, *Neisseria gonorrhoeae* has adopted unique methods of obtaining nutrients, including hijacking human proteins to acquire the essential nutrient iron. Although *N. gonorrhoeae* can use several host proteins to fulfill its iron needs, the most ubiquitously expressed system is the human transferrin-iron acquisition system, composed of an integral, outer-membrane, TonB dependent transporter (TbpA), and a surface-exposed lipoprotein (TbpB). Expression of the Tbps is necessary to initiate infection in human males, and these iron-regulated proteins are not subject to antigenic or phase variation, making them ideal vaccine candidates. In addition, the Tbps have been crystallized, which provides valuable information towards the goal of using these proteins as vaccine antigens. With a nutritional vaccine in mind, this project aims to assess a putatively key functional domain, the TbpA loop 3 helix, to determine if specific residues of this region are essential for iron piracy from the host. This study employs site specific mutagenesis of TbpA, followed by ELISA based ligand binding assays and radioactive iron uptake assays to characterize the role of the loop 3 helix. Thus far, all single amino acid substitution mutants remained capable of growing on Tf as the sole iron source. However, some single point mutations reduced binding of Tf to TbpA by greater than 50%. Iron uptake assays are ongoing. So far, the data supports the hypothesis that the TbpA loop 3 helix plays a significant role in iron hijacking from human transferrin. However, this region is not the sole contributor to Tf binding or iron acquisition. The results suggest that the loop 3 region of TbpA could be a good vaccine target in combination with other antigens, and that blocking this and other functional epitopes of TbpA could limit nutrient access and hinder bacterial growth.

P82

Binding of the RmpM to porin complexes depends on a six amino acid peptide of its N-terminal domain

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The *Neisseria meningitidis* RmpM is considered to be a peptidoglycan-binding protein anchoring the outer membrane protein complexes to the peptidoglycan layer. The RmpM sequence can be divided in a 22-residue signal sequence, an N-terminal domain (RMn) of approximately 40 amino acids, followed by a 20-residue hinge region and a C-terminal domain (RMc) of approximately 150 amino acids. Structural homology to E.coli OmpA has indicated the RmpM C-terminal domain contains a putative peptidoglycan binding region (Grizot S. et al, 2004). Although the structure and function of the N-terminal domain is not yet determined it is hypothesized to be an involved in binding to outer membrane proteins. We have previously shown that RmpM binds to outer membrane porin complexes through the PorB (Freixeiro P. et al, 2013). We aimed to investigate the domains in the RmpM protein responsible for interaction with PorB and identify regions essential for binding.

To study the potential interaction between RmpM and porin, recombinant RmpM, PorA and PorB were incorporated into liposomes. Protein complexes in these proteoliposomes were analyzed by chemical cross-linking, Sulfo-SBED. Results confirmed that porin complexes bind to RmpM through PorB. To identify which RmpM domain was responsible for formation of the complex, recombinant rRMc and rRMn domains were incorporated into liposomes with rPorA or rPorB. Subsequent analysis of the liposomes by hrCNE identified a protein complex containing PorB and rRMn but there was no evidence of any interactions with rRMc. To further characterize the PorB-RMn interaction, two truncated N-terminal domain proteins, (rRMnt1 and rRMnt2) were obtained by deleting the first 9 and 15 amino acids respectively. These truncated proteins were expressed and incorporated into liposomes together with rPorB. Following hrCNE, rRMnt1:rPorB complexes were detected but rRMnt2:rPorB complexes were not observed.

In conclusion, RmpM binds to porin complexes through interaction between PorB and its N-terminal domain, where a six amino acid sequence is crucial for this interaction.

P83

Structural characterisation of HpuA

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To utilise heme from haemoglobin(hb)-haptoglobin(hp) complexes as an iron source, pathogenic *Neisseria* species express a bipartite outer membrane receptor called HpuAB. HpuB is a TonB dependent receptor predicted to form a plug domain within a 22-stranded beta barrel that facilitates active transport of heme across the outer membrane in combination with the TonB complex and the inner membrane proton gradient. HpuA is an associated lipoprotein shown to be required for heme uptake by the receptor. While unable to interact directly with the host proteins, HpuA is essential for the high-affinity interaction between the receptor and hb and hb-hp complexes. We have determined and will present the crystal structure of an HpuA homologue at 1.7 Å resolution. The protein comprises a single globular domain with the C-terminal 160 residues forming a small beta barrel. One strand of the barrel contains a 16 residue insertion extending as a loop almost perpendicular to the barrel axis. The N-terminal half of the protein forms a beta sandwich, with one sheet of the sandwich packing against the surface of the C-terminal barrel and the outer sheet exhibiting a highly twisted structure with two long strands extending 20 Å from the core of the protein. This component and a similarly protruding loop at one end of the beta-barrel are key features of the structure; they show a high degree of variability, indicative of being under immune selection, and presumably act to draw the attention of the host's adaptive immune system away from the functioning core of the protein. Three dimensional similarity searches suggest transferrin receptor component TbpB is the closest structural homologue to HpuA, reinforcing the theory that the TbpAB and HpuAB systems share a common ancestor. However, in contrast to HpuA, TbpB acts by preferentially binding iron loaded human transferrin, thereby providing specificity of the receptor complex and reducing unproductive binding of apo-transferrin. Further microbiology and biochemical studies of HpuA including structure-guided mutagenesis will be required to elucidate its role in vivo.

P84

SLAM2: an outer membrane transporter required for the display of surface lipoprotein HpuA

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Surface lipoproteins (SLP's) are a class of proteins that have an N-terminal lipid head group, which anchors them to the outer surface of the outer membrane. They are found in certain species of gram-negative bacteria, including *Neisseria meningitidis* (Nme) and *Neisseria Gonorrhoeae* (Ngo), and are involved in key pathogenic processes such as nutrient acquisition and immune evasion. Hence, SLP's have been studied extensively as vaccine targets in *Neisseria*. SLP's, like other bacterial lipoproteins utilize the Lol system to traverse the periplasm, however it is not known how the SLP's are trafficked to the cell surface in *Neisseria* [1]. Herein we report an outer membrane protein that is required for transporting a *Neisseria*l SLP, called HpuA, to the *Neisseria*l cell surface. Hemoglobin-haptoglobin binding protein A or HpuA, is involved in acquisition of iron from hemoglobin and hemoglobin-haptoglobin complex [2]. The HpuA transporter we identified contains an N-terminal soluble domain with tetratricopeptide (TPR) repeats and a C-terminal membrane bound beta-barrel domain with 14 predicted beta strands. It shares significant sequence similarity and similar domain architecture as surface-lipoprotein assembly modulator (SLAM), a TbpB transporter previously identified in our lab. Hence, we have named the HpuA transporter SLAM2 and TbpB transporter as SLAM1. We have further confirmed that SLAM2 can transport HpuA to the outer surface of *E. coli*, however it is unable to transport TbpB, which is the substrate of SLAM1 and vice versa. This suggests that SLAM1 and SLAM2 transport different SLPs and hence may be required at different stages of Nme infection. Using crystallography and functional transport assays we have begun to elucidate the structure, mechanism and specificity-determining factor present on SLAM1 and SLAM2 that allow them to differentiate between different SLPs. References: 1. Okuda S, Tokuda H, Lipoprotein sorting in Bacteria, Annual Review of Microbiology, Vol. 65: 239-259 (Oct 2011) 2. Rohde KH, Dyer DW, Analysis of haptoglobin and hemoglobin-haptoglobin interactions with the *Neisseria meningitidis* TonB-dependent receptor HpuAB by flow cytometry, Infect Immun, 72(5): 2494-506 (May 2004).

P85

The role of gonococcal TonB-dependent transporters, TdfH and TdfJ in heme and zinc acquisition

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The function of gonococcal TonB-dependent Transporters, TdfH and TdfJ has remained elusive since their identification. Although TdfH shares limited sequence similarity with the hemophore receptor, HasR of S. marcescens, it is not regulated by iron in *Neisseria gonorrhoeae*. Conflicting reports regarding gonococcal TdfJ and its meningococcal homologue ZnuD have suggested that TdfJ is iron and zinc regulated and capable of binding heme. To explore the potential of redundant heme receptors, we constructed double mutants unable to express either tdfH or tdfJ. We found that the double and isogenic mutants were not defective for heme-dependent growth. Growth of tdfH and tdfJ isogenic and double mutants with heme was also assessed in a hemH mutant background incapable of heme biosynthesis. The mutants did not display a heme-dependent growth defect compared to the parental strain. We evaluated TdfJ and TdfH expression in the presence of zinc and the zinc specific chelator, TPEN, and have found that both TdfH and TdfJ are zinc-repressed. We evaluated the growth of gonococcal tdfH and tdfJ isogenic mutants under conditions of zinc limitation and found that neither mutant was defective for growth when compared to wild type. We also evaluated the growth of tdfH and tdfJ mutants in the presence of Zn-loaded calprotectin, a protein highly expressed in neutrophils with anti-microbial activity due to its Zn-chelating ability. We found that only the tdfH mutant was defective for growth in the presence of calprotectin. We are currently constructing a complemented derivative of the tdfH mutant and will evaluate its ability restore growth in the presence of calprotectin. We are also evaluating the ability of TdfH to specifically bind calprotectin. These results suggest that TdfH may be important for overcoming calprotectin mediated nutritional immunity in the human host.

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Applications of high-resolution MALDI-TOF mass spectrometry to analysis of intact lipooligosaccharides (LOS) from *Neisseria meningitidis*

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Background: The LOS of *N. meningitidis* is a major cell-surface component that is antigenic and inflammatory. Previous studies have shown that LOS aids the bacteria in escaping surveillance of the immune system. Our work has focused on understanding the relationship between the structure of the LOS and its bioactivity. Characterizing intact LOS is challenging from an analytical perspective due to the amphipathic nature of the molecule, that bears a hydrophobic lipid A moiety and a hydrophilic oligosaccharide.

Methods: We have recently adapted our methods for analysis of intact *Neisseria*l LOS by negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) using a nitrogen gas UV laser and linear TOF in order to incorporate reflectron-based TOF mass analysis using a state-of-the-art Waters' Synapt G2 HDMS with a solid-state UV neodymium-doped yttrium aluminium garnet (Nd:Yag) laser. The high-resolution capability and high sensitivity afforded by the orthogonal TOF mass analyzer produces monoisotopic mass resolution of intact and fragment ions of the LOS. This instrument also is equipped for ion mobility spectrometry (IMS) enabling gas phase resolution of molecules by size and shape, which can be followed by subsequent fragmentation for MS/MS analysis.

Results: The methods we have adapted enable acquisition of spectra that contain molecular ions for intact LOS, and prompt fragment ions produced in the source for the lipid A and oligosaccharide. Using the Synapt, MS/MS analysis can be performed on selected parent ions directly after selection or following IMS. Fragmenting after IMS provides cleaner MS/MS spectra of separated components, free of peaks arising from interfering species that would otherwise be in the selection window. Using high mass resolution and accuracy coupled with knowledge of previously described constituents, has enabled us to propose compositions for *Neisseria*l LOS from a single spectrum. We will present examples of spectra illustrating the utility of high-resolution MALDI-TOF in deciphering LOS structures as part of our quest to correlate them with the pathogenicity of *Neisseria* species.

Conclusions: Application of highly sensitive, high-resolution MALDI-TOF mass spectrometry to analysis of intact LOS produces content rich spectra that enable rapid assignment of structure composition.

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Biochemical and biophysical analysis of the interactions between lactoferrin-binding protein B and lactoferrin

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The iron-binding glycoproteins transferrin (Tf) and lactoferrin (Lf) are captured by specific surface receptors in pathogenic *Neisseria* in order to acquire iron that is essential for their growth and survival. The surface receptors are comprised of a bi-lobed surface accessible lipoprotein, transferrin or lactoferrin binding protein B (TbpB or LbpB) and a TonB-dependent, integral outer membrane protein, transferrin or lactoferrin binding protein A (TbpA or LbpA). A combination of biochemical, biophysical and molecular biology approaches have provided considerable insights into the role of TbpB in the iron acquisition process. TbpB initially captures holo Tf by binding to the C-lobe with the 'cap region' of the N-lobe and delivers Tf to TbpA through an interaction involving the N-terminal anchor peptide region. Recently it has been proposed that LbpB plays a similar role in the process of iron acquisition from Lf, in spite of its demonstrated function to protect *N. meningitidis* from cationic antimicrobial peptides. This study was initiated to probe the interaction between Lf and LbpB and determine the extent to which it parallels the interaction between Tf and TbpB. The interaction of intact LbpB, the individual C- and N-lobes and derivatives lacking one or more of the clusters of negatively charged regions with Lf was probed by affinity capture and biophysical approaches. In contrast to TbpB, there was no preferential association of Lf with the N-lobe of LbpB. The interaction between LbpB and Lf is more sensitive to binding conditions (pH, salt concentrations) and is optimum with intact LbpB compared to the individual lobes. These results indicate that the Tf-TbpB interaction differs dramatically from the Lf-LbpB interaction and raises the question of how critical LbpB is to the iron acquisition process from Lf.

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Neisserial Opa protein interactions with human receptors in vitro and in vivo

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Background: *Neisseria*l opacity-associated (Opa) proteins are β-barrel integral outer membrane proteins that bind to human host cell receptors, the most common being members of the CEACAM (carcino-embryonic antigen-like cellular adhesion molecule) family. Within the Opa family, the 8-stranded barrel of the protein has a highly conserved sequence, the first loop exhibits slight sequence diversity, and the fourth loop is conserved. Within the second and third loops there is high sequence variability, which is primarily responsible for host cell receptor specificity.

Objective: While the Opa protein family has conserved structural elements, the molecular determinants of the receptor interactions are unknown. We seek to compare the function of a variety of Opa proteins in vivo (expressed natively in *Neisseria gonorrhoeae* (Gc), to the outer membrane of *Escherichia coli*), and in vitro (reconstituted into liposomes) in order to define how changes in Opa sequence and structure inform receptor binding.

Methods: Opa proteins from Gc strains MS11 and FA1090 were expressed in Gc by selection for phase-ON Opa expression states or by expressing non-phase-variable opa alleles in an opa-deficient Gc background. The same Opa proteins were expressed both to the outer membrane of *E. coli*, and recombinantly expressed and purified from *E. coli* and reconstituted into predominantly 1,2-dimyristoyl-sn-glycero-3-phosphocholine liposomes. Opa protein binding to the N-terminal domain of human CEACAMs was qualitatively assessed with centrifugal pull-down assays and western blots, and binding affinities were quantitated by fluorescence polarization.

Results and Conclusions: Opa60, a CEACAM binding Opa from MS11, was efficiently refolded into liposomes. Akin to Opa60-expressing Gc and recombinant Opa60-*E. coli*, Opa60 liposomes retained the ability to interact with N-CEACAM1. Opa60 displayed a tight binding affinity to the N-terminal domain of CEACAM1 (approximately 5 nM). Similar measurements are being made for other Opa proteins and the N-terminal domains of other human CEACAMs. We will determine how these measured affinities correspond to differences in bacterial adhesion and/or invasion of human CEACAM-expressing cells. This work establishes a platform for investigating the specificity and selectivity of Opa proteins for their cognate receptors, in order to reveal how the varied expression of these virulence factors contributes to *Neisseria*l infectivity.

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Analysis of *Neisseria meningitidis* PorB extracellular loops potentially implicated in TLR2 recognition.

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Among *Neisseria*e species, *N. meningitidis* and *N. gonorrhoeae* are human pathogens and the causative agents of bacterial meningitis and gonorrhoea, respectively. PorB is a pan-*Neisseria*e outer-membrane trimeric porin with a β-barrel structure and 8 surface-exposed hydrophobic regions (Loops). In the bacterial membrane, PorB mediates diffusive transport of essential molecules. When interacting with host cells, PorB induces host innate immunity responses via Toll-like receptor 2 (TLR2)-mediated signalling. The molecular mechanism of PorB/TLR2 interaction is still under investigation, but it is thought that it occurs though electrostatic interactions between the receptor ectodomain region and the surface-exposed loop regions of PorB. Strain-specific PorB loop sequence variability influences its binding affinity for TLR2 and subsequent cell activation levels. Our crystal structure studies of PorB from *N. meningitidis* serogroup B, strain 8765, and of a hybrid PorB molecule, in which amino acids in L7 have been mutated based on the corresponding L7 sequence of *N. lactamica* PorB, show dramatic conformation and charge changes. These changes not only affect the conformation of L7, but also extend to L5, possibly influencing the overall charge “environment” of the hypothetical PorB/TLR2 interaction site. We hypothesize that such changes explain the reduced magnitude of TLR2-dependent cell responses in response to the PorB mutants in vitro. Structure analysis of additional PorB mutants in which loop charges have been altered, and the observation of variable levels of TLR2-dependent activity in vitro further describes the importance of loop structure for PorB-mediated TLR2-dependent signalling.

P90

Glycan binding by *Neisseria meningitidis* and the meningococcal serogroup B vaccine antigen NHBA

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A multicomponent vaccine for meningococcal serogroup B (MenB) was recently approved for use in Europe, Australia and Canada. One of the key antigens in this vaccine is a glycan binding lectin known as neisserial heparin binding antigen (NHBA). Understanding the molecular nature of such glycan-lectin interactions between *Neisseria meningitidis* and host cells is necessary for a better understanding of meningococcal virulence mechanisms.

Previous studies have shown that NHBA binds the glycosaminoglycan heparin, through an Arginine rich region. Two proteases, the phase variable meningococcal NalP and human lactoferrin have been shown to cleave NHBA upstream and downstream of the central Arg-rich region respectively. Using glycan array technology we have investigated NHBA binding to other, non-heparin, glycans. The full-length wild type protein, mutant proteins lacking the arginine-motif, and N-terminal and C-terminal fragments, were used to determine the binding affinities and kinetics of these interactions as well as the regions of NHBA involved in glycan binding.

Glycan array analysis ofthe encapsulated MC58 wild type strain revealed binding of the organism to more than 30 of the 364 structures printed on the glycan array. Comparison of glycan binding profiles of *N. meningitidis* wild type and ΔNHBA mutant strains revealed a loss of binding to heparin by the NHBA mutant cells. However, the ΔNHBA mutant strain still bound several glycan structures, including sulpho Lewis a and H blood group antigen, indicating that other lectins are responsible for interactions with these glycans. A series of mutant strains lacking major outer membrane structures are being analysed to identify the lectins responsible for these interactions.

P91

Compensatory mutations in *Neisseria meningitidis* factor H binding protein: implications for immune escape and evolution

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Background: Factor H binding protein (FHbp) is the major ligand in *Neisseria meningitidis* for human complement component H (hFH), which is a down-regulator of the complement alternative pathway. Antibodies to FHbp elicit complement-dependent bactericidal activity. Antibodies directed towards the hFH-FHbp interacting surface are also able to inhibit the binding of hFH, rendering the bacteria more susceptible to killing. FHbp is a variable antigen that exists in two sub-families (A and B) or three variant groups (v.1-3). Furthermore, as FHbp variability is mostly located in the surface that interacts with hFH, ligand binding determinants and critical epitopes targeted by bactericidal antibodies are sub-family or variant group specific. We hypothesized that variation that would decrease hFH binding could be compensated by mutations elsewhere in the protein that allow retention of hFH binding.

Methods: We used a previously described mutation (R41S) as a model case. We created mutants of a variant group 2 fHbp sequence, two engineered chimeric sequences, Chimera I (Beernink et al I&I, 2008) and G1 (Scarselli et al Sci Transl Med, 2011), and one natural sub-family A/B FHbp hybrid (ID 207). Recombinant mutant proteins were expressed in *Escherichia coli*, purified by Ni2+ affinity chromatography and tested for hFH binding.

Results: We re-confirmed that in sub-family A (v.2), R41S has no discernible effect on hFH binding. In contrast, we found that R41S eliminated hFH binding to all three hybrid sequences. Sequence and structural analysis suggested that the region between amino acid positions 99 and 135 was important in retaining binding of hFH on v.2 R41S mutants. We then replaced residues within this region in the R41S A/B hybrid FHbp ID 207, with those residues found in sub-family A sequences. We found that replacement of a segment from residues 128-135 restored hFH binding activity when R41S is present in ID 207.

Conclusions: We provide experimental evidence that compensatory mutations can occur in FHbp. The evolutionary and practical implications are that advantageous variability that is selected to escape immune pressure can be offset by compensatory mutations that restore hFH binding, and aid the process of emergence of escape variants.

P92

Investigating host specificity in the bacterial transferrin receptors

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Background: Understanding the molecular basis of host specificity is crucial for the design of better animal models of colonization and infection, as well as for modeling potential host-switching events. The bacterial transferrin receptor is used by pathogens such as *Neisseria* to pirate iron in the blood. This system binds the iron-transporting serum protein transferrin (Tf) and removes iron from its C-lobe. It is composed of Transferrin binding protein A (TbpA), a TonB-dependent transporter, and Transferrin binding protein B (TbpB), a surface-anchored lipoprotein that preferentially binds holo-Tf. The Tbps demonstrate a remarkable specificity for the Tfs of their host species, which is observed both on the level of Tf binding and utilization. Additionally, mouse infection experiments have shown that this specificity restricts the ability of *Neisseria* to cause mortality in the presence of non-human Tf iron sources. However, the molecular features that mediate the specificity of Tbp-Tf interactions have not been identified, and high levels of sequence variability impede inference of these features through sequence and structure analyses.

Methods: To investigate this problem I have chosen a system in which a pathogen, *Histophilus somni*, is able to distinguish between two very similar Tfs - the cattle and sheep Tfs - which share 93% sequence identity. I am exploring the specificity of the bovine Tf-HsTbpB/HsTbpA interaction through whole-cell binding and protein-protein interaction assays. To identify specificity-altering mutations in the Tbps I am developing a screening method that tests for the ability of Tbp mutants to utilize Tf-bound iron in a neisserial background.

Results: I have confirmed that the *H. somni* Tf receptors selectively bind cattle Tf. Both TbpA and TbpB display host-specific binding to Tf, but TbpA is more selective and is likely the main determinant of specificity. In addition, certain strains of *H. somni* have been found to encode a phase variable gene called TbpA2. Strains containing this phase-variable TbpA-like gene have been shown to have a broader range of Tf recognition. I have shown that TbpA2 binds the N-lobe of Tf rather than the C-lobe and am working to identify the species range of its Tf targets.

P93

Neisseria gonorrhoeae and complement receptor 3: probing the lectin functions of the I-domain of CD11b

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Background: *Neisseria gonorrhoeae* is an exclusive human pathogen and the causative agent of the disease gonorrhea. It has been previously shown that the type IV pili of gonococci have an important role in mediating adherence to human epithelial cells. Pili of *N. gonorrhoeae* are post-translationally modified with a disaccharide, Gal(α1-3) 2,4-diacetamido-2,4,6-trideoxyhexose, at serine 63. This O-linked glycan on the gonococcal pilin is part of a protein-glycan interaction with the I-domain of complement receptor 3 (CR3; integrin αmβ2 or CD11b/CD18) which is a key receptor that mediates gonococcal adherence to primary human cervical epithelial (pex) cells.

Methods: A glycan binding profile of the I-domain was achieved using recombinant human and glycan microarray analysis. Surface plasmon resonance (SPR) was performed to verify the microarray results with a selection of glycans. Array and SPR experiments were further verified by fluorescently labeling glycans and incubating on CHO cells over expressing CR3 and pex cells to see if the glycans bound CR3 ex vivo.

Results and Conclusion: It was found that the recombinant human I-domain has extensive lectin function as it bound over 100 structures on the microarray including structures containing α-linked galactose, similar to the terminal sugar found on gonococcal pilin (terminal Galα1-3). With SPR analysis, it was found that the recombinant human I-domain and the human CR3 complex bound to glycans with affinities ranging from 0.7 to 2.5μM and with off rates ranging from 0.01 to 1 x 10-9 1/s. The affinities achieved from SPR agreed with the level of labelled glycan binding measured on the pex and CHO cells. These glycans, especially the high affinity interacting structures, could potentially be used to block gonococcal pilin glycan binding to CR3.

P94

Meningococcal disease among men who have sex with men – United States, 2012-2013

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Background: In 2012-2013, New York City (NYC) reported an outbreak of meningococcal disease among men who have sex with men (MSM). Additionally, Los Angeles County (LAC) reported 4 cases among MSM within a 5 month period during 2012-2013. MSM have not previously been considered at increased risk for meningococcal disease.

Methods: Health departments reviewed meningococcal disease cases among males aged 18-64 years occurring from January 2012-May 2013 to determine MSM status. Demographic, clinical, and risk factor information were abstracted for known MSM case-patients. Rates of meningococcal disease among MSM were calculated using national surveillance data, 2012 census data, and estimates of the proportion of MSM in the U.S. Passive reports of meningococcal disease among MSM have continued to be received by CDC since June 2013.

Results: From Jan 2012-May 2013, 235 male cases aged 18-64 years were reported; of these 33(14.0%) were reported as MSM, including 17 NYC, 4 LAC, and 12 sporadic meningococcal disease cases. HIV prevalence was higher among NYC MSM and MSM-sporadic cases compared to cases among other men (50.0%, 42.9%, and 6.3% among those with known HIV status, respectively); no LAC case-patients were HIV-positive. The rate of meningococcal disease for MSM in NYC was 11.6/100,000 person-years (rate ratio [RR]: 59.8, 95% confidence interval [CI]: 52.8-67.0, compared to other men), and in LAC was 2.3/100,000 person-years (RR: 14.1, CI: 12.4-15.8, compared to other men). The rate for MSM-sporadic cases was 0.24/100,000 person-years (RR: 1.6, CI: 1.4-1.8, compared to other men). Serogroup C accounted for all NYC and LAC MSM cases and 75.0% of MSM-sporadic cases. Since June 2013, 15 cases have been reported from 7 states; serogroup C accounted for 60% of these cases. Six of these cases were reported from LAC including 4 serogroup C, 4 HIV-positive, and 3 fatal cases.

Conclusions: The increased risk observed among NYC MSM is consistent with a community outbreak. The LAC MSM and MSM-sporadic rates, while elevated, are lower than rates observed among high-risk groups routinely recommended for meningococcal vaccination, and absolute risk is low. Continued study is needed to better understand transmission and risk factors for the MSM population.

P95

Understanding factors affecting University of California Santa Barbara students' decision to get vaccinated with Bexsero™, an unlicensed meningitis B vaccine: a survey on students' knowledge, attitudes and practices

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Background: Public health responses to meningococcal serogroup B (menB) outbreaks have been limited since there is no licensed menB vaccine available in the U.S. During March–November 2013, five cases of menB disease occurred in University of California Santa Barbara (UCSB) undergraduates. The Centers for Disease Control and Prevention (CDC) sought approval from the Food and Drug Administration (FDA) to use Bexsero™, an investigational menB vaccine, to control the outbreak. Two doses of vaccine were offered to all UCSB undergraduates at clinics held by UCSB Student Health between February and May, 2014. Clinics were promoted through a multi-faceted approach including emails, campus posters, digital media and peer-peer communication.

Methods: To determine which factors impacted a student's decision to get vaccinated with Bexsero™ and which public health messages had an impact on this decision, a short anonymous online survey was sent electronically to all 19,702 UCSB undergraduates. The survey included basic demographics, menB vaccination decision, sources of information, and meningitis knowledge. The survey was implemented June 2, 2014; responses will be analyzed following the survey's closure on June 30, 2014.

Results: In total, 9,825 (51%) of undergraduates received at least one dose of vaccine and 7,195 (37.4%) received the recommended two doses. More freshmen were vaccinated than students in other class years, and the proportion vaccinated decreased with increasing class year (50% freshman–28% seniors). As of June 3, 7159 (37.5%) students have read the invitation to participate in the survey and of these, 515 (7.2%) have completed the survey.

Conclusions: College students are at risk for meningococcal disease and are routinely recommended MenACWY vaccine against non-menB serogroups. In the absence of a licensed menB vaccine, FDA approval will be needed to use Bexsero™ in future campus outbreaks. This survey will help CDC understand factors impacting a students' decision to get vaccinated and improve CDC's guidance for future large scale campus vaccination campaigns.

P96

Whole genome sequence analysis of a representative collection of disease-associated isolates from the Republic of Ireland, epidemiological years 2010-11 to 2012-13.

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Background and Methods: The Republic of Ireland experiences a higher incidence of meningococcal disease relative to other European countries. Although the incidence has decreased from 14.8/100,000 in 1999 to 2.7/100,000 in 2010, partly due to the effect of the introduction of Meningococcal C Conjugate (MCC) vaccines, it remains nearly four times the European average. Serogroup C-related invasive meningococcal disease (IMD) has remained at very low levels since 2000 and since 2003 and serogroup B has accounted for at least 80% of IMD cases each year. Whole Genome Sequencing (WGS) was carried out on 87 disease-associated *Neisseria meningitidis* isolates from the epidemiological years 2010-11 to 2012-13. The genomes are hosted on the publically accessible BIGSdb and analyses were performed using the embedded database analysis tools.

Results: The majority of isolates were serogroup B (82%) with serogroup C, W and Y accounting for 4.5% each. There were 16 clonal complexes (ccs) identified, with five accounting for 65% of isolates. Together the ST-41/44cc and ST-269cc accounted for 48% of the isolates. There was variable allelic diversity amongst the four antibiotic resistance associated loci rpoB (rifampicin), penA (penicillin G), gyrA (ciprofloxacin)and folP (sulphonamide): 25, 12, 21 and 13 alleles respectively. No resistant gyrA and rpoB alleles were detected; 26% of isolates had resistant folP alleles, and 33% had resistant penA alleles. There was an uneven distribution of ccs between the 1yr age groups. ST-41/44cc accounted for nearly 50% (13/27) of the 1 year group, with only 3/18 (17%) recovered from patients <1yr. Several complexes were absent from the <1yrs, including the most virulent lineages, the ST-11 and ST-8cc (n=5). Bexsero® coverage estimates using exact peptide matches (PorA, NHBA) including possible cross protective response for fHbp and NadA, and the presence of at least one target peptide were as follows: all ages: 65.51% (57/87); under 1 year: 74.04% (20/27); over 1 year: 61.7% (37/60).

Conclusions: This collection of genomes offers a valuable resource and exemplar for the analysis of meningococcal disease in Ireland including monitoring disease lineage trends and antibiotic resistance, detection of emerging types and estimates of vaccine coverage.

P97

Invasive bacterial disease in Croatia and the role of *Neisseria meningitidis*

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Background: It passed almost 15 years since vaccine preventable IBD in Croatia are not any more caused by bacterial trio *Haemophilus influenzae*, *Streptococcus pneumoniae* or *Neisseria meningitidis*. Thanks to introduction of vaccine against *H. influenzae* type b in 2002 IBD in Croatia is mainly caused by *S. pneumoniae* and *N. meningitidis*. Nowadays pneumococcal IBD has higher incidence especially in elderly. Incidence of IMD is around 1/100 000. Both pneumococcal and meningococcal IBD caused fatal outcome. However a single fatal outcome of a child patient with IMD creates feeling of public helplessness.

Material and methods: Data were obtained from database of Croatian University Hospital for Infectious Diseases (UHID) Zagreb. Since 2005 microbiology examination is performed simultaneously by gold standard-culture and in house multiplex real time PCR for *S. pneumoniae*, *N. meningitidis*, *H. influenzae* including *L. monocytogenes*. Real time PCR is used for serogrouping of *N. meningitidis* isolates too. Antibiotic susceptibility was done by commercial gradient diffusion test using EUCAST interpretation recommendations. Results Routine monitoring of vaccine preventable IBD in UHID reveals that the major bacterial pathogen in two third of cases is *S. pneumoniae* causing mainly IBD in adults (≈70%). *N. meningitidis* causes IMD mainly in children presenting mostly as sepsis. Comparing two analyzed periods from 2008 - mid 2012 and from 2005 - mid 2013 it was observed that more than half cases of IMD was confirmed only by rt-PCR (53,2% respectively 56,1%). In both periods even 85% of *N. meningitidis* isolates were serogroup B, while serogroup C slightly decreased (10,3% to 9,4%) and the proportions of serogroup Y slightly increased (3% to 3,3 %). Penicillin resistance is increasing having MIC90 0,094 μg/mL.

Conclusion: Implementation of multiplex real time PCR in IBD diagnostics doubled confirmed cases of IMD. IMD in Croatia is almost exclusively caused by *N. meningitidis* group B what is of particular importance for future vaccine policy. In recent years increase of IMD cases caused by *N. meningitidis* group Y was recorded, while serogroup C cases are constantly stabile. It should be noted that, although rare, fatal cases caused by *N. meningitidis* in children are recorded every year. Increasing penicillin resistance emphasizes importance of regular monitoring of PenA gene for PCR isolates.

P98

National surveillance of serogroups and antimicrobial resistance of *Neisseria meningitidis* (Nme) isolates causing invasive disease in Argentina: Period 2006-2013

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Background: Meningococcal disease (MD) is a serious public health problem. From 2006 to 2013, 1138 strains from invasive disease were submitted to the National Reference Laboratory by the National Lab Network for Meningitis and Bacterial Respiratory Infections distributed across 23 provinces and Buenos Aires City to evaluate the serogroup distribution and antibiotic resistance.

Methods: Isolates were recovered from normally sterile body sites. Serogroup was determined by PCR and MIC by agar dilution (CLSI).

Results: The clinical presentations were meningitis, 51.7%, meningococcemia, 26.7%, meningitis and meningococcemia, 11.8%, bacteremia, 4.6%, arthritis, 3.4%, pneumonia, 1.6% and epiglottitis, 0.2%. Arthritis was more common in children under 2 year. The highest frequency of isolations corresponded to children under 5 years, 62.2% (34.2% under 1 year). The incidence of MD was higher in children under 1 year than in adolescents (9.6/100.000 vs. 0.2/100.000 in 2013). In 2006 and 2007 serogroup B was prevalent, 72.2% and 66.9% respectively. In 2008 serogroup W increased dramatically and since 2010 it became prevalent reaching 57% in 2012. In 2013 W accounted for 47.6%, B 44.01%, Y 2.4%, C 5.4% and X 0.6%. Arthritis and pneumonia were mainly associated with W (16/23 and 6/7 respectively). The proportion of W isolates since 2008 was greater in older than 20 years compared to other age groups (64%). No susceptibility to penicillin (Ns-PEN) (MIC≥0.12 mg/L) and ampicillin (MIC≥0.25 mg/L) was detected in 47.3% and 43.5% of the isolates, respectively. Ns-PEN was 74.0%/71.2%/16.9% in serogroups B/C/W isolates, respectively. Ns-PEN decreased from 78.5% in 2006 to 45.2% in 2013 caused by the reduction of B and C serogroups and the increase of W. All strains were susceptible(MIC90mg/L) to ceftriaxone(0.002), chloramphenicol(1), tetracycline(0.25) and rifampicin(0.03). 93.5% were resistant to trimethoprim-sulfamethoxazol(4). Ciprofloxacin(CIP) no-susceptibility (MIC≥0.06 mg/L) emerged in our country in 2003, reaching 4.1% in 2013. Eighteen isolates with CIP MIC 0.06-0.25 mg/L were detected, 17 W and 1 B.

Conclusions: Since 2008 serogroup W was predominant. Arthritis, pneumonia, older age and no-susceptibility to CIP were mainly associated with W. As common in our country, no peak of incidence of MD in adolescents was observed.

P99

Asymptomatic carriage of *Neisseria meningitidis* (nm) among 18-21 year old students attending the "Universidad Nacional de la Plata" (UNLP)-Buenos Aires- Argentina between September 2012 and March 2013

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Background: Meningococcal asymptomatic carriage studies conducted in Europe demonstrated their importance for improving our understanding of the bacterium's epidemiology and monitoring the implementation of preventive measures against meningococcal disease (MD). Since these studies have rarely been conducted in Latin America, here we present data for the first meningococcal carriage study conducted in Argentina.

Methods: Oro-pharyngeal swabs were taken from 696 students aged 18-21attending the UNLP. Samples were plated within 4h, and meningococci were identified by conventional microbiology methods. Serogroup were determined by antibody agglutination and PCR, and molecular typing (MLST) results are available for 48 isolates. Participants filled in a questionnaire regarding well-known risk factors for meningococcal carriage and transmission.

Results: Ninety-six meningococci were isolated (overall carriage 13.79%). Odds-ratio calculations showed that male gender (OR:1.79,CI[1.14-2.86],p=0.008), intimate kissing (OR:2.20,CI[1.10-4.37],p=0.022) and attendance to social venues (OR:4.40,CI[1.06-18.14],p=0.024) where all associated with carriage. Consumption of mate in groups, a tradition unique to South America, also favored carriage, but the association was not statistically significant (OR:1.52,CI[0.92-2.53], p=0.102). Serogroups were distributed as follows: B 34%, C 4%, Y 5%, W 6%, E 3%, X 2% and cnl 44%. Clonal complex was determined for 48 isolates, of which 7 (14.2%) were ST-41/44 complex and mainly belonged to serogroup B. Six isolates (12.5%) were ST-461 complex, all serogroup B. Four serogroup W isolates belonged to ST-41/44, ST-23, ST-22 and ST-11 complex respectively. The cnl was seen in 16 isolates belonging to ST-198 (4), ST-1136 (7) and ST-53 (5) complexes.

Conclusions: Our carriage rate is comparable to those reported from Europe. Serogroup W, the most common cause of MD in Argentina, represented only 6% of our dataset, and only one W:ST-11 strain was detected. The results from this study will be very valuable to evaluate the effects of any future preventive measure that might be implemented against MD in Argentina, but larger nationwide investigations would be needed to confirm our results and provide a detailed insight on the composition of the population of carried meningococci in the country.

P100

Epidemiology and surveillance of meningococcal disease in England and Wales.

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Background and Aims: Public Health England (PHE) performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers, characterise strains and inform vaccine policy.

Materials and Methods: Clinicians notify suspected cases of meningococcal meningitis/septicaemia to the Office for National Statistics. Hospital microbiology laboratories in England and Wales routinely submit invasive meningococcal isolates to PHE for phenotypic characterisation and, since October 2007, porA sequencing. Clinical samples (from nearly all hospitals in England) are received for non-culture detection and capsular group confirmation by PCR.

Results and Conclusions: Laboratory confirmed cases rose from 1,448 (1995) to peak at 2,804 (1999) falling to 756 in 2013. During 2013, 329 cases (44%) of invasive meningococcal disease were confirmed by PCR alone.

The major reduction in cases was due to the decrease in serogroup C infections, ranging 10 - 39 cases per year from 2005; the consequence of direct and indirect protection afforded by the UK serogroup C conjugate vaccine programme since November 1999. There has also been a sustained decrease in serogroup B cases from 1,710 cases (2001) to 560 (2013), in the absence of any vaccine intervention.

In 2013 serogroup B accounted for 74% of all confirmed cases whereas only 4% (29 cases) were confirmed as serogroup C. Serogroup Y accounted for 10% (79 cases) in 2013 a reduction in the number (but similar proportion) since the 2011 peak of 93 cases. Serogroup W represented 10% (79) of cases in 2013, an increase from 3% (26 cases) in 2010. There has been a marked increase observed in phenotype W:2a:P1.5,2 (CC11) from 2 in 2009 to 54 in 2013: where W:2a accounted for 66% (33) of W case isolates in epidemiological year 2012/13. The cases have been observed nationwide and across all ages, requiring continued close monitoring.

Phenotypic and genotypic shifts have been observed since 1999: specifically the relative contributions of serogroup B associated clonal complexes ST-41/44 (stable), ST-269 (increasing), ST-32 (reducing), ST-213 (low rise and fall) and the reduction of the previously predominant serogroup C ST-11 CCs to meningococcal epidemiology.

P101

Re-emergence of a *Neisseria meningitidis* serogroup A ST2859 clone in Northern Ghana after transient replacement by serogroup W ST2881 meningococci

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Meningococcal meningitis is a major health problem in the 'African Meningitis Belt' where recurrent epidemics occur in irregular cycles every five to ten years during the dry season. The periodicity of these epidemics is not well understood, nor is it possible to predict them accurately. Within the framework of a longitudinal meningococcal colonization and disease study in the Kassena Nankana District (KND) of northern Ghana initiated in 1998 (1), we are analysing cerebrospinal fluid samples from suspected meningitis cases. In addition, the dynamics of carriage of *N. meningitidis* in the KND is monitored by twice yearly colonisation surveys.

Between 2007 and 2009 serogroup A ST2859 meningococci dominated both as colonizers and as causative agent of bacterial meningitis in the KND. However, in spring 2009 first W ST2881 cases were observed, although the carriage rate of these bacteria was still under the detection limit of the regular surveys. Subsequently, the ST2881 W bacteria dominated in 2010 and 2011 both as meningitis causing agent and as colonizer. Both W carriage and disease was associated with a broad age spectrum. While no serogroup A carriers were found in fall of 2009 and spring of 2010 and no A cases were observed in the meningitis seasons of 2009 and 2010, the A ST2859 clone re-emerged as colonizer in fall of 2010 and as meningitis causing agent in the meningitis season of 2011. A comparative genomic analysis of A carriage and disease strains isolated before and after the transient replacement by the W meningococci revealed purifying selection by the expansion of a particular ST2859 subclone in 2010/11.

(1) Leimkugel J, Hodgson A, Forgor AA, Pfluger V, Dangy JP, Smith T, Achtman M, Gagneux S, Pluschke G (2007) Clonal waves of *Neisseria* colonisation and disease in the African meningitis belt: eight- year longitudinal study in Northern Ghana. PLoS Med 4, e101.

P102

Retrospective characterization of meningococcal serogroup B and C outbreak strains in the United States

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Background: Meningococcal disease incidence is at a historic low in the United States. Approximately 98% of meningococcal cases are sporadic; however, outbreaks and clusters continue to occur. During 2012-13, two *Neisseria meningitidis* serogroup B (NmB) outbreaks occurred on university campuses, and a serogroup C (NmC) outbreak was reported among men who have sex with men. During these outbreaks, questions were raised about differences in virulence between sporadic and outbreak strains, genetic linkage between outbreak strains, and the value of molecular typing for outbreak investigations.

Methods: Of the 47 NmB and NmC outbreaks and clusters reported by states between 2009-13, we characterized 3 NmB and 3 NmC outbreaks (21 isolates) using multilocus sequence typing and PorA and FetA typing. Outbreaks were defined as the occurrence of ≥3 meningococcal cases of the same serogroup in 3 months' time. We compared molecular profiles of these outbreak strains to isolates from sporadic cases collected through Active Bacterial Core surveillance (ABCs) during 2000-10 (426 NmB, 439 NmC isolates). PFGE patterns of the outbreak strains were also compared to other strains in the CDC collection.

Results: Five different genotypes (clonal complex [CC]:PorA:FetA) were detected among the outbreak strains. One NmC (CC11:P1.5-1,10-8:F3-6) and one NmB strain (CC32:P1.7,16-20:F3-3) caused two outbreaks during 2009-13; one outbreak was caused by strains of more than one genotype. Three outbreak genotypes were detected among sporadic cases from ABCs (1-9%). Outbreak strains had 7 unique PFGE patterns; 2 of which were unique to the CDC collection. Remaining patterns were identified in outbreak-related cases, in the same region, or as part of outbreaks prior to 2009. Some sporadic cases during 2009-13 had the same genotype as outbreak strains; their PFGE patterns differed.

Conclusions: Genotypes of outbreak strains were determined among strains causing sporadic cases, indicating that these strains are not uniquely associated with outbreaks. Some strains were associated with more than one outbreak. Whether these genotypes are more likely to cause outbreaks is not understood. The addition of PFGE allowed us to determine that outbreaks were caused by the same or multiple strains, and to differentiate between outbreak and sporadic cases in our investigations.

P103

Factors affecting vaccine uptake during mass-vaccination with the serogroup A meningococcal conjugate vaccine, MenAfriVac in Burkina Faso

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Background: Obtaining high vaccination coverage is an important objective in any vaccination program. Socio-demographic factors within the eligible population can influence an individual's or their parent's decision to accept vaccination, and the identification of these factors may help in planning vaccination campaigns.

Methods: In a repeated cross-sectional carriage study conducted in Burkina Faso, we collected information about MenAfriVac vaccination status among a representative selection of the eligible population (1-29-year) living in one urban district, Bogodogo, and two rural districts, Dandé and Kaya. We also collected socio-demographic data, such as age, gender, smoking habits, occupation, and for pupils, which type of school they attended. The association between these factors and the receipt of MenAfriVac was estimated as odds ratios (OR) and 95% confidence intervals in bivariable logistic regression accounting for the cluster sampling design (STATA v.12).

Results: To minimize recall bias we report from the first survey after country-wide mass-vaccination. Of 5169 persons surveyed, 89.7% had received MenAfriVac. Vaccine uptake was higher in Bogodogo and Kaya compared with Dandé (OR, 2.85 [1.41-5.74] and 2.15 [1.01-4.58], respectively). Variation between villages in the same district was highest in Dandé where coverage ranged from 55.5-91.9%. Vaccine uptake was slightly higher in households with 7-10 persons (OR, 1.62 [1.21-2.16] compared to households with 1-6 persons) but did not differ by gender (OR female:male, 0.94 [0.76-1.16]). Smoking was negatively associated with vaccination (OR, 0.43 [0.23-0.81]) among participants > 15 years. Attending private schools was associated with lower vaccine uptake than attending public schools (OR, 0.13 [0.07-0.27]). Vaccine uptake was lower in the age group 15-29 years. In later survey time points we observed that the primary factors affecting vaccine uptake remained consistent.

Conclusion: In our study, people living in small households, smokers and those > 15 years were less likely to receive MenAfriVac. Among school children, those attending private schools were also less likely to be vaccinated. These findings can be utilized for targeted sensitization prior to future mass-vaccination campaigns in sub-Saharan Africa.

P104

Transmissibility of recent isolates of *Neisseria gonorrhoeae* from Nanjing, PRC

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Background: Transmission of *Neisseria gonorrhoeae* (Ng) is complex; previous studies reported that ~25% of females exposed to Ng-infected partners remain uninfected. Pre-existing immunity, subversive immune responses, hormonal status, the microbiome (including co-infections), host genetic factors and strain differences may all contribute to this phenomenon. We analyzed 42 monogamous females exposed to Ng-infected male partners in Nanjing, China and similarly found that 21.4% remained uninfected. To define strain attributes that may be associated with transmission, we compared transmitted and non-transmitted Ng strains.

Methods: Serum resistance (>50% survival in 10% pooled normal human serum (PNHS)) was determined using a bactericidal assay. Binding of the complement inhibitors, C4BP and FH was detected by FACS. LOS epitopes were determined by lgt sequencing, ELISA and western blot. Genomic sequences were determined by next-generation sequencing. Ng were genotyped by Ng-MAST (*porB* and *tbpB*) and MLST.

Results: Ng-MAST analysis of isolates demonstrated 100% concordance between partner strains. Phylogeny of porB or *tbpB* identified single clusters that were statistically associated with non-transmission (PorB, $P < 0.02$ and TbpB, $P < 0.03$). Genome sequence (26 strains) revealed 13 MLST and 18 Ng-MAST types. MLST 7827 and 7828 (35% all, 67% non-transmitted) and Ng-MAST 2318 (19% all, 44% non-transmitted) were associated with non-transmission. The gonococcal conjugal plasmid, found in 54% of all strains, correlated with transmission; 76.5% transmitted versus 11% non-transmitted strains contained this element ($P = 0.004$). The gonococcal genetic island and β-lactamase plasmids did not correlate with transmission. Fifty-one percent of transmitted and 78% of non-transmitted Ng strains resisted killing by 10% PNHS; the LOS profiles of all strains were similar and all serum-resistant strains bound FH. Sixty-percent of serum-resistant/transmitted, but none of the serum-resistant/non-transmitted, strains bound C4BP ($P = 0.02$). C4BP binds to PorB; PorB Ng-MAST allele 1053 was found in 67% of non-transmitted but only 6% of transmitted strains.

Conclusions: Non-transmitted Ng were related by Ng-MAST and MLST and possessed similar PorB and TbpB. Notably, non-transmitted strains lacked the conjugal plasmid and tended to be serum-resistant but did not bind C4BP. These studies have identified bacterial variables associated with heterosexual transmission of Ng and enhance our understanding of disease acquisition.

P105

Epidemiology of serogroup B meningococcal disease—United States, 2005-2012

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Background: Since 2005, quadrivalent (serogroups A, C, W, Y) meningococcal conjugate vaccines have been licensed and recommended routinely for adolescents in the United States. In 2012, coverage with at least one dose of quadrivalent meningococcal conjugate vaccine in adolescents was 74.0%. Serogroup B vaccines are not yet licensed in the United States, but have recently been approved in Europe, Australia, and Canada. Understanding the burden of serogroup B disease will inform future vaccination policy.

Methods: Meningococcal cases reported to the National Notifiable Diseases Surveillance System between 2005 and 2012 were analyzed. State health departments supplemented serogroup and outcome information to maximize data quality. Incidence rates were calculated using US population estimates and the chi-square test was used to compare proportions of disease.

Results: During 2005–2012, 600-1200 cases of meningococcal disease were reported annually in the United States, representing an incidence of 0.3 per 100,000 persons. Of cases with known serogroup, 35% of cases were due to serogroup B. Approximately 11% of serogroup B cases were fatal, and case-fatality ratios did not vary substantially by age. In recent years (2010–2012), the highest burden of disease was in infants (1.09 cases/100,000), 1–4 year olds (0.20 cases/100,000) and 19–24 year olds (0.10 cases/100,000); a pattern consistent throughout the study period. Following the introduction of the quadrivalent vaccine, no changes in the proportion of serogroup B disease occurred between 2005–2007 and 2010–2012 in these age groups ($P > .05$). During 2009–2012, CDC consulted on 5 clusters of serogroup B meningococcal disease in universities, ranging from 2–13 cases.

Conclusions: Serogroup B meningococcal disease incidence is currently at historic lows in the United States. Infants remain at highest risk for serogroup B meningococcal disease, however outbreaks have recently been reported in university aged persons. Future vaccination policy decisions will be challenging, and will need to weigh the current low burden of meningococcal disease against the severity and potentially devastating effects of this disease.

P106

Epidemiology of serogroup B meningococcal outbreaks and interim guidelines for the use of an unlicensed serogroup B vaccine under a CDC-sponsored IND for the control of outbreaks in organizational settings

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Background: In 2013, two universities in the United States responded to outbreaks of serogroup B meningococcal (MenB) disease with mass vaccination campaigns using an unlicensed MenB vaccine under a CDC-sponsored Investigational New Drug (IND) program. An evaluation of the current epidemiology of meningococcal disease outbreaks and development of guidelines for use of an unlicensed MenB vaccine under a CDC-sponsored IND are important to guide decision-making until a licensed MenB vaccine is available in the US.

Methods: A retrospective review of meningococcal cases reported since January 1, 2009, was conducted by state health departments and CDC to identify clusters of meningococcal disease. A cluster was defined as 2 or more cases of the same serogroup in an organization in <3 months (organization-based) or a two-fold increase in disease rates in a community (community-based). A Meningococcal Outbreak Work Group was convened to evaluate the epidemiological data and literature, consider options for updating the current meningococcal disease outbreak guidelines, and develop guidance for the use of meningococcal vaccines in an outbreak setting.

Results: Preliminary review of the epidemiologic data revealed 250 primary meningococcal cases from 62clusters identified from 18 states: 18 organization-based and 40 community-based. The majority of organization-based clusters were due to serogroup B disease (10, 56%), and five of them were associated with universities. The median number of cases in an organization-based MenB cluster was 2 (range: 2-10) and cases had a median age of 19 years (range: 1-55). The median number of days from onset of the first to last case in organization-based MenB outbreaks was 21 days (range: 2-616).The Work Group developed interim guidelines for the use of an unlicensed serogroup B vaccine under a CDC-sponsored IND in response to organization-based MenB outbreaks.

Conclusions: With high coverage of quadrivalent meningococcal vaccine among adolescents, serogroup B is now the most common cause of meningococcal outbreaks in organization-based settings. Interim guidance for the evaluation and control of MenB outbreaks will assist in the decision for mass vaccination, clarify the process for obtaining the vaccine, and improve timeliness of implementation of a vaccination campaign.

P107

Meningococcal disease in ethiopia 2012-13, prior to MenAfrivac vaccination

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Background: Ethiopia has experienced several large epidemics of serogroup A meningococcal disease and outbreaks of serogroup C. However, there is a lack of recent data on the bacterial etiology of suspected meningitis cases. The aim of this study was to provide such data from three hospitals in Ethiopia (Gondar, Addis Ababa and Hawassa) during one year (March 2012 to April 2013).

Methods: Cerebrospinal fluid (CSF) samples were collected from 163 meningitis patients. Standard culture methods were used to identify the causative organisms. CSFs were further analyzed by multiplex species-specific PCR for *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* and genogrouping (A, W and X) of *N. meningitidis* at AHRI. CSFs were further analyzed at NIPH by a porA nested PCR assay and by RT-PCR for species and genogroup verification.

Results: Of the 163 patients, 13 (8%) died, and among the survivors sequelae (mainly seizure) were reported during the admission period for 45150 patients (30%). Antibiotic treatment was taken prior to admission for 46/163 (28%) patients. Conventional multiplex PCR performed in Ethiopia resulted in 7/163 (4.3%) cases verified as caused by *N. meningitidis*, 11/163 (6.7%) by *S. pneumoniae* and 1/163 (0.6%) by *H. influenzae*. By multiplex RT-PCR the aetiology could be identified in 47 of the 163 (28.8 %) samples, with 29 (17.8%) *N. meningitidis*, 19 (11.7%) *S. pneumoniae* and 1 (0.6%) *H. influenzae*. Genogrouping of the 29 *N. meningitidis* positive samples showed 13 serogroup A, 7 serogroup W, 1 serogroup C and 1 serogroup X. In 7 samples the serogroup could not be ascertained. Serogroup W cases were dominant in Gondar, whereas serogroup A dominated in Hawassa.

Conclusions: *N. meningitidis* and *S. pneumoniae* were the major causes of bacterial meningitis. To our knowledge, this is the first study to demonstrate serogroups W and X as causes of meningococcal disease in Ethiopia. The findings indicate that implementation of novel meningococcal vaccines in Ethiopia warrants close monitoring and that new multivalent affordable vaccines are needed.

P108

Neisseria meningitidis serogroup B (NmB) clones circulating in Argentina: impact on vaccination strategies

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Background: Laboratory-based surveillance of Invasive Meningococcal Disease (IMD) in Argentina is conducted by a nationwide laboratory network, and coordinated by the National Reference Laboratory (NRL), which participates in the SIREVA-II network coordinated by the Pan-American Health Organization. The incidence of IMD in Argentina declined progressively from 2.6/100,000 in 1993 to 0.46/100,000 in 2012. Serogroup B was the most prevalent from 2001 until 2008, when serogroup W rose and reached 56,8% of all disease-associated isolates characterized through the net working 2012. However, NmB remains the second most common cause of disease.

Methods: Multilocus Sequence Typing (MLST), sequence determination of the porA variable regions (VR) and fHbp, were performed on a representative panel of disease-associated isolates obtained in 2006, 2010 and 2011 from throughout the country. Additionally fetA of 2006 and 2010 isolates were characterized.

Results: NmB represented 72% of isolates in 2006, 42.1% in 2010 and 38.4% in 2011. Most isolates belonged to the ST-865 Complex (36.0%, 43.0% and 33.5% in 2006, 2010 and 2011 respectively). ST-35, ST-32, ST-41/44 and ST-461 complex isolates were also detected, and their prevalence was variable across the three years. A strong association between ST-865 Complex and PorA 21, 16-36, fetA F5-8 and fHbp 2.16 was observed. The ST-35 complex was associated with PorA 22-1,14, fetA F4-1, and also fHbp 2.16. ST- 461 Complex was associated with the PorA 19-2,13-1. The association ST-41/44 Complex with PorA VR1 18 family was found in 2010 and 2011 but not in 2006. The fHbp variant 2 was the most frequent in our dataset (32%-2006, 56%-2010 and 64%-2011) followed by variant 1 (38%, 22% and 18% respectively) and variant 3 (26%, 22% and 14%).

Conclusions: Disease-associated NmB isolates circulating in Argentina belong mainly to the ST-865 complex also detected in other countries in South America, and differ from those found in Europe and USA. PorA, FetA and fHbp variants are strongly associated to clonal-complex and the associations appear stable over the three years. More information on the genotypic and antigen composition of the meningococcal population is needed if the use of one of the currently licensed vaccines is to be considered in Argentina.

P109

Clinical course and mortality of meningococcal infections in the Netherlands between June 1999 and June 2011: results of a national representative surveillance study

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Background: Invasive meningococcal disease (IMD) can be fatal within 24 hours and survivors can suffer from serious sequelae. Vaccination is considered the best strategy against IMD. Many effective (conjugate) vaccines against the capsular polysaccharide of serogroups A, C, W and Y have become available. Recently, a four-component MenB vaccine (4CMenB, Bexsero®) has been licensed in Europe, Canada and Australia. Besides incidence rates, information on clinical course and mortality of IMD is useful to evaluate the cost-effectiveness of implementing a meningococcal vaccination.

Aim: To provide national representative information on disease burden of meningococcal infections in a Western European country.

Methods: A retrospective study using surveillance data on meningococcal infections in the Netherlands between June 1999 and June 2011. The surveillance data covered approximately 25% of the Dutch population and were representative for the total Dutch population.Information on comorbidity, clinical manifestation, disease course, treatment, sequelae and fatality was retrieved from hospital records.

Results: A total of 939 cases of meningococcal infection were included in this study.

The incidence of IMD was highest among children aged 0-5 years. 47.8% of the patients presented with meningitis, 16.6% with septic shock and 22.4% with both septic shock and meningitis. Patients aged <15 years and >65 years more often developed septic shock compared to the other age groups. The median (IQR) number of days in the hospital was 10 (8-13). 38.4(%) of the patients required admittance to the ICU with a median (IQR) ICU stay of 3 (2-5) days. Overall mortality was 8.0% with the highest CFR among patients with septic shock (18.6%) and the lowest among patients with meningitis (2.2%). Among surviving patients 28.7% had either mild or severe sequelae at discharge.

Conclusions: Meningococcal infections coincide with a considerable disease burden and mortality.The results of this study can be used for cost-effectiveness analyses on implementation of meningococcal vaccines.

P110

Comparison of two trans-isolate media for transport of CSF and growth of bacterial meningitis pathogens to improve culture confirmation

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Bacterial meningitis is a significant cause of morbidity and mortality worldwide, particularly in sub-Saharan Africa. Rapid and reliable identification of pathogens is essential for making informed decisions regarding vaccination campaigns and for monitoring disease trends. While cases of *Neisseria meningitidis* (Nm) serogroup A have decreased across the meningitis belt after the introduction of MenAfriVac™, serogroup determination remains important to detect the emergence of new epidemic strains and to monitor molecular epidemiology. Standard Trans-Isolate medium (T-I) currently used to transport cerebrospinal fluid (CSF) and grow Nm, *S. pneumoniae* (Sp), and *H. influenzae* (Hi) is only produced in two laboratories worldwide; is relatively expensive (approximately $2.00 USD/bottle for production and trans-Atlantic shipping); and requires a skilled laboratorian, a clean room for production, and an established quality assurance/quality control program. The need remains high for an affordable and rapidly available method of transporting and culturing CSF specimens from patients with suspected bacterial meningitis obtained under adverse conditions in the meningitis belt. T-I produced at Inter-Country Support Team for West Africa (IST-WA) in Burkina Faso uses the same procedure and formulation as CDC (Ajello, et al.), but replaces IsoVitaleX with Vitox, which lowers the cost by $0.28 USD/bottle. An intra-laboratory comparison of CDC- and IST-WA-produced T-I was performed. T-I were inoculated in duplicate with 3 inoculum sizes (1,500, 15,000, and 150,000 CFU/mL), placed at 3 temperatures (25°C, 37°C, and 40°C), and sampled for up to 2 weeks. Preliminary results demonstrate that both media provide adequate support for growth and survival of Nm and Sp for up to 2 weeks at tested temperatures, including ambient temperature across the meningitis belt (40°C). Comparison data for Hi and detailed analyses are pending. IST-WA T-I is an affordable medium that can be produced in the meningitis belt and should be used for outbreak response and surveillance in the African region.

P111

Regulation of the *Neisseria gonorrhoeae* misSR two component system

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Third generation cephalosporin resistance of *Neisseria* gonorrheaeo (Ngo) has emerged as a world-wide problem. Understanding pathogenesis and gene regulation of Ngo has become ever more important in the development of novel antimicrobials and vaccines. Our recent studies indicate that Ngo has the ability to differentially acetylate lysine residues of a wide variety of peptides in the proteome. These include the two-component system (TCS) homologs to MisS and MisR. TCS are composed of histidine kinases (HKs) and cognate response regulators (RRs) that allow bacteria to sense and respond to a wide variety of signals. HKs phosphorylate and dephosphorylate their cognate RRs in response to stimuli. Most RRs regulate gene expression via DNA-binding domains that are covalently attached to the phosphorylateable receiver domain at their N-terminus. Analysis of the genomes of the sequenced Ngo strains indicates that a very limited number of TCS are encoded in the Ngo genome. Acetylation studies show that the response regulator, MisR, has differentially acetylated lysines at residues 143 and 224 raising the possibility that acetylation of MisR may modify DNA binding and subsequent gene expression. As we began our studies of the role of acetylation on MisR function, we found evidence that expression of *misSR* itself might be regulated. We constructed a deletion in *misR* that allowed the *misR* promoter to drive expression of *misS*. This *ΔmisR* mutant was unable to grow on Gc medium base but grew on Chocolate plates. Protophoryin IX did not rescue the growth of the ΔmisR mutant in supplemented Gc medium, thus the defect does not appear to be related to heme production but to some other factor in lysed red blood cells. Complementation of the *ΔmisR* mutant in cis recovered the wild-type phenotype and enabled the organism to grow significantly better than the parent strain Ngo 1291. These phenotypes were not seen in a *ΔmisR* mutant where misS expression was driven by the promoter for the selectable marker. These studies indicate that the expression of Ngo *misSR* is regulated and alterations in this TCS substantially modify growth characteristics.

P112

DprA is required for natural transformation, limits phase and pilin antigenic variation, and is dispensable for DNA repair in *Neisseria gonorrhoeae*

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Natural transformation is the main means of horizontal genetic exchange in the obligate human pathogen *Neisseria gonorrhoeae* and drives the spread of antibiotic resistance and virulence determinants. Transformation can be divided into four steps, 1) DNA binding, 2) DNA uptake, 3) DNA processing, and 4) DNA recombination into the chromosome. Much is known about DNA binding, DNA uptake, and DNA recombination during transformation of *Neisseria* spp., although few studies have investigated DNA processing during transformation of *Neisseria* spp. The DNA processing enzyme DprA has been shown to shuttle incoming ssDNA to the recombination enzyme RecA during transformation in Bacillus subtilis and *Streptococcus pneumoniae*. Here, we investigated the role of DprA during transformation in two strains of *N. gonorrhoeae*. Inactivation of dprA in strains FA1090 and MS11 completely abrogated transformation of *gyrB1*-encoding DNA which confers Nalidixic acid resistance. The presence of the DNA uptake sequence, a 10 or 12 nucleotide non-palindromic sequence (DUS10 or DUS12, respectively), enhances DNA uptake and transformation by binding to the minor pilus protein ComP. Loss of transformation in the *dprA* null mutants was independent of the presence or absence of the DUS10/12. DprA mutants exhibited increased PilC-dependent phase variation and pilin antigenic variation which occurs through DNA recombination by RecA. Unlike the exquisite UV sensitivity of a *recA* mutant, inactivation of *dprA* did not affect survival following UV irradiation. These results demonstrate that DprA has a conserved function during transformation, and reveal additional functions of DprA in *N. gonorrhoeae* in limiting or modulating pilin variation.

P113

Quantitation of proteins regulated by the RNA chaperone protein Hfq of *Neisseria meningitidis* using LC-MSE

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Background: The conserved RNA-binding protein Hfq has many regulatory roles within the prokaryotic cell, facilitating duplex formation between sRNAs and mRNAs, influencing their stability. Consequently, *hfq* mutants have pleiotropic phenotypes (Vogel and Luisi, 2011). Previous research on the proteome (Pannekoek et al., 2009; Fantappie et al., 2009) and transcriptome (Mellin et al., 2010) of *N. meningitidis* has only generated partial insights into differential expression resulting from the loss of Hfq. To further elucidate the differentially expressed proteome of an Hfq mutant we employed LC-MSE; a method allowing rapid high-resolution proteomic analysis.

Methods: Whole cell lysates of *N. meningitidis* H44/76 wildtype (wt) and H44/76Δhfq cultured in GC medium containing 1% Vitox were subjected to tryptic digestion. The resulting peptide mixtures were separated by reversed-phase liquid chromatography (LC) prior to analysis by data independent alternate scanning mass spectrometry (MSE). Differential expression was analyzed by Student's t-Test with False Discovery Rate (FDR) correction according to Benjamini-Hochberg at $p \leq 0.05$.

Results: Of 2,480 annotated Open Reading Frames (ORFs) in H44/76 (Piet and Huis in 't Veld et al., 2011), 937 proteins (38%) were detected. Reliable quantification of relative expression between H44/76 wt and H44/76Δ*hfq* was achieved with 506 proteins (20%). Using a 1.5 fold ratio as a cutoff, 45 up- and 55 downregulated proteins were identified. Of these, 73 were identified as novel Hfq-regulated candidates, while 15 proteins were previously found by SDS-PAGE/ peptide mass fingerprinting, 8 with microarray analysis, and 4 were (putative) phase variable genes. The majority of proteins were involved in general metabolism. Twenty proteins involved in oxidative phosphorylation, DNA replication/ repair, membrane biogenesis and cell division were downregulated. Four proteins from the TCA cycle that catalyze reactions converting malate to 2-oxoglutarate were upregulated, as are four proteins involved in converting propanoyl-CoA to succinate.

Conclusions: Compared to earlier reports the collection of Hfq dependent proteins was expanded two-fold using LC-MSE with rigorous control of FDR and strict fold ratio requirements. The majority of Hfq regulated proteins were involved in general metabolism, showing its role in adaptation of the meningococcus to variations in availability of nutrients.

P114

Identification and characterization of novel pil RNAs and promoters of *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae possesses a vast repertoire of previously characterized transcriptionally silent pil genes denoted as pilS. While it is believed that the role of *pilS* is to undergo unidirectional recombination with the pilE expression locus during antigenic variation, the mechanism remains unclear. However, recent transcriptome analysis has revealed the presence of *pilS*-derived RNAs originating from the majority of *pilS* loci in both *N. gonorrhoeae* strains MS11 and FA1090. Analysis of RNA procured from *N. gonorrhoeae* MS11 cell lysates and supernatant extractions revealed the presence of remarkably stable *pilS* transcripts. Consequently, the small pil-derived RNAs may be an additional component of the *Neisseria* biofilm mattrix. Investigation with Northern blot and real-time quantitative PCR analysis into transcription from the *pilS6* loci within *N. gonorrhoeae* MS11 revealed the presence of a functional sense promoter through both exclusion and mutagenesis constructs. The verified pilS6sense promoter is located within a constant region of pil and is fully functional within two areas of the *pilS6* loci; directly upstream of copy 2 and within copy 1. Computational analysis of several *N. gonorrhoeae* and *N. meningitidis* strains also verified the presence of this promoter within every encoded pil gene, implying that the functionality of this promoter may extend beyond MS11. Further mutagenesis and real-time quantitative PCR analysis of the *pilS6* loci also revealed a functional antisense promoter located near the 3' end of the loci directly downstream of the stop codon of copy 1. Computational investigation of regions downstream of all pil loci encoded by *N. gonorrhoeae* strains MS11 and FA1090 identified complete homology of this promoter as well. The presence and apparent conservation of these promoters within the supposed silent genes of pathogenic *Neisseria* species adds further complexity to these important virulence genes.While the role of *pilS*-derived RNAs remains unclear, the presence of these verified promoters within the conserved region of all *pil* genes may provide transcription bubbles within pil that allow strand invasion leading to homologous recombination and antigenic variation.

P115

Characterization of *pilE* gene regulation in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae employs a variety of gene expression strategies to allow successful invasion, survival and proliferation within host cells. PilE polypeptide is the major component of the pilus structure which provides the initial attachment to host epithelial cells. Previous studies have shown that the small DNA-binding protein, integration host factor (IHF), is a cofactor of pilE transcription, with binding of IHF to a region upstream of the pilE promoter significantly enhancing transcriptional efficiency. In this study, we show that pilE transcript levels are potentially modulated through the formation of secondary structures within its 5' untranslated region (5' UTR) and by transcription of cis-acting anti-sense RNAs within the *pilE* coding sequence. Three stem loops are predicted to form within the *pilE* 5' UTR, with the third stem loop containing the ribosomal binding site. The formation of the stem loops, which was confirmed by real-time quantitative PCRs (qPCR), was shown to prolong mRNA lifetime as deletion of each loop caused a significant reduction in transcript levels. Further analysis revealed the presence of three additional promoters embedded within the pilE gene (one sense; two anti-sense). Transcription efficiency is apparently influenced through the presence of the 2 cis-anti-sense RNAs that are transcribed from promoters located within the middle portion of the gene as well as at the 3' end of *pilE*. Transcription from these anti-sense promoters appears to proceed with a similar efficiency as observed for the cognate sense promoter. Site-directed knock-out of the 3' anti-sense promoter resulted in an 8-fold decrease of anti-sense RNA amount while the level of sense transcript was approximately 16-fold higher compared to the wild-type construct, suggesting that pilE messages are degraded at a higher rate in the presence of antisense RNAs . Deletion of the mid gene promoters also significantly impeded antigenic variation despite the fact that the G quartet structure and small associated RNA was detectable. In conclusion, we propose that expression of *pilE* is maintained by regulating mRNA levels through the formation of secondary loop structures in the 5' UTR as well as through the coordinated action of cis-acting anti-sense RNAs.

P116

Translational regulation of the respiratory electron transport chain of *Neisseria meningitidis* by the Fur controlled small non-coding RNA NrrF

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Background: NrrF is a small non coding RNA (sRNA) from *Neisseria meningitidis*, previously shown to mediate post-transcriptional repression of succinate dehydrogenase (sdhCDAB) under control of the ferric uptake regulator (Fur) (Mellin et al, 2007; Metruccio et al, 2008).

Methods and results: To find novel candidates regulated by NrrF in *N. meningitidis*, we combined a biocomputional target mRNA prediction with experimental approaches. In silico, mRNA of *cycA* and *petABC*, encoding cytochrome c4 and cytochrome bc1 respectively, both functionally involved in respiration, were identified as putative NrrF targets. Using FLAG-tagged versions of cytochrome c4 and cytochrome bc1 we show decreased expression of both in meningococci overexpressing NrrF. Direct interaction between NrrF and the 5'- untranslated region (5'-UTR) of the mRNAs of *cycA* and *petABC* was assessed in vivo by using an *Escherichia coli gfp*-reporter system for translational control via sRNA target recognition: both targets were confirmed. The precise region of NrrF essential for the interaction with petABC was identified by site-directed mutagenesis.

Conclusions: Together, our results are the first to provide insight into the mechanism by which components of the respiratory chain are regulated by Fur. Suppressing expression of these components under iron limitation is mediated at the post-transcriptional level through the action of the small regulatory RNA NrrF.

P117

A two-component system regulates *pilE* transcription in *Neisseria elongata*

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Two-component systems (TCS) are signaling mechanisms that allow bacteria to sense and respond to changing environments. A TCS is composed of a membrane-bound sensor kinase and a cytoplasmic response regulator. We recently reported that Npa, the response regulator in commensal *Neisseria elongata*, positively regulates transcription of *pilE*, the gene encoding the major subunit of the Tfp.Npa binds upstream of the *pilE* promoter and, together with IHF, activates the alternate sigma factor RpoN to initiate transcription. We have identified the partner sensor kinase of Npa in *N. elongata* and have named it *Neisseria* pilus sensor (Nps). *nps* is present in the genome of all commensal *Neisseria*. *nps* is located between a gene with locus tag NEIELOOT_00057 and *npa*; by using RT-PCR we have confirmed that these genes form an operon. An in-frame deletion of nps in *N. elongata* significantly decreased *pilE* transcription and abolished production of PilE protein; mutant colonies have a nonpiliated phenotype. Phosphorylation of specific conserved residues is vital during signal transduction in TCS. A point mutation in the putative phosphorylation sites of nps (H325A), and npa (D58A) yielded nonpiliated colonies that were unable to transcribe *pilE*.
Incontrast, *pilE* transcription in pathogenic *Neisseria* requires the housekeeping sigma factor RpoD. Truncated nps and npa sequences are present in *N. meningitidis*; in *N. gonorrhoeae* Npa and Nps are fused into a single protein, called Rsp. Whether Rsp regulates *pilE* transcription in *N. gonorrhoeae* is unclear.
That *pilE* transcription in commensal and pathogenic *Neisseria* is controlled by two different mechanisms implies that this locus is regulated by different environmental cues. We tested the influence of pH and iron on *pilE* transcript levels in *N. gonorroheae* and *N. elongata*. pH had no effect on *pilE* mRNA levels in either species. However, iron affected *pilE* transcription in *N. elongata* but not *N. gonorrhoeae*. Whether this effect is due to Nps sensing iron is currently under investigation.
The data to date indicate that commensal and pathogenic species of *Neisseria* have evolved two different systems to regulate *pilE* transcription to adapt to changing microenvironments.

P118

GdhR belongs to the gonococcal MtrR regulon and is a transcriptional activator of the genes encoding GdhA glutamate dehydrogenase and GltT glutamate symporter

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Neisseria gonorrhoeae, the causative agent of gonorrhea, is the most prevalent bacterial sexually transmitted infection globally, and the associated medical and socioeconomic consequences of gonorrhea make it a major public health issue worldwide. *N. gonorrhoeae* often develops resistance to antibiotics used in therapy within a few years of introduction of a new drug. Mutations that increase *mtrCDE* efflux pump gene expression due to loss of the MtrR repressor or *mtrCDE* promoter mutations can decrease gonococcal susceptibility to a broad range of antibiotics and host-derived antimicrobials. In addition to controlling *mtrCDE* expression, MtrR also serves as a global regulator of other gonococcal genes. In this study, we found that GdhR (NGO1360), a GntR family protein, also belongs to the MtrR regulon. We determined that a *gdhR*-negative mutant of wild-type strain FA19 was deficient for growth when glucose, but not lactate, was added to the growth media. mRNA levels of both gdhA (NGO1358) and gltT (NGO2117), which respectively encode a glutamate dehydrogenase and a glutamate symporter, were decreased in a *gdhR*-negative mutant. Transcriptional regulation of *gdhA* and *gltT* by GdhR was carbon source-dependent, but independent of growth phase. We showed that purified GdhR could specifically bind to the *gdhA* and *gltT* promoter regions, suggesting that GdhR directly controls transcription of these genes. Hence, the results from our study link glutamate metabolism to antibiotic resistance in gonococci by virtue of joint control by MtrR.

P119

The Correia Enclosed Repeat Element: how it is affected by temperature, pH, CO2, and non-coding RNAs in the *Neisseria* spp

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Correia Repeat Enclosed Elements (CREE) have been found most commonly in intergenic regions and are often near virulence, metabolic, and transporter genes. With CREE being found in great abundance throughout the entirety of the *neisseria*l chromosome, their presence supports the notion that they may play a role in genome organization, function, and evolution. In 2012, the World Health Organisation warned that gonorrhoea poses a global threat, as it may become virtually untreatable due to antibiotic resistance and attempts to develop novel therapeutic agents have proven unsuccessful. There has never been more of a reason to shift from the use of chemical compounds targeting protein structures and processes to a new era of RNA-based therapeutics by manipulating gene expression. It is becoming increasingly evident that ncRNAs have a very wide repertoire of biological functions, in particular as a means of gene regulation. Understanding ncRNAs, CREE, and regulatory network controls is key to discovering virulence and genetic differences between *neisseria*l species. An investigation into a possible relationship between them was undertaken by comparing the locations of ncRNAs to the locations of CREE. Genome analysis showed most locations either overlapped or were found to join end-to-end. Statistical analysis has shown that there is indeed a significant relationship between CREE and ncRNAs. Transcriptomic analysis of experimental manipulation of temperature, pH, and CO2 growth conditions highlighted similarities and differences in the gonococcal strain NCCP11945 related to CREE.

P120

DNA uptake sequences in *Neisseria gonorrhoeae* as intrinsic transcriptional terminators and markers of horizontal gene transfer

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DNA uptake sequences (DUS) are widespread throughout the *Neisseria gonorrhoeae* genome. DUS allow endogenous DNA to be exchanged between *Neisseria* species, and also occur as inverted repeats (IR-DUS), which have been suggested previously to play a role in rho-independent termination and attenuation. Here we show that IR-DUS occur both where the DUS precedes the inverted DUS and also in reverse order. IR-DUS can potentially act as bi-directional terminators, therefore affecting transcription on both DNA strands. Using both bioinformatics predictions and experimental evidence, we demonstrate which IR-DUS are transcriptional terminators and in which direction. This work also provides evidence that gaps in DUS density in the gonococcus coincide with areas of DNA that are foreign in origin, such as prophage.

P121

Genome sequence assembly, annotation, and comparative analysis of a potential novel serogroup of *Neisseria meningitidis*

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University students have a propensity to carry a rich bacterial nasopharyngeal flora, with a higher carriage rate of *Neisseria meningitidis* compared with the average population. To explore this further, in the Spring of 2012, 64 Kingston University student volunteers were assessed on three occasions for carriage of *N. meningitidis*. A single *N. meningitidis* isolate was positively identified and its genome sequenced using the Ion Torrent PGM next-generation genome sequencing system. Data analysis and interpretation were undertaken. In place of the meningococcal serogroup-specific capsular biosynthesis genes, the Kingston University isolate contains 8 genes which have no nucleotide similarity to any gene in *N. meningitidis*. Blast analysis of the translated sequences revealed significant homologies to glycolsyl transferases as well as other proteins from non-pathogenic *Neisseria* spp., suggesting horizontal gene transfer. The first two genes display 45% similarity with a family of glycosyl transferases from non-pathogeneic *Neisseria* sp. The third and fourth genes show 88% and 100% identity, respectively, to glycosyl tranferases of *Neisseria* mucosa. The fifth gene shows 100% identity to a hypothetical protein in N. mucosa. Genes 6 and 7 have 100% and 99% identity, respectively, to D-alanine-D-ligase of N. mucosa and gene 8 has 100% identity to a hypothetical protein in *Neisseria subflava*. We hypothesise these genes are involved in the biosynthesis of a novel capsule. In addition, some of the known virulence genes for both the meningococcus and the closely related species *Neisseria gonorrhoeae* are present in duplicate copies in the chromosome. While some of these are tandem duplications, others are present remotely in the genome. In all, this additional whole genome sequence is revealing new insights into the nature of chromosomal diversity compared to those previously studied in *N. meningitidis*.

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Epigenetic gene regulation of DNA methyltransferase in *Neisseria meningitidis*

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DNA methyltransferases catalyse the addition of a methyl group to recognition sequences within genomic DNA. Methylation of these sequences is a mechanism of self recognition utilised by multiple host adapted bacterial pathogens in restriction-modification systems. Additionally, multiple genes are up-regulated or down-regulated through methylation of overlapping DNA methyltransferase recognition sites and promoters. Phase variation is the ON/OFF switching of gene expression resulting from changes in the number of short DNA repeats within an open reading frame or promoter region. ON/OFF switching of DNA methyltransferase expression epigenetically regulates gene expression in a phase variable manner. In *Neisseria* spp. and other bacterial pathogens, phase variation of gene regulators such as DNA methyltransferases has been identified as a novel system known as phasevarions. Phasevarions are phase variable regulons. The methylation target sites of DNA methyltransferase ModA11 and ModA12 of *N. meningitidis* have recently been identified. In this study we conducted a bioinformatic analysis of the ModA11 and ModA12 DNA methyltransferase recognition sequences that are observed at the promoter regions of genes which are regulated by ON/OFF switching of Mod expression. These promoter regions containing DNA methyltransferase target sites were fused to the 5' end of a promoter-less *lacZ* gene in *N. meningitidis* chromosome to confirm ModA dependent phase variation of expression. To understand the mechanism of epigenetic regulation of the genes in these phasevarions, mutagenesis of the *modA* site in these fusions are used to confirm ModA dependence at that particular site as an adjunct to current and future hypothesis driven promoter level investigations.

P123

MtrA is a global regulator of genes in *N. gonorrhoeae* with roles in iron acquisition and glutamine metabolism

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Neisseria gonorrhoeae is a strict human pathogen that causes the sexually transmitted infection gonorrhea. Unfortunately, gonorrhoea may soon could become untreatable with current antibiotics due to the emergence of strains expressing resistance to multiple antibiotics. One of the main reasons for the decreased susceptibility of gonococci to antibiotics is increased expression of the resistance nodulation division (RND)-efflux pump, MtrCDE. Expression of the operon encoding this efflux pump is regulated by a repressor, MtrR, and a conditional activator, MtrA, whose binding to the *mtrCDE* promoter is enhanced in the presence of a known inducer, Triton X-100. It has previously been shown that MtrR is a global regulator of genes, including those involved in glutamine biosynthesis (*glnA* and *glnE*) and stress response (*rpoH*). Here we report that MtrA is also a global of regulator of gonococcal genes, even in the absence of the known inducer Triton X-100. Using a microarry to compare gene expression between FA19 and FA19 *mtrA::kan* grown at mid and late-log in the absence of the known inducer triton X-100, we found 46 genes that were differentially expressed between the parent and mutant strain. These genes included *glnE*, a glutamine synthetase adenylytransferase/deadenyltransferase, and *mpeR*, an iron-responsive regulator of antibiotic resistance and iron uptake, whose regulation by MtrA we confirmed by reverse-transcriptase PCR (RT-PCR). To verify these observations, we replaced the non-functional *mtrA* allele in FA1090 (containing a 11 bp deletion) with the full-length version from FA19, generating the strain FA1090 mtrAFA19. Using RT-PCR , we found that MtrA is an indirect activator of *fetA*, which encodes a xenosiderophore receptor, and that this regulation is mediated through MtrA's repression of *mpeR*. These results confirm that MtrA is involved in a complex regulatory network in *N. gonorrhoeae*, whose regulation is independent of the known inducer Triton X-100. Hence, MtrA has dual regulatory properties, one in the presence of an inducer (the activation of the mtrCDE expression involved in high level resistance to hydrophobic compounds) and another when it is absent wherein it contributes to regulating genes important for gonococcal metabolism.

P124

Lipooligosaccharide (LOS) heptose I glycan extensions modulate the bactericidal efficacy of a monoclonal Ab directed against a gonococcal LOS vaccine epitope

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Background: *Neisseria gonorrhoeae*(Ng) lipooligosaccharide(LOS) glycans are altered by phase-variation of *lgtA, lgtC, lgtD* (modulate heptose (Hep) I glycans) and *lgtG* (extends HepII). Almost all clinical Ng isolates express HepII lactose (*lgtG* ON). HepII lactose is part of an epitope recognized by mAb 2C7 (the 2C7 epitope). Abs elicited against the 2C7 epitope following natural infection or immunization with a peptide mimic of the 2C7 epitope are bactericidal and attenuate Ng infection in mice. We asked how HepI glycans affected complement activation and bactericidal activity of mAb 2C7.

Methods: *lgtG* was locked ON in strain MS11 by introducing synonymous point mutations in the polyC tract. Four isogenic HepI glycan mutants were created in this HepII lactose backgroundby either deleting or fixing ON *lgtA, lgtC* and *lgtD* in various combinations to yield: i) GalNAc→lacto-N-neotetraose(LNT) (5-Hex), ii) LNT (4-Hex), iii) PK-like LOS (3-Hex) and iv) lactose (2-Hex). Human complement lacking IgG and IgM was prepared by immunodepletion over protein A/G and anti-human IgM, respectively, and used in serum bactericidal assays (20% complement). Complement C3 deposition, complement inhibitor (factor H[FH] and C4b-binding protein[C4BP]) binding and mAb 2C7 binding were measured by flow cytometry.

Results: Hep I glycans modulated mAb 2C7 binding (2-Hex>>4-Hex>5-Hex>3-Hex). The 2-Hex mutant was most susceptible to killing by 2C7 plus complement (>90% killing in 0.2 µg/ml 2C7), while 3-Hex was fully resistant (>100% survival in 6 µg/ml 2C7). The two remaining mutants were resistant to 2, but killed by 4 µg/ml of 2C7. mAb 2C7 mediated C3 deposition on all mutants and the amount of C3 deposited correlated inversely with survival. HepI glycans influenced binding of FH (2-Hex bound the most), but not C4BP. Complement-dependent killing correlated only with binding of mAb 2C7 and was not restricted by regulator binding.

Conclusions: HepI glycan extensions affect the binding and bactericidal efficacy of mAb 2C7 that is directed against a Ng LOS epitope currently being investigated as a vaccine candidate. The opsonophagocytic efficacy of mAb 2C7 and its ability to attenuate infection caused by LOS variants in the mouse model merits investigation.

P125

A new approach to gonococcal vaccine development based on local induction of Th1-driven immune responses

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We have demonstrated in the mouse model of genital gonococcal infection that the suppression of Th1/Th2-governed adaptive immune responses to *Neisseria gonorrhoeae* can be reversed by intravaginal treatment with IL-12 encapsulated in sustained-release biocompatible polymer microspheres (IL-12/ms), resulting in accelerated clearance of the infection and protection against future re-infection. The treatment promotes Th1-driven immune responses including anti-gonococcal antibodies in serum and genital secretions, interferon (IFN)-γ-secreting CD4+ T cells in the genital tract and its draining iliac lymph nodes (ILN), and establishment of immune memory that can be recalled upon re-exposure to *N. gonorrhoeae* in vivo and in vitro. Protection against re-infection persisted for at least 6 months, and extended to unrelated heterologous challenge strains of *N. gonorrhoeae* (FA1090 vs MS11 and FA19). Although protective immunity was not induced by IL-12/ms in the absence of gonococcal infection, gonococcal outer-membrane vesicles (OMV) could substitute for live *N. gonorrhoeae* as a non-viable gonococcal vaccine. Intravaginal immunization (3 doses at 7-day intervals) with gonococcal OMV (40μg protein) plus IL-12/ms (1μg IL-12) induced responses that were essentially similar to those obtained by treatment of gonococcal infection with IL-12/ms. Serum (IgG) and vaginal (IgG and IgA) anti-gonococcal antibodies were generated. CD4+ T cells isolated from the ILN secreted IFN-γ but not IL-4 and maintained the production of IL-17 induced by the infection. Intravaginal challenge of immunized mice with live *N. gonorrhoeae* led to rapid clearance of the infection up to 12 days earlier than in control mice. Immunization with OMV plus control (blank) microspheres generated no protection against challenge with live *N. gonorrhoeae*, and induced only weak anti-gonococcal antibody responses but no significant IFN-γ production by ILN T cells. This affords a mouse model in which protective immunity against genital gonococcal infection can be reliably induced. The findings suggest a new approach to developing a vaccine against *N. gonorrhoeae*, that beneficially redirects the host's immune response away from the ineffective responses induced by the pathogen for its own benefit. However, important questions remain about the mechanism of protection against diverse gonococcal strains and how these findings can be translated into human application.

P126

Refinement of immunising antigens to produce antibodies capable of blocking function of the AniaA nitrite reductase of *Neisseria gonorrhoeae*

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Background: Recently we have identified the AniA nitrite reductase of the pathogenic *Neisseria* species as a surface exposed glycoprotein. Furthermore, we have shown that the monosaccharide of the AniA glycan is immunodominant and that removal of the AniA glycan generates a non-native immune response against the core of the protein. We were able to produce antibodies capable of blocking nitrite reductase activity by immunising animals with a recombinant, truncated form of AniA completely lacking the glycosylated C-terminus. The aim of this study is to produce antibodies with a greater ability to inhibit AniA function by further refining the antigens used for immunisation.

Methods: Seven peptides encompassing regions critical to AniA function were designed based on the AniA crystal structure. These peptides were synthesized conjugated to the keyhole limpet hemocyanin carrier protein. Antisera were raised by immunising rabbits and mice with the conjugated AniA peptides. An *E. coli* strain over expressing recombinant AniA from *N. gonorrhoeae* 1291, which is functional, is being used to determine if these antisera can block AniA nitrite reductase activity.

Results and Conclusions: Preliminary data indicates that a number of these antisera are capable of inhibiting AniA activity. As AniA is essential for the growth and survival of *N. gonorrhoeae* under oxygen-limited conditions and for biofilm formation, we propose that modified AniA has potential as a vaccine antigen for *N. gonorrhoeae*.

P127

Antibody to reduction modifiable protein (Rmp) increases the bacterial burden and the duration of gonococcal infection in a mouse model

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Antibodies directed against reduction modifiable protein (anti-Rmp) in *Neisseria gonorrhoeae* block killing by bactericidal mAb 2C7 directed against gonococcal lipooligosaccharide (LOS). We have shown previously that passive transfer of mAb 2C7 to BALB/c mice reduces the duration and bacterial burden of gonococcal infection.

We tested the efficacy of mAb 2C7 in clearing gonococcal vaginal colonization in mice that possessed anti-Rmp Abs resulting from: (1) active immunization with purified recombinant gonococcal Rmp (rRmp; three doses ip of rRmp in alum at 3-weekly intervals) or (2) passive transfer of affinity-purified mouse polyclonal anti-Rmp Abs. Median anti-Rmp Ab levels in serum and vaginal washes of actively immunized mice were 43.14 mg/ml and 0.27 mg/ml, respectively. We administered mAb 2C7, 10 mg ip twice daily for 3 days to 20 rRmp immunized mice in the diestrus phase of the estrous cycle and treated them with water soluble 17β–estradiol. To study effects of passively transferred anti-Rmp, 10 female mice were given affinity-purified mouse anti-Rmp Abs (30 mg) together with mAb 2C7 (10 mg), both ip twice daily for 3 days. 15 naïve female mice given mAb 2C7 alone served as controls. All mice were challenged intravaginally with strain FA1090.

Mice receiving only mAb 2C7 cleared gonococci significantly faster than mice actively immunized with rRmp or mice given anti-Rmp Ab; both groups having been administered mAb 2C7 (median times to clearance 7 and 8.5 days, respectively, vs. 4 days in control '2C7-only' animals; $P<0.0001$). Bacterial burden for the "2C7-only" group was significantly lower than burdens in each of the Rmp groups ($P\leq0.006$ in each group). In actively immunized mice, serum anti-Rmp concentrations correlated directly with vaginal anti-Rmp levels and with time to clearance ($P<0.0001$). Time to clearance correlated inversely with the ratio of vaginal 2C7 Ab/anti-Rmp Ab ($P<0.0001$). Clearance by vaginal 2C7 was completely abrogated at a ratio of 1.5 but was restored at a ratio 4.0 (vaginal 2C7 Ab mainitaned constant throughout).

Anti-Rmp Ab blocks the disease attenuating effects of mAb 2C7 in a dose-dependent manner in mice. Pre-existing anti-Rmp Abs will be an important consideration in evaluating the efficacy of gonococcal vaccines.

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Pre-existing *Chlamydia* infection is associated with an increased risk of gonococcal infection

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We conducted epidemiologic studies of *Neisseria gonorrhoeae* (Ng) and *Chlamydia trachomatis* (Ct) infection in heterosexual partners in Boston and Nanjing STD clinics. The design of the studies enhanced the likelihood that transmission of Ng and Ct infection occurred from men to women because men had ≥ 2 female sex partners in the 30 days prior to the onset of urethral symptoms. 84% of American and 100% of Chinese women stated they were monogamous with the male index case dating back 4 weeks. Self-reported exposure histories were validated in American women by repeat questioning on a subsequent date. 98% of Chinese women were married to their infected sex-partners. Both studies included a subset of men with only Ng infection, thereby excluding men with 'dual' Ct+Ng infection as a possible source of Ct in women. Thus, we were able to isolate the effects of 'background' Ct infection in women in acquiring Ng from their male partners.

In Boston, 89% of women with pre-existing Ct acquired Ng from their Ct-negative male partners compared to 69% without pre-existing Ct (p=0.02). In Nanjing, 89% of women with pre-existing Ct acquired Ng from their Ct-negative male partners compared to 65% without pre-existing Ct (p=0.16) indicating that pre-existing Ct in women facilitates Ng acquisition. Furthermore, Nanjing women with pre-existing Ct were more susceptible to Ng infection when exposed to low numbers (≤120 cfu/ml) of Ng (p=0.04).

To recapitulate this phenomenon in mice, we used a *C. muridarum* (Cm)/Ng co-infection model developed previously (Vonck et al, Infect and Immun, 2011); mice given only Ng served as controls. Gonococci were cleared significantly faster from 'only Ng' group compared to Cm+Nginfected mice (median clearance time was 6 vs. 7 days, respectively; P=0.002). Ng burden was higher in the Cm+Ng group (P=0.0002) and consistent with findings by Vonck et al.

Pre-existing Ct infection in women is associated with an increased risk of acquiring Ng from male sex partners. The mouse Cm/Ng co-infection model is a promising tool to elucidate the mechanism(s) of enhanced Ng acquisition and bacterial burden in the context of Ct infection.

P129

Functional analysis of the anti-factor H binding protein antibody repertoire of adults immunized with 4CMenB vaccine

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Background: 4CMenB vaccine (Bexsero, Novartis Vaccines) recently was licensed in Europe, Australia and Canada for prevention of serogroup B meningococcal disease. One of the key antigens in this multicomponent vaccine is Factor H-binding protein (FHbp), which recruits complement Factor H (FH) to the bacterial surface, and contributes to evasion of complement. Binding of FH to FHbp is specific for human FH. Inhibition of binding of FH to FHbp by anti-fHbp antibodies can augment complement-mediated bactericidal activity.

Methods: To investigate human anti-FHbp antibody repertoire to 4CMenB, we cloned recombinant anti-FHbp antibody fragments (Fabs) from plasmablasts isolated from post-immunization blood samples from three adults. From more than 70 anti-FHbp Fab clones, we selected ten with unique germline immunoglobulin gene rearrangements, which were expressed as recombinant Fabs in *Escherichia coli*.

Results: All ten Fabs recognized surface-exposed epitopes on live meningococci. Many of the Fabs had high affinity for FHbp as measured by surface plasmon resonance (KD values between 20 pM and 72 nM). By ELISA, two of the human Fabs reacted only with FHbp ID 1 (the amino acid sequence variant of the group 1 antigen in 4CMenB); six Fabs showed various degrees of cross-reactivity with other sequence variants within variant group 1; and two Fabs cross-reacted with FHbp from all three variant groups. None of the Fabs inhibited binding of FH to the bacterial surface, whereas a control murine anti-FHbp Fab inhibited binding of FH. In previous studies ~50% of anti-FHbp mAbs from mice immunized with FHbp vaccines inhibited binding of FH.

Conclusions: In humans, 4CMenB vaccine elicited anti-FHbp antibodies with multiple distinct specificities. A broader analysis of the anti-FHbp antibody repertoire in humans is needed to evaluate whether the presence of human FH that binds to FHbp might direct the antibody repertoire to FHbp epitopes predominantly outside of the FH binding site.

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N. gonorrhoeae activates caspase 1 in epithelial cells

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Gonorrhea, caused by *Neisseria gonorrhoeae* (GC), has now become a significant public health problem due to recent emergence of resistance against third-generation cephalosporins. Most GC infections in women are asymptomatic, which hinders early diagnosis and prevention of complications. The asymptomatic infection in female indicates that GC can avoid immune detection when establishing infection. However, the underlying mechanism is not known. In this study, we examined the impact of GC infection on the activation of caspase 1, the key effector of inflammasome, and the production of IL-8, a chemokine for neutrophils and T cells, in polarized epithelial cells. Activated caspase 1 was detected by fluorescent FAM-YVAD-FMK that specifically binds to activated caspase 1 and inhibits its activity, and the production of IL-8 by ELISA. After polarized epithelial cells (T84 and HEC-1-B) were incubated with GC apically for 6 h, we detected both activated caspase 1 and IL-8 secretion, but the IL-8 level was relatively low. When purified GC DNA was included during GC inoculation, the level of IL-8 production by epithelial cells was significantly higher than those induced by either GC or GC DNA alone. The secreted IL-8 was detected predominantly in the basolateral medium, indicating basolaterally polarized secretion. Inhibiting caspase 1 using FAM-YVAD-FMK significantly reduced the IL-8 production in epithelial cells induced by both GC and GC DNA. These results suggest that the GC infection alone does not induce a significant amount of IL-8 secretion by polarized epithelial cells and the production of IL-8 requires additional signals, including GC DNA and inflammasome activation.

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Development of an asymptomatic gonorrhea/chlamydia coinfection model

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Gonorrhea/chlamydia coinfection is very common, yet very little is known about the biology of these infections. An estimated 30-40% of patients infected with *Neisseria gonorrhoeae* (Gc) are also infected with *Chlamydia trachomatis*, and a high percentage of these coinfections are inapparent and thus under diagnosed. Coinfection models are needed to study differences in susceptibility, disease and host responses during gonorrhea/chlamydia coinfection compared to single infections. We recently developed a Gc/*C. muridarum* (Cm) coinfection mouse model in which BALB/c mice were first infected with Cm then treated with 17β-estradiol to increase susceptibility to Gc, and challenged with Gc. Higher numbers of Gc were recovered from Cm-infected BALB/c mice compared to mice infected with Gc alone, and coinfected mice exhibited a greater polymorphonuclear leukocyte (PMN) influx than mice infected with either single pathogen. To study the effect of host genetic factors on coinfection and to potentially establish a model of asymptomatic infection, here we conducted coinfection studies in C57BL/6 mice, which are known to exhibit significantly less inflammation than BALB/c mice when infected with Gc or Cm alone. C57BL/6 mice were inoculated vaginally with Cm 10-14 days before inoculating with Gc. Vaginal swabs were cultured for both organisms for 10 consecutive days following inoculation with Gc, and the percentage of PMNs among vaginal cells on stained vaginal smears was determined. C57BL/6 mice infected with only Gc exhibited the characteristic cyclical colonization pattern in which a drop in Gc colonization occurred around days 6-7, followed by a return to initial colonization levels; in contrast, coinfected mice maintained a consistent level of Gc colonization throughout the experiment. Furthermore, although colonization levels of co-infected and singly infected mice were similar on day 1, on subsequent days, co-infected mice had CFUs 0.5-1 log higher than those in singly infected mice. Importantly, both the single and dual infected C57BL/6 mice did not demonstrate a PMN influx, supporting the possible use of these mice as a model of asymptomatic infection. Coinfection experiments using mice with other genetic backgrounds are underway with the goal of developing a range of models to study different aspects of of these infections.

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Neisseria gonorrhoeae induces a M2 polarization of human macrophages

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Introduction: *Neisseria gonorrhoeae* is the etiological agent of gonorrhoea, a sexually transmitted disease widespread throughout the world, with an estimated 60 million new cases per year. Infections with gonococcus do not improve immune response in re-infected patients, suggesting that gonococcus displays several mechanisms to evade immune response. Macrophages are an essential component of innate immunity with a central role in host defense. In response to various signals, macrophages may undergo classical-M1 activation or alternative-M2 activation. Until now there are no reports of the gonococcus´ effect on human macrophages polarization and its consequences in the infection.

Methods: We assessed the phagocytic ability of monocytes-derived macrophages by immunofluorescence and gentamicin protection experiments. Then we evaluated by flow cytometry M1/M2 specific-surface markers on macrophages challenged with *N. gonorrhoeae* and proliferative capacity of effectors T cells.

Results: We demonstrated that in gonococcus exposed macrophages there were up-regulation of M2-asocciated markers and down-regulation of M1-asocciated markers. Moreover, *N. gonorrhoeae* exposure leads to up-regulation of a number of secreted and cell surface proteins with immunosuppressive properties, particularly IL-10 and Programmed Death Ligand 1 (PD-L1). We also show that *N. gonorrhoeae* is able to inhibit macrophage-induced proliferation of human T-cells and it can be reversed using blocking antibodies.

Conclusion: We think that macrophage M2 phenotype can favour gonococcus against host defence during infection and this effect could explain in part the immune evasion observed in *N. gonorrhoeae* infected patients.

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Immunity elicited by *neisseria meningitidis* carriage confers broader protection than anticipated by serum antibody cross-reactivity in CEACAM1-humanized mice

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Introduction: During *Neisseria meningitidis* infection, individuals mount an immune response against the infecting strain. Hitherto, we know little about the breadth of immunity induced by natural carriage of a single strain, or its implications for subsequent infectious challenge. Here, we monitored the induction of strain-specific immunoglobulin during asymptomatic colonization, measured the cross-reactivity of serum against heterologous strains, and tested the ability of these responses to provide sterilizing immunity against other meningococcal strains.

Methods: Human CEACAM1-expressing transgenic mice were infected by intranasal inoculation with a variety of *N. meningitidis* strains to allow persistent asymptomatic colonization. The cross-reactivity of serum immunoglobulin against a panel of meningococcal strains was assessed using whole bacterial ELISA. Finally, the impact of the observed cross-reactivity on nasal colonization was tested by infecting mice with ST-11 strain 90/18311 (serogroup C) followed by nasal challenge with the isogenic strain; ST-11 strain 38VI (serogroup B); ST-32 strain 196/87 (serogroup C); or ST-32 strain H44/76 (serogroup B).

Results: A broad range of *N. meningitidis* isolates successfully colonize mice in a human CEACAM1-dependent manner. Upon intranasal exposure to *N. meningitidis* strains 90/18311 (ST-11, serogroup C) or H44/76 (ST-32, serogroup B), respectively, CEACAM1-mice mount a mutually exclusive strain-specific immune response, however one that does not strictly correlate with the serogroup of the heterologous strain. As expected, we observed protection of 90/18311-sensitized mice against subsequent challenge by the isogenic strain. These mice were also protected against the closely related serogroup B strain 38VI (ST-11), but remain fully susceptible to the more distantly related H44/76 (ST-32, serogroup B). Somewhat unexpectedly, despite little apparent cross-reactivity of the immune sera generated by 90/18311 infection, we observed cross-protection against strain 196/87 (ST-32, serogroup C).

Conclusions: The CEACAM1-humanized mice can be colonized by a broad variety of different meningococcal strains. Natural immunity in mice appears similar to that in humans with respect to limited cross-reactivity of serum antibodies. Despite the lack of cross-reactive serum antibodies against strain 196/87, 90/18311-sensitized animals displayed mucosal protection. Considered together, our data suggest that we currently underestimate the actual breadth of mucosal protection gained through natural exposure to *N. meningitidis* strains.

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The role of lipooligosaccharide phosphoethanolamine transferase A, a lipooligosaccharide-modification enzyme, in gonococcal defense against human neutrophils

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Background: Infection with *Neisseria gonorrhoeae* (Gc) is marked by a rapid influx of neutrophils to the site of infection. Despite a robust immune response, viable Gc are recovered from neutrophil-rich gonorrheal secretions. We hypothesize that Gc has defensive measures that help confer resistance to neutrophils. Lipooligosaccharide (LOS) has been implicated in gonococcal resistance to human antimicrobial defenses, but its role in interactions with human neutrophils is underexplored. Gc enzymatically modifies the lipid A portion of LOS by the addition of phosphoethanolamine (PEA) moieties to phosphate groups at the 1' and 4' positions. Loss of LptA, the enzyme catalyzing this reaction, increases bacterial sensitivity to killing by human complement1 and cationic antimicrobial peptides (CAMPs)2.

Methods and Results: To investigate the importance of LptA modification of LOS to Gc interactions with neutrophils, we generated an lptA mutant and complement in piliated, opacity protein-negative Gc of strain FA1090. The lptA mutant was significantly more sensitive to killing by adherent, chemokine-primed primary human neutrophils than the parent or *lptA* complement. The *lptA* mutant was more susceptible to neutrophil killing both intra- and extracellularly, as shown with fluorescent dyes that reveal the viability of individual bacteria. Loss of *lptA* did not affect Gc internalization by neutrophils. We have identified two mechanisms underlying the increased sensitivity of *lptA* mutant Gc to neutrophils. 1) *lptA* mutant Gc is significantly more likely to reside in mature phagolysosomes than parent bacteria. We previously showed that LptA-expressing Gc survives inside neutrophils by avoiding residence in mature phagolysosomes3. 2) *lptA* mutant Gc is more sensitive to killing by selected components found in neutrophil granules, including the serine protease cathepsin G. Sensitivity of *lptA* mutant Gc to neutrophils was overcome by inhibiting neutrophil proteases.

Conclusions: We conclude that LptA-catalyzed modification of LOS enhances the resistance of Gc to the bactericidal activity of neutrophils, thus enhancing Gc survival against the human inflammatory response during acute gonorrhea.

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Neisseria meningitidis activates NLRP3 inflammasome in human neutrophils

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Introduction: Lipooligosaccharide (LOS) of *Neisseria meningitidis* is an endotoxin that is responsible for activation of immune cells and the release of proinflammatory cytokines. Further, endotoxic shock, characterized by an amplified or harmful response of the host immune system is mediated by LOS. The NLRP3 inflammasome is an intracellular multi-protein complex that triggers caspase-1 mediated maturation of interleukin-1β (IL-1β); possibly the most potent mediators of inflammation and a major cytokine produced during severe infections and unregulated inflammatory conditions, like sepsis. The NLRP3 inflammasome is activated by a number of microbial factors as well as dangers molecules, but there are no data available regarding a role for inflammasome activation in meningococcal disease. The aim of this study was to investigate if *N. meningitidis* activate inflammasome and if so, the role of LOS in this activation.

Methods: LOS-deficient *N. meningitidis* FAM20 serogroup C lpxA mutants and wild-type FAM20 serogroup C bacteria were grown on GC agar plates for 18-20h. Neutrophils were isolated from healthy blood donors (n=5) and stimulated with lpxA mutants and wild-type FAM20 for 2h. Caspase-1 activity of neutrophils was determined by flow cytometry. Relative mRNA levels of NLRP3, pro-caspase-1 and proIL-1β were measured using RT-qPCR.

Results: Caspase-1 activity was significantly increased ($p = 0.001$) in neutrophils stimulated with wild-type FAM20 strain when compared with caspase-1 activity of unstimulated neutrophils or in cells stimulated with the lpxA mutant. Similarly, significant ($p = 0.001$) increase in mRNA levels of NLRP3, pro-caspase-1 and proIL-1β was detected in neutrophils stimulated with wild-type FAM20 strain, whereas non-significant increase was observed in neutrophils stimulated with the lpxA mutant when compared to unstimulated cells.

Conclusion: LOS of *N. meningitidis* plays a major role in the up-regulation as well as the activation of the NLRP3 inflammasome in human neutrophils.

P136

Distinct outcomes upon transcervical *Neisseria gonorrhoeae* infection of the female mouse upper genital tract during different phases of the reproductive cycle

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Background: In women, *Neisseria gonorrhoeae* ascends from the endocervix, where infection is mainly asymptomatic, to the upper genital tract in up to 20% of cases. This outcome can cause inflammation of the uterus, fallopian tubes and ovaries to varying degrees, often leading to persistent pain, ectopic pregnancy and infertility. A mouse model that allows long-term colonization of the lower genital tract, reflecting uncomplicated infections, is now well-established. Herein, we aim to describe the pathobiology of ascending gonococcal infections, and to determine how the structural and immunological changes that occur in the endometrium during the reproductive cycle affect gonococcal infection, immunity and disease.

Methods: We transcervically instilled gonococci into the uterine horns of wild type FvB mice that were at either at estrus or diestrus stages of the cycle, and studied bacterial burden and tissue penetration, inflammation and disease pathology using immunostaining, qPCR, cytokine and whole-bacterial ELISAs.

Results: Gonococci triggered a robust inflammatory response, as evidenced by the rapid migration of phagocytes within a few hours of infection in the uterus. This response did not occur in the vagina, highlighting the compartmentalized nature of immune responses along the genital tract. Although infection during both estrus and diestrus led to tissue inflammation, remarkable differences in bacterial localization and immune cell recruitment were observed. In particular, mice infected at diestrus display a more severe pathology characterized by extensive gonococcal penetration, tissue damage and formation of purulent neutrophil-containing exudates. Notably, uterine infection in naturally cycling mice at diestrus induced stable titres of systemic gonococcal-specific IgG, while those in estrus stage did not, suggesting that the female sex hormones and/or tissue structural changes dramatically affect immune induction within the upper genital tract. Our ongoing studies aim to investigate how *N. gonorrhoeae*-specific immunoglobulin affects subsequent infection and the pathology of ascending gonococcal disease.

Conclusions: We have established a new model in which to study gonococcal physiology and the host response to ascending gonococcal infection. Our findings highlight that the reproductive cycle markedly affects both *N. gonorrhoeae* association with the endometrial mucosa, and the innate and adaptive immune responses elicited during upper genital tract infection.

P137

Use of GMMA for the generation and characterisation of monoclonal antibodies specific for the Neisserial Adhesin A (NadA)

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Background: *Neisseria meningitidis* is a major cause of meningitis epidemics in Sub-Saharan Africa. One attractive vaccine option is an outer membrane particle-based vaccine termed GMMA (Generalized Modules for Membrane Antigens). GMMA are made from bacterial stains genetically-engineered for over-production of these particles and are highly immunogenic in mice. To help understand the fine specificity of the humoral immune response, we produced monoclonal antibodies (mAbs) against GMMA, which were screened for reactivity to vaccine candidate antigen NadA.

Methods: We generated GMMA from a recombinant serogroup A ST2859 case strain expressing NadA allele 3. B cell hybridomas were generated with spleen cells of mice immunized three times with GMMA alone or with GMMA formulated on alum. Screening of the hybridoma cell culture supernatants was performed by ELISA and Western blot analysis using both GMMA and recombinant NadA as target antigens.

Results: We obtained 25 hybridoma clones producing GMMA-binding mAbs. Four of the clones produced anti-NadA mAbs of immunoglobulin subclasses IgG1, IgG2a/b and IgG3. The other 21 mAbs were specific for seven other GMMA antigens. Three anti-NadA mAbs of IgG1 or IgG2a/b subclass showed broad reactivity with differences in fine specificity and binding pattern in Western blotting analysis using a panel of *N. meningitidis* serogroup A and W strains. One mAb of IgG3 subclass showed specific binding to two serogroup A and 4 W isolates. In flow cytometry analysis using two isolates of serogroup A, one W and X isolate all four anti-NadA mAbs produced bound to the vaccine serogroup A strain with allele 3 and cross-reacted with a W strain with NadA allele 2. One of the antibodies bound to the second serogroup A with low expression of NadA allele 3. When tested in serum bactericidal assays using human complement all four anti-NadA mAbs were bactericidal for the vaccine A strain with high NadA allele 3 expression. One of the antibodies targeting non-NadA antigens showed broad cross-reactivity with serogroup A, W and X isolates.

Conclusion: Immunization of mice with GMMA derived from an engineered *N. meningitidis* strain elicits bactericidal antibodies to NadA with differing fine specificities.

P138

Structural analysis of lipooligosaccharides (LOS) of *Neisseria meningitidis* from patient and carrier strains reveals multiple facets of correlation of structure with pathobiology

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Background: Among the virulence factors in *Neisseria*l infections, the LOS is a major inducer of proinflammatory cytokines. The LOS is composed of a primarily hexaacylated lipid A (LA) with phosphate (P) and phosphoethanolamine (PEA) substitutents and an oligosaccharide moiety.

Methods: Herein we report on structural analysis of meningococcal LOS from 40 patient and 25 carrier strains from Norway. Patient strains were characterized as causing (i) distinct meningitis, (ii) severe septicemia, or (3) distinct meningitis and septicemia. Analyses of intact LOS were performed by high-resolution matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry with monoisotopic mass resolution of ions.

Results: The data showed that the substitution pattern of LA in patient and carrier strains was almost invariably hexaacyl with from 1-3 and sometimes up to 4 P, and 0-1 PEA. This differs from a previous structural analysis of *Neisseria*l LA that reported 1-2 PEA. Exceptions were among the carrier strains where 6 serotype Y strains expressed pentaacylated LA, which has previously been reported in disease-causing *N. meningitidis* with reduced pathogenicity. Analysis of relative ion ratios of hexaacylated phosphorylated LA fragment ions revealed an increase in expression of 3P PEA relative to 2P LA in patient strains that caused meningitis without causing septicemia, compared to strains causing septicemia with or without meningitis. We also found that incubation of LOS from the more highly phosphorylated LA-expressing patient strains causing meningitis but not septicemia induced higher expression of TNF-alpha in human THP-1 monocytes.

Conclusions: This is the first report to our knowledge of an association of LA pentaacylation with carriage of *N. meningitidis*. The results indicate that the ability of *N. meningitidis* to cause septicemia is correlated with the expression of a relatively less highly phosphorylated, less inflammatory LA. Our analyses also revealed that the oligosaccharide of disease strains was more highly sialylated than that of the carrier strains. This finding supports previous results showing that the presence of sialic acid facilitates meningococcal invasion by preventing complement-mediated lysis. Overall, our findings indicate that the structure of the LOS has direct bearing on the invasion and pathogenicity of *N. meningitidis*.

P139

Secretion of a nuclease by *Neisseria gonorrhoeae* enhances bacterial escape from killing by neutrophil extracellular traps

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Background: Symptomatic infection by *Neisseria gonorrhoeae*, the gonococcus(Gc),results in a highly inflammatory environment caused by a potent infiltration of neutrophils, especially in males. Neutrophils possess a diverse antimicrobial arsenal that can be directed to the phagolysosome, or released by exocytosis, to kill microbes. Another approach used by neutrophils to trap and kill microbes is the release of neutrophil extracellular traps (NETs). NETs are web-like structures comprised of chromatin, decorated with antimicrobial proteins, and can be released from neutrophils undergoing a unique form of cell death. In spite of neutrophils' robust antimicrobial activities, viable bacteria are recovered from gonorrheal disease exudates. We seek to define the bacterial virulence factors that contribute to gonococcal survival after exposure to neutrophils. The NG0969 open reading frame encodes a thermonuclease (nuc) that contributes to gonococcal biofilm architecture and remodeling (Steichen et al., Infect Immun 79:1504, 2011). We hypothesize that Nuc contributes to Gc resistance to neutrophils by degrading NETs.

Methods and Results: In support of this hypothesis, we found by confocal microscopic analyses that parent FA1090 Gc and and isogenic Δ*nuc* bacteria induced equivalent NET release early after exposure to primary human neutrophils. However, significantly more intact NETs were observed in neutrophils infected with Δ*nuc* mutant Gc over time, compared with cells exposed to parent or nuc-complemented bacteria. Moreover, purified Nuc protein cleaved human neutrophil DNA and the DNA backbone of chemically-induced NETs. Conditioned media from Nuc-expressing Gc degraded NETs that were induced by Δnuc Gc or by chemical stimulation. When exposed to NETting neutrophils, the Δnuc mutant survived significantly less well than parent or nuc complement bacteria as evidenced by viable CFU enumeration. Survival of the Δ*nuc* bacteria was rescued by addition of exogenous DNAse or Nuc.

Conclusion: These studies support a model in which Gc secretes Nuc to escape trapping and killing by NETs during symptomatic infection, revealing Nuc as an important virulence factor in Gc pathogenesis.

P140

Phase variable expression of *lptA* modulates resistance of *Neisseria gonorrhoeae* to cationic antimicrobial peptides

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Background: Cationic antimicrobial peptides (CAMPs) are a crucial part of the human innate immune response to gonococcal infection. However, the gonococcus (Gc) has evolved several mechanisms to resist CAMP killing action. For instance, decoration of lipid A by phosphoethanolamine (PEA), carried out by the lipid A PEA transferase LptA encoded by the gene *lptA*, has been established to be important in CAMP-resistance. Moreover, Gc with lipid A decorated by PEA were recently found to have a competitive advantage during experimental infections using murine and human models. Accordingly, we characterized the *lptA* gene and its expression.

Methods/Results: Primer extension revealed a transcriptional start point 61 bp upstream of the LptA start codon and 4 bp downstream of a consensus -10 element (TATAAT). We confirmed that this promoter could drive expression of lptA using the pLES94 promoterless *lacZ* system. In addition to transcription from a nearby promoter, results from RT-PCR experiments demonstrated that *lptA* is co-transcribed as part of an operon consisting of *serC* (which encodes a putative phosphoserine aminotransferase) and *nfnB* (which encodes a putative nitroreductase). We also found that production of a full length LptA enzyme is subject to phase variation due to a T-8 polynucleotide tract in the *lptA* coding sequence. This T-8 tract was contained by 73 Gc clinical isolates that were analyzed by whole genome sequencing as well 13 Gc strains that were independently sequenced by the Broad Institute. Using a polymyxin B (PB) screen/selection protocol, phase-off and phase-on *lptA* variants were found to arise at a frequency of approximately 10-5, which was three logs more frequent than a spontaneous mutation for resistance to erythromycin. Spontaneous *lptA* phase variants differed by >100-fold in their resistance to PB and were found to contain single nucleotide changes in the poly T tract that placed the coding sequence in or out of frame.

Conclusion: We propose that exposure of Gc to CAMPs during infection provides a selective pressure for phase-on *lptA* variants.

P141

Molecular basis of the human antibody repertoire to meningococcal factor H binding protein

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Factor H binding protein (fHbp) represents a key antigen in the recently licensed multicomponent meningococcal vaccine (4CMenB). To investigate the human antibody repertoire to fHbp, we used single B cell/PCR cloning to isolate and sequence the immunoglobulin variable (V) regions of native monoclonal Fab fragments from 4 adults immunized with 4CmenB vaccine. For 3 of the 4 subjects, blood samples were obtained at a single time point, 8 days, after 2 injections of vaccine. On the fourth subject, blood samples were obtained 30 days following the first injection and 7, 30 and 180 days after the third injection. A total of 77 sequence-unique Fab fragments were isolated representing the product of 38 distinct rearrangements/clones. Collectively, the findings demonstrate a complex repertoire where the capacity for recognition of fHbp is distributed over a substantial proportion of the germline genes (16 VH, 14 D, 8 Vκ and 7 Vλ). Further diversity is achieved through extensive CDR-3 heterogeneity and hypermutation. Thus, the potential for combining diversity is high. IgG and IgA were the predominant H chain isotypes. Thus, 4CMenB vaccination elicits polyclonal antibody responses to fHbp that have class-switched and have undergone affinity maturation.

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Human transferrin increases gonococcal colonization in the lower genital tract of female mice and the neutrophil response to infection

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Transferrin (TF) is the major glycoprotein involved in the transport of iron from absorption sites to all tissues. In humans, only 30% of the TF iron-binding sites are saturated. The low TF saturation and high affinity for iron allows TF to limit free iron available to microorganisms. The pathogenic *Neisseria*e circumvent this host defense by expressing cell membrane receptors specific for human transferrin (hTF). The importance of the gonococcal TF receptor for urethral infection in men has been clearly demonstrated in male volunteers. The importance of TF as an iron source in the female genital tract is not known, however, and is likely less critical based on the presence of other usable iron sources in the female genital tract and the ability to establish a productive gonococcal genital tract infection in female mice. In this study we examined the consequence of parenteral administration of hTF on experimental murine infection. Estradiol-treated female BALB/c mice were given daily injections of hTf (8 mg) beginning on the day of bacterial inoculation or left untreated. Mice were inoculated with 104 or 105 colony forming units (CFU) of *N. gonorrhoeae* strain FA1090 and the number of CFU recovered from vaginal swabs and the percent of PMNs in vaginal smears was determined daily for 10 days. In both experiments, higher numbers of gonococci were recovered from hTF-supplemented mice compared to untreated mice on days 5-10 of infection, although the difference was not statistically significant. Consistent with hTF supplementation supporting a higher gonococcal bioburden, an earlier and greater influx of vaginal neutrophils occurred in hTF-supplemented mice compared to untreated mice and hTF-supplemented, uninfected mice. Our results from this surrogate model system suggest that while hTF is not critical for infection of the lower female genital tract, it provides a growth advantage to *N. gonorrhoeae* in this body site. The use of hTF supplementation is an improvement over the existing mouse model and has the advantage over transgenic technology of being easily applicable to mice of different genetic backgrounds. Experiments using a transferrin receptor knockout mutant are underway to confirm the basis of our results.

P143

Two lytic transglycosylases are important for *Neisseria gonorrhoeae* survival from human neutrophils

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Background: Symptomatic infection by the obligate human pathogen, *Neisseria gonorrhoeae* (Gc), induces a potent inflammatory response involving a neutrophil-rich exudate. Neutrophils are innate immune cells which effectively kill microbes. However, a population of Gc resists neutrophil killing. The ability to resist neutrophil killing may be influenced by the extracellular release of immunomodulatory peptidoglycan (PG) by Gc. PG is a macromolecular network composing bacterial cell walls. PG monomers consist of a disaccharide with an attached peptide stem. During growth and cell wall remodeling, Gc releases PG monomers extracellularly. Monomer release is dependent on the lytic transglycosylases A (LtgA) and D (LtgD) (Cloud-Hansen, et al., 2008). Notably, the majority of released PG monomers are agonists for the proinflammatory pattern recognition receptor NOD1. While PG monomers can damage fallopian tube epithelium akin to the damage in female patients with ascending infection (McGee & Rosenthal, 1984), their influence on neutrophils is largely unknown.

Methods: Primary human neutrophils were infected with mid-log phase parent or mutant Gc. Infected neutrophils were either lysed to plate for viable bacteria or prepared for immunofluoresence. Complemented or mutant Gc were also treated with increasing concentrations of cationic antimicrobial peptides.

Results: We found an *ltgA ltgD* double mutant survived significantly less well than parent Gc when challenged with primary human neutrophils. Deletion of both genes was required to abrogate the release of PG monomers and to yield a survival defect in neutrophils. Complementation with wild-type copies of *ltgA* and *ltgD* rescued the survival of the mutant after exposure to neutrophils. Parent and *ltgA ltgD* bacteria were similarly internalized by neutrophils, but the viability of *ltgA ltgD* was decreased both intracellularly and extracellularly compared to parent bacteria. Importantly, *ltgA ltgD* was not more susceptible to killing by the antimicrobials LL-37 or polymxyin B.

Conclusions: These findings suggest extracellularly released PG may contribute to the ability of Gc to stimulate, yet survive, the human inflammatory response in gonorrhea. We are testing two non-exclusive hypotheses to explain the mutant defect: (1) differential activation of neutrophils and/or (2) increased sensitivity to neutrophil antimicrobials via changes in cell wall architecture.

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Neisseria gonorrhoeae modulates immune cell survival through pyroptosis

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Cell death is a common mechanism for maintaining immune cell homeostasis and is induced in response to both endogenous and exogenous stimuli. Classically, cell death occurs through either apoptosis or necrosis. However, recent studies have defined additional cell death pathways including pyroptosis and pyronecrosis. In macrophages exogenous stimulation with some Gram-negative bacteria has been demonstrated to induce pyroptosis. Due to inflammasome activation and the spilling of intracellular components pyroptosis is highly pro-inflammatory. Recent studies have reported that the Gram-negative pathogen *Neisseria gonorrhoeae* induces pyronecrotic cell death in monocytic cells. In contrast, other immune cells, such as phagocytic neutrophils, are resistant to cell death following stimulation with *N. gonorrhoeae*. We have previously shown *N. gonorrhoeae* inhibits apoptosis in human endocervical cells and results in increased levels of cIAP2, an inhibitor of apoptosis protein, both intracellularly and extracellularly. In this study we have further characterized the cell death pathways induced by *N. gonorrhoeae* in THP-1 like-macrophages. We demonstrate that stimulation of macrophage-like cells with *N. gonorrhoeae* induces cell death in a lytic manner. *N. gonorrhoeae* stimulation did not activate caspase-3, but did activate caspase-1. We conclude that *N. gonorrhoeae* stimulates cell death in human macrophages by pyroptosis and postulate that this contributes to both bacterial persistence and the induction of inflammatory pathways.

P145

Neisseria gonorrhoeae induces changes in MMP-2 and MMP-9 levels upon infection of human fallopian tube epithelial cells

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Background: Extracellular matrix (ECM) is a complex protein network composed of collagens, proteoglycans, elastins and glucosaminoglycans. In the Fallopian tube (FT), ECM is critical for maintaining reproductive function through tissue remodelling. This cellular event is mainly performed by metalloproteases (MMPs), a group of zinc-dependent endopeptidases that cleave most of the ECM proteins. During infection of FT mucosa by *Neisseria gonorrhoeae* (Ngo), tissue damage and subsequent repair processes are triggered by bacterial-host cell interactions, and ECM function is critical. However, changes in MMPs pattern in FT tissue following Ngo infection have not been studied.

Methods: Samples of primary cultures of human FT epithelial cells were obtained from fertile donors after informed consent and were used for infection assays with Ngo (n=8 independent experiments). ELISA was used to measure MMP-2, MMP-3, MMP-8 and MMP-9 in supernatants and cell lysates, gelatin zymography was carried out to visualize proteolytic bands from samples, and cell viability was monitored by measurement of lactate dehydrogenase (LDH) release.

Results: MMP-2 levels in extracellular supernatants from infected cultures were not significantly different to the levels observed in uninfected cells at 8, 12 and 24 h post-infection. However a time-dependent increase in intracellular MMP-2 levels was detected. By contrast, extracellular MMP-9 levels were increased in supernatants from infected cell cultures, whereas intracellular levels were reduced. Cell viability was not affected during gonococcal infection of FT epithelial cells.

Conclusions: Our data suggest that *N. gonorrhoeae* may modulate MMP-2 and MMP-9 patterns in FT epithelial cells by promoting MMP-9 secretion, while MMP-2 is accumulated in the cytoplasm. Further studies are required to decipher the molecular mechanisms involved in modulating MMP release upon gonoccocal infection of FT epithelial cells.

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NADPH oxidase assembly in primary human neutrophils infected with *Neisseria gonorrhoeae*

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Background: Infection with *Neisseria gonorrhoeae* (Gc) promotes a robust inflammatory response driven by neutrophils, yet some Gc remain viable. In activated neutrophils NADPH oxidase catalyzes conversion of oxygen into superoxide, the substrate for other reactive oxygen species (ROS). NADPH oxidase subunits are located at the granule membrane (gp91phox and p22phox) and in the cytoplasm (p47phox, p67phox and p40phox). Upon neutrophil stimulation and phosphorylation of the p47phox, cytoplasmic subunits and the GTPase Rac2 translocate to phagosome or/and plasma membranes to assemble the active enzyme. CEACAM-binding Opa+ Gc induce oxidative burst, while Opa- Gc suppress ROS production. We hypothesized that Opa-dependent differences in neutrophil ROS production are attributable to differential assembly of NADPH oxidase.

Methods: Primary human neutrophils were exposed to *S. aureus*, Opa-negative or the CEACAM-binding OpaD expressing Gc. NADPH oxidase subunits were examined by immunofluorescence and immunoblotting of membrane and cytosolic neutrophil fractions. Cell lysates were tested for p47phox phosphorylation, and activation of Akt and MAPK kinases.

Results: gp91phox and p22phox membrane subunits were present at bacterial phagosomes , regardless of Opa protein expression. In contrast, infection with Opa- Gc did not promote p47phox, p40phox, or p67phox translocation to neutrophil membranes, whereas these proteins translocated to membranes after infection with OpaD+ Gc and S. aureus. Similarly, there was limited recruitment of the p47phox and p67phox subunits to Opa- Gc phagosomes, compared to phagosomes containing OpaD+ Gc and *S. aureus*. p47phox was less phosphorylated in neutrophils exposed to Opa- Gc vs. OpaD+ Gc, which was associated with less activation of ERK1/2, p38 and Akt.

Conclusion: Gc modulates the activation state of human neutrophils in an Opa protein-dependent manner. Unlike Opa+, Opa- Gc does not initiate signaling events required for activation of p47phox. Consequently, cytosolic subunits do not translocate to membranes and NADPH oxidase does not assemble, thus preventing the oxidative burst. Opa-dependent differences in kinase activity and ROS production may contribute to the presentation of gonorrheal disease at different mucosal sites in men and women.

P147

Seroprevalence of antibody-mediated, complement-dependent opsonophagocytic activity to *Neisseria meningitidis* serogroup B in England

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The correlate of protection for the licensure of meningococcal vaccines is serum bactericidal activity. However, there is evidence that this situation is complex and other mechanisms may play a role and should be investigated to understand immunity to this disease. One alternative protective mechanism is antibody-mediated, complement-dependent opsonophagocytosis (OP), which is the correlate of protection for *Streptococcus pneumoniae* vaccines.

In this study a high-throughput flow-cytometric opsonophagocytic assay (OPA) has been optimised and qualified. The assay uses cultured HL60 cells, which are differentiated into granulocytes, as the phagocytic effector cells. These are incubated with heat-inactivated test sera, an exogenous source of human complement (IgG-depleted pooled human plasma) and killed fluorescently-labelled *N. meningitidis*. The presence of fluorescent bacteria within the granulocytes is then measured by flow cytometry. This method has previously been shown to correlate well with a more labour-intensive opsonophagocytic killing assay and showed low levels of inter- and intra-assay variation and low intra-operator variation. The OPA was used to measure OP activity in a panel of 1878 sera from individuals ranging from 0-99 years of age obtained between 2000 and 2001, against strain NZ98/254 (B:4:P1.7-2,4). Similar strains were responsible for approximately 20% of all UK laboratory-confirmed cases of invasive group B meningococcal disease at this time.

Levels of OP activity in individual sera varied greatly. OP activity was found to be very low in infants below 6 months of age but then increased in those 6-12 months of age. Low OP activity was then observed until the early teenage years, apart from a slight increase between 3-6 years. Greatest OP activity occurred in the 30-39 year age group ($P<0.05$). From age 50, OP activity declined and the lowest levels were observed in adults above 60 years of age ($P<0.05$). Thus it appears that peak OP activity occurs later than peak SBA titres (20-24 year-olds). The OP activity seen in children aged 3-6 years may indicate a protective role for this mechanism of immunity in primary school age children where the incidence of serogroup B meningococcal disease is low.

P148

Lipopolysaccharide engineering in *Neisseria meningitidis*: structural and functional analysis of novel lipid A variants obtained by expression of heterologous modifying enzymes

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Lipopolysaccharide (LPS) from *Neisseria meningitidis* is a potent activator of TLR4/MD-2 leading to strong activation of innate immunity. Its presence in outer membrane vesicle (OMV) vaccines is therefore an important factor in determining both immunogenicity and reactogenicity. Engineering the lipid A biosynthesis pathway offers the potential to obtain modified LPS molecules with altered TLR4 agonist properties. We have previously shown how inactivation of the lpxL1 gene results in the synthesis of penta-acyl LPS with strongly reduced activity. However, for vaccine applications this may not be the optimal form of LPS because its activity is too much reduced. In order to fine-tune the agonist activity of meningococcal LPS, a broader range of modifications than can be obtained by solely inactivating the known biosynthesis steps would be desirable. In the present study, we have expanded the possibilities for lipid A engineering in *N.meningitidis* by expressing lipid A modifying enzymes from other bacterial species, in particular *Bordetella sp*.

N.meningitidis strain H44/76 LPS was modified by expression of the pagL gene encoding lipid A 3-O-deacylase from *Bordetella bronchiseptica*. Mass spectrometry analysis of purified mutant LPS was used for detailed compositional analysis of all present molecular species. This determined that the modified LPS was mainly pentaacylated, demonstrating high efficiency of conversion from the hexaacyl to the 3-O-deacylated form by heterologous PagL expression. However, small amounts of hexa-acyl LPS were still present and shown to be contributing to overall activity. The immunomodulatory properties of PagL-deacylated LPS were compared to LpxL1 mutant LPS, also a pentaacyl form but of different structure as it lacks the 2' secondary acyl chain. While both LPS mutants displayed impaired capacity to induce production of IL-6 in the monocytic cell line Mono Mac 6, induction of the TRIF-dependent chemokine IP-10 was largely retained only for the PagL-deacylated mutant. These results demonstrate a qualitatively different response of human innate cells to different pentaacyl LPS forms. Other modifications now under study by biosynthetic engineering include the phosphate and phosphoethanolamine groups, as well as deacylations at other positions and various combinations of these alterations.

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Host cytokine responses to *Neisseria gonorrhoeae* infection within the female genital tract of humanized mice

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Gonorrhea, caused by *Neisseria gonorrhoeae* (Ng), is a major sexually transmitted disease (STD) that leads to pelvic inflammation, endometritis and preterm births in women and urethritis in men. Upon infection, Opa proteins on the surface of Ng interact with CEACAM receptors on human epithelial cells to facilitate attachment. The lack of an effective vaccine against Ng infection in the face of increasing incidence of antibiotic resistance is a major public health concern. Limitation in development of vaccines has been attributed in part to its host specificity. The aim of this study was to evaluate host immune responses to Ng infection in vivo using humanized mouse model expressing human CEACAMs to compare to Wt mice not expressing these molecules.

Wild type (Wt) or transgenic FvB mice expressing either human CEACAM3, CEACAM5 and CEACAM6 (CEABAC2) or CEACAM1 (Tg418) were treated with β-estradiol to induce estrous, similar to previous mouse models. Mice were infected by injecting 107 Ng strain MS11 in PBS directly into the uterine horn. Mice were euthanized at 6, 12 and 24 h post infection, and serum, along with upper or lower genitourinary tract (UGU, LGU, respectively) tissues were collected. Levels of cytokines and chemokines in these tissues were measured by multiplex immune assays.

Analysis indicated that there was a relatively stronger immune response in CEABAC2 and hCEACAM1 mice compared to Wt. Levels of pro-inflammatory and effector cytokines and chemokines including TNFa, IL-1a, IL-1b, IL-5, IL-6, IL-12, MCP1, MIP1a, MIG, and IP-10, all mainly in the UGU, were significantly higher in the CEABAC2 mice at 6 h compared with Wt, hCEACAM1 mice and levels generally decreased later time points. Interestingly we observed no difference in the levels of anti-inflammatory cytokine IL-10 to Ng infection in all mouse groups. In conclusion, transgenic mice harboring human CEACAM receptors (CEABAC2 mice) elicited significantly increased inflammatory cytokine response to Ng infection,at early time points, as compared to Wt, indicating an induction of an acute innate immune response. These results suggest that human CEACAMs, which promote infection, facilitate induction of inflammatory responses to Ng infection in mice.

P150

Phosphoethanolamine (PEA) modification on the lipid A moiety of *Neisseria gonorrhoeae* lipooligosaccharide reduces autophagy formation in macrophages

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Background: Autophagy, an ancient homeostasis mechanism for macromolecule degradation, has recently been recognized to play an important role in host defense by facilitating pathogen elimination. Bacterial pathogens have evolved a variety of mechanisms to avoid autophagy, including the inhibition of autophagy signaling, masking of bacterial surfaces, blocking of autophagosome fusion with lysosomes, escape from autophagosomes or hijacking autophagy for bacterial replication. *Neisseria gonorrhoeae* is a strictly human pathogen that causes the sexually transmitted disease, gonorrhea. It is not known if gonococci can evade autophagy. PEA modification on the lipid A moiety of *N. gonorrhoeae* increases resistance to host-derived cationic antimicrobial peptides and complement-mediated bacteriolysis, and impacts the host immune response to gonococcal infection. The aim of this study was to investigate if PEA modification of *N. gonorrhoeae* lipid A affects autophagy formation.

Methods: Autophagy induction was investigated using RAW264 macrophages stably transfected with a GFP-tagged construct encoding the autophagy marker LC3 protein. Macrophages were infected with live gonococcal strain FA19, the isogenic PEA-deficient *lptA::spc* mutant, or complemented mutant *lptA::spc/lptA+* at an MOI of 50, then incubated at 37°C + 5% CO2 for 16 hrs. Macrophage nuclei were stained with DAPI and autophagy induction was visualized using confocal microscopy imaging. The autophagic index reflecting GFP-puncta formation was calculated for at least 16 fields.

Results: Infection with FA19 significantly reduced autophagy formation in RAW264 macrophages when compared to the PEA-deficient mutant (p value=0.002). The complemented mutant induced similar autophagy formation to that of wild type FA19 strain. The data suggest that PEA modification on the lipid A moiety of LOS decreases autophagy formation. Further, PEA modification impacts bacterial survival in macrophages. Our data confirm that the PEA-deficient gonococci were killed more rapidly in a macrophage bactericidal assay when compared to the WT and complemented PEA-sufficient strains. Since nitric oxide exerts complex inhibitory effects on autophagy, we also measured nitric oxide release from infected macrophages and found that the PEA-deficient mutant induced significantly less nitric oxide than PEA-sufficient FA19 gonococci.

Conclusions: PEA modification of lipid A reduces autophagy of *N. gonorrhoeae* and promotes intracellular survival in macrophages.

P151

Extended glycan diversity in the O-linked protein glycosylation system linked to allelic polymorphisms and minimal genetic alterations in a glycosyltransferase gene

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Glycans manifest in conjunction with the broad spectrum O-linked protein glycosylation in species within the genus *Neisseria* display intra- and interstrain diversity. Variability in glycan structure and antigenicity are attributable to differences in content and expression status of glycan synthesis genes. Given the high degree of standing allelic polymorphisms in these genes, the level of glycan diversity may exceed that currently defined. Here, we identify unique protein-associated disaccharide glycoforms that carry N-acetylglucosamine (GlcNac) at their non-reducing end. This altered structure was correlated with allelic variants of pglH whose product was previously demonstrated to be responsible for the expression of glucose (Glc) -containing disaccharides. Allele comparisons and site-specific mutagenesis showed that the presence of a single residue, alanine at position 303 in place of a glutamine, was sufficient for GlcNac versus Glc incorporation. Phylogenetic analyses revealed that GlcNac-containing disaccharides may be widely distributed within the pgl systems of *Neisseria* particularly in strains of *N. meningitidis*. Although analogous minimal structural alterations in glycosyltransferases have been documented in diversification of lipopolysaccharide and capsular polysaccharides, this appears to be the first example in which such changes have been implicated in oligosaccharide diversification of a protein glycosylation system.

P152

Spatial organization of the endothelial receptors for meningococcal type IV pili governs bacterial adhesion and signaling events

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A crucial step in the pathophysiology of invasive bloodborne meningococci is the colonization of peripheral and brain microvessels that leads to deregulated inflammatory and coagulation processes, endothelial dysfunction and, ultimately, bacterial dissemination into perivascular tissues. Establishment of a tight interaction of meningococci with endothelial cells is determinant to resist the hemodynamic forces and promote vascular colonization. We previously showed that this process relies on the primary attachment of meningococcal type IV pili to the transmembrane receptor CD147 [1]. This interaction is further strengthened by the activation of the G-protein-coupled β2-adrenergic receptor (β2AR) that subsequently triggers cell signaling events stabilizing meningococci at the endothelial cell surface [2]. Here we addressed whether CD147 and β2AR directly interact and/or cooperate in a same complex to promote the pilus-mediated colonization of meningococci onto human endothelial cells. We found that CD147 and β2AR engage in lateral cis-interactions that are required for the initiation of the signaling networks. Upon bacterial adhesion, these receptors organize in large clusters arranged in 3D hexagonal arrays centered on adhering bacteria. Looking for CD147/β2AR complex-interacting partners in endothelial cells that can cooperate to potentiate adhesion, we identified the actin crosslinking and molecular scaffold protein alpha-actinin-4 (Actn4). Actn4-deficient cells display a defective organization of CD147/β2AR receptor complexes at bacterial adhesion sites and fail to strengthen bacterial adhesion under shear stress. Our results show that, beyond the presence of receptor binding sites for meningococcal type IV pili at the cell surface, the efficiency of meningococcal adhesion to endothelial cells also relies on the spatiotemporal organization of CD147/β2AR multimolecular complex receptors to ensure sufficient avidity for initial interaction, specific signaling and strengthening of bacterial adhesion.

[1] Bernard et al, Nature Medicine 2014

[2] Coureuil et al Cell 2010.

P153

Exhaustive identification by high throughput screening of new meningococcal and host factors required for meningococcal pathogenesis

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In some unknown circumstances, *N. meningitidis* is able to invade the bloodstream and causes meningitis. The specificity of meningococcal infections is the consequence of a very uncommon interaction of the bacteria with the brain and peripheral microvasculature. A tight interaction of meningococci with endothelial cells has been highlighted during meningococcemia. To achieve this task, *N. meningitidis* adhere to endothelial cells via the human receptor CD147 and signal to the cells via the β2-adrenenergic receptor. The type four pili of *N. meningitidis* are responsible for these interactions. To identify new meningococcal factors required for meningococcal pathogenesis, we set up a "high-throughput insertion tracking by deep sequencing" strategy that uses a whole-genome transposon mutant library in combination with massively parallel sequencing. A transposon mutant library of approximately 70,000 mutants of invasive serogroup A strain Z5463 that is being analyzed by Transposon-Directed Insertion-Site Sequencing (TRADIS). In a preliminary experiment, this meningococcal transposon mutant library has been screened using an in vitro colonization model in order to identify mutants displaying diverse capacity to colonize human epithelial cells. Ibidi microslides containing epithelial cell monolayers were infected with the whole mutant library under defined shear stress conditions that mimic blood flow. Comparison of input/output pools will allow us to identify genes involved in *N. meningitidis* colonization of epithelial cells. Bacteria collected after the screening through the epithelial cells (output pool) are also being analyzed by TRADIS. This innovative high throughput screening technology may provide new insights into the meningococcal functions necessary for the bacterium to adapt to growth on host cells.

P154

Characterization of Neisserial autotransporter lipoprotein (nalp)

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Neisseria meningitidis is a human nasopharyngeal commensal capable of causing life-threatening septicaemia and meningitis. One important class of *neisseria*l virulence factors are the autotransporter (or type V secreted) proteins. Eight autotransporters have been identified in meningococci: IgA1 protease, NhhA, AutA, AutB, NadA, App, MspA and NalP (also known as AspA). Autotransporters consist of three domains: an N-terminal signal peptide which directs translocation across the inner membrane; the biologically active passenger domain; and a C-terminal domain that forms a β barrel required for translocation of the passenger domain across the outer membrane. Following export, the passenger domain of some autotransporters undergoes proteolytic cleavage, resulting in the release of biologically active fragments from their respective cell-bound translocator domain. NalP is a phase-variably expressed serine protease which cleaves a number of cell surface proteins including itself, MspA, App, IgA1 protease, Lactoferrin-binding protein B (LbpB) and *Neisseria*l heparin-binding protein A (NhbA). The consequences of this proteolytic activity on meningococcal pathogenesis are yet to be fully determined, but have already been shown to influence the sensitivity of meningococci to killing by human whole blood and the ability of meningococci biofilm formation. To enhance our understanding of the role of NalP during meningococcal pathogenesis, functional recombinant NalP passenger domain has been purified under non-denaturing conditions using immobilized nickel chromatography. NalP will enable functional studies to better understand the role of NalP during meningococcal-host interaction. Purified recombinant NalP passenger domain was shown to be proteolytically functional in in vitro assays and demonstrated to cleave a number of host proteins likely to play important roles in host-pathogen interactions.

P155

Molecular characterization of two capsule null locus meningococci causing invasive disease in South Africa

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Background: Encapsulation is an important virulence determinant for invasive meningococcal disease (IMD) and is encoded by genes in the capsular polysaccharide synthesis (*cps*) locus. Capsule null locus (cnl) isolates lack regions A, B and C of the *cps* locus, and therefore do not express a capsule. Such isolates are common among distinct lineages of colonizing meningococci, but are rare among invasive isolates. The clinical significance of these isolates remains unclear. To date, three countries have reported cases of IMD due to cnl isolates: Germany (1 case, ST-845), Burkina Faso (3 cases, ST-192) and Canada (2 cases, ST-198). We aimed to characterize nongroupable *Neisseria meningitidis* isolates, causing IMD in South Africa.

Methods: From 2003-2013, 4770 cases of IMD were reported through national laboratory-based surveillance, of which 2981 (62%) were available for serogrouping. Six isolates were phenotypically and genotypically negative for serogroups A, B, C, W, X and Y. Four of these were *ctrA* negative and *sodC* positive. These genomes were sequenced and two isolates were cnl. The *cps* locus, multilocus sequence typing loci, *porA, fetA, nadA, nhba* and *fHbp* genes were analyzed using the CLC Bio Genomics Workbench software and PubMLST *Neisseria* BIGS database.

Results: The first isolate was cultured in 2006, from the pleural fluid of a 46-year-old male, who was HIV-negative, had diabetes mellitus, and subsequently recovered. The isolate was ST-53 of clonal complex ST-53, contained porA and fetA types P1.7-2,30 and F1-2, respectively, and the *cnl-1* allele. The second was isolated in 2010, from the cerebrospinal fluid of a 15-year-old male, whose HIV status, underlying medical conditions and outcome were unknown. The isolate was ST-192, contained *porA* type P1. 18-11,42-2, a *cnl-2* allele, and was *fetA* negative. Analysis of vaccine targets showed the presence of *nhba* and *fHbp* in both isolates, whilst neither contained *nadA*.

Conclusions: This is the first report of cnl isolates causing IMD in South Africa. The isolates belonged to two lineages: ST-53 has previously been associated with carriage and has not been described in invasive disease whereas ST-192 has previously been described in IMD in Burkina Faso although with a *cnl-3* allele.

P156

Molecular analysis of the Type IV pilus motor proteins in commensal and pathogenic *Neisseria*

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Type IV pili (Tfp) of pathogenic *Neisseria* mediate twitching motility, DNA uptake, and adhesion and signaling to host cells. The macromolecular Tfp structure consists of an extracellular polymeric fiber, an outer and inner membrane-spanning assembly complex, and cytosolic proteins that assemble and retract the Tfp fiber. PilF assemble/elongates the Tfp fiber, while PilT and PilT2 cause it to retract. In *N. gonorrhoeae* (Ngo), a *pilT* mutant cannot retract pili (Merz), while a *pilT2* mutant retracts pili at half the speed of the wt parent (Kurre). PilU is also thought to affect Tfp retraction, as its gene is co-transcribed with pilT. Whether PilU affects Tfp retraction, and how PilT and PilT2 retract the fiber, are unknown.

Commensal *Neisseria* also produced Tfp (Marri; Higashi), although their Tfp differ in several important respects from pathogen Tfp. They use a different transcriptional system to regulate expression of *pilE*, the Tfp fiber subunit (IPNC abstract from Rendon et al.). Nel and Ngo retract their Tfp with different dynamics (IPNC abstract from Biais et al.).

We sought to gain a better understanding of the Tfp motor proteins in the *Neisseria* genus, and to determine whether differences in amino acid sequence between commensal and pathogenic *Neisseria* PilT, PilT2 and PilU may influence Tfp retraction dynamics. We over-expressed Nel and Ngo PilT, PilT2 and PilU separately in *E. coli* and purified the proteins to near homogeneity. Each protein purified as a hexamer, and hydrolyzed ATP in a temperature-dependent manner. Each set of orthologues hydrolyzed ATP at similar rates, with PilT2Ngo and PilT2Nel having the highest rates. We mutated select residues of Nel and Ngo PilT, PilT2 and PilU, including the lysine residue at 117 in PilTNgo, which, according to mass spectrometry studies, is acylated. The PilTNgo K117Q substitution, which mimics the presence of an acyl group (acylation “ON”), results in cells that form colonies with aberrant morphology and are deficient in DNA uptake. Experiments are underway to further characterize these retraction mutants.

P157

Pilus mediated bacterial aggregation is critical for optimal meningococcal endovascular colonization in vivo

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Background: Type IV pili (TFP) are key virulence factor that allow *N. meningitidis* (Nm) to target human vessels and to induce subsequent tissular lesions. TFP also mediate bacterial aggregation in vitro. The minor pilin PilX is required to stabilize bacterial aggregation at the time of pilus retraction under the control of the PilT ATPase.

Methods: To address the specific role of meningococcal aggregation in vascular colonization in vivo, we infected human skin xenografted SCID mice with the wild type (WT) capsulated 2C4.3 strain and with three 2C4.3 isogenic mutants: a non-aggregative Δ*pilX* mutant, an hyper-aggregative Δ*pilT* mutant and a non-piliated Δ*pilE* mutant as control. Mice were infected intravenously with 5.106 CFU of bacteria and sacrificed 4 hours later to study early colonization events. Nm endovascular human graft colonization was quantified by bacterial cultures, double anti-Nm and anti-collagen IV immunostaining to measure a vascular colonization index (VCI), and standard histopathology.

Results: Four hours after infection, bacterial loads within the grafts were similar in mice infected with the WT and the Δ*pilT* mutant (8.5 ± 0.2 and 8.4 ± 0.4 log10 CFU/g respectively). In comparison, grafts bacterial loads obtained with the Δ*pilX* and Δ*pilE* mutants were 3 and 5 logs lower (5.3 ± 0.3 and 3.6 ± 0.4 LogCFU/g respectively, $p < 0.05$). Immunofluorescence studies demonstrated that the ΔpilX strain adhered to human blood vessels, but with a very low VCI (0.12 ± 0.004 arbitrary units), only in small microvessels (< 50 µm in diameter). The VCI of the ΔpilE mutant was null. Comparatively, the VCI of the Δ*pilT* strain was significantly higher (17.9 ± 1.8 AU) but remained below the VCI of the WT strain (27.9 ± 2.0 AU, $p < 0.05$). Histopathological analysis showed widespread graft thrombotic lesions in mice infected with the WT and Δ*pilT* strains, whereas no significant lesions were observed with the Δ*pilX* and Δ*pilE* strains.

Conclusion: These data demonstrate that PilX-mediated bacterial aggregation is essential for meningococcal colonization of microvessels, and that pilus retraction is required for optimal bacterial spreading through the vasculature.

P158

Adhesion of *Neisseria meningitidis* to endothelial cells impairs the generation of the potent anticoagulant activated protein C through the cleavage of the endothelial protein C receptor

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Meningococcemia are associated with a high level of endothelial colonization and are often complicated with thrombosis, which are part of the pathological process leading to purpura fulminans. A recently developed humanized mouse model has clearly demonstrated that thrombosis is a consequence of the adhesion to microvessels endothelial cells. Our goal is to identify an adhesion-specific endothelial cell prothrombotic response.

The activated protein C (aPC) is a very potent anticoagulant protein preventing blood clotting within the vessels. APC is generated from circulating PC by thrombin, and this activation requires two endothelial cell receptors, among which the Endothelial Protein C Receptor (EPCR). The physiological importance of this anticoagulant system is attested by the thrombotic manifestations encountered in congenital or acquired deficiencies. Interestingly, homozygous protein C deficiency is associated with purpura fulminans in newborns. Thus, we hypothesized that a defect in the activation of PC following meningococcal adhesion was responsible for the thrombotic events leading to meningococcal purpura fulminans.

Using Human Dermal Microvessels Endothelial Cells (HDMEC), we showed that adhesion of *Neisseria meningitidis* (Nm) to endothelial cells induced a strong decrease of the endothelium-generated aPC. This was the consequence of a strong decrease of the EPCR exposure. A non-adherent non-piliated mutant strain had no effect on the endothelial aPC generation or EPCR expression. On the other hand, a non-capsulated non-piliated Opa-positive adhesive strain was still able to induce the loss of EPCR, demonstrating that it is not a consequence of a pilus-mediated signaling. We then showed that following meningococcal adhesion, the EPCR was cleaved from the cellular membrane by the TNFa activating protein (TACE), a process known as shedding. TACE is known to be activated in monocytes by TLR-dependent signaling. However specific transfection of siRNA against Myd88 or TRIF, two TLR-signaling adapters, had no effect on the Nm-induced EPCR shedding, suggesting that a yet unidentified pathway is responsible for this phenomenon.

The aPC has a major role in limiting the procoagulant activity of endothelium. The local defect of its activation is a likely explanation for the thrombosis that occurs following meningococcal adhesion to endothelial cells.

P159

TLR2-dependent epithelial cell activation increases cellular up-take of *Neisseriae*

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Neisseria meningitidis (NM) is an opportunistic gram-negative human pathogen. While asymptomatic carriage is common, nasopharyngeal epithelial cells colonization can be followed by bacterial invasion and severe infections with high morbidity and mortality in some patients or mild and banal infections in others. The mechanistic understanding of this dichotomy is limited. Many bacterial factors and host cell cognate receptors for bacterial recognition contribute to meningococcal invasiveness. PorB, a trimeric β-barrel outer membrane protein with 8 surface-exposed loop regions, is not a classical virulence factor but exchange of *N. gonorrhoeae* PorB with that of the commensal *N. lactamica* (NL) reduces gonococcal cell up-take in vitro and PorB sequence variants have been linked to hyper-invasive meningococcal strains. Purified PorB activates cells in vitro by binding to and signaling via Toll-like receptor 2 (TLR2), a host cell surface receptor involved in innate and adaptive immunity. The TLR2/PorB binding site has not been identified yet but it is likely located in the PorB surface-exposed loop regions. Amino acid sequence differences in these regions of PorB influence the magnitude of TLR2-dependent cell activation and intracellular signaling pathways, i.e. NF-κB and MAPKs. Modulation of these pathways can affect host cell susceptibility to bacterial up-take, thus establishing a link between TLR signaling and host cell invasion processes. Furthermore, some TLR polymorphisms have also been correlated to the clinical course and disease severity in bacterial meningitis patients, including TLR2, TLR4, TLR9 and CD14.

Here we show that stimulation of TLR2-competent human airway epithelial cells, BEAS-2B cells, and TLR2-over-expressing HEK cells with PorB or Pam3CSK4 prior to incubation with live bacteria, increases up-take of NM, of NL (non-invasive commensal) and of NM-[Nlac PorB] (hybrid NM strain expressing NL PorB, less invasive than NM likely due to porin switch). By contrast, pre-incubation with a TLR2-inactive PorB loop 7 mutant does not enhance bacterial up-take, similar to the effect of NF-κB and MAPKs inhibitors. This supports a general mechanism by which, via interaction with TLR2, PorB loop sequence variants may contribute to enhance airway epithelial cells susceptibility to organisms' uptake and subsequent disease onset (see also Poster from A. Neri).

P160

Typing and loop charges of *porB* VR of *Neisseria meningitidis* carriage and invasive isolates

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PorB, a pan-*Neisseria*e outer-membrane surface trimeric porin, mediates diffusive transport of essential molecules across the bacterial outer membrane and induces TLR2-dependent cellular responses in epithelial and immune cells. Sequence variability in the loop regions leads to differential TLR2-dependent host cell activation levels. We have performed a comparative genomic and protein structure/charge analysis of PorB from invasive strains of *N. meningitidis* isolated in Italy and from *N. meningitidis* carriage strains available on the website www.pubmlst.org.We have characterized 26 invasive meningococcal isolates by porB VR typing and MLST through the meningococcal typing database and compared PorB from these strains. Computer-based electrostatic surface analysis was performed using Adaptive Poisson-Boltzmann Solver (APBS) based on the crystal structure of PorB from *N. meningitidis* serogroup B strain 8765. A total of 9 porB alleles have been identified among invasive isolates, 3 of which as new alleles. The porB allele 2-2 was primarily associated with ST-11 complex serogroup C isolates (81%) and the porB allele 2-227 with ST-334 complex (100%). From serogroup B invasive meningococci, the porB allele 3-36 was associated mainly to ST-41/44 complex (80%). The 5 porB alleles from carriage were: 3-64, 3-106, 3-25, 3-16 and 3-84. Analysis of electrostatic surface potential shows segregation of PorB from invasive serogroup B into a negatively-charged L7 region and concomitantly positively-charged L6 region. A similar segregation was not observed for PorB from invasive serogroup C strains. PorB from carriage strains present a uniformly predominant negative charge for L6 and L7 regions throughout all the strains examined, which lack the strong opposite-charge environment between the (positive) L6 and the (negative) L7. These results suggest association between PorB alleles, serogroup and clonal complex mainly among invasive meningococci, while higher genetic variability is observed among PorB from carriage isolates. Thus, PorB sequence variants might be linked to pathogenicity or carriage status, reflecting the effect observed for different loop surface electrostatic charges on TLR2-dependent cell activation. Additional studies are needed, especially in the context of serogroup C meningococci.

P161

Investigating the role of type IV pili retraction forces of pathogenic *Neisseria gonorrhoeae* and commensal *Neisseria* elongata during their interaction with human host cells

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Type IV pili (Tfp) are long polymeric fibers produced on the surfaces of a wide variety of bacteria and archaea. In *N. gonorrhoeae* (Ng) and *N. elongata* (Ne) Tfp are involved in DNA uptake, adhesion and motility, with essential roles in biofilm formation and virulence as in the case of Ng. Tfp are dynamic and can generate pulling forces that are up to 1 nN. We investigated the role of Tfp retraction force during infection using a combination of genetics, fluorescence microscopy, optical and magnetic tweezers, and digital image analysis. We generated various deletion mutants in both Ng and Ne* for the Tfp retraction machinery: PilT the main protein responsible for Tfp retraction, and two other associated motors, PilT2 and PilU respectively. In addition, we generated Ng strains in which either or both the pilT promoter and the pilT ORF were replaced with that of Ne (and vice versa for Ne) to investigate the nature of the different retraction speeds measured between Ng and Ne and to determine their contribution to the bacteria interaction with host cells. The actin recruitment in human cells at the infection site was monitored and the Tfp pulling force on cells was manipulated either by direct infection with the bacterial mutant strains or by 'artificial' infection with magnetic beads which were subjected to variable magnetic forces. Direct measurement of the forces produced by Tfp retraction of bacteria was also recorded using flexible polyacrylamide micro-pillars. Preliminary results suggest that i) the force produced by Tfp retraction depends on PilT and PilT2, specifically that pilT2 deletion in Ng decreases Tfp retraction speed, as previously reported, and that deletion of *pilT2* in Ne also decreases by half the retraction speed, and ii) actin recruitment at the infection site directly correlates to the presence and speed of pulling forces. Our investigation further elucidates the role of the Tfp retraction machinery and the forces exerted by Tfp during bacterial interaction with human cells. *see abstract " Quick molecular techniques to generate mutants in the *Neisseria* genus" for the specifics on the methodology for generating the mutants.

P162

Neisseria meningitidis infection causes cell cycle arrest at S phase in continuous and primary human brain endothelial cells

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Recent studies have shown that some bacterial pathogens produce and secrete compounds, e.g. toxins, and effectors that interfere with the host cell-cycle. These factors, also named cyclomodulins, have been proposed to be a new class of virulence-associated factors. Previous transcriptomic data showed that *N. meningitidis* is able to alter host cell cycle gene expression, although the signaling events were not clearly understood. In this study, we analysed cell cycle alterations using an immortalised cell line of human brain microvascular endothelial cells (HBMEC/ciβ) and primary HBMEC (pHBMEC) in response to *N. meningitidis* infection.

To characterize cell cycle alterations during meningococcal infection of HBMEC/ciβ and pHBMEC, we analysed cell cycle changes using propidium iodide and 5-ethynyl-2'-deoxyuridine (EdU) pulse-labeling, which precisely establishes the cell cycle pattern based on both cellular DNA replication and nuclear DNA content. We found that *N. meningitidis* arrested both HBMEC/ciβ and pHBMEC at S phase. S phase arrest was only induced after infection with live bacteria or supernatants of *N. meningitidis* culture. Importantly, infection of cells with *E. coli* recombinantly expressing the major adhesins/invasins Opc or the opacity (Opa) proteins resulted in a S phase arrest, indicating that Opc and the Opa proteins may act as proposed cyclomodulins.

In addition, a more detailed transcriptomic analysis of *N. meningitidis*-infected HBMEC/ciβ and pHBMEC was conducted using the human cell cycle RT2 profiler PCR array and revealed alterations in the abundances of many mRNAs encoding cell-cycle regulatory molecules, including cyclin-dependent kinase inhibitors, genes involved in DNA damage responses and several S phase regulators. In contrast, there was no change in the abundances of cyclins as also confirmed by western blot analyses.

Taken together, our results confirmed that *N. meningitidis* infection induces cell cycle arrest at S-phase in immortalised and primary brain endothelial cells, which is independent of cyclin expression, but relies on Opc and Opa protein expression.

P163

Investigating the role of the non-integrin laminin receptor in the pathogenesis of meningococcal meningitis

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Despite major advances in medical treatment, bacterial meningitis caused by Group B meningococci (*Neisseria meningitidis*) is a medical emergency. Initial attachment of the meningococcus to human cells, particularly those constituting the blood brain barrier (BBB), plays a key role in the development of meningitis. *N. meningitidis*, *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* are the main causes of bacterial meningitis and each target laminin receptor (LamR), a receptor present on the surface of human brain microvascular endothelial cells, to invade the central nervous system. We aimed to investigate putative regions of LamR that may responsible for binding the N. meningitids ligands- PorA- (specifically its 4th extracellular loop) and PilQ. Targeted mutations were introduced into recombinant LamR (rLamR) and wild type and mutated rLamR was expressed in Human Embryonic cells (HEK 293T) and purified under native condition. Enzyme-linked immuno-sorbent assays (ELISA) were used to examine the interaction of the LamR proteins with meningococcal the outer membrane proteins PorA and PilQ, a peptide corresponding to PorA Loop4, and intact *N. meningitidis* bacterial cells. We identified residues required for optimal binding of PorA and PilQ. We also demonstrated that meningococcal PorA and PilQ act synergistically as ligands for LamR. These results provide a more detailed understanding of the role of LamR in the pathogenesis of meningitis.

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Neisseria meningitidis sensing of host cells induces microcolony dispersal

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Initial colonization of the human pathogen *Neisseria meningitidis* to host cells is a key factor in the development of disease. The bacteria colonize the epithelial layer in the nasopharynx asymptomatically but occasionally cross the mucosal barrier and blood-brain barrier, causing sepsis and meningitis, respectively.

In encapsulated strains, the type IV pilus (Tfp) is one of the most important virulence factors and plays a major role in the initial adhesion. A single bacterium uses the pilus tip adhesin to bind to host cells. Both in liquid and on host cells, meningococci aggregate through pilus-pilus interactions, forming structures termed microcolonies. After several hours, the bacteria detach from the microcolonies. On host cells, the bacteria can then form intimate adhesion, which is highly important for bacterial traversal of the epithelial barrier. The dispersal of microcoloniesis a highly concerted event but the causative factor is unknown.

In this study, we analyzed the mechanisms behind synchronized microcolony dispersal. Formation and subsequent dispersal of microcolonies was much faster in the presence than in the absence of host epithelial cells. However, direct contact with host cells was not necessary since similar results were obtained at cell confluences of 50% and 100%, i.e. microcolonies that attached to the cell culture dish itself also showed synchronized dispersal. Also, a non-adherent mutant showed identical pattern of dispersal as the wild type. Further, medium incubated with uninfected host cells and added to microcolonies in liquid triggered dispersal. This indicates the presence of a soluble signaling compound derived from host cells. In summary, these findings indicate that meningococci sense molecules secreted by host cells, which triggers the dispersal of microcolonies.

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Neisseria gonorrhoeae breaches the epithelial barrier by inducing calcium flux and calcium-dependent activation of non-muscle myosin II for tissue invasion

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Neisseria gonorrhoeae (GC) infects epithelial cells lining the female reproductive tract, which can lead to pelvic inflammatory and disseminated diseases. GC interactions with epithelial cells are known to induce signaling, including Ca2+ flux in host cells. This study examined the mechanism by which GC-induced signaling contributes to GC infection in polarized epithelial cells. We demonstrated that the interactions of GC (MS11) with the apical surface of polarized epithelial cells induce Ca2+ flux and the activation and accumulation of non-muscle myosin II (NMII) at the apical junction and the apical surface of epithelial cells. GC-induced activation and redistribution of NMII is dependent on Ca2+ and a myosin light chain kinase that is activated by Ca2+-bound calmodulin. Inhibition of either GC-induced Ca2+ flux or NMII activation blocks GC-induced disruption of the apical junction and reduces GC transmigration, but has no significant effect on GC adherence and invasion into polarized epithelial cells. Compared to wild type MS11 GC, a derivative of MS11 strain where all 11 Opa genes are deleted induces the disassembly of the apical junction and the activation and redistribution of NMII to significantly greater magnitude, consequently transmigrating at a much higher efficacy. Electron microscopic analysis showed that unlike wild type GC, the Opa deletion mutant alters the apical morphology and forms extensive interaction with the apical membrane of polarized epithelial cells. Taken together, our results reveal that GC disrupts the apical junction of the epithelial barrier by inducing Ca2+ flux and Ca2+-dependent activation and reorganization of junction-associated actomyosin, which facilitates GC invasion into the mucosal tissue. These results suggest that phase-variation of Opa proteins can change the level and nature of GC infectivity via modifying the interaction of GC with the epithelium and induced signaling.

P166

Quick molecular techniques to generate mutants in the *Neisseria* genus

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We have developed simple and highly efficient mutagenesis protocols based on PCR products to obtain mutants in multiple *Neisseria* species. Thus far, generating mutants in pathogenic *Neisseria* has predominantly relied on the construction of plasmids bearing flanking sequences of the gene or region targeted for deletion. These homologous regions enable recombination at desired genomic loci. Inspired by similar techniques used to obtain gene deletions in S. cerevisiae, then modified in *E. coli* and more recently for *Vibrio cholerae* our techniques bypass the need for subcloning into plasmid vectors by using PCR products to directly transform bacteria. We have designed vectors bearing various antibiotic resistance cassettes under the control of a consensus promoter and including a *Neisseria* DNA uptake sequence. PCR utilizing long primers containing upstream and downstream regions of the gene targeted for deletion along with complementary sequences to the antibiotic resistance vector produces a PCR product that can be directly transformed into *Neisseria* species by spot transformation on agar plates. With the typical one day of selection on antibiotic plates, mutants can be obtained in as little as two days. As few as 80 base pairs of homology to both the upstream and downstream sequences were sufficient to obtain mutants, but homology of 150 base pairs was sometimes necessary. In addition, as an alternate approach to the use of long primers, we have used our plasmids as the base to leverage the combinatorial possibilities offered by Gibson assembly protocols and used this DNA to transform *Neisseria*. We have successfully applied these techniques to generate mutants in both *Neisseria gonorrhoeae*, the causative agent of gonorrhea, and the human commensal *Neisseria elongata* - In particular, we have generated Type IV pili motor protein deletion mutants for both of these species1. These protocols provide both modularity and speed to *Neisseria* mutagenesis, allowing for the development of complex systematic mutant libraries in the *Neisseria* genus.
1. See accompanying abstracts “Mechanobiology of a Commensal *Neisseria* species” and “Investigating the role of type IV pili retraction forces of pathogenic *Neisseria gonorrhoeae* and commensal *Neisseria elongata* during their interaction with human host cells” for details.

P167

The moonlighting functions of meningococcal fructose 1,6-bisphosphate aldolase: adhesion and plasminogen binding

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Background: Fructose 1, 6 bisphosphate aldolase (FBA) is a glycolytic enzyme in the Embden-Meyerhof-Parnas (EMP) pathway (an inactive pathway in *Neisseria meningitidis*). Previously, FBA has been shown to be localised to the meningococcal cell surface and to have non-glycolytic (moonlighting) functions related to adhesion to host cells. This study further explores the moonlighting functions of FBA in the pathogenesis of meningococcal disease.

Methods and Results: Wild-type rFBA and rFBA with mutations in the active cation-binding site (D83A and H81A/H84A) were cloned, overexpressed and purified. A coupled enzyme assay confirmed the aldolase activity of wild-type rFBA. In contrast, rFBA with mutation(s) in the active (cation-binding) site (D83A and H81A/H84A) had no detectable enzymatic activity. Employing flow cytometry, FBA could be detected on the surface of wild type *N. meningitidis* MC58 cells but not onMC58Δ*cbbA* (FBA-deficient mutant). Complementation of MC58Δ*cbbA* with an ectopic copy of *cbbA* (either wild type or D83A and H81A/H84A variants) restored the ability to express FBA on the surface suggesting the lack of involvement of the active site in the transportation of FBA to the cell surface. Moreover, *N. meningitidis* MC58ΔcbbA showed impaired adherence to human brain endothelial (HBME) cells compared with its wild type parent. Complementation of MC58Δ*cbbA* with an ectopic copy of *cbbA* (either wild type, or D83A and H81A/H84A variants) restored the ability to adhere to HBME cells.

Furthermore, we demonstrate that rFBA binds human Glu-plasminogen. No significant difference was observed between the plasminogen binding by wild type rFBA and rFBA lacking aldolase activity. Plasminogen binding was inhibited by the lysine analogue, ε-aminocaproic acid, indicating the involvement of lysine residue(s) in this interaction. Substitution of the terminal lysine residue of rFBA with alanine dramatically reduced the binding of plasminogen.

Conclusions: Taken together, our data suggest that the moonlighting functions of meningococcal FBA on the bacterial surface include the binding of human plasminogen and the facilitation of optimal adhesion to host cells, and that both of these functions are independent of its aldolase activity.

P168

Molecular characterization of *Neisseria meningitidis* isolates collected through Active Bacterial Core surveillance and an enhanced surveillance in the United States, 2010-11

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Background: The *Neisseria meningitidis* (Nm) population is very dynamic due to frequent horizontal gene transfer events. Therefore, it is crucial to continuously monitor changes in the molecular epidemiology and the emergence of new invasive strains over time. In the United States, the molecular epidemiology of Nm is monitored through the population-based Active Bacterial Core surveillance (ABCs) program. Nm isolates are also collected from several states that conduct enhanced surveillance to monitor additional circulating meningococcal strains.

Methods: Nm isolates collected from ABCs (which covers 10 states and approximately 13.6% of the population) during 2010 (n=79, 91.9% of cases) and 2011 (n=101, 97.1% of cases) and from enhanced surveillance (10 states, 31% of the population) during 2010 (n=162, 50.6% of cases) were characterized using multilocus sequence typing (MLST) and genotyping of outer membrane proteins and serogroup B (NmB) vaccine antigens. The molecular profile of isolates collected from ABCs and enhanced surveillance were compared. Analyses of NmB isolates collected through ABCs were weighted to reflect a prolonged NmB outbreak in Oregon.

Results: Three predominant clonal complexes (CC11, CC23, CC32) accounted for at least 60% of the isolates in each of the three collections, both ABCs years and the enhanced surveillance program. PorA showed the most diversity among the genotypes determined. The 5 predominant PorA types account for >45% of the isolates. There were no significant differences in the distribution of serogroup B subvariants between ABCs and enhanced surveillance. Although new genotypes with low frequency were detected among isolates from both ABCs and enhanced surveillance, enhanced surveillance detected additional genotypes not observed in ABCs collection.

Conclusions: ABCs and enhanced surveillance both capture common genotypes currently in circulation in the US and therefore provide useful systems to monitor the molecular epidemiology of *Neisseria meningitidis*. Enhanced surveillance also captures genotypes that are rare or not detected among the ABCs collection, that are associated with high rates of disease in other countries. Given the low incidence rate of meningococcal disease in the US and the highly dynamic structure of the circulating meningococcal population, the enhanced surveillance system improves monitoring for the emergence of new meningococcal strains.

P169

Restriction endonucleases from invasive *N. gonorrhoeae* cause DNA double-strand breaks and distort mitosis in epithelial cells during infection

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Neisseria gonorrhoeae infections in non-tumorigenic vaginal epithelium disrupts host cell cycle regulation machinery. The infection causes DNA double strand breaks that delay progression through the G2/M phase. We show in qPCR assays that gonococcal restriction endonucleases are upregulated during invasion. Bacterial lysates containing restriction endonucleases were able to fragment genomic DNA as detected by PFGE. Lysates were also microinjected into the cytoplasm of the cell and after 20 hours, DNA double strand breaks were identified by 53BP1 staining. These data show that the restriction endonucleases can damage human chromosomal DNA and access the nucleus through nuclear pores. In addition, by using live-cell microscopy and DyLight NHS-ester 594 stained live gonococci we visualized the intracellular location of the bacteria upon mitosis. We detected a direct interaction between intracellular gonococci and host cell chromatin during mitosis potentially causing a sterical hindrance for anaphase progression but also enabling direct nucleomodulatory distortion of the chromatin. Taken together, infected cells show impaired and prolonged M-phase, nuclear swelling and lagging chromosomes resulting in micronuclei formation and chromosomal instability of the infected cell. These data highlight basic molecular functions of how gonococcal infections affect host cell cycle regulation, cause DNA double strand breaks and predispose cellular malignancies.

P170

Fibroblast Growth Factor1 is essential for meningococcal invasion into human brain microvascular endothelial cells

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Background: *Neisseria meningitidis* (meningococcus) is an obligate human commensal bacterium that can cause meningitis and sepsis. Crossing the Blood-Brain Barrier (BBB) is a crucial step in the development of meningitis, but the mechanisms used by the meningococcus to achieve this are not fully understood. The aim of this study was to investigate the role of the Fibroblast Growth Factor1-IIIc isoform (FGFR1-IIIc) in the attachment to, and invasion of, Human Brain Microvascular endothelial cells (HBMECs) by *N. meningitidis*.

Methods: Confocal microscopy was employed to investigate the possible association of meningococcal microcolonies with the receptor FGFR-1-IIIc. ELISA assays were employed to demonstrate a direct interaction between the receptor and bacterial cells. siRNA knock-down was employed to investigate the influence of FGFR-1-IIIc on meningococcal adhesion to and invasion into HBMECs.

Results: Confocal microscopy showed that micro-colonies of adhered *N. meningitidis* recruit activated FGFR1. Direct interaction between meningococci and the extracellular domain of FGFR1-IIIc was demonstrated by ELISA confirming the ability of this bacterium to bind FGFR1-IIIc. Other bacterial meningeal pathogens, including *Streptococcus pneumoniae* and *Haemophilus influenzae*, were unable to bind to this receptor confirmed specificity. siRNA Knock down of FGFR1 showed that expression of this receptor is required for invasion of bacteria into HBMECs as the number of invaded bacteria into endothelial cells significantly reduced in FGFR1 knock down HBMECs compared to controls.

Conclusions: This study identified a novel receptor for meningococci, FGFR1, which plays an important role in the pathogenesis of this pathogen, and may constitute a new therapeutic and prevention target for disease caused by these bacteria.

Oral Abstracts

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O1

A mouse model for studying the genetic and immunologic mechanisms of *Neisseria* commensalism

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Our body is home to a large number of bacteria. A fundamental but unexplored question is how our native microbiota, a.k.a. commensal bacteria, colonize and persist in the body in the face of a healthy immune response.

We believe that *Neisseria* and the laboratory mouse are an ideal pair for addressing this issue. In vitro studies have generated a large body of literature on the genetic, biochemical, and cell biological mechanisms by which human-adapted *Neisseria* interact with host cells. The mouse is the sine qua non for modeling disease. Accordingly, we undertook to develop an animal model for studying the genetic and immunological mechanisms of bacterial commensalism that is based on inoculating mice with a species of *Neisseria* native to this animal. This model circumvents the issues of host specificity that hamper many animal models; and because of the genetic relatedness of animal- and human-adapted *Neisseria*, the model has the potential to help us understand how pathogenic *Neisseria* establish asymptomatic infection, an aspect of pathogenesis that has been intractable to experimentation.

Neisseria species are cultured from a variety of animals, from rodents to man. We isolated *Neisseria spp* from wild mice in two distinct regions of North America. One of them, AP2031, is most closely related to *Neisseria dentiae*, a bovine isolate. AP2031 is amenable to genetic manipulation, and encodes orthologues of pathogenic *Neisseria* determinants that mediate host cell interactions in vitro, as well as orthologues of *Neisseria meningitidis* vaccine targets.

AP2031 colonizes lab mice without inducing symptoms of illness, and persists in the animals for at least six months. Mice with different genetic backgrounds and immune deficiencies are being tested for differences in their susceptibility/resistance to *Neisseria* colonization, and bacterial mutants for their ability to colonize, persist and engage in horizontal gene transfer in the animal.

O2

The dynamic 'acetylome' of *Neisseria gonorrhoeae* in biofilm formation

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Recently there has been a growing appreciation for the role of lysine acetylation in prokaryotes as an important posttranslational modification (PTM). Acetylation changes the charge state of lysine residues, resulting in structural changes that can alter enzymatic function, protein-protein interactions, and localization. To assess the landscape of protein acetylation in *Neisseria* and understand how this PTM affects signaling networks and metabolism, especially with respect to biofilm formation, we carried out a global proteomic study to identify the proteins and sites that undergo acetylation. Cultures of wildtype and an acetate kinase (ackA) mutant of *N. gonorrhoeae* strain 1291 were prepared and proteins isolated, proteolytically digested, and acetyllysine-containing peptides enriched by immunoprecipitation. Using quantitative proteomics, we then compared these populations to assess the sites and proteins that appeared to be regulated by the levels of acetylphosphate (acP), an acetyl donor whose concentration is increased in the ackA mutant compared to wildtype. Based on these data, the acetylation states of various mutants deficient in biofilm formation were studied, including luxS and a putative acetyltransferase. These studies show that a number of proteins are dynamically acetylated and also impact the acetylation status of other proteins in networks that control biofilm formation. Together, we are now assessing the functional roles of these genes with respect to the gonococcal acetylome when grown as biofilms using an in vitro model. For example, in the wildtype strain, we identified 617 unique acetylated sites in a biofilm sample compared to 451 from the planktonic (runoff) control sample. We have begun comparing the wildtype findings with our various mutants. These studies suggest that a number of proteins show differential acetylation in the wildtype compared to the biofilm deficient mutants including porin and ClpB, and number of pilus associate proteins, including PilT, PilM, PilQ, PilU, RegF, PilD and PilZ. Our longer-term goal is to understand the effect of variable acetylation on the biology of *Neisseria gonorrhoeae* by moving into a human cervical epithelial cell model, which will require a more targeted proteomic approach due to the complexity of this co-cultured system.

O3

Localization and substrate specificity of lytic transglycosylases LtgA and LtgD contribute to high levels of peptidoglycan monomer production

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Peptidoglycan (PG) fragments are generated during normal cell wall growth and division. *N. gonorrhoeae* and *N. meningitidis* release these soluble PG fragments during growth, with *N. gonorrhoeae* releasing significantly more PG fragments. In Fallopian tube organ culture, the addition of monomeric PG fragments recapitulates the ciliated cell damage seen in patients with pelvic inflammatory disease. Previous studies demonstrated that two lytic transglycosylases, LtgA and LtgD, generate these toxic PG monomers. We seek to understand the mechanisms involved in PG monomer generation and release, the reasons that *N. gonorrhoeae* releases more of these toxic fragments than related species or other Gram-negative bacteria, and the consequences for different types of host cells during infection. Inefficiency in PG recycling by *N. gonorrhoeae* was found to account for part, but not all, of the differences in PG release between species. This result suggests that aspects of PG fragment generation and/or release are important in determining amounts of fragments released. We examined LtgA and LtgD function by determining the biochemical activities and localization of these proteins. HPLC and LC/MS analyses demonstrated that LtgA and LtgD act to degrade sacculi to 1,6-anhydro PG monomers with di, tri, tetra, or penta-peptide side chains as well as PG dimers with various peptides attached. In contrast with the activities of related enzymes in *E. coli,* both enzymes were able to degrade a synthetic glycosydically-linked PG dimer to PG monomers, suggesting one explanation for increased PG monomer production in gonococci. Fluorescence microscopy demonstrated that LtgA and LtgD are found at the septum, but LtgD can also be seen at other locations around the cell. Subcellular localization studies found that LtgA and LtgD are localized to the outer membrane, and for LtgD it was demonstrated that this localization was required for high levels of PG fragment release. Overall, these studies indicate that aspects of lytic transglycosylase substrate specificity and localization combine with inefficiency in PG recycling to cause *N. gonorrhoeae* to show increased PG monomer production and release.

O4

The two-component system NtrYX is a key regulator of respiratory gene expression in *Neisseria gonorrhoeae*

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NtrYX is a sensor-histidine kinase/response regulator two-component system that is extensively distributed across the bacterial Domain. It is present in a variety of beta-Proteobacteria, including *Neisseria gonorrhoeae*. Microarray analysis and enzyme activity measurements revealed that the expression of key respiratory chain components was reduced in an *N. gonorrhoeae* ntrX mutant compared to that in the isogenic wild-type (WT) strain 1291. Respiratory enzymes affected included cytochrome oxidase subunit (cytochrome cbb3), nitrite reductase (AniA) and nitric oxide reductase (NorB). *N. gonorrhoeae* ntrX mutants had reduced capacity to survive inside human primary cervical cells compared to the wild type and, although they retained the ability to form a biofilm, they exhibited reduced survival within the biofilm compared to wild-type cells, as indicated by LIVE/DEAD staining.

NtrY sequences in the database are highly conserved, being recognized as members of COG5000. Analysis of the conserved domains present in NtrY showed that this ~ 730 amino acid protein contains an N-terminal transmembrane anchor, a putative N-terminal signalling domain as well as a HAMP domain, a Per-ARNT-Sim (PAS) domain and the typical His-Kinase catalytic domain towards the C-terminus. The entire soluble domain of NtrY has been expressed in E. coli and purified. It binds heme in a 1:1 stoichiometry suggesting that this PAS domain may be involved in the sensing of oxygen and/or nitric oxide.

O5

A genetic screen reveals a periplasmic copper chaperone required for nitrite reductase activity in pathogenic *Neisseria*

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Background: Bacterial denitrification is an important respiratory process that is present in a physiologically and taxonomically diverse range of bacterial species, including pathogenic bacteria. *Neisseria meninigitidis* and *N. gonorrhoeae* can use nitrite as a terminal electron acceptor to grow anaerobically. Nitrite reductase (AniA) is a copper-containing protein that converts nitrite (NO2-) to nitric oxide (NO.). Nitric oxide is then reduced to nitrous oxide (N2O) by nitric oxide reductase NorB. Since nitrous oxide reductase is not active in these pathogenic bacteria then N2O is the end product of nitrite production. Unlike other Gram-negative bacteria, it appears that the nitrite reductase AniA of pathogenic *Neisseria*is expressed on the surface of the outer membrane rather than in the periplasm. The unique location of AniA provides a challenge in relation to protein folding, copper loading and electron transport to the outside of the outer membrane and the role of accessory proteins required for these processes has not been defined.

Methods: In the presence of nitrite in growth medium, a NorB mutant strain of pathogenic *Neisseria* accumulates nitric oxide leading to bacterial death. This lethal phenotype of the NorB mutant can be suppressed by loss of AniA function. This observation allowed development of a genetic screen in which cells could only survive if they lost AniA activity.

Results/Discusions: Screening a random mutant library of *N. meninigitidis* strain ¢3norB::kan under nitrite conditions revealed a gene encoding a periplasmic copper chaperone that is essential for AniA activity, called AniA Copper Chaperone A (AccA). Cu stoichiometry measured using a bathocuproine disulfonate assay confirmed that AccA contains a Cu(I) centre and surface plasmon resonance revealed a direct and high affinity protein-protein interaction between AccA and AniA in vitro. Apart from the *Neisseria* genus, homologues of AccA can only be found in environmental diazotrophic bacteria. Parallel studies *N. gonorrhoeae* strain 1291 reveal that the same AccA chaperone is required for AniA function. We conclude that *Neisseria meninigitidis* and *N. gonorrhoeae* require this novel, periplasmic copper chaperone due to the unique localisation of AniA.

O6

Global analysis of HPr role in *Neisseria meningitidis* physiology and virulence and its implication during experimental infection in mice

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Background: HPr (encoded by the ptsH gene) is part of the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS), a carbohydrate transport system that allows simultaneously the sugar uptake and its phosphorylation. In several bacteria, besides sugar transport, HPr is also involved in the regulation of several other cellular processes including the expression of virulence factors in response to carbon source availability, a phenomenon called carbon catabolite repression (CCR) (Deutscher et al., 2014). *Neisseria meningitidis* (Nm) possesses an incomplete PTS with an HPr. Previously, several genes essential for meningococcal bacteremia were identified that encoded for metabolic enzymes, including HPr, suggesting a tight correlation between carbon metabolism and virulence in Nm (Sun et al., 2000).

Methods and Results: Growth of ΔptsH strain was not impaired in vitro either in rich medium or in presence of limited carbon sources. We hence constructed wild type and ΔptsH strains that were bioluminescent and used them in experimental infection in transgenic mice expressing the human transferrin. Six hours after intraperitoneal challenge both wild-type and ΔptsH strains were recovered at similar levels from the peritoneal cavity. However, ΔptsH strains provoked lower levels of septicemia than the wild-type strain in transgenic mice. We therefore analyzed transcriptomic profiles and observed pleiotropic differential gene expression between wild-type and ΔptsH strains recovered from mice 6h post-infection. The innate immune response of the host was also altered as shown by differential cytokines production, inflammatory cell recruitment and bacterial survival in mice sera.

Conclusions: Our data suggest that HPr plays a pleiotropic role in Nm that impacts the host-bacteria interactions. The lack of ptsH modifies the interaction of bacteria with the innate immune response enhancing the clearance of ΔptsH strain.

O7

NagZ triggers gonococcal biofilm disassembly

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Neisseria gonorrhoeae is capable of forming biofilms on glass surfaces and human cells. They are dynamic structures, with bacteria constantly leaving and joining the biofilm. However, the mechanism by which gonococci form a biofilm, the process gonococci use to leave it, and the role of biofilms in gonococcal disease is largely unknown. We identified a gene encoding a beta-hexosaminidase (NagZ) in *N. gonorrhoeae* that modulates biofilm formation by *N. gonorrhoeae* by functioning as a dispersing agent. In comparison to the parental strain FA1090, a strain with this gene deleted (FA1090DNagZ) produced a biofilm with increased mass under both static and dynamic conditions. The biomass of biofilms made by FA1090 peaked at 48 hrs, and then began to disperse, while the FA1090DNagZ biofilm continued to enlarge. Erosion of the biofilm by wild type FA1090 correlated with death of the bacteria. Scanning electron microscopy and confocal laser microscopy were able to visualize differences in the biofilms formed by the two strains. No differences in membrane blebbing or extracellular DNA were observed between the parent and isogenic mutant at early time points in biofilm formation, suggesting that the differences in biofilm are not due to the deficiency of blebbing or lack of extracellular DNA. Biofilms formed by FA1090DNagZ were destroyed by addition of exogenously added purified NagZ. NagZ was characterized biochemically and defined as a hexosaminidase, able to act on *neisseria*l peptidoglycan fragments and other substrates containing ²-1-4-N-acetylhexosamine. The enzyme lacked β-1-6 endoglycosidase, β-1-6- and b1-3 exoglycosidase and a1-2 endoglycosidase activity. The purified enzyme failed to act on *neisseria*l lipooligosaccharide. This is the first study to demonstrate that an enzyme thought to be restricted to peptidoglycan recycling is able to moonlight to modulate biofilm formation. The action of this enzyme can explain why gonococci do not accumulate on epithelial surfaces during infection.

O8

The neisserial outer membrane protein SLAM is required for translocation of surface lipoproteins across the outer membrane of Neisseria

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Lipoproteins decorating the surface of many obligate host restricted Gram-negative bacterial pathogens play essential roles in immune evasion and nutrient acquisition. In particular, surface lipoproteins (SLPs) such as Factor H binding Protein (fHbp) and Transferrin binding protein B (TbpB) inserted into the outer leaflet of the outer membrane of *Neisseria meningitidis* elicit bactericidal antibodies and are required for virulence. As such, these SLPs are primary targets for broad-spectrum vaccine development. However, the machinery and mechanism that lead to the surface display of these SLPs is not understood.

Herein, we describe an essential component in the translocation machinery required to deliver these SLPs to the extracellular surface of *Neisseria meningitidis*, which we have named Surface Lipoprotein Assembly Modulator (SLAM). Using FACS, proteinase K and solid phase binding assays we illustrate how SLAM is required for surface display of TbpB, LbpB and fHbp in *Neisseria meningitis*. Highlighting the essential nature of this function, SLAM-deficient neisserial strains are avirulent within a mouse sepsis model. Finally, the *Neisseria* SLAM protein alone is sufficient to reconstitute surface lipoprotein transport in laboratory strains of *E. coli,* which do not otherwise display lipoproteins on their surface, suggesting it is the key translocation component required for the proper display of SLPs.

SLAM thus represents a key but previously unrecognized factor in virulence of *Neisseria*, providing a novel new therapeutic target to prevent *neisseria*l infections and a novel biotechnological approach to display proteins on the Gram negative cell surface.

O9

Concerted spatio-temporal dynamics of imported DNA and ComE DNA uptake protein during gonococcal transformation

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Competence for transformation is widespread among bacterial species. In the case of Gram-negative systems, a key step to transformation is the import of DNA across the outer membrane. Although multiple factors are known to affect DNA transport, little is known about the dynamics of DNA import. Here, we characterized the spatio-temporal dynamics of DNA import into the periplasm of *Neisseria gonorrhoeae*. DNA was imported into the periplasm at random locations around the cell contour. Subsequently, it was recruited at the septum of diplococci at a time scale that increased with DNA length. We found using fluorescent DNA that the periplasm was saturable within minutes with ~ 40 kbp DNA. The DNA-binding protein ComE quantitatively governed the carrying capacity of the periplasm in a gene-dosage-dependent fashion. As seen using a fluorescent-tagged derivative protein, ComE was homogeneously distributed in the periplasm in the absence of external DNA. Upon addition of external DNA, ComE was relocalized to form discrete foci colocalized with imported DNA. We conclude that the periplasm can act as a considerable reservoir for imported DNA with ComE governing the amount of DNA stored potentially for transport through the inner membrane.*

*Gangel H, Hepp C, Mueller S, Oldewurtel ER, Aas FE, et al. (2014) Concerted Spatio-Temporal Dynamics of Imported DNA and ComE DNA Uptake Protein during Gonococcal Transformation. PLoS Pathog 10(4): e1004043. doi:10.1371/journal.ppat.1004043

O10

Mechanobiology of a commensal *Neisseria* species

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During the last decade the study of physical forces in biology, also called mechanobiology, has shed light on eukaryotic phenomena in a diverse set of fields such as embryonic development, cancer, and cell signaling. The importance of mechanobiology is becoming acknowledged in prokaryotic systems as well and is exemplified in the human pathogen *Neisseria gonorrhoeae* (Ng). The retraction of Ng Type IV pili (Tfp) and the forces they exert on their surroundings have been demonstrated as crucial in most aspects of Ng behavior such as: motility, DNA uptake, infection…etc. A comprehensive genomic study of human *Neisseria* species highlighted the fact that all members of the genus (including commensals) have the genomic capacity to produce Tfp. This prompted us to study a commensal member of the genus *Neisseria elongata* (Ne). We generated an in-frame deletion mutant of the AAA-ATPase PilT responsible for Tfp retraction along with a complemented strain in Ne1. Using an array of force measuring devices, fluorescence microscopy, and in vitro invasion assays we were able to show that Ne possesses fully retractable Tfp which modulate the interaction of Ne with human epithelial cells. Tfp retraction force amplitudes are comparable to those exerted by pathogenic Ng (~70 pN) with pull speeds that are 2 fold slower than that in Ng (~0.5 µm/s for Ne compared to ~1 µm/s for Ng). This study demonstrates the utility of the genus *Neisseria* as a model for studying how physical forces can determine pathogenicity or commensalism. Understanding the role of force in both commensalism and pathogenicity will provide insights in the disease process while demonstrating the evolution of the Tfp machinery.

1. See abstract " Quick molecular techniques to generate mutants in the *Neisseria* genus" for the specifics on the methodology for generating the mutants.

O11

Modulation of gonococcal type IV plus expression and function

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The type IV pilus is a major virulence factor of *Neisseria gonorrhoeae* (Gc) and is established as a critical determinant for adherence, motility, and genetic transfer. We have demonstrated that pilus-mediated motility and adherence to epithelial cells is modulated by interactions between components of seminal plasma (semen without insoluble material) and the Gc pilus [1]. We have defined molecules responsible for this alteration of pilus function, probed the molecular basis of the binding to the pilus and have also shown an effect of these substances on Gc biofilm formation. Additionally, production of a functional Gc pilus requires the peptidoglycan-reactive, zinc-metalloprotease, Mpg [2], and pilus expression is required for the resistance to oxidative and non-oxidative killing mechanisms of PMNs [3]. We are examining the mechanistic basis for the role of Mpg in pilus expression as well as pilus-dependent resistance to PMN killing. These studies expand our understanding of type IV pilus function in the context of host interactions provides to Gc, and show that environmental factors influence pilus function and pathogenesis.

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O12

Structural and functional investigations of the DUS (DNA uptake sequence) receptors in the neisseriaceae family

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A remarkable feature of *Neisseria* species is their competence for natural transformation. This is a powerful mechanism for generating genetic diversity through horizontal transfer of genes, and a key virulence property. For example *N. meningitidis*, the causative agent of meningococcal disease, displays extensive genetic diversity with as much as ten percent of its gene content differing between isolates. However, because transformation by foreign DNA is most of the time deleterious, *Neisseria* species have developed a contingency mechanism to limit this by displaying a marked preference for their own DNA that contains specific DNA uptake sequence (DUS) motifs. This preference is at the level of DNA uptake, the first step of natural transformation, which was known to be mediated by type IV pili (Tfp). Our group has recently verified that the DUS receptor in the meningococcus is a minor pilin subunit of Tfp, ComP, which is not only able to bind DNA but demonstrates an exquisite preference for DUS. We have confirmed that a similar mechanism, through co-evolution of ComP orthologs and variant DUS motifs, is likely to be used by all *Neisseria* species and other members of the Neisseriaceae family species to promote transformation by their own DNA. We will present evidence which, using in vitro and in vivo experimental approaches, highlights important features of DUS variants and their cognate ComP receptors. Importantly, our high-resolution structure of *N. meningitidis* ComP paints a picture of an unusual type of DNA binding protein, and suggests an elegant mechanism whereby competent Neisseriaceae are able to modulate horizontal gene transfer while sharing the same ecological niche.

O13

The number of *Neisseria meningitidis* type IV pili determines host cell interaction

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As mediators of adhesion, autoaggregation and bacteria-induced plasma membrane reorganization, type IV pili are at the heart of *Neisseria meningitidis* infection. Previous studies have proposed that two minor pilins, PilV and PilX, are displayed along the pilus structure and play a direct role in mediating these effects. In contrast with this hypothesis, combining imaging and biochemical approaches we found that PilV and PilX are located in the bacterial periplasm rather than along pilus fibers. Furthermore, preventing exit of these proteins from the periplasm by fusing them to the mCherry protein did not alter their function. Deletion of the *pilV* and *pilX* genes led to a decrease in the number, but not length, of pili displayed on the bacterial surface indicating a role in the initiation of pilus biogenesis. By finely regulating the expression of a central component of the piliation machinery, we show that the modest reductions in the number of pili are sufficient to recapitulate the phenotypes of the *pilV* and *pilX* mutants. We further show that specific type IV pili-dependent functions require different ranges of pili numbers.

O14

Sexual transmission of meningococci may account to an outbreak of meningococcal disease among men who have sex with men

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Introduction: An increase in group C meningococcal disease incidence among men who have sex with men (MSM) was recently observed in Europe and the United States. We aimed to explore bacterial specific factors that might explain this outbreak.

Methods: Invasive meningococcal isolates were extensively analysed using whole genome sequencing (WGS), transcriptomic and proteomic analysis. Experimental infection in transgenic mice was performed to evaluate invasiveness of the isolates.

Results: Meningococcal isolates from MSM were identical by genotyping, and belonged to clonal complex cc11. WGS showed emergence of a new branch (clade) within cc11. While close to the branch of other ET-15 isolates, MSM isolates expressed new functional fHbp alleles. Interestingly, group C/cc11 isolates from urethritis cases in men also clustered in the new branch based on WGS. However, all urethritis isolates harboured a non-functional fHbp allele with a frame-shift mutation. Experimental infections in transgenic mice expressing human factor H (fH), a complement regulatory protein, suggested higher invasiveness of invasive isolates from MSM compared to urethritis isolates. Moreover, transcriptomic and proteomic analyses showed consistent expression of aniA gene in invasive MSM and urethritis isolates when compared to other cc11 isolates. Lack of AniA expression was caused by point mutations as evidenced by genome data. Biochemical and growth tests confirmed the enzymytic activity of AniA and functionality of the anaerobic respiration pathway in MSM isolates, but not from isolates of an adolescent outbreak of group C disease.

Discussion and conclusions: Altered expression of AniA may have conferred a selective advantage on urethral mucosal surfaces and sexual spread. AniA is essential for gonococcal growth under oxygen limiting conditions that may prevail on the genitourinary pathway. The consecutive acquisition of functional fHbp by invasive isolates found in MSM in contrast to urethritis isolates may explain, at least in part, the recovery of invasiveness, as these isolates as shown by enhanced blood stream survival during experimental infection. Our data provide evidence for specific patho-adaptation of meningococcal serogroup C cc11 isolates from MSM and warrant targeted preventive measures (MenC vaccination) for persons closely associated with this community.

O15

Genomic analysis of the evolution and global spread of hyperinvasive meningococcal lineage 5

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The predominant model for bacterial pandemics is the emergence of a virulent variant that diversifies as it spreads in human populations. We investigated a 40-year meningococcal disease pandemic caused by the ET-5/ST-32 complex (defined here with genomic data as Lineage 5). Analysis of whole genome sequence data from representative isolates identified a 'Lineage 5 pan genome' of ~2,080 genes, 1,887 of which were present in all isolates (Lineage 5 'core genome'). Genetic diversity, which was mostly generated by horizontal gene transfer, was unevenly distributed in the genome; however, genealogical analysis of diverse and conserved core genes, accessory genes, and antigen encoding genes, robustly identified a star phylogeny with a number of sub-lineages. Most European and American isolates belonged to one of two closely related sub-lineages, which had diversified before the identification of the pandemic in the 1970s. These sub-lineages were antigenically distinct, each with a stable antigenic repertoire including vaccine components, but contained appreciable genetic diversity. A third, genetically more diverse sub-lineage, was associated with Asian isolates, some of which had acquired DNA from the gonococcus. These data were inconsistent with a single point of origin followed by pandemic spread, rather suggesting that the sub-lineages had diversified and spread by asymptomatic transmission, with multiple distinct strains causing localised hyperendemic outbreaks. Why many of these occurred in a 'pandemic' is unclear but was presumably a consequence of interactions of individual strains with local human populations. These findings have implications for controlling meningococcal disease by vaccination.

O16

The ST-11 clonal complex: Core genome MLST reveals a complex population structure

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Background: The meningococcal ST-11 clonal complex (cc11) is associated with high morbidity/mortality and has been responsible for large outbreaks and epidemics including the global Hajj-related group W outbreak in the early 2000s, the Burkina Faso group W epidemic in 2002, and a protracted episode of hyperendemic group C disease across Europe prompting the introduction of the group C glycoconjugate vaccines. Recently, endemic group W disease has increased on several continents, whilst group C cases among MSM in Europe and North America have fuelled speculation of a genitourinary reservoir. Multilocus Sequence Typing (MLST) has formed a benchmark for meningococcal population surveillance and, in conjunction with eBURST analysis, provides excellent estimations of the population structure of most invasive lineages. For cc11, however, the resolving power of MLST (alongside routine typing schemes) is poor suggesting that a single clone (ST-11) causes the vast majority of disease, albeit with differing capsules. Multilocus Enzyme Electrophoresis distinguished the ET-15 subgroup, hinting at greater underlying complexity.

Methods: To elucidate cc11 population structure and relate past and present incidences we performed core genome MLST (cgMLST; >1500 genes) on a comprehensive panel of invasive cc11 isolates

Results: The isolate panel included >200 isolates each for groups C and W and >30 group B isolates. Most were collected since the late 1990s in England, Wales and Northern Ireland (EWNI) with smaller panels dating back to the early 1970s. Non-UK isolates were obtained to represent noteworthy episodes including MSM cases, pre/post-Hajj-outbreak African W cases and Canadian ET-15/non-ET-15 cases (early to late 2000s). Also included were a panel of GUM carriage isolates, recent W throat carriage isolates from EWNI, and all remaining cc11 genomes on the PubMLST *Neisseria* database, representing 14 additional countries and dating back to 1964.

We observed an essentially linear population structure along which were distributed at least six major clusters representing episodes of clonal expansion. In this context we discuss the distribution of past and present incidences of cc11 disease.

Conclusions: cgMLST determines the complex population structure of cc11 enabling accurate differentiation of relatively unrelated strains and improved characterisation of endemic disease and outbreaks.

O17

Ancestral acquisition of the capsule locus in *Neisseria meningitidis* occurred multiple times

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Genome-based phylogenetic reconstructions suggest that *N. meningitidis* emerged from a common ancestor with *Neisseria gonorrhoeae* and *N. lactamica* when a 25 kb capsule synthesis locus (*cps*) locus was acquired via horizontal gene transfer. The *cps* island inserted into an ancestral gene, *galE*, encoding UDP-galactose 4-epimerase activity. This event resulted in the duplication of *galE* on either side of the *cps* island with the creation of an expression locus, a functional *galE1* which synthesizes UDP-galactose and a non-functional locus termed *galE2*. By comparison, *N. gonorrhoeae* contains a single *galE1*. Examination of the biochemical function of gonococcal *GalE1* revealed that this enzyme is bi-functional with the capacity to synthesise both UDP-galactose and UDP-N-acetylgalactosamine. Bi-functionality of the epimerase was determined to be the result of the presence of a conserved serine residue at position 299. Examination of a library of 150 isolates including non-pathogenic and pathogenic *Neisseria* species, revealed that *N. mucosa*, *N. animalis, N. weaveri, N. gonorrhoeae, N. polysaccharеae* and *N. lactamica* carried bi-functional *galE1*, whilst *N. subflava* carried a mono-functional allele. Both *N. meningitidis* and *N. cinerea* possessed GalE1 alleles of both types. Phylogenetic clustering of galE alleles was species specific, except for the acquisition of bi-functional *galE* alleles from *N. animalis* in meningococci expressing serogroup E and Z polysaccharides. Non-encapsulated meningococci that contained no *cps* locus carried a bi-functional allele that was closely related to that of gonococci. Consistent with the hypothesis that the *cps* locus horizontal transfer event resulted in the duplication of *galE* in the ancestral recipient, the non-functional galE2 in meningococci was bi-functional (98.2%, n=1256). In contrast, the functional *galE1* locus was mono-functional except for clonal complex 8 which has retained a bi-functional allele in the expression locus. Each meningococcal clonal complex was dominated by the carriage of a specific *galE2* allele (84-100% in 11 different clonal complexes, n=1241) and a specific allelic pair of *galE1/galE2*. These observations are consistent with multiple acquisition events of the *cps* locus into commensal meningococci and the evolution of a specific sequence type as the progenitor of each clonal complex.

O18

Length modulation of horizontal gene transfer in in-silico evolution explains *Neisseria meningitidis* population structure

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Background: In 1994 John Maynard Smith and co-workers challenged the clonality of asexually reproducing organisms, proposing three paradigms for bacterial populations: clonal, panmictic and an intermediate, "epidemic" structure. The intermediate paradigm where cohesive clades co-exist with high rates of HGT – particularly frequent in pathogenic bacteria – is still debated. In a recent pan-genomic study, *Neisseria meningitidis* (Nm) was found to be structured in phylogenetic clades associated with pools of restriction modification systems (RMSs). Gene-conversion events were shown to be longer within- than between-clades, suggesting a DNA cleavage mechanism associated with the species phylogeny.

Methods: To test this hypothesis we simulated in-silico a forward-in-time Wright-Fisher model of haploid individuals with genetic drift, mutation and HGT. Model parameters were calibrated to mimic genome size, mutation and HGT rates of Nm. Simulations were performed with and without modulating the length of horizontally transferred DNA according to the proposed RMS model. We defined a Metric Index (MI) that generalizes the inner-to-outer branch length ratio, and a Topological Index (TI) that quantifies the compatibility between the reconstructed tree and the real phylogeny.

Results: The transition from the clonal to the panmictic state was driven with continuity by the rate of HGT imposed to the system. The Metric analysis showed that, without length-modulation of HGT, HGT rates estimated from experimental data generate panmictic populations. Lower HGT rates could produce "intermediate" population structures. However, the Topological analysis showed that the phylogenetic groups (clades) generated in this way are highly incongruent with the actual evolutionary relationships. Length-modulation of HGT as proposed in the RMS model was sufficient to generate "intermediate" population structures metrically compatible with the Nm experimental results and topologically congruent, i.e. with clades comprising evolutionary related organisms.

Conclusions: We show with in-silico evolution experiments that the length-modulation of DNA exchange provided by RMSs is sufficient to generate the "intermediate" population structure observed in Nm.
These findings have general implications for the emergence of lineage structure and virulence in recombining bacterial populations, and could provide an evolutionary framework for the population biology of other species showing contradictory population structure and dynamics.

O19

Epidemic meningococcal meningitis in Africa: Success using a Group A conjugate vaccine and a development update on a new pentavalent vaccine (A/C/Y/W/X)

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Background: Group A meningococcal meningitis has long been a vexing public health problem in Sub Saharan Africa. In 2010 MenAfriVac™, a WHO prequalified PsA-TT conjugate vaccine, was successfully introduced and has had a dramatic impact. The vaccine when used in well conducted campaigns aimed at 1-29 year olds has generated herd protection and Group A meningococcal disease has rapidly disappeared. Over 158 million Africans in 14 countries have received a dose of MenAfriVac™ and no major safety issues have been reported. Campaigns will continue through 2016 and birth cohorts will be protected through EPI based routine immunizations and/or catch up campaigns that will begin in 2015. In short, the prospect of eliminating Group A meningococcal infections in Africa is a distinct possibility. Nonetheless, meningitis outbreaks caused by non A meningococcal strains remain a threat for this region and the availability of an affordable, heat stable polyvalent meningococcal conjugate vaccine that includes Group X would be an important product for Africa.

Methods: Meningococcal polysaccharide (PS) manufacturing processes for A, C, Y, W and X were developed and optimized at Serum Institute (SIIL). Purified and sized PS were individually conjugated to either TT or CRM using cyanylation chemistry. Immunogenicity of monovalent and polyvalent meningococcal conjugate vaccines was evaluated in mice and rabbits using a bead based immunoassay for IgG titers and a serum bactericidal assay (rSBA). Conjugate vaccines were thermally stressed for extended periods at 40 C .

Results: Purified PS complied with WHO specifications and NMR analyses confirmed structural identity of the individual PS. Purified cyanylate conjugate vaccines gave yields ≥ 20% and murine immunogenicity studies demonstrated that all conjugates, including Men X, generated strong bactericidal activity. The rabbit SBA and IgG studies consistently showed that a candidate polyvalent vaccine was equivalent or better than a licensed comparator for Groups A, C, Y and W. The PsX-TT conjugate also generated robust SBA titers. An alum adjuvant improved the immunogenicity of the polyvalent vaccine. The freeze dried vaccine formulation was stable at 40 C and retained its ability to elicit robust immune responses.

Conclusions: MenAfriVac™ has had a profound effect on Group A meningococcal infections and a new thermostable polyvalent meningococcal conjugate vaccine that includes Group X will begin clinical trials in 2015.

O20

Safety and immunogenicity of a meningococcal serogroup B outer membrane vesicle vaccine with constitutive expression of the iron receptor FetA: a phase I, open-label, dose escalation clinical trial in healthy adult volunteers

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Background: Outer membrane vesicles (OMVs) have been used successfully during strain-specific outbreaks of serogroup B *Neisseria meningitidis* (MenB) and are included in the vaccine 4CMenB. A mechanism to broaden the protection of OMVs may be achieved by the judicious association of PorA and iron receptor FetA variants, which has the potential to protect against a high proportion of circulating invasive strains. The aim of this trial was to demonstrate that constitutive expression of FetA in an OMV vaccine induces a FetA-specific bactericidal response. The B-cell responses underlying the protective responses were also investigated.

M**ethods:** Al(OH)3-adjuvanted OMV vaccine were obtained from a genetically modified H44/76 strain constitutively expressing FetA. Fifty-two healthy adult volunteers received three doses of either 25 or 50 µg OMVs at 8 week intervals in a phase I, open-label trial. Safety was assessed by record of local and systemic adverse events. Immunogenicity was assessed by serum bactericidal activity assay with human complement (hSBA) against the strain H44/76, a panel of PorA and-FetA mutants. The vaccine-specific plasma cells, memory B-cells, serum antibody levels and opsonophagocytic activities were measured.

Results: The vaccine was well tolerated with mostly non-severe, self-limited symptoms. The vaccine induced a FetA-specific SBA response, shown by the 77% seroprotection rate after the third dose versus 35% at baseline against the PorA off-FetA on strain. As expected, the vaccine elicited a high seroprotection rate against wt44/76 strain (98% versus 43% at baseline). Robust plasma and memory B cell responses were elicited, but there was no correlation between the two populations and with serum bactericidal titres, likely indicating that most B cell responses are directed against non-bactericidal epitopes.

Conclusions: Constitutive expression of FetA in an OMV vaccine induced bactericidal FetA-specific responses in addition to PorA-specific responses. This provides a proof-of-concept that a PorA – FetA combination design is a promising approach towards a vaccine providing broad protection against invasive MenB disease.

O21

Exploring the capsule biosynthesis machinery of *Neisseria meningitidis* a with regard to its suitability for in vitro vaccine production

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Neisseria meningitidis serogoup A (NmA) is a major cause of epidemics in the sub-Saharan meningitis belt. The capsular polysaccharide (CPS) of NmA consists of N-acetylmannosamine-1-phosphate units linked together by phosphodiester linkages [→6)-α-D-ManNAc-(1→OPO3→]n and with O-acetylation in O-3 (to a minor extend in O-4) position. In the capsule gene cluster (*cps*) of Nm, region A contains the genetic information for CPSA biosynthesis. Thereby, the open reading frames *csaA, -B,* and *-C* are thought to encode the UDP-N-acetyl-D-glucosamine-2-epimerase, poly-ManNAc-1-phosphate-transferase, and O-acetyltransferase, respectively.

In order to produce immunologically active CPSA using a minimal number of recombinant enzymes, the genes *csaA, csaB* and *csaC* were cloned and the recombinant proteins were purified and functionally characterized. By using a combination of recombinant CsaA and CsaB in one reaction tube, the priming CPSA oligosaccharides were efficiently elongated with UDP-GlcNAc as donor substrate, thus confirming that CsaA is the functional UDP-Nacetyl-D-glucosamine-2-epimerase and CsaB the functional poly-ManNAc-1-phosphatetransferase. Subsequently, CsaB was shown to transfer ManNAc-1P onto O-6 of the non-reducing end sugar of priming oligosaccharides, to prefer non-O-acetylated over O-acetylated primers, and to efficiently elongate the dimer of ManNAc-1-phosphate.

The in vitro synthesized CPSA was purified, O-acetylated with recombinant CsaC, and proven to be identical to the natural CPSA by 1H NMR, 31P NMR and immunoblotting.

This work establishes the basis for the development of cost efficient vaccine production strategies.

O22

Use of a novel serogroup B meningococcal vaccine in response to two university outbreaks in the US

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Background: Serogroup B *Neisseria meningitidis* accounts for a third of all meningococcal disease cases in the US. Increased risk of disease in persons 16-21 years can lead to institutional outbreaks on college campuses. Although a serogroup B meningococcal (MenB) vaccine is available elsewhere, there is no licensed MenB vaccine in the US. To control two MenB outbreaks on US college campuses in 2013, the Food and Drug Administration (FDA) authorized use of an investigational MenB vaccine at these universities.

Methods: An Investigational New Drug (IND) protocol for recombinant MenB+OMV NZ (rMenB) vaccine was submitted to FDA in November 2013 for University A and January 2014 for University B. Vaccination campaigns were held from December 2013 to May 2014. Monitoring for serious adverse events (SAEs) was conducted from receipt of the first dose to 30 days after the second dose. SAEs were defined as death, life-threatening adverse event, inpatient hospitalization, inability to conduct normal life functions, and congenital anomalies and classified as related, possibly related, unlikely to be related, or not related to rMenB by a safety committee. SAEs were collected passively by phone, email, university clinic visits, and local hospital reports, and actively via questionnaires prior to the second dose and electronic surveys 30 days after the second dose.

Results: Between December 2013 and May 2014, 15,346 participants were vaccinated and 28,229 doses of rMenB were administered. The rate of SAEs reported was 3.3/1000 vaccinees, including 1 death, 1 life-threatening adverse event, and 48 hospitalizations. Causal association with rMenB was suspected for two SAEs: rhabdomyolysis (possibly related) and anaphylaxis (related) (0.13/1000 vaccinees); both participants recovered. All other SAEs were not related to rMenB. No cases of MenB disease have occurred among vaccinated persons.

Conclusions: We describe the first use of an unlicensed MenB vaccine to successfully control two institutional outbreaks in the US. Safety monitoring demonstrated no concerning or unexpected patterns of SAEs and supports the use of rMenB in response to future MenB outbreaks. Until a licensed MenB vaccine is available, prompt multi-agency coordination to facilitate the IND process should be established when a MenB outbreak is suspected.

O23

Factor H binding protein as a meningococcal vaccine candidate: Are we there yet?

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About a decade ago, two groups (Novartis Vaccines and Pfizer) used independent approaches and reported that a meningococcal lipoprotein antigen (originally called Genome-derived Neisserial Antigen 1870 [GNA1870] by Novartis and LP2086 by Pfizer) elicited bactericidal antibodies and represented a promising vaccine candidate against group B meningococci. Subsequently this antigen was discovered to bind to an inhibitor of the alternative complement pathway called factor H (FH) and hence was renamed FH binding protein (FHbp). Subsequently, several groups have shown that FHbp plays an important role in complement evasion by meningococci. Elegant structural studies have shed light on how human FH interacts with FHbp. This talk will summarize: i) the biological role of FH, ii) the importance of FH in meningococcal pathogenesis, ii) the effects of FH on the efficacy of anti-FHbp antibodies and iv) the possible effects of FH on the immunogenicity of FHbp. The Novartis vaccine (4CMenB) is licensed in Europe Canada and Australia, while the Pfizer vaccine is in late-stage clinical trials. The lessons learned from FHbp have considerable translational importance. Several medically important bacteria bind complement inhibitors. The use of such proteins as vaccine candidates is an attractive concept because antibodies elicited against such antigens could potentially block a key bacterial virulence mechanism(s). However, the effect of binding of the endogenous human protein to the vaccine antigen on the immune response is an important consideration when evaluating and designing such vaccines.

O24

Identification of several bactericidal epitopes on factor H binding protein, a meningococcal vaccine component using deuterium-hydrogen exchange mass spectroscopy

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Objectives: The Factor H Binding Protein (FHBP) of group B *Neisseria meningitidis* (MnB) comprises a family of surface exposed outer membrane lipoproteins with two subfamilies (A and B). A vaccine composed of two lipoproteins, one from each subfamily, elicits broad bactericidal antibodies to diverse invasive MnB strains in humans. This bivalent rLP2086 vaccine has recently obtained Breakthrough Therapy designation by the FDA and a BLA filing under accelerated approval regulations is underway. The primary objectives of our studies were to identify and characterize several monoclonal antibodies (mAbs) that would: a) preferentially recognize either LP2086 subfamily A or B; b) initiate complement mediated bacteriolysis; c) recognize different domains of LP2086. Our secondary objective was to evaluate deuterium-hydrogen exchange mass spectroscopy (DXMS) as an alternative to co-crystallization or NMR.

Methods: mAbs were generated to both FHBPs included in the bivalent rLP2086 vaccine. mAbs that demonstrated specificity for either subfamily A or B by ELISA were further binned by their ability to compete and recognize native or stressed LP2086, in Biacore or Octet analyses. Members of these groups were screened for the ability to initiate bacteriolysis in a serum bactericidal assay using human complement (hSBA). From the resultant bins, six mAbs were selected for epitope mapping studies using DXMS.

Results: Two bactericidal subfamily A and two bactericidal subfamily B mAbs mapped to their respective C-domains on LP2086. One subfamily A and one subfamily B mAb each mapped to bactericidal epitopes on their respective N-domains. Five of the six mAbs mapped to discontinuous epitopes and the specific peptides involved in the binding of the mAbs will be presented. The mapping results for a mAb that was used to evaluate both NMR and DXMS approaches were in good agreement.

Conclusions: Three bactericidal epitopes were identified on each of the two LP2086 subfamily proteins. Bactericidal epitopes on both N- and C-domains were identified. DXMS is a useful tool for defining both continuous and discontinuous bactericidal epitopes of FHBP and requires less protein than co-crystallization or NMR experiments. A comparison of the epitopes identified in this study with those identified on other LP2086 variants will be presented.

O25

Human complement FH impairs protective serum anti-FHbp antibody by skewing antibody repertoire and enhancing FH binding

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Background: 4CMenB vaccine (Bexsero®, Novartis) contains four antigens that can independently elicit serum bactericidal activity (NHba, NadA, PorA P1.4, and Factor H binding protein, or FHbp). Binding of FH to FHbp is specific for human FH. When humans are immunized, FHbp is expected to complex with FH, which could adversely affect immunogenicity and safety.

Methods: Wildtype (WT) BALB/c mice (whose mouse FH doesn't bind to FHbp) and human FH transgenic (Tg) BALB/c mice (serum human FH >240 µg/ml), were immunized with three IP injections of the 4CMenB vaccine, and their serum responses compared.

Results: There were no significant differences in human complement-mediated serum bactericidal responses between Tg and WT mice against serogroup B strains with all of the vaccine antigens mismatched except for PorA 1.4, or NadA. In contrast, against a strain mismatched for all of the vaccine antigens except FHbp, the Tg mice had 28-fold lower serum bactericidal antibody responses after dose 2 ($P<0.0001$), and 15-fold lower after dose 3 ($P=0.004$). One explanation for the lower bactericidal activity in the Tg mice is their post-immunization sera enhanced binding of FH to FHbp by both ELISA and flow cytometry, whereas the sera from the WT mice inhibited FH binding. Control antisera from Tg mice immunized with a low FH binding mutant FHbp (R41S) vaccine inhibited FH binding. Two of 11 4CMenB-vaccinated Tg mice developed serum IgM antibodies reactive with human FH. Binding could be quenched with soluble recombinant human FH domains 6 and 7 fused to Fc (FH67/Fc) and partially by domains 18 to 20 (FH18-20/Fc). None of 6 control Tg mice immunized with aluminum hydroxide alone developed IgM antibodies to human FH; nor did 4 control unvaccinated Tg mice.

Conclusions: Human FH impairs protective anti-FHbp antibody responses, in part by directing the antibody repertoire to FHbp epitopes outside the FH combining site, which enhance, and do not inhibit FH binding. Vaccination with FHbp in a complex with human FH also may elicit FH autoantibodies, which should be investigated in humans. Mutant FHbp antigens with low FH binding could improve protection in humans and, potentially, vaccine safety.

O26

Impact of reducing complement inhibitor binding on the immunogenicity of an outer membrane vesicle-based vaccine against serogroup B *Neisseria meningitidis*

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Background: Outer membrane vesicles (OMVs) have been used successfully during outbreaks of serogroup B *Neisseria meningitidis* (MenB) and are also included in the multicomponent MenB vaccine Bexsero®. However OMVs have limitations: the immune responses are weak, strain-specific and short-lived. Therefore improving their immunogenicity may contribute to the design of more potent MenB vaccines or vaccine components. *Neisseria meningitidis* recruits human complement inhibitors such as factor H (fH) to the surface of the bacteria, in order to down-regulate complement activation and enhance bacterial survival in human serum. Binding of the same complement inhibitors may occur during vaccination with (OMVs), and we investigated whether this has an impact on the immunogenicity of OMVs.

Methods: OMVs with reduced human fH binding (nOMVdis) were generated by deletions of the lpxl1, factor H binding protein and Neisserial surface protein A-encoding genes. Serum bactericidal antibody responses of mice immunized with the modified nOMVdis were compared to mice immunized with the wild-type counterpart nOMVwt in the presence of human fH, by ELISA and serum bactericidal assays.

Results: As expected, the mutations led to a total suppression of fHbp and NspA expression in nOMVdis. Despite a drastic reduction of hfH binding to the nOMVdis vaccine, its immunogenicity in mice was not significantly different from nOMVwt in the presence of human fH, either pre-incubated with the OMVs prior to injection or injected into mice.

Conclusions: Inhibition of factor H binding to produce enhanced immunity is unlikely to be an appropriate solution to the limited immunogenicity of OMV vaccines.

O27

Resistance of meningococci to anti-FHbp bactericidal activity can be mediated by functional binding of complement FH to PorB3 and overcome by non-bactericidal anti-NspA antibody

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Background: Factor H binding protein (FHbp) is sparsely-expose. A critical determinant of anti-FHbp bactericidal activity is the ability for low-level C3b deposited by the classical pathway (CP) to be amplified by the alternative pathway. Methods: We investigated basis of resistance (N=4, titer <1:10) or susceptibility (N=6, titer >1:1000) of serogroup B isolates to human complement-mediated bactericidal activity of mouse antisera to recombinant FHbp.

Results: All ten isolates showed similar susceptibility to anticapsular mAb-mediated bactericidal activity. By flow cytometry, all isolates bound similar respective amounts of antibody to capsule, FHbp, and NspA (a FH ligand), and similar low-level CP activation (C4 deposition) by anti-FHbp antisera. Anti-FHbp antisera were less effective in preventing bacteremia in human FH transgenic infant rats challenged with a resistant than a sensitive strain ($P<0.001$). To investigate complement down-regulation by binding of human FH to ligands other than NspA or FHbp in the resistant strains, we created double NspA/FHbp knock-out mutants of two resistant and two susceptible isolates. The mutants from the resistant strains bound >10-fold more recombinant human FH domains 6 and 7 fused to Fc (FH67/Fc) than the mutants from the sensitive strains. In a previous study PorB2, but not PorB3, functionally bound FH domains 6 and 7; however all 10 isolates studied here expressed PorB3. To investigate a role of PorB3 variants in anti-FHbp resistance, we replaced PorB3 from a wild-type resistant isolate with PorB3 from a susceptible isolate, which rendered the strain susceptible to anti-FHbp bactericidal activity. Conversely, replacing PorB3 of a susceptible isolate with PorB3 from a resistant isolate decreased anti-FHbp bactericidal activity. Further, adding 5 µg/ml of a non-bactericidal anti-NspA mAb to the anti-FHbp antisera resulted in bacteriolysis of all four resistant wild-type strains whereas anti-NspA or anti-FHbp antisera alone were negative.

Conclusions: Some meningococci resist anti-FHbp bactericidal activity through binding of human FH to PorB3. Resistance can be overcome by antibodies to a second sparsely-exposed antigen such as NspA, which together resulted in more CP activation than either antibody alone and blocked FH binding to NspA. A vaccine that targets both FHbp and NspA should elicit broader protection than FHbp alone.

O28

Molecular epidemiology and global expression profiling of *Neisseria meningitidis* factor H binding protein (fHbp) by quantitative mass spectrometry

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fHbp is a surface-exposed lipoprotein of *N. meningitidis* and a protective antigen used as a component of the recently licensed Bexsero vaccine. fHbp is a highly variable meningococcal protein: to reflect its remarkable sequence variability, fHbp has been classified in three variants (or two subfamilies), with limited cross-protection. Furthermore the level of fHbp expression varies significantly among strains, and this has also been considered an important factor for predicting MenB strain susceptibility to anti-fHbp antisera. Different methods have been used to assess fHbp expression on meningococcal strains, however all these methods use anti-fHbp antibodies, and for this reason the results are affected by the different affinity that antibodies can have to different antigenic variants. To overcome the limitations of an antibody-based quantification, we developed a quantitative Mass Spectrometry (MS) approach. Selected Reaction Monitoring (SRM) recently emerged as a powerful MS tool for detecting and quantifying proteins in complex mixtures. SRM is based on the targeted detection of “ProteoTypicPeptides (PTPs)”, which are unique signatures of a protein that can be easily detected and quantified by MS. This approach, proven to be highly sensitive, quantitatively accurate and highly reproducible, was used to quantify the absolute amount of fHbp antigen in total extracts of 105 serogroup B strains, evenly distributed among the three main variant groups and selected to be representative of the fHbp circulating subvariants around the world. We extended the study at the genetic level investigating the correlation between differential level of expression and polymorphisms present within the genes and their promoter sequences. The implications of fHbp expression on the susceptibility of the strain to killing by anti-fHbp antisera are also presented. To date this is the first comprehensive fHbp expression profiling in a large panel of strains driven by an antibody-independent MS-based methodology.

O29

Native outer membrane vesicle vaccine with over-expressed factor H binding protein confers protection against meningococcal colonization in human CEACAM1 transgenic mice

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Background: Capsular polysaccharide-protein conjugate vaccines confer protection against invasive meningococcal disease and decrease nasopharyngeal carriage. In contrast, detergent-extracted outer membrane vesicle vaccines given alone or with recombinant protein antigens protect against disease but appear to have minimal effect on colonization in humans. We are developing a meningococcal native outer membrane vesicle (NOMV) vaccine from mutants with genetically attenuated endotoxin (lpxL1 knockout) and over-expressed Factor H binding protein (NOMV-FHbp). In mice, the NOMV-FHbp vaccine elicited broad serum bactericidal activity against genetically diverse strains. However, the effect of vaccination on nasopharyngeal carriage has been difficult to investigate experimentally because the receptors important for meningococcal colonization such as carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are human-specific.

Methods: We immunized human CEACAM1 transgenic (Tg) mice with a NOMV-FHbp vaccine prepared from a mutant of group B strain H44/76 or, as a control, aluminum hydroxide adjuvant only. Two weeks after the third dose, the animals were challenged intranasally with 107 CFU of wild type strain H44/76. We measured CFU in nasotracheal washes 72 hours after the challenge. To investigate the role of antibodies in protection against colonization, we treated naïve human CEACAM1 transgenic mice on days 0 and 1, with 100 µl IP of a 1:5 dilution of post-vaccination serum pools. Three hours after the first dose of serum the animals were challenged intranasally and we measured CFU in washes at 72 hrs.

Results: In active immunization, all 14 negative control mice were colonized, compared to 1 of 14 mice given the NOMV-fHbp vaccine ($P < 0.001$). In passive immunization experiments, mice treated with the anti-NOMV antisera had lower levels of CFU in nasotracheal washes than mice treated with negative control antisera ($P < 0.05$ in Expts 1 and 3; $P=0.1$ in Expt 2). In all three experiments, 17 of 33 (51%) of anti-NOMV antisera treated mice had undetectable CFU in the washes compared with 5 of 34 (14%) in the control groups ($P < 0.002$).

Conclusions: NOMV-FHbp vaccination protects against meningococcal serogroup B colonization, and serum antibodies contribute to protection. Thus, meningococcal NOMV-FHbp vaccines have the potential to protect against both invasive disease and interrupt transmission in the population.

O30

Antimicrobial resistance in *Neisseria gonorrhoeae*–crucial public health actions and research to retain gonorrhea treatable

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Neisseria gonorrhoeae has during the latest 70-80 years developed antimicrobial resistance (AMR) to all drugs used for treatment of gonorrhea. In vitro and clinical resistance to ceftriaxone, the last option for first-line empiric monotherapy, has now evolved. Crucial actions to combat this worrying situation include using dual antimicrobial therapy that has been introduced in USA and Europe; enhancing surveillance of gonorrhea, gonococcal AMR (e.g. WHO's initiated global surveillance programme), treatment failures and antimicrobial use/misuse; and improving prevention, early diagnosis, and contact tracing. However, the knowledge regarding emergence, evolution, and spread, including effects on biological fitness, of gonococcal AMR remain limited. It is essential to improve our understanding of previously identified and new AMR determinants, their effects on resistance and fitness, and their interplay including with epistatic mutations and compensatory mutations emerging to restore fitness. This is important for prediction of emergence and spread of future AMR and development of molecular assays for detection/prediction of AMR (or susceptibility), ideally point-of-care with simultaneous detection of gonococci, to supplement culture-based methods and guide individually-tailored treatment. This in combination with enhanced understanding of the dynamics of national and international emergence, transmission and evolution of AMR gonococcal strains will be important ways forward. Genome sequencing combined with AMR and additional epidemiological metadata will clarify several of these issues and might also revolutionize the molecular AMR prediction. Genomics together with transcriptomics and proteomics might additionally elucidate several issues regarding gonococcal evolution and strain populations; associations with pathogenesis/virulence; and novel targets for diagnostics (ideally rapid point-of-care tests), treatment and vaccine. However, these molecular technologies need to be supplemented by traditional phenotypic assays, biochemistry and structure-biology including crystallography (e.g. to study the structural impact of different mutations on antimicrobial targets), and in vivo studies, e.g. using mice models to predict the fitness, virulence and further spread of AMR gonococcal strains. Ultimately, to retain gonorrhea a treatable infection novel antimicrobials are essential. Several new compounds have shown potent in vitro activity against gonococci and deserve further attention.

O31

Whole genome sequencing of *Neisseria gonorrhoeae* isolates with reduced cephalosporin susceptibility collected in Canada from 1989 to 2012

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Background: To demonstrate the utility of genomic epidemiology for investigating the spread of antimicrobial resistant *N. gonorrhoeae*, we carried out a large-scale whole-genome comparison of Canadian isolates with decreased susceptibility to the extended spectrum cephalosporins (ESC), cefixime and ceftriaxone.

Methods: Whole genome sequencing (WGS) was carried out using the MiSeq Illumina platform on 170 isolates of *N. gonorrhoeae* with a range of MICs for cefixime and ceftriaxone, including 60 isolates with MIC < 0.063 ug/mL for both drugs, 100 isolates with MIC >= 0.063 µg/ml - 0.5 µg/ml for either drug; one European isolate with very high MICs; and 9 international reference strains (WHO F, G, K, L, M, N, O and P and ATCC-49226). Single nucleotide polymorphisms (SNPs) were found by mapping reads to the reference strain, NCCP11945, and contigs were assembled de novo with the SPAdes algorithm. Antimicrobial susceptibility testing was carried out using agar dilution following CLSI guidelines.

Results: The collection of 170 isolates represented 71 different *N. gonorrhoeae* multi-antigen sequence types (NG-MAST) and grouped into 12 major phylogenomic clades (A – L). NG-MAST types were strongly associated with individual phylogenomic clades. Elevated ceftriaxone MICs (≥ 0.032 µg/ml) were observed in 91.6% of isolates (109/119) in Clades A, B, C, E, F, and G; whereas lower ceftriaxone MICs (≤0.016 µg/ml) were observed in 95.7% of isolates (45/47) in Clades D, H, I, J, K and L. The mosaic allele of the penA gene, which is a major resistance determinant, was observed in Clades A, C and G and represented 41.2% (n=70) of all isolates, with penA mosaic type XXXIV being predominant (n=58). Other mutations associated with elevated MICs for cephalosporins or fluoroquinolones were identified, including: mtrR -35 A deletion (n=110); ponA L421P (n=119); porB mutations (n=122); and gyrA and/or parC mutations (n=112).

Conclusions: WGS offered enhanced discrimination in the analysis of highly clonal *N. gonorrhoeae*. The clades correlated strongly with sequence types and MICs for cefixime and ceftriaxone. WGS can improve surveillance of antimicrobial resistant *N. gonorrhoeae* and inform treatment guidelines.

O32

Structural analysis of penicillin-binding protein 2 from the cephalosporin-resistant *N. gonorrhoeae* strain H041 – molecular mechanism underlying treatment failures in the clinic

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The emergence of *N. gonorrhoeae* strain H041 in Japan that exhibits resistance to most antibiotics, including extended-spectrum cephalosporins (ESCs), has caused considerable alarm globally as a harbinger of untreatable gonorrhea. Against this clinical background, it is imperative to understand and ultimately combat mechanisms underlying antibiotic resistance in *N. gonorrhoeae*. The primary difference between strains exhibiting intermediate resistance to ESCs and H041 is the penA gene (penA41), which encodes penicillin-binding protein 2 (PBP2). PBP2 is an essential transpeptidase, forming peptide cross-links during peptidoglycan synthesis. Compared to PBP2-WT from FA19, PBP2-H041 contains 70 amino acid mutations. Strikingly, penA41 can transform the β-lactam-susceptible strain FA19 to full resistance for cefixime and ceftriaxone in the absence of any other resistance determinants such as mtrR or penB.

To address the central question of how resistance to β-lactams is achieved at the molecular level in H041, we have conducted a biochemical and structural investigation of PBP2-H041. Using purified PBP2-H041, we measured the second-order acylation rate constants for cefixime and ceftriaxone to be 12,500- and 2,000-fold lower, respectively, than PBP2-WT. To determine the impact of mutations on structure, we solved the crystal structure of a construct of PBP2-H041 to 1.9 Å resolution. Commensurate with the dramatic kinetic differences, there is significant remodeling in the active site compared to PBP2WT. Specifically, the positions of two loops has changed significantly and the β3-β4 loop exhibits greater order. The overall effect is to widen and rigidify the active site. In support of a mechanism for antibiotic resistance that involves increased rigidity, a crystal structure of a PBP2 variant in complex with meropenem (the first acylated structure of PBP2) shows only minimal conformational changes. Furthermore, titrations of PBP2 with β-lactams, monitored by NMR, show fewer conformational changes in PBP2 derived from cephalosporin-resistant strains compared to PBP2WT. Together with our recent demonstration of a high-throughput assay for PBPs, our new understanding of the structural impact of mutations responsible for the clinical resistance observed in H041 paves the way to develop new antimicrobials that target this and other resistant strains of *N. gonorrhoeae* via inhibition of PBP2.

O33

In vivo-selected compensatory mutations increase fitness of ceftriaxone-resistant *Neisseria gonorrhoeae*

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Increasing resistance of *Neisseria gonorrhoeae* (Gc) to ceftriaxone (CRO), the last remaining monotherapy for gonorrhea, has amplified the threat of untreatable gonorrhea. CRO resistance in Gc is conferred primarily by mosaic *penA* alleles that encode multiple amino acid substitutions within penicillin-binding protein 2. Whether these mosaic alleles also confer a fitness cost is unknown. Investigation of this question is needed to predict the spread of CROR Gc.

Here we examined the impact of mosaic *penA* alleles from CRO^R strains H041 and F89 on Gc fitness. The *penA41* and *penA89* alleles were introduced into wild-type laboratory strain FA19 (CRO^S) by allelic exchange to create mutants FA19penA41 and FA19penA89, respectively (CRO resistance, ≥ 500-fold increase). The mutants grew significantly slower than strain FA19 and were also out-competed by the CRO^S parent strain when co-cultured in broth. Strain FA19 also out-competed the CRO^R mutants during competitive genital tract infection in BALB/c mice; however, CRO^R compensatory mutants of strain FA19penA41 were isolated from several mice. Three classes of compensatory mutants were defined. Class I mutants were fully compensated for growth in vitro, and more fit than FA19penA41 and FA19 in vivo. Class II mutants exhibited a modest decrease in fitness in vitro, and were less fit than FA19 but more fit than FA19penA41 in vivo. Class III mutants were characterized by a rapid logarithmic phase of growth that fully compensated for growth in vitro, and were significantly more fit than FA19 and FA19penA41 in vivo. None of the compensatory mutants carried mutations in the *mtr* locus or the quinolone resistance-determining region of *gyrA*, which we showed previously increase Gc fitness in vivo, nor were there additional mutations in *penA*. We hypothesize that the compensatory mutations are in other genes important in cell wall synthesis or division, or genes that affect metabolism.

We conclude that compensatory mutations can be selected during infection with CRO^R Gc that increase fitness, which may facilitate the persistence and spread of CROR Gc strains. Compensatory mutations may be in novel genes that could be targeted in future drug design. Identification of the compensatory mutations by high-throughput sequencing is underway.

O34

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

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Neisseria gonorrhoeae is the etiologic agent of the sexually transmitted infection gonorrhea. Antibiotics are the mainstay in treating infections, but widespread resistance to antibiotics previously recommended for treatment of gonorrhea, coupled with the recent emergence of strains with high-level resistance to ceftriaxone, portends the loss of the only remaining antibiotic currently available for monotherapy of gonococcal infections. Thus, new antibiotics against novel targets are desperately needed to stem the tide of emerging gonococcal resistance that is becoming a major threat to public health.

N. gonorrhoeae is a species of Gram-negative bacteria that are characterized by the enrichment of lipid A, the hydrophobic anchor of lipopolysaccharide (LPS) or lipoologosaccharide (LOS), in the outer monolayer of the bacterial outer membrane. The biosynthesis of lipid A is a highly conserved pathway that is required for the viability of virtually all Gram-negative bacteria, including *N. gonorrhoeae*, but has never been exploited by commercial antibiotics. Using bacterial genetics, we have established that LpxC, the second enzyme in the lipid A biosynthetic pathway, is an essential enzyme in *N. gonorrhoeae*, making it an attractive target for drug discovery. We report the synthesis and optimization of potent inhibitors of LpxC as novel antibiotics against susceptible and drug-resistant *N. gonorrhoeae*, and show that disruption of lipid A biosynthesis by inhibition of LpxC is bactericidal for *N. gonorrhoeae*. Potent LpxC inhibitors display impressive antibiotic activity against susceptible and drug-resistant *N. gonorrhoeae* in vitro. These compounds, which are well tolerated in mice at the solubility limit, clear gonococcal infections in the female mouse model, demonstrating the therapeutic potential of LpxC inhibitors. We have also discovered a potential mechanism of resistance to LpxC inhibitors in *N. gonorrhoeae*, which is distinct from those reported in *E. coli*.

O35

Utilizing sialic acid analogues to define the molecular basis of complement resistance mediated by sialylation of *Neisseria gonorrhoeae* lipooligosaccharide and to design novel therapeutics

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Substitution of *Neisseria gonorrhoeae* lacto-N-neotetraose (LNT) lipooligosaccharide (LOS) with Neu5Ac confers on the bacterium the ability to evade complement-mediated killing by enhancing binding of the alternative pathway inhibitor, factor H (FH), and also reducing binding of specific IgG. We utilized the CMP salts of six sialic acid analogues, Neu5Gc, Neu5Ac9Ac, Neu5Ac9Az, Neu5Gc8Me, Leg5Ac7Ac and Pse5Ac7Ac, to characterize the substrate specificity for gonococcal LOS sialyltransferase (Lst) and define the structural requirements of sialic acid-mediated complement resistance. All analogues, except Pse5Ac7Ac, served as substrates for gonococcal Lst and were incorporated on to LNT LOS. Only Neu5Gc incorporation yielded FH binding and high-level serum resistance comparable with Neu5Ac-substituted LOS. Neu5Ac9Ac and Neu5Gc8Me permitted resistance only to low, but not high complement concentrations, while Neu5Ac9Az and Leg5Ac7Ac did not confer any resistance to complement. Neu5Ac9Ac addition resulted in low-level FH binding (~20% of the levels seen with Neu5Ac and Neu5Gc), but addition of none of the remaining analogues showed detectable FH binding by FACS. The extent of classical pathway inhibition (measured by IgG binding and C4 deposition) was proportional to serum resistance. Adding CMP-Neu5Ac9Az or CMP-Leg5Ac7Ac to bacteria within 15 min of adding CMP-Neu5Ac did not block FH binding facilitated by Neu5Ac, but blocked classical pathway suppression and serum resistance mediated by Neu5Ac on strain F62 and a high-level ceftriaxone-resistant isolate called H041, even when Neu5Ac was present at a 10- to 100-fold molar excess. Administration of CMP-Leg5Ac7Ac intravaginally significantly reduced the duration of infection (median time to clearance 6 and 10 days in treated and untreated mice, respectively; $P<0.0001$) and bacterial burdens ($P=0.0005$) in the murine vaginal colonization model of gonorrhea. Collectively, these data shed light on substrate specificity of gonococcal Lst and reveal critical roles for carbon 7, 8 and 9 substitutions on sialic acid for complement inhibition on gonococci. The use of sialic acid analogues to counter gonococcal virulence provides a novel therapeutic strategy against the global threat of multi-drug resistant gonorrhea.

O36

Characterization of a novel outer membrane protein, NGO1985, as a potential target for the development of pharmacological interventions against gonorrhea

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Infections caused by *Neisseria gonorrhoeae* (GC) remain a significant health and economic burden globally. The lack of appropriate vaccines to prevent gonorrhea makes antimicrobial compounds the sole line of pharmacological intervention. Therefore, there is an immediate need for the development of drugs with new mechanisms of action. To identify novel, potential therapeutic targets, we performed proteomic analyses of cell envelopes and naturally released membrane vesicles derived from GC strains FA1090, MS11, 1291, and F62 (1). A plethora of ubiquitously expressed proteins, including 11 novel outer membrane proteins were identified. We chose to evaluate NGO1985 for its potential as a new drug target. The comparison of the predicted amino acid sequence of NGO1985 using the completed genome sequences of 16 different GC strains demonstrated a high degree of conservation. Corroborating these findings, the anti-NGO1985 antisera cross-reacted with 22 geographically diversified GC isolates. Interestingly, a lipobox motif and two putative membrane-binding BON domains were identified in NGO1985. The presence of these domains suggested that NGO1985 functions in GC cell envelope homeostasis. To test this hypothesis, the Δ*ngo1985* mutant and its isogenic wild-type strain FA1090 were examined for susceptibility to a variety of compounds. The lack of NGO1985 resulted in an altered cell envelope protein profile and caused dramatically decreased GC viability upon exposure to detergents, polymyxin B, chloramphenicol, and ceftriaxone. These studies demonstrated that NGO1985 contributes to the integrity of GC cell envelope, which underscore its potential as a novel molecular target for the development of therapeutics against gonorrhea. Accordingly, we utilized phage-display approach to identify peptide ligands with binding affinities for NGO1985. The recombinant version of NGO1985 was used in affinity capture method as a bait during panning experiments with a phage-display library expressing randomized linear dodecameric peptides. After four rounds of biopanning, the DNA of 20 randomly selected phages was subjected to sequencing. These studies revealed that all peptides shared a similar sequence motif. The high binding affinities of individual peptides were further confirmed in quantitative ELISA assays. Studies are under way to examine the ability of these peptides to inhibit NGO1985 function in vitro and in vivo.

O37

Novel factor H-Fc chimeric immunotherapeutic molecules against pathogenic *Neisseria*

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Antimicrobial resistance is a global threat to human health and several pathogens may soon be 'untreatable' with currently available antibiotics. Many pathogens bind the complement inhibitor, factor H (FH) to dampen complement activation on their surfaces. Most microbes bind FH through domains 6 and 7 and/or the C-terminal domains 18 through 20. Because the microbial binding FH domains are distinct from its complement inhibiting domains (domains 1–4), we explored the utility of fusing the microbial-binding FH domains with IgG Fc (the 'effector' region of antibody) to create novel anti-infective immunotherapeutics. We created two recombinant proteins by fusing FH domains 18-20 or domains 6 and 7 with human IgG1 Fc (FH18-20/Fc or FH6-7/Fc) and provided proof-of-principle for its activity against *Neisseria gonorrhoeae* and *N. meningitidis*.

Sialylation of gonococcal lipooligosaccharide, as occurs in vivo, augments binding of human FH through domains 18-20. However, FH domains 18-20 bind to select host glycosaminoglycans and limit unwanted complement activation on host cells ('self-nonself discrimination'). We introduced a mutation in FH domain 19 (D1119G) that is associated with a disease called atypical hemolytic uremic syndrome and decreases binding of FH to host cells, thereby causing uninhibited hemolysis and renal damage. FHD1119G/Fc retained binding and complement-dependent killing of gonococci, but unlike unmodified FH18-20/Fc, did not lyse human erythrocytes. FHD1119G/Fc bound to all of 16 sialylated clinical isolates of *N. gonorrhoeae* tested, including three contemporary ceftriaxone-resistant strains, mediated complement-dependent killing of 10/16 isolates and enhanced C3 deposition at least 10-fold over baseline levels in the six isolates that were not killed. FHD1119G/Fc facilitated complement-dependent opsonophagocytic killing of serum-resistant strain FA1090 (an Opa-negative mutant was used to abrogate non-opsonic killing) by human polymorphonuclear neutrophils.

N. meningitidis bind FH through domains 6 and 7. Binding of FH6-7/Fc enhanced C3 fragment deposition on, and mediated complement-dependent killing of *N. meningitidis* in a dose-dependent manner.

In summary, FH/Fc fusion proteins show promising activity in vitro against pathogenic *Neisseria*. Studies to evaluate these molecules in vivo are underway. These data provide a novel approach to combat multi-drug resistant pathogens that bind FH and pose a threat to human health worldwide.

O38

Development of novel 2-pyridones for the treatment of *Neisseria gonorrhoeae* infections

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Drug resistant *Neisseria gonorrhoeae* is one of three bacteria named by the CDC to be an urgent threat to public health. The rise of cephalosporin-resistant *N. gonorrhoeae* presents a significant medical need and new antibiotics to treat infections caused by this organism are urgently needed. PTC has discovered and developed a novel class of small molecule 2-pyridones that are orally bioavailable and selectively target *Neisseria* species. These novel molecules are equipotent against wild-type and multidrug resistant strains. They act by inhibiting bacterial DNA synthesis and exhibit a time dependent bactericidal effect. There is no pre-existing resistance to the PTC compounds, nor is there cross resistance with other commercially available antibacterials. To evaluate the in vivo efficacy of these novel anti-gonococcal small molecules, a mouse model of gonorrhea was optimized and established at PTC Therapeutics. This model utilizes ovariectomized female mice, supplemented with estrogen and then infected vaginally with *N. gonorrhoeae*. Multiple strains of *N. gonorrhoeae* have been adapted to grow in vivo including the WHO F (TetIR) and WHO K (MDR) isolates. A single oral dose of novel PTC compounds clear infection in this mouse model of GC infection. A fAUC/MIC ratio of 70 was determined to be optimal for in vivo efficacy (100% clearance of infection at 5 days-post dosing). The 2-pyridone compounds have desirable pharmaceutical attributes including predictable pharmacokinetics, a low volume of distribution and clearance across multiple pre-clinical species. The molecules have no drug-drug interaction liabilities, no observed off-target activities and are well-tolerated when dosed daily in rat safety studies. A clinical development candidate will be selected for the single dose treatment and cure of wild type and drug resistant gonorrhea.

O39

Connection between the twin sRNA regulon and the stringent response in *Neisseria meningitidis*

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Background: Small RNAs (sRNAs) are often involved in the regulation of adaptation to physiological changes. Many sRNAs are synthesized upon nutritional stresses encountered by pathogens. They generally regulate expression of target mRNAs forming part of a single nutritional regulatory circuit or network, or target mRNAs that act as transcriptional regulators. sRNAs usually act by occupying or freeing-up ribosomal entry sites of target transcripts or by regulating accessibility of transcripts for RNases by base-pairing.

Methods and results: Using transcriptome analysis of *Neisseria meningitidis*, two structurally nearly identical sRNAs were identified: encoded together, having 70% sequence identity ('twin sRNAs') and found adjacent to the gene coding for the stringent response transcriptional regulator Leucine responsive protein (Lrp). Direct translational control by one of the twin sRNAs via basepairing to ribosomal entry sites was proven for 6 enzymes involved in the TCA cycle (PrpB, PrpC, SdhCDAB, GltA, SucC and FumC) in an *Escherichia coli* gfp-reporter system. Overexpression of the twin sRNAs did not impair meningococcal growth in nutrient-rich medium or fresh human blood, whilst growth was drastically inhibited in medium with glucose as sole carbon source or human CSF, suggesting crosstalk between the twin sRNA regulon and the stringent response. Based on homology of its 5'- untranslated region (5'-UTR) with those of the 6 enzymes mentioned, Lrp was identified as a possible target. Direct interaction between one of the twin sRNAs and the 5'-UTR of *lrp* mRNA was assessed and validated in vivo using the same gfp reporter. Site directed mutagenesis of the twin sRNA showed that its complimentary sequence interacts with the 5'-UTR of *lrp* mRNA. Connection with the stringent response was also indicated by differential twin sRNAs expression in a *relA* knockout versus wild type strain.

Conclusions: The twin sRNA regulon is connected to the stringent response. By orchestrating the stringent response in meningococci via antisense mechanisms, this twin sRNA connects metabolic status of meningococci to virulence and hence pathogenesis.

O40

Regulation of the gonococcal type IV secretion system involves two transcriptional repressors, two proteases, and an RNA switch

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Eighty percent of gonococcal strains and seventeen percent of meningococcal strains carry a genetic island, the gonococcal genetic island, inserted at the chromosomal replication terminus. The region encodes a type IV secretion system (T4SS) that, in gonococci, secretes single-stranded DNA effective in transforming other gonococci in the population as well as initiating biolfilm formation. Furthermore, the T4SS can function in iron acquisition during intracellular growth in the absence of TonB. In order to understand more about the function of the T4SS during infection, we have made monoclonal antibodies to outer-membrane proteins as well as epitope-tagged constructs for T4SS proteins and evaluated T4SS expression using western blots and qRT-PCR. Subcellular fractionation demonstrated that T4SS proteins TraK, TraN, and TraB are localized to the outer membrane. However, these proteins were produced in very low amounts, not detectable by standard western blot methods. Furthermore, significant amounts of the proteins were degraded by periplasmic proteases. Thin-section immuogold electron microscopy detecting TraK demonstrated a single gold particle on some gonococci, suggesting that the T4SS may be present at one copy per cell. Three of the four T4SS operons were found to be repressed. A survey of environmental conditions identified Mn, Zn, Fe, and H2O2 as well as ferric uptake regulator Fur as affecting the traI operon. DNA-binding studies and mutational analyses demonstrated that FarR represses the operon that includes *traK*. There was increased expression of *traK* when the bacteria were grown in human whole blood. The third operon was found to include an RNA switch controlling translation of traH and *traG*. Translation of a LacZ reporter fused to the TraH start was found to be affected more than 1000-fold by mutations affecting the RNA structure. Together these results demonstrate that the T4SS is regulated at multiple levels, transcription, translation, and proteolysis. Conditions were identified that increase expression, and these include conditions related to oxidative damage, low iron, or growth in blood.

O41

The ModD1 epigenetic methyltransferase and transcriptional regulator from pathogenic *Neisseria meningitidis*

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Phase variation is the high frequency, reversible switching of genetic elements, such that the gene product is either produced (said to be ON) or not produced (OFF). This phenomenon is typically caused by errors in DNA replication, when slippages occurs in nucleotide repeat tracts, and consequently alters promoter strength or shifts the reading frame of the gene. In cases where the phase-variable element is a regulator protein, phase variation is also applicable to the members of the regulon – which is consequently known as a phasevarion (phase-variable regulon).

Neisseria meningitidis strains can possess up to three independent, epigenetic, phase-variable regulators, encoded by the *modA, modB* and *modD* genes. Each of these genes possesses a number of allelic variants that differ in the DNA recognition domain sequence, and hence the corresponding Mod proteins regulate different phasevarions. The distribution and specific allele frequency of the mod genes varies in *N. meningitidis* sequence types and clonal complexes, however some associations between allele and pathogenic sequence types are seen. Most notably, the modD1 allele has been shown to specifically and significantly associate with hypervirulent, clonal complex 41/44 strains, but is absent from all screened carriage strains.

Our studies demonstrate that ModD1 is a functional adenine methyltransferase that methylates the sequence motif 5'-CCm6AGC-3' (as identified by single molecule, real-time (SMRT) sequencing analysis). In addition, ModD1 regulates a phasevarion of over 20 genes, many of which are associated with colonisation and virulence of *N. meningitidis*. To further clarify the role of ModD1, a number of phenotypic assays have been conducted, including adherence and invasion of airway epithelial cells (HBE14 and A549 cells), and survival assays in human blood and sera. These studies will help elucidate the role that phase-variable regulators play in the virulence by *N. meningitidis* isolates.

O42

Characterization of the complete gonococcal transcriptome during natural mucosal infection reveals expression of numerous gonococcal regulatory, phage, and hypothetical proteins

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Gonorrhea is a high morbidity disease worldwide with ~106 million cases reported annually. *Neisseria gonorrhoeae*, the causative agent of the disease, infects the human genital tract and often evades host immune mechanisms to persist until antibiotic intervention. The alarming increase in antibiotic-resistant strains of *N. gonorrhoeae*, the asymptomatic nature of this disease in women, together with the lack of a vaccine will require continued study of this organism during natural infection. Here, we examined the global transcriptome of *N. gonorrhoeae* during natural mucosal infection in women and identified infection-specific gonococcal gene expression profiles. RNA was collected and sequenced from 5 vaginal lavage samples from female asymptomatic patients exposed to infected male partners. On average, 2.67% of the total RNA aligned to the *N. gonorrhoeae* TCDC-NGO8107 genome and ~45% aligned to the human genome. Of 1577 gonococcal genes transcribed, categorization based on functional pathways of the top 100 most highly expressed revealed that during in vivo infection the gonococcus expresses genes involved in iron transport, metabolism, respiration and genes encoding phage associated proteins with particularly high expression of outer membrane, and hypothetical proteins. We also detected expression of 48 transcripts that did not align to any known gene. The corresponding gonococcal strains isolated from 4/5 patients were grown in vitro and the resulting transcriptome was compared to that expressed during natural infection in vivo. This comparison identified 226 ORFs, 31 tRNAs and 75 other non-coding sRNAs differentially regulated during infection. Major differences in the genes involved in metabolism, iron scavenging, cell wall biogenesis and lipid metabolism were observed. We observed increased expression of the iron regulated genes *tbpAB*, and *fbpAB* during natural infection as compared to growth in vitro, suggesting that the female genital tract is an iron deplete environment. These studies are the first to examine the global transcriptome of *N. gonorrhoeae* during natural in vivo infection. Knowledge of highly expressed genes during infection may lead to the identification of novel vaccine targets while profiles of regulated genes during infection will lead to new paradigms regarding molecular mechanisms of infection in this human pathogen.

O43

Comparative genome sequencing reveals within-host evolution of *Neisseria meningitidis* during invasive disease

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The mechanism of pathogenesis of invasive meningococcal disease (IMD) and the genetic factors on the side of the causative agent *Neisseria meningitidis* (Nme) are still elusive. Why these commensal bacteria cause IMD is even more puzzling since disease is a dead end for these bacteria. Consequently, it has been hypothesized that virulence and thus IMD is a consequence of short-sighted within-host evolution that provides no benefit to the pathogen beyond the host, and that in particular rapid phase-shifting at phase variable genes (PVGs) is expected to increase the likelihood that colonizing bacteria will cause IMD (Meyers LA et al. (2003) Proc Biol Sci 270: 1667).

To put this hypothesis to an experimental test we sequenced the genomes of throat-blood isolate pairs from four patients. Whole-genome sequences were obtained for one sequence type (ST)-42 serogroup B, two ST-11 serogroup C and one ST-23 serogroup Y throat isolate by combining sequencing with the Roche GS FLX Titanium Series Chemistry and short-read sequencing on a Illumina Genome Analyzer IIx. The genomes of the corresponding blood isolates were re-sequenced at over 1000-fold coverage on a Illumina Genome Analyzer IIx and mapped onto the reference genomes of the throat isolates. All sequence differences between the genomes of the throat and blood isolates were finally verified using standard Sanger sequencing.

Computational analysis of a set of 18 complete genomes showed that Nme contains about 250 potential PVGs per genome with a putative “core PVGenome” of 90 genes. Genome comparisons of throat-blood isolate pairs further revealed that three of the four pairs differed in the sequence of at least one PVG. In addition, in two genome pairs we identified (additional) differences due to gene conversion and non-homologous recombination events, respectively. Altogether, we could detect between 1 and 3 genetic differences in all four isolate pairs.

Therefore, our data indicate that in addition to phase-variation at PVGs also recombination contributes to within-host genetic diversity, and by considering recombination, our data support the hypothesis of within-host evolution of Nme during acute infection. The phenotypic consequences if any await further experimental investigation yet.

O44

Neisseria gonorrhoeae infection and female hormonal risk factors: menstruation and ovulation

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We are rapidly approaching the very real prospect that we will not be able to treat *Neisseria gonorrhoeae* (GC) infections with any antibiotic. Because we have only a limited understanding of the biologic parameters that affect risk of GC infection, particularly cervical infections in women, we have little hope of controlling infection by non-antibiotic means without further investigations. From 40-60% of individuals who are exposed to the organism resist infection, but the nature of resistance is not known for any anatomic site. Our ongoing studies have sought to define resistance by comparing various personal, demographic and immunologic attributes of those who resisted infection during exposure with those infected individuals. Previous studies have shown that a woman is more likely to be diagnosed with gonococcal infection during active menstruation. However, no correlation has been established between risk of infection at the time of exposure and the day of a women's menstrual cycle. We studied this risk in a cohort of women who reported to an Inner City STI Clinic because they had been identified by a heterosexual partner as having been exposed to an active GC infection and correlated the risk of subsequent infection with their menstrual cycle phase. We found risk of GC infection is not uniform over the menstrual cycle and that risk appears highest during the peri-menstrual and peri-ovulatory phases. Since iron is necessary for GC growth and proliferation, it is not surprising that the risk of infection appears higher during the perimenstrual phase, given the markedly lower levels of host lactoferrin and the presence of menstrual blood. The possible increase in GC infection risk seen during the periovulatory phase could be due to a variety of mechanisms: increased expression of the gonococcal Opa, which is involved in adhesion and up-regulated when the GC is shed from infected cervical epithelial cells during ovulation; host expression of CEACAM (CD66), an Opa receptor; or up-regulation of the Lutropin receptor, which has been implicated in GC attachment. Expansion and refinement of these data should allow for the focusing of prevention methods during the periods of greatest risk in a woman's cycle.

O45

Neisseria gonorrhoeae-mediated immune suppression: mechanisms and consequences in coincident chlamydia infection

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As a highly adapted pathogen that persists only in its human host, *N. gonorrhoeae* has acquired multiple mechanisms to evade the host immune system. We recently reported that *N. gonorrhoeae* suppresses the capacity of antigen-presenting dendritic cells to induce CD4+ T cell proliferation. Conditioned media from *N. gonorrhoeae* has a capacity to inhibit T cell proliferation induced by antigen-pulsed dendritic cells equivalent to that of live *N. gonorrhoeae*. The majority of this suppressive activity is contained in large molecular weight fractions that contain outer-membrane vesicles (OMVs) shed by the bacteria during growth. Recently, similar immune suppressive properties have been observed in OMVs from GI tract commensal Bacteroides fragilis. While capsular polysaccharide is the primary factor from B. fragilis responsible for these suppressive properties, *N. gonorrhoeae* does not produce a polysaccharide capsule. Gonococcal OMVs contain lipids, including lipooligosaccharide, and outer-membrane proteins. *N. gonorrhoeae* PorB is the most abundant protein in these vesicles, and treatment of dendritic cells with highly purified recombinant PorB recapitulated the inhibitory phenotype of treatment with *N. gonorrhoeae* or conditioned media. *N. gonorrhoeae*-mediated inhibition was partially relieved by anti-PorB antisera, suggesting that gonococcal PorB-containing OMVs play a role in suppression of the adaptive immune response.

Co-infection with *Chlamydia trachomatis* is common in individuals with *N. gonorrhoeae*, but the effects of these pathogens on host immune response to one another is not well understood. We found that just as *N. gonorrhoeae* suppresses the immune response to well characterized experimental antigens, it also suppresses the capacity of dendritic cells to stimulate chlamydia-directed CD4+ T cells. Furthermore, *N. gonorrhoeae*-treated dendritic cells caused co-cultured, chlamydia-directed CD4+ T cells to produce increased IL-17 and pro-inflammatory cytokines. These data suggest that *N. gonorrhoeae* can enhance pathologic inflammatory responses that drive symptoms and reproductive tract damage by co-infecting STI pathogens.

O46

N. gonorrhoeae induces localization of the inhibitor of apoptosis protein cIAP2 to Exosomes

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The family of inhibitors of apoptosis proteins (IAPs) has been implicated in the establishment of microbial infection in host target cells and in cancer progression, due to their dual role in apoptosis and inflammation. It has been reported that survivin, an IAP family member, has both an intracellular and extracellular role in cancer cells by inhibiting apoptosis while promoting proliferative and metastatic potential in neighboring cells when secreted in exosomes. Exosomes are small lipid vesicles released from various cell types and deliver messages in the form of protein and mRNA to neighboring cells near and far. We have previously established that *Neisseria gonorrhoeae* protects against staurosporine-induced apoptosis in transformed human endocervical epithelial cells (End/E6E7 cells) as well as HeLa cells. The ability of *N. gonorrhoeae* to inhibit apoptosis correlates with the upregulation of cIAP2. In this study, we have further characterized the role of cIAP2 in the host inflammatory response to gonococcal infection. In End/E6E7 cells, we demonstrate that gonococcal infection induces a significant increase followed by a loss of intracellular cIAP2, yet a stable increase in extracellular cIAP2. Extracellular cIAP2 localization is not due to membrane disruption or cell death during infection. Notably, extracellular cIAP2 is located in exosomes released after N.gonorrhoeae infection, supporting that cIAP2 is released in a controlled mechanism. Furthermore, we demonstrate that cIAP2 has a role in the response to gonococcal infection as it is required for cytokine production and protection from cell death. Collectively, our studies reveal significant alterations in exosome production and cIAP2 expression following gonococcal infection. Such changes may affect both immune signaling and apoptosis of infected epithelial cells and potentially, uninfected neighboring cells.

O47

An LD-carboxypeptidase (LdcA) controls the release of NOD1 agonist peptidoglycan from *Neisseria gonorrhoeae*

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Inflammation is a defining feature of infection with *Neisseria gonorrhoeae* (GC) and is generated in large part by lipooligosaccharide and released peptidoglycan (PG) fragments. The largest proportion of released PG fragments is 1,6-anhydro disaccharide tripeptide monomers, a potent agonist of the human NOD1 receptor. Previously, we identified LdcA (NGO1274, NGFG_02152), an LD-Carboxypeptidase responsible for the generation of tripeptide stems from the tetrapeptide stems of PG that predominate in the sacculus. Purification of LdcA confirms the predicted activity on tetrapeptide monomer and reveals additional activity on peptide-linked PG dimers, with mutation of residues in the predicted serine protease catalytic triad eliminating both activities. In strain MS11, deletion of *ldcA* or mutations of the active site residues increases PG dimer release and causes a complete shift from generation of tripeptide monomer to tetrapeptide monomer. The resulting shift in monomer release results in a loss of NOD1-dependent NF-kB activation in HEK-BLUE reporter cells, while not affecting TLR4 (LOS) or TLR9 (DNA) signaling. Since inflammatory PG fragments have been shown to contribute significantly to the damage observed during ascending infection of human fallopian tubes, we have employed an ex vivo infection model of human fallopian tube organ cultures to explore the significance of this change in PG fragment release. Preliminary results indicate that PG-containing supernatant from a strain without *ldcA* induces less IL-6 and IL-8 production than wild-type MS11 or a strain overexpressing *ldcA*. The fallopian tube model is also being used to explore how PG monomers impact the pathways leading to the death and sloughing of ciliated cells that are characteristic of the pathology of pelvic inflammatory disease. Since subcellular fractionation of MS11 reveals localization of LdcA to the outer membrane, via a TAT-dependent signal sequence, this work represents the first characterization of an extracytoplasmic LD-Carboxypeptidase responsible for the tetra-to-tri conversion normally necessary for PG fragment recycling. In this case, LdcA localization appears to be critical for GC to generate inflammatory PG fragments that are human NOD1 agonists, fragments that ultimately enhance the ability of GC to cause damage during fallopian tube infection.

O48

Neisserial-derived heptose is a novel microbial-associated molecular pattern that elicits a TIFA-dependent innate immune response

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Background: Bacteria of the *Neisseria* species secrete a heptose-based metabolite that drives HIV expression from co-infected human T cells. Because heptose is a unique 7-carbon sugar that is solely of microbial origin, we speculated that it represents a novel microbial associated molecular pattern (MAMP) that is sensed by the innate immune system.

Methods: To further elucidate the identity of the agonist, a bacterial genetics approach was taken to isolate *Neisseria meningitidis* mutants in the heptose biosynthesis pathway that do not secrete the pro-inflammatory heptose. In parallel, a human genome-wide RNAi screen was performed to isolate host cellular proteins that specifically mediate signaling downstream of the heptose sugar. Biochemical and genetic approaches were performed to validate candidate effectors potentially involved in the heptose-specific innate response.

Results: We demonstrate that in addition to being secreted by *Neisseria* sp., heptose phosphate (HP) is present in a variety of Gram-negative bacteria. However, in contrast to the efficient liberation of HP from *Neisseria*, other bacteria must be lysed in order to elicit an HP-dependent signal. Once HP passes into the host cell cytosol, it activates an NF-κB-dependent pro-inflammatory response that is independent of the pattern recognition receptors responsible for detecting other bacterial MAMPs. A human genome-wide RNAi screen revealed that signaling was dependent on the human gene TRAF-interacting forkhead associated protein (TIFA). Treatment of mammalian cell lines with HP resulted in TIFA recruitment to lysosomal compartments, phosphorylation, oligomerization, and complex formation with the ubiquitin ligase TRAF6. This pathway is apparent in a wide variety of human cells, and HP elicits an inflammatory response in otherwise sterile 'air pouch' chambers in mice, validating its identity as a MAMP.

Conclusions: Heptose phosphate is a novel MAMP, present in Gram-negative bacteria, yet uniquely secreted from *Neisseria* species. Mammalian cells sense the presence of HP in their cytosol via a previously unrecognized innate-immune signaling pathway dependent on the protein TIFA.

O49

Modeling the potential impact of gonococcal vaccines

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Gonorrhea is one of the most commonly reported sexually transmitted infections (STI) worldwide. Development of a gonococcal vaccine is of increasing importance given the continuing emergence of antibiotic resistance, the high levels of asymptomatic infection in women, and the serious implications that infection can have on female and newborn health. To effectively evaluate candidate antigens, a clear understanding of the key requirements of a vaccine is needed (i.e., does vaccination need to induce sterilizing immunity, would a vaccine induced decrease in infection levels or transmission have a significant impact on disease prevalence and incidence, and what is the ideal target population?). The "Global Action Plan to Control the Spread and Impact of Antimicrobial-Resistance in *Neisseria gonorrhoeae*" (WHO,2012) recommended the use of mathematical modeling to analyze the feasibility of interventions. To this end, we aimed to investigate the prospect that even a moderately effective vaccine may have a substantive impact on the reduction of disease. For example, modeling of vaccines for the STI Chlamydia trachomatis indicated vaccine strategies should focus on women and that even partially effective vaccines can greatly reduce the incidence of chlamydia. Similar mathematical models were used to simulate gonococcal transmission by considering the biology of susceptible (non-infected) and infected individuals, as well as their sexual behaviors and partnership dynamics. The model tracks parameters critical to incidence and prevalence rates, including: length of infection, disease progression, the dynamics of an individual's infectivity, and transmission rates. Subsequently, these data were applied to investigation of the population-level impact of vaccines that 1) protect uninfected persons by raising the infectivity threshold required for successful transmission, 2) alter the natural course of disease (e.g., changing the gonococcal growth rate, peak bacterial load, or duration of infection), 3) increase the duration of post-infection immunity, and 4) provide finite sterilizing immunity. A thorough understanding of the potential ability of a *N. gonorrhoeae* vaccine to reduce the incidence and prevalence of infections is critical to the future development, implementation, evaluation, and regulation of candidate vaccines and will help determine appropriate parameters to measure in vaccine studies using models of infection.

O50

Proteomics-driven reverse vaccinology for gonorrhea

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With 106 million new cases of gonorrhea reported annually worldwide, this ancient human disease remains a threat to public health. Because of a dire possibility of untreatable gonorrhea, there is a critical need for expanded efforts to develop preventive anti-*Neisseria gonorrhoeae* (Ng) vaccines.

Reverse vaccinology, which includes genome and proteome mining, has proven very successful in the discovery of vaccine candidates against many pathogenic bacteria. In particular, these methodologies paved the way to the development of group B meningococcal vaccines, which was a formidable effort for many years. However, progress in this research area in the Ng field remains in its infancy. To identify novel vaccine targets, we compared cell envelopes and naturally released membrane vesicles derived from four different Ng isolates using a comprehensive proteomic platform, isobaric tagging for absolute quantification coupled with 2D-LC/MS/MS. These studies enabled the identification of numerous proteins including 11 novel potential vaccine candidates. Initial characterization of several of these proteins demonstrated that LptD, BamA, NGO1956, NGO1985, NGO2054, and NGO2139 were expressed in various clinical Ng isolates; were surface exposed, and elicited bactericidal antibodies. In addition, depletion of LptD caused a loss of Ng viability, while the lack of NGO1985 and NGO2121 altered the cell envelope permeability barrier. These promising results suggest that the proteomics-driven approach can be successful in the identification of potential antigens. In addition, an effective vaccine with a broad spectrum of protection would be expected to consist of antigens that are not only highly conserved among different isolates, but also composed of proteins utilized by Ng to persist at anatomically distinct sites within the human host. Therefore, we have begun high-throughput proteomic experiments to broaden the array of potential antigens to these surface proteins, which are specifically utilized by Ng upon stimuli encountered at different infection sites including: oxygen availability, iron deprivation, and the presence of human serum. Together, these studies provide a foundation for the development of anti-Ng vaccine(s), which will be essential to prevent gonorrhea.

O51

Development of MtrE, the outer membrane channel of the MtrCDE and FarAB,MtrE active efflux pump systems, as a gonorrhea vaccine

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Worldwide, gonorrhea occurs at high incidence with more than 106 million estimated cases each year. Rising antibiotic resistance underscores the urgent need for a gonorrhea vaccine. MtrE, the surface-exposed component of the MtrCDE and FarAB,MtrE active efflux pump systems, is stably expressed and has two surface-exposed loops that are predicted to be conserved amongst all strains examined. Due to its role in protection against host antimicrobial factors and importance in establishing experimental genital tract infection in mice, MtrE is an attractive vaccine target. Here we tested the vaccine potential of a full-length His-tagged MtrE protein (rMtrE3-447) designed to begin at residue 3 of the mature peptide and lacking the N-terminal signal sequence. rMtrE3-447 was over-expressed in Escherichia coli, purified from inclusion bodies and used to produce antiserum in BALB/c mice. Anti-rMtrE3-447 serum recognized MtrE produced by eleven different *Neisseria gonorrhoeae* (Gc) strains and bound the Gc surface as assessed by flow cytometry. Additionally, in the presence of human complement, the anti-rMtrE3-447 serum was bactericidal against three Gc strains tested, including two highly serum resistant strains, but not against an MtrE-deficient mutant. In preparation for active immunization/challenge studies, we tested the capacity of rMtrE3-447 to induce high level serum and mucosal antibodies against MtrE when delivered to BALB/c mice by a subcutaneous prime/intranasal boost regiment with Th1-inducing (CpG, R848, Pam3CSK4, MPLA) or Th2-inducing (cholera toxin (CT)) adjuvants. All mice exhibited high-titer anti-rMtrE3-447 serum and vaginal responses (total Ig, IgG1, IgG2a, and IgA). Th2 responses were dominant in all groups; however CpG elicited the strongest IgG2a (Th1) response when administered with rMtrE3-447. The protective capacity of rMtrE3-447 administered with either CT or CpG was then tested in the mouse model. Protection was not observed when CT was utilized as the adjuvant. In contrast, the percentage of animals colonized over time in mice immunized with rMtrE3-447+CpG was significantly decreased compared to mice given CpG alone [41% and 83% colonized at day nine post-inoculation, respectively ($p = 0.04$, log-rank test)]. We conclude that rMtrE3-447, when combined with a Th1-inducing adjuvant, is a promising gonorrhea vaccine antigen. Examination of the mechanism(s) of protection is underway.

O52

N. gonorrhoeae methionine receptor GNA1946 confers protection from host mediated killing

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted disease gonorrhoea, which afflicts an estimated 100 million people each year. Approximately 50% of gonococcal infections in women are asymptomatic, which leads to an onset of serious conditions such as pelvic inflammatory disease and subsequently infertility. Development of a gonococcal vaccine has been challenging, as the bacterium is known for its high rate of antigenic variation as well as its ability to suppress protective immunity. However, vaccination against *N. gonorrhoeae* is feasible and requires identification of suitable candidates. This study looked at characterisation of gonococcal outer membrane proteinsin terms of their role during infection and their potential use as vaccine antigens. Here we describe one of the candidates, the highly conserved gonococcal methionine receptor GNA1946, its role in infection and interactions with host cells. We have demonstrated that GNA1946 is a surface exposed molecule and that is not essential for gonococcal growth, likely due to the fact that methionine can be synthesised endogenously from several precursors. However, GNA1946 is involved in serum resistance (24 fold less survival of the knockout mutant (KO) vs. the wild type (WT) in 90% human serum; 15 fold less survival of KO vs. WT in 50% serum), and provides protection against killing by primary monocytes and mature macrophages (2 fold less survival of KO vs. WT). Furthermore, the GNA1946 mutant strain has significantly reduced adherence and invasion of cervical epithelial cells (9 fold less adherence and 4 fold less invasion of KO vs. WT). Antibodies against GNA1946 alone are unable to block infection of cultured cervical epithelial cells with *N. gonorrhoeae*, which may be due to low level of expression of this receptor on the surface of the bacterium. However due to its role in protection from host mediated killing, GNA1946 may be a valuable vaccine antigen in combination with other vaccine targets.

O53

Inflammatory micro RNAs induced by neisserial OMPs support adjuvant activity

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Recent outbreaks of meningococcal disease, caused by *Neisseria meningitidis*, illustrate the need of an efficient vaccine. Current conjugate and polysaccharide vaccines have proven effective, although with a lack of long-lasting immunity. A highly potent vaccine triggers both a humoral as well as a cellular immune response, which possesses also memory characteristics. Initially, cells of the innate immune system encounter surface or outer membrane proteins (OMPs) from invading pathogens and mount an inflammatory response. Afterwards the adaptive immune response establishes and prevents future infections with the same pathogen. Our group utilizes the adjuvant capability of the outer membrane protein Porin B (PorB) purified from *Neisseria meningitidis*. PorB is a well-characterized pattern recognition receptor (PRR) ligand and binds to TLR1/TLR2 heterodimers, activating the NF-κB signaling cascade in a MyD88 dependent manner. Antigen presenting cells (APCs) up regulate co-stimulatory molecules, e.g. CD86, after encountering PorB. In vivo PorB induces substantial upregulation of genes upregulation in genes involved in inflammation, immune signaling and lysosomal degradation. Here we elucidate the underlying mechanisms and the potential role of PorB in inducing regulatory micro RNAs in different murine APC types. A well-studied miR involved in the regulation of early inflammation is mir-155. Stimulation of primary B cells, bone marrow derived macrophages (BMDMs) and bone marrow derived dendritic cells (BMDCs) with PorB triggered mir-155 expression in vitro. The stimulatory effect of PorB is partially TLR2 independent, since TLR2 deficient BMDMs showed also an increased mir-155 expression. Furthermore, using microarray techniques we characterized the differentially expressed miRs triggered by various PRR stimulants in vitro. We also demonstrated novel upstream-regulated proteins that might be affected by PorB compared to other PRR adjuvants, e.g. LPS and PAM3CSK4, using Ingenuity Pathway Analysis (IPA). Our findings could explain the role of these miRs in the immune stimulating effect of PorB. A more generalized understanding of the induced and altered miR expression pattern will be crucial for the incorporation of PRR ligands in future adjuvant containing vaccine preparations.

O54

Global analysis of neutrophil responses to *Neisseria gonorrhoeae* reveals a self-propagating inflammatory program

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Background: An overwhelming neutrophil-driven response causes both acute symptoms and the lasting sequelae that result from infection with *Neisseria gonorrhoeae*. Neutrophils undergo an aggressive opsonin-independent response to *N. gonorrhoeae*, driven by the innate decoy receptor CEACAM3. CEACAM3 is exclusively expressed by human neutrophils, and drives the potent binding, phagocytic engulfment and oxidative killing of Opa-expressing bacteria. In this study, we sought to explore the contribution of neutrophils to the pathogenic inflammatory process that typifies gonorrhea.

Methods: To investigate the contribution of neutrophils to the inflammatory response, we used a combination of genome-wide microarray and biochemical profiling of gonococcal-infected neutrophils from wild type mice or a CEACAM-expressing transgenic mouse line. To complement this, an 'air pouch' model was used to address the role of CEACAM3 in the context of in vivo infection.

Results: We reveal that CEACAM3 engagement triggers a Syk-, PKCδ- and Tak1-dependent signaling cascade that leads to the activation of an NF-κB-dependent transcriptional response, with consequent production of pro-inflammatory cytokines capable of recruiting more neutrophils. Furthermore, we show that human CEACAM-expressing neutrophils have heightened migration toward the site of gonococcal infection in vivo, where they become further activated upon binding Opa-expressing *N. gonorrhoeae*.

Conclusions: This study establishes that the role of CEACAM3 is not restricted to the direct opsonin-independent killing property of neutrophils. Instead, CEACAM3 binding also drives a vigorous pro-inflammatory response that recruits more neutrophils to the site of infection. By carrying the potential to mobilize increasing numbers of neutrophils, CEACAM3 effectively aids in the rapid response of bactericidal neutrophils to the site of infection. However, the potency of CEACAM3-dependent signals also represents a tipping point, which, once surpassed, can drive the self-propagating and eventually pathogenic neutrophil responses typifying gonococcal disease.

O55

Insights into *Neisseria meningitidis* infection and immunity from the CEACAM-humanized mouse model

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Background: We recently established that transgenic mice expressing human CEACAM1 can be colonized by Opa protein-expressing *N. meningitidis* upon nasal challenge. Herein, we provide a perspective on the utility of this model for understanding the innate and adaptive immune response elicited by infection, and the pre-clinical assessment of vaccines with potential to confer sterilizing immunity.

Methods: Transgenic mice expressing human CEACAM1 or their wild type littermates were infected intranasally with *N. meningitidis* to allow persistent colonization. Bacterial burdens were measured by quantifying colony forming units recovered from infected tissues. Kinetic cytokine responses in nasal tissue were measured by ELISA, while specific immunoglobulin responses elicited during infection or immunization were measured using meningococcal-based ELISA.

Results: *N. meningitidis* colonize the nasopharynx of CEACAM1-humanized mice, leading to induction of a rapid innate inflammatory cytokine response and eventual emergence of *N. meningitidis*-specific IgA within nasal secretions and IgG-dependent bactericidal activity in the blood. These responses rely upon intimate mucosal attachment and the extended persistence of viable bacteria, with little detectable meningococci-specific humoral response emerging after intranasal inoculation of wild type mice. While infection with either serogroup B or serogroup C strains can confer complete protection against subsequent challenge with the identical strain, cross-reactivity of the humoral responses did not strictly correlate with the serogroup of the heterologous strains. Despite this fact, the currently-licensed carbohydrate-conjugate NeisVac-C vaccine (Baxter) was sufficient to provide 100% protection against colonization by a group C strain without affecting the ability to colonize the animals with a group B strain of *N. meningitidis*. Immunization of mice with the Bexsero (Novartis), recently released in Canada, has been initiated to measure the ability of this vaccine to affect colonization by group B meningococcal strains.

Conclusions: Human CEACAM1 expression allows intimate association of *N. meningitidis* with the nasal mucosa and penetration into underlying tissues, driving innate and adaptive immune responses reflecting those thought to occur in humans. The model provides an exciting opportunity to study meningococcal physiology and the host response during infection of the nasal mucosa, as well as to test how prophylactic and therapeutic strategies affect colonization by this deadly pathogen.

O56

Recruitment of CD46 to the cortical plaque serves to confer resistance to serum killing on *Neisseria gonorrhoeae*

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The complement cascade is crucial for innate immune responses to infection. Bacterial pathogens frequently utilize complement regulatory proteins to protect themselves from complement killing. The complement regulatory protein CD46 is a type I transmembrane protein that acts as a cofactor for Factor I-mediated proteolysis of opsonins C3b and C4b. *Neisseria gonorrhoeae* (Ngo) manipulates CD46 during infection of epithelial cells by redirecting its trafficking. We previously demonstrated that CD46 is recruited to the cortex of infected epithelial cells underneath adherent Ngo microcolonies. Infection also induces shedding of CD46-positive vesicles and CD46 proteolysis. All three Ngo infection-induced CD46 phenotypes - recruitment, shedding and proteolysis, - are dependent upon retraction of Type IV pili (Tfp). Mutating PilT, the Tfp retraction motor, blocks these phenotypes without reducing Ngo adhesion. Images obtained using a superresolution structured illumination microscope show that CD46-positive puncta permeate wild-type bacterial microcolonies during infection. The presence of CD46 throughout Ngo microcolonies could provide a means by which the bacteria evade complement killing, through inhibiting complement activation near bacterial surfaces. As a first step towards testing this hypothesis we conducted serum bactericidal assays during epithelial cell infection. Epithelial monolayers were infected with wild-type or *pilT* Ngo for five hours, then treated with normal human serum (NHS) or heat-inactivated NHS (HI-NHS). Wild-type Ngo was more resistant to killing by NHS than *pilT* Ngo, whereas both strains were viable in the presence of HI-NHS. When the assay was repeated on bacteria infecting glutaraldehyde-fixed cells, wild-type and *pilT* Ngo were equally sensitive to killing by NHS. Thus, live epithelial cells are required for the highest levels of serum resistance by wild-type Ngo. Our findings strongly suggest that Ngo recruitment of host cell protein CD46 into its microcolonies allows the pathogen to avoid complement killing. A prediction of our model is that downregulation of CD46 will reduce the resistance of wt Ngo to complement killing. We are currently testing this hypothesis.

O57

Resistance to serum and antibody-mediated bacteriolysis dependent on neisserial immunoglobulin-binding protein TspB

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Background: Prophage DNA carried by *Neisseria meningitidis* (Nm) isolates associated with invasive disease (Bille et al 2005, J Exp Med 201:1905) contains a gene (ORF6) coding for TspB. We showed TspB is an immunoglobulin-binding protein that mediates the formation of bacterial aggregates enveloped in a matrix of TspB, IgG, and DNA (Müller et al, 2013, J Immunol 191:1153). The objective of this study was to determine whether TspB contributes to survival of group B strain H44/76 in human serum and, if so, by what mechanism.

Methods: Mutants of H44/76 with combinations of the 3 full-length tspB genes knocked out were tested for resistance to human serum and IgG-depleted human serum. The effect of suppressing TspB surface exposure on bactericidal activity mediated by anticapsular, anti-porin and anti-factor H binding protein (fHbp) antibodies was determined by bactericidal assay with human serum. IgG- and DNA-binding activities of recombinant TspB subdomains were measured by ELISA and gel mobility shift assay, respectively. Complexes formed by mixtures of purified TspB, IgG, and DNA were observed by fluorescence microscopy.

Results: Nm resistance to normal human serum was dependent on functional tspB genes. Survival was enhanced with increasing number of tspB genes and the ability to promote survival varied between given genes, with nmbh4476_0681 being most important. Suppressing TspB surface exposure decreased resistance to complement-dependent bacteriolysis mediated by antibodies against PorA and fHbp but had mixed effect on anticapsular antibodies to representative group A, B, and C strains. Resistance to bacteriolysis was indirect through formation of a matrix containing TspB, IgG, and DNA that enveloped aggregates of bacteria. Recombinant proteins corresponding to subdomains of TspB had human IgG Fcγ- and/or DNA-binding activities but only TspB derivatives containing both domains formed a large film-like matrix when combined with purified IgG and DNA. Overall, the results show that TspB promotes serum survival by suppressing complement activation via the classical/lectin pathways.

Conclusion: Recognizing that TspB is important for serum resistance may lead to a better understanding of why isolates that carry *tspB* genes are associated with causing invasive meningococcal disease as well as new approaches to prevention and treatment of disease.

O58

Molecular epidemiology of serogroup A meningococcus in South Africa, 2003-2012

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Background: In South Africa, invasive meningococcal disease (IMD) is caused by serogroups A, B, C, W and Y (MenA, -B, -C, -W, -Y, respectively). The ST-5/ST-7 (subgroup III) pandemic clone was first detected in South Africa during a meningitis outbreak in 1996 where it represented 13.5% of outbreak isolates. 50% belonged to the ST-1 complex/ subgroup I/II clone, which was previously identified in South Africa in 1968. Endemic MenA, collected through our IMD surveillance from 1999 to 2002, was mostly ST-1. ST-7 isolates were also circulating albeit at a much lower frequency. We aimed to characterise MenA using whole genome analysis.

Methods: IMD cases reported through national, laboratory-based surveillance, from 2003 to 2012, were reviewed, and genomes of randomly selected serogroup A isolates (n=39) were sequenced. Using the PubMLST *Neisseria* BIGS database, ST-1 complex (subgroup I/II) genomes were compared with available ST-1 MenA genomes from Africa and Asia. ST-1 genomes were analysed further using the typing schemes available on the BIGSdb.

Results: 4535 IMD cases were reported and 63% (n=2865) had viable isolates available for serogrouping. MenA, -B, -C, -W and -Y represented 6% (179), 22% (628), 9% (252), 51% (1457) and 11.5% (330), respectively, with an average annual incidence of 1/100,000 population. Incidence of MenB, -C and -Y disease remained consistent, however, MenW disease increased from 0.06 to 0.64/100,000 population ($p<0.0001$) from 2003 to 2006, subsequently declining to 0.1 in 2012 ($p<0.0001$). MenA disease declined from 0.2 to 0/100,000 population ($p<0.0001$) with no MenA cases reported in 2011 or 2012. Genome data were available for 34/39 isolates: 30 were ST-1, three were ST-7 and one was ST-6709. Our ST-1 isolates clustered separately from other countries, and formed three groups by whole genome MLST, stratified by time. Individual analysis, by scheme, showed that these differences were potentially located in the core genome.

Conclusions: Prior to its decline and disappearance in 2010, almost all MenA isolates represented ST-1. At the whole genome level, however, some heterogeneity, linked to time, appears to be evident. More in-depth analyses are required to determine the degree of heterogeneity and the affected regions of the core genome.

O59

Capsular switching and global spread of *Neisseria meningitidis* serogroup W ST-11

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Background: The Hajj 2000 outbreak was the first known epidemic caused by *N. meningitidis* serogroup W, which has emerged as a leading cause of meningococcal disease globally. The Hajj clone belongs to ST-11 lineage and is believed to have acquired the W capsule through a C to W capsular switching event. We applied whole genome sequence (WGS) analysis to study W ST-11 capsular gene sequences and generate a model of genetic relatedness of W ST-11 strains.

Methods: Twenty-five global W ST-11 isolates collected from disease cases from 1970-2012 were sequenced using Pacific Biosciences and/or short read WGS platforms. Capsular gene sequences were studied using phylogenetic and BLAST analyses. Full length antigen gene sequence analysis for *porA, porB, fetA, nadA, nhbA* and *fHbp* was combined with whole genome phylogenetic and single nucleotide polymorphism (SNP) analyses to study genetic relatedness among invasive W ST-11 isolates.

Results: We identified two distinct recombination events within the W ST-11 capsule locus of all 25 strains – a 15.5kb recombination from ST-185 cc-174 (serogroup W) which included the sialic acid transferase gene (*csw*) and an 11.2kb recombination from ST-23 involving neighboring non-capsular genes.

W ST-11 strains formed two distinct clusters based upon antigenic and genomic comparison to the Hajj 2000 clone: 1) Seven strains from the African meningitis belt (2000-2005) and 1 strain from the US (2000) clustered based on identical antigenic profile, phylogeny and 3 to 406 SNP differences as compared to the Hajj 2000 clone and 2) Seven sporadic strains from 1970-1999 and 10 strains from Brazil (2001), Chile (2008-2012), France (2002), USA (2008-2009), and Burkina Faso (2001-2002) had at least 1200 SNP differences and differed at 2 or more antigenic loci compared to the Hajj 2000 clone.

Conclusion: The W ST-11 capsular switch involved two separate recombination events and current global W ST-11 meningococcal disease is caused by strains that are descendants of a strain that underwent this capsular switch. In addition, there is persistence and spread of W ST-11 strains highly related to the Hajj 2000 outbreak strain within the African meningitis belt. Importantly, there is co-circulation of W ST-11 strains that are phylogenetically and antigenically distinct from the Hajj clone and are also causing disease in the African meningitis belt and globally.

O60

New hypervirulent clones of *Neisseria meningitidis* evade herd immunity through homologous replacement of loci for cell surface protein antigens and protein glycosylation

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Epidemics of meningococcal meningitis in the African 'meningitis belt' occur in irregular cycles initially proposed to be primarily related to herd immunity to capsular polysaccharides. However, during our longitudinal meningococcal colonization and disease studies we observed waves of clonal replacement with the same capsule type, suggesting that natural immunity to non-capsular antigens may play a significant role in the dynamics of meningococcal epidemics. Here we describe a high-resolution comparative genomic view of the evolutionary changes that occurred during the clonal replacement of one meningococcal clone (ST7) by a descendent clone (ST2859) during the period of 2001-2009. We found that the majority of genetic changes were due to homologous recombination of laterally acquired DNA. Markers of adaptation to evade herd immunity were indicated by genomic hotspots of recombination and point mutation. These hotspots are encoding enzymes involved in glycosylation patterns of major protein antigens, proteins regulating pilus expression and Maf3 adhesins. These results are highlighting the importance of these surface structures in host-pathogen interaction and immune evasion. Our findings show that, for meningococcal populations, evasion of herd immunity can be achieved though recombination events at a set of key loci.

O61

Relationship between carriage of *Neisseria meningitidis* and meningococcal disease in Burkina Faso, 2009-2012

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Background: The relation between population meningococcal carriage and disease in the African meningitis belt is poorly understood. To better understand this association, we compared disease incidence and carriage prevalence in three districts of Burkina Faso in 2009–2012 using an ecological analysis.

Methods: Carriage prevalence was assessed through cross-sectional, multistage cluster sample surveys in two rural (Dandé, Kaya) and one urban (Bogodogo) district. Oropharyngeal swabs were collected and meningococcal culture and identification performed. Disease incidence was determined using Ministry of Health case-based surveillance data. The temporal relationship between carriage and disease was characterized by evaluating carriage prevalence at time points before, during, and after peaks in disease. Carriage per case ratios were calculated for each district, comparing carriage in October–November to disease incidence in the preceding January–June of each year.

Results: The analysis focused on two serogroups, X (NmX) and W (NmW), that contributed substantially to both carriage and disease during this time period. NmX carriage prevalence increased 24-fold in the three districts from 2009 to 2010 while NmW carriage increased 13-fold from 2011 to 2012. These changes were due to increased carriage of sequence types (STs) that were infrequently carried at prior sampling time points: NmX ST-181 in 2010 and NmW ST-11 in 2012. In all but one case, peaks in disease occurred before sampling time points with highest carriage prevalence. Higher NmX carriage in 2010 was associated with increased NmX disease in the preceding epidemic season in Bogodogo (5168 carriers/case, 95% interval estimate (IE): 2772–8759) and Dandé (378 carriers/case, 95% IE: 85–1053) but not Kaya (0 cases). Higher NmW carriage in 2012 was associated with increased NmW disease in the preceding epidemic season in Bogodogo (1111 carriers/case, 95% IE: 566–1952), Dandé (718 carriers/case, 95% IE: 554–907), and Kaya (1527 carriers/case, 95% IE: 470–3634).

Conclusions: In this analysis, high carriage prevalence did not precede increased disease incidence; rather, disease appeared to increase prior to or simultaneously with carriage. These findings suggest that expansion of specific clones in a susceptible population contributes to increases in meningococcal disease incidence and carriage prevalence.

O62

Association of meningococcal type with disease outcome

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Background: We present analyses based on a matched dataset containing 4,590 laboratory confirmed cases of invasive meningococcal disease, processed over a twelve year period (2002 to 2013), and representing 63.9% of notifications to the Robert Koch-Institute. Of 4,590 cases, data on full finetype (i.e. serogroup, PorA, FetA), fatal outcome, and disease manifestation ("meningitis", "sepsis", and "fulminant sepsis") were available in 96.9%, 99.3%, and 79.3%, respectively. We explored whether particular finetypes were more commonly associated with septic disease (i.e. sepsis and fulminant sepsis) or death.

Methods: Finetypes represented less than 50 times were collectively defined as the reference group in logistic regression models testing the association of finetype with death, and in multinomial logistic regression models analyzing the association of finetype with clinical manifestation. While all models were adjusted for age group and sex, models predicting death were fitted with and without a variable representing clinical manifestation. Of 1,002 finetypes within the dataset only 11 occurred more than 50 times and were thus tested for associations.

Results: Of three finetypes significantly associated with death (B:P1.7-2,4:F1-5; C:P1.5-1,10-8:F3-6; C:P1.5,2:F3-3) only the two serogroup C types were also associated with septic disease. Accordingly, inclusion of manifestation into the model predicting death lowered odds ratios of serogroup C types, but not of B:P1.7-2,4:F1-5; significant associations, however, remained for all three types, suggesting an independent effect on death. In addition, two types (B:P1.17,9:F1-7; B:P1.7,16:F3-3) were associated with septic disease, without, however, causing significantly higher case fatality.

Conclusions: These analyses suggest that while disease manifestation remains a strong predictor of death, some finetypes are associated with death independently of clinical manifestation. Also, some types are associated with sepsis without a concomitant association with death. Higher case fatality, but also increased risk of sepsis, might be due to higher bacterial load attained during disease caused by above types. To test this hypothesis, we are currently quantifying meningococcal DNA in a subset of 280 samples (mainly cerebrospinal fluid and serum) collected over the last decade, in which culture-independent typing of meningococcal DNA was successful. Results will be presented and discussed at the conference.

O63

MDAΦ the invasive filamentous bacteriophage of *Neisseria meningitidis*, increases bacterial colonization onto epithelial cells by mediating bacteria-bacteria interaction

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The mechanism by which *Neisseria meningitidis* becomes invasive is not well understood. Comparison of the genome of strains revealed the presence of an 8kb island in strains belonging to invasive clonal complexes. Epidemiological investigations confirmed that the presence of this island is associated with the ability of bacteria to be invasive (Bille et al. JEM 2005; Bille et al. PlosONE 2008). This island was designated MDA (Meningococcal Disease Associated). MDA is highly conserved among meningococcal isolates and its analysis revealed a genomic organisation similar to that of a filamentous prophage such as CTXΦ of *Vibrio cholerae*. Subsequent molecular investigations showed that the MDA island has indeed the characteristics of a filamentous prophage which can enter into a productive cycle and is secreted using the type IV pilus (tfp) secretin PilQ.

The analysis of the genome content of the phage did not reveal any obvious virulence factor encoded by the prophage. The use of isogenic derivatives deleted in the prophage did not show any role of the MDAΦ in phenotypes associated with the bloodstream phase of meningococcemia (growth in serum, interaction with endothelial cells). On the other hand, when interacting with epithelial cells, phage production increased as microcolonies grew on the apical surface of the monolayer, thus leading to a thicker biofilm than in deleted isogenic variants. This effect on biofilm was dependent on the presence of phage particles, as mutations in genes responsible for phage production abolished this effect. Immunogold labelling on biofilm demonstrated that bacteria inside the biofilm produced large amount of phage. The phages are long filamentous fibers with a structure similar to that of tfp. They are in close interaction with the bacteria and able to form small bundles connecting two bacteria. Usually bacteria-bacteria interaction are mediated by tfp, surprisingly the production of tfp in biofilm was almost abolished. We hypothesized that inside biofilm, phage production in place of pili maintain bacteria-bacteria interactions favouring bacterial colonization onto a monolayer of epithelial cells, thus suggesting that the gain of invasiveness of strains producing this phage rely on its ability to increase colonization at the port-of-entry.

O64

Neisserial phage protein contributes to neisserial pathogenesis

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Neisseria gonorrhoeae possesses four genomic regions that are associated with the production of filamentous bacteriophages, and these regions are highly conserved in all gonococcal strains (Orthologous phage are found in *N. meningitidis*). However, their function in gonococcal infection is unknown. We demonstrated that while two of these regions contain all of the genes needed to express a functional phage during in vitro growth, the expression of all phage genes is highly repressed. Our DNA sequence analysis shows that one of the phage genes (orf9), with five copies in the gonococcal genome, has significant homology with Zot, a *Vibrio cholera* toxin with a capability of reversibly disassembling tight junctions between epithelial cells. The presence of specific antibody against this protein in gonococcal infected women indicates the expression of this protein occurs in vivo. We identified the likely insertion sites for these phages in the gonococcal genome and used this information to create a strain that was genetically deleted for all phage regions. The successful construction of this strain demonstrates that the bacteriophage are non-essential for gonococcal in vitro growth. We cloned the DNA region encoding Orf9 and expressed it in *Escherichia coli*. We found that while expression of Orf9 was toxic and rapidly killed the expressing strain, we were able to purify the protein to homogeneity. When this protein was added to polarized human epithelial (T84) monolayers, after 30 min the level of the transepithelial electric resistance (TEER) of the monolayer was dramatically reduced in a dose dependent manner. The reduction in TEER was concurrent with the redistribution of junctional proteins ZO1 and occludin from the apical junction. Immunofluorescence microscopy analysis found that Orf9 appeared to localize to the apical junctions. Incubation of epithelial cells with high concentrations of Orf9 resulted in significant cell killing, as measured by trypan blue exclusion assays. Taken en toto, these data suggest that the expression of the phage proteins during in vivo growth can lead to the disruption of the apical junction and the epithelial integrity, providing the gonococcus with a mechanism for invading into host tissues.

O65

Gonococcal restriction endonucleases cause double-strand breaks and distort mitosis in epithelial cells

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The host epithelium is both a barrier against and the target for microbial infections. Maintaining regulated cell growth ensures a intact protective layer towards microbial-induced cellular damage. *Neisseria gonorrhoeae* infections disrupt host cell cycle regulation machinery. The infection causes DNA double strand breaks that delay progression through the G2/M phase. We show in qPCR assays that gonococcal restriction endonucleases are upregulated during invasion. Bacterial lysates containing restriction endonucleases were able to fragment genomic DNA as detected by PFGE. Lysates were also microinjected into the cytoplasm of the cell and after 20 hours, DNA double strand breaks were identified by 53BP1 staining. We show that intracellular gonococci release restriction endonucleases that enter the nucleus and damage human chromosomal DNA. In addition, by using live-cell microscopy and NHS-ester stained live gonococci we visualized the subcellular location of the bacteria upon mitosis. We detected a direct interaction between intracellular gonococci and host cell chromatin during mitosis potentially causing hindrance for anaphase progression but also enabling nucleomodulatory distortion of the spindle assembly checkpoint proteins MAD1 and MAD2. Taken together, infected cells show impaired and prolonged M-phase, nuclear swelling and lagging chromosomes resulting in micronuclei formation and chromosomal instability of the infected cell. These data highlight basic molecular functions of how gonococcal infections affect host cell cycle regulation, cause DNA double strand breaks and predispose cellular malignancies.

O66

Gonococcal association with human CEACAMs during infection of the female genital tract

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Background: In vitro studies have demonstrated that CEACAM glycoproteins are the primary target of gonococcal Opa adhesins, implicating epithelial CEACAM1, CEACAM5 and CEACAM6 as targets during bacterial colonization. On the other hand, the neutrophil-restricted decoy receptor CEACAM3 mediates effective bacterial engulfment and killing in vitro. Herein, we aim to establish the physiological relevance of Opa-CEACAM binding interactions within the female genital tract.

Methods: Minimally-cultured clinical isolates were obtained from a longitudinal study of female sex workers and male patients in an STD clinic in Nairobi, Kenya. Each isolate was phenotyped with respect to Opa protein expression and binding specificity in vitro. Human hysterectomy samples and female genital tissues from CEACAM-humanized mice were analyzed for human CEACAM expression. Gonococci were either vaginally or transcervically administered to β-estradiol treated transgenic mice to emulate lower and upper genital tract infections, respectively.

Results: While each isolate has the capacity to bind each CEACAM receptor, the primary isolates display selective expression of Opa variants that bind CEACAM1 and CEACAM5 but not CEACAM3, reflecting the beneficial versus detrimental outcomes of their binding in vitro. Upon staining for each CEACAM in the human female genital tract, we observed that CEACAM1 is restricted to columnar cells of the endometrium and endocervix, whereas CEACAM5 on the ectocervix, suggesting that both are well-positioned to contribute to gonococcal attachment. Supporting this, CEACAM1 and CEACAM5 transgenic mice, which mirror the CEACAM-expression pattern of the human genital tract, show increased gonococcal association with tissues in the uterine lining and lower genital tract, respectively. Moreover, we observed that Opa-expressing *N. gonorrhoeae* elicit a more robust phagocytosis, killing and inflammatory neutrophil response, explaining the strong negative selection against CEACAM3 binding in the context of natural infection.

Conclusions: When considered together, our data establish that CEACAM1 and CEACAM5 are expressed on tissues targeted by *N. gonorrhoeae* within the female genital tract, with differential Opa specificities needed to facilitate primary colonization versus ascending infection. Moreover, the selection for Opa variants that allow attachment to epithelial-expressed CEACAMs but not the highly related CEACAM3 highlights the exquisite adaptation of this pathogen to life in humans.

O67

Neisseria meningitidis differentially activates the acid sphingomyelinase-ceramide system to induce its uptake into brain endothelial cells

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The interaction with brain endothelial cells is central to the pathogenicity of *Neisseria meningitidis* infections. Recent studies demonstrated that distinct membrane microdomains, named lipid rafts, and ceramide play an important role in infectious biology. Ceramide forms larger ceramide-enriched membrane platforms that are required for segregation of receptors and diverse signal transduction. In this study, we show that *N. meningitidis* causes transient activation of acid sphingomyelinase (ASM) followed by ceramide release in brain endothelial cells. In response to *N. meningitidis* infection, ASM and ceramide are displayed at the outer leaflet of the cell membrane and condense into large membrane platforms wherein ErbB2, an important receptor involved in bacterial uptake, clusters. Mechanistically, *N. meningitidis*-mediated ASM activation relied on binding of the outer membrane protein Opc to heparan sulfate proteoglycans followed by activation of phosphatidylcholine-specific phospholipase C. Pharmacologic or genetic ablation of ASM abrogated meningococcal internalization without affecting bacterial adherence. In accordance, the restricted invasiveness of a defined set of pathogenic isolates of the ST-11/ST-8 clonal complex into brain endothelial cells directly correlated with their restricted ability to induce ASM and ceramide release. In conclusion, ASM activation and ceramide release are essential for internalization of Opc-expressing meningococci into brain endothelial cells, and this segregates with invasiveness of *N. meningitidis* strains.

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