Abstracts oral presentations O01 – O69

O01

A cis-acting DNA element upstream of the pilin expression locus is required for pilin antigenic variation

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To help avoid immune surveillance, *Neisseria gonorrohoeae* promotes high frequency gene conversion events between many silent pilin loci and the single expressed pilin locus, pilE; resulting in the production of variant pilin proteins. Transposon insertions have been isolated in the non-coding region upstream of pilE that block pilin antigenic variation (Av) [1, 2]. To define which DNA sequences in the non-coding region upstream of pilE are required for pilin Av, this region was randomly mutagenized using error prone PCR, and the mutations were introduced into the Gc chromosome by DNA transformation. 103 transformants unable to undergo pilin Av were selected by screening for stable piliated colony morphology, and DNA sequence analysis revealed that each mutant carried a point mutation in a specific region upstream of pilE. Further characterization of this DNA element by site directed mutagenesis revealed that mutating any one of 12 specific bases in this region blocks pilin Av. We conclude that this 12 bp sequence in the noncoding region upstream of the pilus expression locus defines a cis-acting DNA element required for directing recombination from the silent loci to the expressed pilin gene and may define the initiation site for pilin Av.

References

1. Kline, K.A., et al., Transposon mutagenesis identifies sites upstream of the *Neisseria gonorrhoeae* pilE gene that modulate pilin antigenic variation. J Bacteriol, 2007. 189(9): p. 3462-70.
2. Sechman, E.V., M.S. Rohrer, and H.S. Seifert, A genetic screen identifies genes and sites involved in pilin antigenic variation in *Neisseria gonorrhoeae*. Mol Microbiol, 2005. 57(2): p. 468-83.

O02

DNA processing and secretion by the gonococcal type IV secretion system

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Most strains of *N. gonorrhoeae* and some strains of *N. meningitidis* encode a type IV secretion system (T4SS) on the chromosome. The T4SS facilitates the transfer of genes by secreting chromosomal DNA into the extracellular milieu, where it can be taken up by other cells through natural transformation. In order to better understand the process of gene transfer in Neisseria, we have examined DNA processing and secretion. *N. gonorrhoeae* requires the putative relaxase TraI for DNA secretion. This requirement suggested that secreted DNA would have similarities to DNA transferred by conjugation systems, specifically that the DNA would be cut at a specific sequence, that the relaxase would remain bound to the 5′ end of the DNA, and that the DNA would be single-stranded. Treatment of gonococcal supernatants with specific nucleases showed that the secreted DNA was degraded by single-strand specific nucleases, but not double-strand specific nucleases. However, a single-strand specific exonuclease that degrades from the 5′ end failed to degrade secreted DNA. Furthermore DNA transfer in coculture was diminished by a single-strand specific exonuclease but not by a double-strand specific exonuclease or an endonuclease. These data indicate that secreted DNA is single-stranded and blocked at the 5′ end.

A non-coding region near to the relaxase gene was found to be necessary for DNA secretion. This region shows multiple similarities to origins of transfer from conjugative systems including a high A-T content, an inverted repeat sequence, and putative binding sites for IHF. Insertion and deletion mutations were used to define the region required for DNA transfer. Insertions outside a 150 bp region did not affect DNA

secretion, whereas an insertion within this region or deletion of the region eliminated DNA secretion. In preliminary experiments, placement of the putative origin on a replicating plasmid conferred the ability of the plasmid to be transferred. These data suggest that the gonococcal chromosome carries a single origin of transfer for DNA secretion.
To determine how common DNA secretion is among strains and examine the effects of allelic variation, DNA secretion was measured from a panel of low-passage clinical isolates of *N. gonorrhoeae* and one *N. meningitidis* strain. DNA sequencing and hybridization studies revealed multiple differences between strains in the T4SS genes. Three alleles of traG, two alleles of yag, and two alleles of traA were identified among gonococcal strains. These genes are predicted to encode an inner-membrane protein, an outer-membrane protein, and a T4SS pilin, respectively. *N. meningitidis* strains carried one of these traG alleles and a traA allele different from either of the gonococcal versions. Half of the gonococcal strains tested secreted DNA, but no correlation was found between traA allele and secretion. The meningococcal strain and one gonococcal strain tested contained a variant traG. These strains did not secrete DNA suggesting that some alleles of traG may not function for DNA secretion. These data indicate that secretion of DNA occurs in some, but not all, T4SS+ isolates of *N. gonorrhoeae* but may not occur in *N. meningitidis.*

O03

Broad spectrum O-linked protein glycosylation in *Neisseria gonorrhoeae*

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Background
Glycoproteins have been documented in both Gram-negative and Gram-positive bacterial species and both N-linked and O-linked glycans have been identified. Most prokaryotic glycosylation systems are dedicated to modification of single proteins or sets of related
proteins that are surface localized (flagellin, pilin etc.)[1]. To date, two generalized protein glycosylation systems have been identified: the *Campylobacter jejuni* (Cje) N-linked system which targets at least 25 proteins found predominantly in the periplasm [2] and a *Mycobacterium tuberculosis* O-mannosylation system acts on at least two surface lipoproteins [3]. Both of these pathways parallel related processes in eukaryotes.
Results
Here we describe the first generalized O-linked glycosylation system in a Gram-negative species. PilE, the pilin subunit of *Neisseria gonorrhoeae* (Ngo) type IV pili undergoes O-linked glycosylation and Pgl (pilin glycosylation) proteins responsible for the synthesis, membrane translocation and transfer of the glycan to PilE have been identified [4]. Genetic analyses and complementation show that this system is remarkably similar to that of Cje save for the use of a Ser-directed oligosaccharyltransferase (Otase) rather than an Asn-directed Otase [4]. We now show that Ngo glycosylates multiple proteins and have gone on to unambiguously identify ten of these additional substrates. Glycosylation of these proteins utilizes the consensus pgl pathway as the proteins bear the same glycans as those found on pilin and loss-of-function pgl mutations abolish modification. Additionally, protein glycosylation can be functionally transferred into *E. coli* by expressing the pgl genes. Thus, the pgl genes are both necessary and sufficient for general glycosylation. Like in Cje, targets of the Ngo glycosylation system are a subset of membrane proteins destined to function periplasmically. Interestingly, several of the glycosylated proteins in Ngo act on common pathways seemingly involved in electron transfer and maintenance of the periplasmic redox state. Furthermore, sites of covalent modification were associated with regions of low complexity and structural features analogous to those influencing target selection in eukaryotic mucin-type glycosylation. We have also gone on to identify broad spectrum protein glycosylation in strains of *N. meningitidis* and *N. lactamica.*
Conclusions
This is the first demonstration of a general O-linked glycosylation system in prokaryotes. Additionally, it is the first instance in which prokaryotic proteins bearing O-glycans are destined to function within the cell.

It follows that systems level Ngo O-linked protein glycosylation fulfills both intracellular and extracellular roles. These findings drive further investigations of neisseria glycobiology as well as highlight previously unappreciated similarities in O- and N-linked systems in prokaryotes and in signals dictating O-linked occupancy sites in prokaryotic and mammalian proteins.

References

1. Szymanski, C. M. & B. W. Wren, (2005) Nat Rev Microbiol 3: 225-237.
2. VanderVen, et al, (2005) Science 309: 941-943.
3. Young et al, (2002) J Biol Chem 277: 42530-42539.
4. Aas et al, (2007) Mol Microbiol 65: 607-624.

O04

Identification of novel DNA binding proteins in *Neisseria meningitidis*

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Objectives

Transformation is the predominant form of horizontal gene transfer in *Neisseria meningitidis*, influencing antigenic variation and antibiotic resistance development. The transformation process is dependent on the presence of the neisserial DNA uptake sequence (DUS), type IV pilus expression and RecA-dependent homologous recombination. The uptake of DNA into the meningococcal cell can be dissected into several steps including crossing of the outer and inner membranes and genome incorporation. Although the neisserial transformation pathway has been described to some extent, little is known about how transforming DNA is taken up. Interestingly, a number of pilus biogenesis components required for transformation have been identified and it is still a conundrum whether the effect of pilus components on transformation is of a direct or only an indirect nature. We hypothesize that meningococcal transformation is coupled to pilus retraction and that exogenous DNA is taken up through non-specific attachment to retracting pili, while other DNA binding components such as the outer membrane protein PilQ promote further entry of DNA into the meningococcal cell. In order to elucidate the next steps of the transformation pathway, we searched for DNA binding proteins within the inner membrane of *N. meningitidis*.

Methods

Meningococcal cells from a representative panel of strains were fractionated and inner membrane proteins were isolated using detergent solubilization and differential centrifugation. Solid phase overlay assays with DNA substrates and subsequent mass spectrometry MALDI-TOF analysis were performed in order to identify DNA binding proteins. The role of candidate proteins in transformation and pilus biogenesis was assessed by competence screening and purification of extracellular pili from the corresponding knock-out mutants, respectively, while the DNA binding activity of components identified was verified by electromobility-shift analysis.

Results

Both annotated as well as hypothetical DNA binding proteins in the meningococcal inner membrane were identified. The DNA binding activity in either of these components was not dependent on the presence of DUS.

Conclusions

A number of DNA binding components co-purify with the *N. meningitidis* inner membrane. The identification and characterization of these DNA binding components and their potential role in transformation will be presented.

O05

The structure of a DsbA-substrate complex: Implications for substrate recognition by oxidoreductases

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Objectives
Periplasmic oxidoreductases (DsbA) introduce covalent disulphide bonds between thiol groups of two cysteine residues, thereby contributing to the stability and function of many virulence proteins. *E. coli* has a single oxidoreductase which donates disulphide bonds to approx. 300 substrates. This has led to the idea that DsbA catalyzed donation of consecutive disulphide bonds, although accurate, is rapid and independent of the structural properties of the substrate. In contrast, *Neisseria meningitidis* contains multiple oxidoreductases, *Nm*DsbA1, *Nm*DsbA2 and *Nm*DsbA3, each of which has a preferred pattern of substrate recognition. To examine the mechanism underpinning the phenomenon of oxidoreductase substrate recognition, we have (a) produced the first crystal structure of a DsbA-substrate peptide complex, (b) created chimeric meningococcal oxidoreductases with different patterns of substrate recognition profiles, and (c) solved the structure of *Nm*DsbA3.
Methods
The *Shigella* autotransporter, SigA, contains a disulphide bond whose formation is catalysed by DsbA. A nine residue peptide encompassing the sequence around one of the cysteines in SigA was synthesised with an acetylated N-terminus, a C-terminal amide and with a homoserine residue in place of the cysteine (Ac-PIPFL-Hse-QKD-NH_2). The hydroxyl of the homoserine was substituted with bromine to generate the homobromoalanine analog, which was reacted with C^{30} of *Ec*DsbA to form a stable covalent thioether complex. Diffraction quality crystals of the complex were formed by soaking the homobromoalanine-peptide into pre-formed crystals of reduced DsbA. The structure of the complex was solved by molecular replacement to a resolution of 1.9Å (R-work = 21.7%, R-free = 25.2%). Descriptions of the construction of chimeric meningococcal oxidoreductases and the solution to the *Nm*DsbA3 structure are described on accompanying posters (Piek et al and Scoullar et al.).
Results
The peptide bound at the interface of the two domains of DsbA, the thiroredoxin and α-helical domains. The interacting surface area between the DsbA and the peptide is relatively low (437 $Å^2$) and the majority of the contacts with DsbA are mediated via backbone atoms of the peptide. The binding interface of DsbA comprises residues adjacent to the active site (F^{29}-H^{32}, Q^{35}) as well the regions linking the α-domain with the thioredoxin domain which are the type IV β turn between β3 and α2 (F^{63} and M^{64}) and the loop connecting α6 with strand β4 (L^{147}-V^{150}). Chimeric oxidoreductases consisting of *E. coli* thioredoxin domains with α-domains from *Nm*DsbA1 and *Nm*DsbA2 which contained different residues in the loop containing L^{147}-V^{150} demonstrated changes in substrate specific recognition. In addition, a comparison of the solved structure of *Nm*DsbA3 to *Ec*DsbA revealed structural changes to this region which could be used to rationalize the narrow substrate recognition profile of *Nm*DsbA3.
Conclusions
This structure suggests a novel site of substrate binding to DsbA, which is distinct from previously described enzyme-substrate complexes in other TRX-family oxidoreductases. This work provides important insights into the structural basis for the interaction of DsbA with substrate proteins and provides a rationale for potential substrate discrimination based upon interdomain flexibility.

O06

Electron transfer to oxygen in *Neisseria gonorrhoeae*

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Although *Neisseria gonorrhoeae* can reduce oxygen rapidly with glucose, succinate and lactate as physiological electron donors and is also a prolific source of c-type cytochromes, little is known about how its electron transfer pathways from physiological substrates to oxygen are organised. Gonococci are able to grow aerobically using a cbb3 cytochrome oxidase to reduce oxygen to water. Eight genes for c-type cytochromes are encoded by the gonococcal chromosome. The aim of this project was to determine whether three of these cytochromes, c2, c4 and c5, are involved in electron transfer from the cytochrome bc1 complex to cytochrome oxidase.
First we will demonstrate that a previously unreported 16.5 kDa cytochrome c is expressed, but the protein product, cytochrome c2, stains poorly following SDS PAGE. Recombinant cytochrome c2 synthesised in E. coli was isolated from the periplasmic fraction, and absorption spectra of the oxidized and reduced forms are reported. The absorption maxima for the reduced cytochrome are at 419, 523 and 552 nm. Single mutants defective in each of these proteins were constructed, and the effects of the mutation on rates of oxygen reduction were determined by comparing respiration rates of the mutants with those of the parent strain. Multiple attempts were made to construct mutants defective in genes for the two c-type cytochromes that are part of the cytochrome c oxidase complex, and for the c1 component of the cytochrome bc1 complex. Although control experiments gave multiple recombinants, no mutants defective in these genes could be isolated. This confirmed that the bc1 complex and cytochrome oxidase are essential for gonococcal survival. In contrast, mutants defective in cytochromes c2, c4 or c5 were readily constructed. Mutations in genes for either cytochrome c4 or c5 resulted in loss of about 50% of the respiratory capacity of the parent. In contrast, a cytochrome c2 mutant reduced oxygen at the same rate as the parent, suggesting that cytochrome c2 is not involved in the gonococcal respiratory chain. Other phenotypes of the cytochrome c4 and c5 mutants will be shown to be secondary consequences of the decreased ability to reduce oxygen. Data on the phenotypes of double mutants defective in genes for two of these three cytochromes will be presented. A scheme for gonococcal respiration based upon these results is proposed in which cytochrome c4 and c5, but not cytochrome c2, provide alternative pathways for electron transfer between the cytochrome bc1 complex and the terminal oxidase.

O07

A generic mechanism in *Neisseria meningitidis* for increased resistance against complement-mediated killing

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Objectives
The complement system is critical for immunity against the important human pathogen, *Neisseria meningitidis* (Nm). The serogroup C Nm, polysaccharide:protein conjugate vaccine elicits bactericidal antibodies that cause complement-mediated lysis, and has been successfully introduced in the UK since 1999. We identified strains of serogroup C Nm isolated in Spain from invasive cases that avoid killing by

bactericidal antibodies in vaccinees' sera. The aim of this study was to characterise the basis for the strains' resistance against complement and to screen for similar strains among meningococcal isolates from UK.

Methods

i) Molecular genetic and phenotypic analyses of the resistant and sensitive strains.
ii) Genetic linkage to define loci responsible for resistance.
iii) Comparison of a collection of over 1,000 disease and carriage isolates of Nm.

Results

Resistance was not caused by changes either in lipopolysaccharide sialylation or acetylation of the α2-9 linked polysialic acid capsule. Instead, resistance of the isolates resulted from the presence of an insertion sequence, IS1301, in the intergenic region (IGR) between the sia and ctr operons, necessary for capsule biosynthesis and export, respectively. The insertion sequence leads to an increase in the transcript levels of surrounding genes, which in turn leads to an increase in the amount of capsule. The increased amount of capsule was associated with down-regulation of the alternative pathway of complement activation, providing a generic mechanism to protect the bacterium against bactericidal antibodies; the strains were also resistant against bactericidal antibodies directed at the outer membrane protein, PorA, or raised against whole cells.

The insertion of IS1301 was strongly associated with disease isolates compared with strains from asymptomatic carriers of the prevalent serogroup B Nm strains circulating in the UK.

Conclusion

We have identified a genetic change in Nm which provides a generic mechanism for resistance against complement-mediated killing. Insertion of IS1301 in the IGR is the first polymorphism in meningococcus that enhances avoidance of this critical aspect of host immunity, and is epidemiologically linked with the development of meningococcal disease.

O08

Characterization of a zinc-regulated TonB-dependent receptor of *Neisseria meningitidis*

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Objectives

The capsular polysaccharide of *Neisseria meningitidis* serogroup B is not suitable as a vaccine. Therefore, we are analyzing various outer membrane proteins for their vaccine potential. One group of outer membrane proteins comprises the TonB-dependent receptors. These receptors consist of a 22-stranded β-barrel and a plug domain that closes the pore. The energy needed for transport of their substrates over the outer membrane is provided by the TonB complex in the inner membrane. In pathogenic Neisseriae, all the TonB-dependent receptors studied so far are involved in the uptake of iron, and their synthesis is de-repressed when iron is limited and repressed when the iron availability is sufficient. Pathogenic Neisseriae have five TonB-dependent receptors that have not been analyzed to date. We report here the characterization of one of them, NMB0964.

Methods

The methods used are SDS-PAGE, followed by Coomassie blue staining or Western blot analysis. Mice were immunized with outer membrane vesicles from a strain over-expressing NMB0964 from a plasmid, and the sera obtained were evaluated in serum bactericidal assays.

Results

Dove et al.[1] showed that transcription of the NMB0964 gene was de-repressed when strain Z2491 was incubated with naïve human serum but not when the serum was heated, hinting to a role of the complement system in the regulation of this receptor. However, we demonstrate that the synthesis of this

receptor is induced under zinc limitation. We further show that this protein is highly conserved among meningococcal strains and that it is expressed in a zinc-dependent manner in all 32 different strains that we tested. In addition, this receptor is capable of inducing bactericidal antibodies. Until recently, all TonB-dependent receptors characterized were involved in the transport of iron and vitamin B12, but a receptor for nickel was recently identified in Helicobacter pylori [2]. This is the first report of a receptor that is regulated by zinc and that will most likely recognize free or liganded zinc. Our results also hint to the presence of additional zinc receptors signifying the importance of zinc for this bacterium.

Conclusion

In the human body, free zinc is bound by albumin and metallothioneins amongst others, thus creating a zinc-limiting environment. NMB0964 will therefore be expressed in the host. This, together with its high conservation and its ability of inducing bactericidal antibodies, makes this protein a good vaccine candidate.

References

1. Dove et al. 2003. Microbiology 149: 1859-1869
2. Schauer et al. 2007. Mol Microbiol 63:1054-1068

O09

Characterization of iron transport systems expressed by *N. gonorrhoeae*

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Neisseria gonorrhoeae can utilize a variety of iron sources, including human transferrin, human lactoferrin and hemoglobin, for essential metabolic functions. The mechanisms by which these iron sources are employed are well characterized and include a requirement for TonB-dependent transporters expressed on the bacterial cell surface. The presence of predicted TonB-dependent transporters in the gonococcal genome suggested that other uncharacterized iron sources might similarly be utilized. Since *N. gonorrhoeae* inhabits complicated ecological niches including the female genital tract, we hypothesize that iron sources provided or made accessible by members of the co-resident microbial flora could provide nutrients that support gonococcal growth.

Objectives

The objectives of this study were to determine the mechanism by which *N. gonorrhoeae* acquires the necessary nutrient iron, in complicated ecological niches such as the female genital tract.

Methods

We have employed two complementary approaches to characterize iron acquisition by *N. gonorrhoeae*. *In vivo* approaches included testing mutants that lack particular iron acquisition systems for ability to colonize the lower murine female genital tract. *In vitro* approaches included evaluation of the ability of various mutants to grow with non-proteinaceous iron sources under defined growth conditions.

Results

We previously demonstrated that mutants lacking the transferrin receptor components remained capable of colonizing the lower female genital tract in a murine infection model. Likewise, strains that did not express the lactoferrin and hemoglobin receptor components were fully competent to colonize mice. In the current study, we determined that mutants without the capacity to express the Ton system, which energizes TonB-dependent transport across the outer membrane, were similar to wild-type in their capacity to colonize the murine lower genital tract. Individual mutants with inactivated predicted transporters remained colonization-competent. *In vitro*, mutants with an inactivated Ton system were prevented from utilizing transferrin, lactoferrin and hemoglobin as expected, but could acquire iron from non-proteinaceous ligands including hemin, ferric citrate, and selected hydroxamate and phenolate siderophores. While use of ferric citrate and ferric siderophores was Ton-independent, acquisition of these iron sources universally required expression of FbpA.

Conclusions
The female genital tract is colonized by a variety of commensal organisms, which could provide nutrients to *N. gonorrhoeae*, including iron. In addition, acid production and the concomitant decrease in pH could enable iron solubilization in this complicated ecological microenvironment. This study demonstrated that gonococcal colonization of the lower genital tract of a female mammalian host did not individually require expression of any known or predicted TonB-dependent transporters. Nor did colonization require expression of the Ton system, which is expected to energize all energy dependent transporters in the outer membrane. This observation suggests that novel and to date uncharacterized iron sources are employed in this environment, via a mechanism that is Ton-independent. In parallel, this study also demonstrated that a variety of non-proteinaceous iron sources could be acquired by *N. gonorrhoeae* in a Ton-independent fashion; however their import required expression of FbpA. A similar Ton-independent, Fbp-dependent iron internalization process has been described in other Gram negative pathogens. A hypothetical model to explain these findings will be discussed.

O10

Colonization of host cells by *Neisseria meningitidis* requires a cross talk between two two-component systems

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The ability to adhere to and grow on the surface of human host cells is an absolute requirement for *N. meningitidis* to colonize new hosts and to disseminate inside its host. The aim of this work is to get insights into the mechanism by which *N. meningitidis* colonizes the host cells. Identification of the function of the meningococcal genes differentially expressed upon initial interaction with host cells and of the regulation pathways controlling the expression of these genes are prerequisites to the understanding of how efficient colonization can be achieved and thereby explain in part the host-specificity of the meningococcus.
The genes of the REP2 regulon are transiently upregulated when the bacteria interact with host cells and encode functions that are potentially crucial for the nasopharyngeal colonization (Morelle et al., 2003). The co-ordinated expression of the genes of the regulon is assumed to rely on the repeated REP2 sequence that constitutes their promoter regions.
A library of mutants of *N. meningitidis* was screened in the search of transcriptional factors involved in the regulation of the expression of the REP2 regulon. The use of both the lacZ reporter gene and quantitative RT-PCR in adhesion assay identified genes NMA0797 and NMA1803, which encode the sensor kinases of 2 two-component systems (TCSs). TCSs are signal transduction systems that have evolved for bacteria to adapt to changing environments through sensing and responding to environmental stimuli. NMA0797 and NMA1803 are involved in the induction and repression of the expression of the genes of the REP2 regulon upon contact with host cells, respectively. Electrophoretic mobility shift assays (EMSAs) revealed that regulatory protein NMA0798 interacts with the REP2 sequence. DNase I protection assays were performed to identify the precise location of NMA0798 binding site(s) in the REP2 sequence. The regulatory NMA1805 protein only binds to its REP2-independent promoter. We also demonstrated that the expression both TCSs is cell contact controlled. A functional NMA1803 / NMA1805 two-component system is required for the adhesion to host cells to proceed efficiently. Thus, we provide evidence that 2 TCSs sequentially control the expression of the REP2 regulon in response to host cell contact. This suggests that 2 out of the 4 TCSs present in *N. meningitidis* could be dedicated to the adaptation to growth on host cells.

O11

Structural studies of CrgA Implications for transcriptional regulation in pathogenic Neisseria

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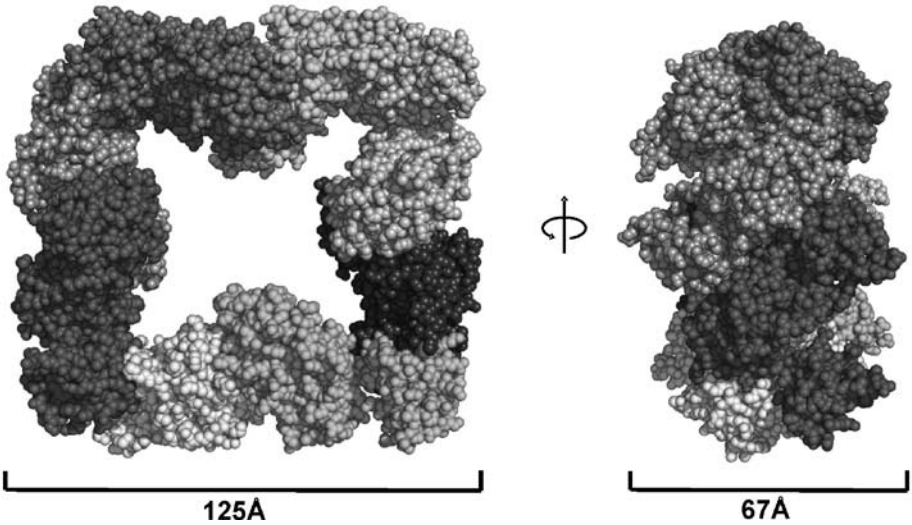
Pathogenesis of Neisseria requires virulence factors that allow survival in the bloodstream and those that mediate cellular interactions. Many of these properties are under direct or indirect transcriptional control. *Neisseria meningitidis* strain MC58 contains forty nine transcription factors of which six are from the LysR family of transcriptional regulators (LTTRs). These include CrgA (contact-regulated gene A) which is induced upon contact with human epithelial cells and appears to have a role in the regulation of intimate adhesion (Deghmane et al. 2000 Embo J 19, 1068). Although LTTRs represent the largest family of bacterial transcription factors little is known about their structure: only one full-length LTTR structure (CbnR of Ralstonia eutropha) has been reported (Muraoka et al. 2003 J Mol Biol 328, 555). Therefore to investigate the structure-function relationships of CrgA we have solved the crystal structure of the full-length and regulatory domain forms of the protein. Analysis of the full-length structure suggests a novel oligomeric assembly quite different to that observed in the only other full-length LTTR (CbnR) structure. Biophysical characterization by analytical ultracentrifugation, non-denaturing mass spectrometry and size exclusion chromatography indicates that this is the biologically relevant form of the protein. These results have important consequences for how CrgA interacts with DNA and controls gene expression.

Figure 1: Structural assembly of full-length CrgA



O12

Identification and functional characterization of sRNAs involved in the RpoE and Fur regulon of *Neisseria meningitidis*

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Objective
Small RNAs (sRNAs), often encoded in intergenic regions (IGRs), alter translation of target genes by interacting with the 5' untranslated region (5'-UTR) of target mRNAs. Expression of genes in *Neisseria meningitidis*, is often regulated by the environmental stress stimuli encountered in the host. Transcription of these genes involves participation of specific factors like RpoE and the ferric uptake transcriptional regulator Fur. We investigated whether the genome of *N. meningitidis* encodes sRNAs that are functionally involved in the RpoE and Fur regulon.
Methods
IGRs of the genome of strain MC58 were queried for sequences that include a promoter sequence characterized by an RpoE signature or a binding site for Fur (Fur box), followed by a 50-500 nt spacer and a potential terminator structure. Expression of sRNA was assessed by Northerns and/or RT-PCR and target mRNAs were identified in silico by putative duplex formation between these sRNA and 5'-UTRs of mRNAs in the genome. Functional interaction of sRNA with target mRNA was assessed *in vivo* using a GFP-reporter system in *E. coli* and further investigated in wild type and hfq knock out meningococcal cells.
Results
Eight sRNAs identified in silico, were shown to be expressed in *N. meningitidis*. The promoter of 6 of these sRNAs contain an RpoE signature, that of one contained a Fur box as well a partially overlapping RpoE signature and that of another a Fur box. The latter sRNA was identical to a previously reported meningococcal sRNA (Melin JBac 189(10):3686-94;2007) designated NrrF and shown to be regulated by iron. Putative targets of sRNAs were identified in silico, among which glucose and iron-responsive genes. The interaction of NrrF and its in silico identified targets, the iron-responsive sdhC and the newly identified target petA was assessed *in vivo*. Expression of NrrF in trans resulted in a major reduction of fluorescence of *E. coli* cells containing the 5'-UTRs of meningococcal sdhC or petA fused in frame to gfp, indicative for translational repression. In this system, the NrrF mediated translational repression of sdhC is dependent on functional Hfq and RNAse E, while that of petA only depends on RNAse E. In meningococci, the transcript levels of sdhC and petA are inversely related to the amount of NrrF. Levels of nrrF and petA are lower in hfq knock-out cells than in wt meningococcal cells, consistent with NrrF and petA mRNA instability in the absence of Hfq. Transcript levels of sdhC were similar in wt and hfq knockout cells, but depending on the iron status of the cell, i.e. the level was lower under iron depletion conditions. However, in the absence of Hfq, transcript levels of petA were marginally lower under iron depletion conditions.
Conclusions
Meningococci express sRNAs characterized by a RpoE signature and a Fur box in their promoter region. Down regulation of translation of sdhC and petA upon expression of NrrF is the first *in vivo* demonstration of functionality of a sRNA of *N. meningitidis*.

O13

Molecular and phenotypic characterization of the Hfq RNA chaperone of *Neisseria meningitidis*

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Objectives
The well conserved protein Hfq has emerged as the key modulator of riboregulation in bacteria. It is thought to function as an RNA chaperone and facilitate base-pairing between sRNA and the mRNA targets, and many sRNAs are dependent on the Hfq protein for their regulatory functions. The goal of this study is to investigate the role of Hfq in modulating ribo-regulated networks and gene expression in *N. meningitidis*.
Methods
We constructed a null mutant of Hfq in the MC58 strain of *N. meningitidis* and a complemented derivative and characterised the phenotypes resulting from the knockout of the hfq gene. In order to investigate the mechanism of action of this protein we purified recombinant meningococcal Hfq protein and we performed *in vitro* binding assays using a recently identified sRNA and its target mRNA.
Results
The knockout mutant shows pleiotropic phenotypes. It has a general growth phenotype in *in vitro* culture media, and it is sensitive to a wide range of stresses including those that it may encounter in the host. Furthermore, the expression profile of a vast number of proteins is clearly altered in the mutant. We performed a proteomic analysis and have identified some of these proteins. All of the phenotypes to date tested are also restored by complementation of Hfq expression in the complemented mutant strain. Results from *in vitro* assays indicate that Hfq binds the sRNA *in vitro* and considerably enhances the efficiency of its interaction with the identified mRNA target. Furthermore genetic studies indicate that Hfq-mediates down regulation of the mRNA target by the sRNA *in vivo*, likely through rapid degradation of the message.
Conclusion
These data indicate that Hfq influences the meningococcal metabolic processes and stress responses. Moreover our results propose a major role for Hfq in regulation of gene expression in meningococcus and suggest that there is a large Hfq-mediated sRNA network as yet unexplored in this important human pathogen.

O14

The impact of the neisserial DNA uptake sequence on genome evolution and stability

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Objectives
Generating genetic variation in the pathogen *Neisseria meninigitidis* is vital for avoiding the specific activities of the immune response of its exclusive human host. Horizontal transfer of DNA by means of transformation has generally been regarded as a major contributor to such variation. However, analysis of the distribution of the abundant DNA uptake sequence (DUS) suggests that the demand for genetic variation is not the main selective pressure that has driven the evolution of the complex process of transformation. Since DUS are required for efficient transformation, their genome-wide distribution provides an unparalleled insight into their evolutionary role and that of transformation itself.

Methods
We aligned six complete genomes from three different neisserial species, defined the core genome and assessed DUS distributions. Every single DUS was accounted for and compared to the corresponding sequence in the alignment. The alignment enabled the detection of recombination events which consequently were correlated to the spacing of DUS elements. A range of genes including DNA repair and phase variable genes and those encoding surface exposed proteins were analysed for their DUS content and also correlated to their expression status by microarray analyses.
Results
We find that DUS clustered in the core genome and surprisingly were completely absent from recently acquired and recently lost sequence. Furthermore, DUS was underrepresented in regions of the core genome that were under selective pressure driving diversification, demonstrating that DUS are not primarily involved in generating variation at these loci. We observed a striking correlation between the distance between DUS elements in the genomes of Neisseria and the length of conversion fragments, suggesting that the DUS distribution is tuned over evolutionary time and can best be explained by a history of recombination. DUS were found to be very stringently conserved, in fact more so than the average conserved sequence, and 71% of the DUS in the multiple alignments were exactly conserved in all genomes. The identification of unique DUS in the alignment, being present in a single genome only, further documented that DUS arise by recombination. These findings are also correlated with analysis of the recombinogenic nature of DUS function.
Conclusions
As many other pathogens, *N. meningitidis* strains have hyperdynamic and adaptable genomes. The results presented here suggest that transformation directly counteracts the deleterious effects of genome instability. Since transformation allows the re-assortment of alleles in populations this process can both reduce clonal interference, the competition between adaptive mutations in different clones, and efficiently purge deleterious mutations. Thus, rather than promoting variation, our data suggests that transformation is predominantly regenerative and in the course of evolution represents a conservative mechanism.
References
1. Ambur OH et al. J Bacteriol 189:2077-2085, 2007
2. Treangen T, Ambur OH et al. Genome Biology 9:3, 2008

O15

Mechanisms *Neisseria meningitidis* utilize to evade the human antimicrobial peptide, LL-37

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Pathogenic bacteria have evolved mechanisms to evade the human immune system and have developed widespread resistance to traditional antibiotics. We study the human pathogen *Neisseria meningitidis* and present novel mechanisms by which these bacteria protect themselves from the human antimicrobial peptide, LL-37. The bacterial endotoxin, lipooligosaccharide, helps protect Neisseria from LL-37 killing, as mutant bacteria lacking lipooligosaccharide bound more LL-37 and were more effectively killed than wildtype bacteria. We also present novel data showing that Neisseria attached to host epithelial surfaces are 10-fold more resistant to LL-37 killing compared to bacteria in solution. Further studies using epithelial cells indicate that human cells are highly resistant to LL-37 exposure compared to bacteria, with 32 μM LL-37 killing most bacteria but not affecting human cells. Our microarray studies indicate that sublethal doses of LL-37 regulate the transcriptome of *N. meningitidis*. Real-time PCR confirmed that resistance, capsule production and outer membrane protein genes are regulated in response to sublethal doses of LL-37. A capsule deficient mutant was constructed and subsequently shown to have reduced resistance to LL-37.

O16

Molecular characterization of the first ciprofloxacin-resistant strains of *Neisseria meningitidis* in the United States

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Objectives
Characterize US isolates of ciprofloxacin-resistant (CIP-R) strains of *N. meningitidis* obtained from three case-patients in Minnesota (MN) and North Dakota (ND) from 1/2007-1/2008; identify the mechanism of resistance in these isolates; and explore the scope of CIP-R *N. meningitidis* in the US.
Methods
N. meningitidis isolated from the MN/ND case-patients were identified by standard reference methods and molecularly typed by PFGE and MLST. We retrospectively screened collections of isolates for antimicrobial susceptibility by Etest and confirmed resistant isolates by broth microdilution. These collections included 40 *N. meningitidis* and 9 *N. lactamica* isolates obtained during a pharyngeal carriage investigation associated with case 3 in MN, a convenience sample of 9 *N. lactamica* isolates from a 2007 carriage study in Georgia, and all *N. meningitidis* isolates sent to CDC from 1/2007-2/2008 through ABCs, an active, population-based surveillance system in 10 US sites. The mechanism of resistance was identified by sequencing gyrA.
Results
All three MN/ND case-patient *N. meningitidis* isolates were serogroup B, had identical PFGE patterns, were sequence-type and clonal complex ST-162, and were CIP-R (MIC 0.25 μg/ml). Epidemiologic investigation revealed no close contact between the patients. Retrospective screening identified an *N. meningitidis* serogroup Y isolate from California (CA-R) and an *N. lactamica* isolate from Georgia which were also CIP-R. A threonine to isoleucine change at amino acid 91 (T91I) of GyrA was detected in the quinolone resistant determining region (QRDR) of all CIP-R isolates. This change has been associated with other CIP-R isolates of *N. meningitidis*.
Comparing the sequence of gyrA from the ND/MN CIP-R cases to sequences from ciprofloxacin-sensitive *N. meningitidis* isolates revealed 94% similarity in the first 1265 nucleotides and ›99.9% similarity in the latter 1486 nucleotides. Conversely, comparing the sequence of the entire gyrA from 4 of the *N. lactamica* isolates from the MN carriage study to sequences from the ND/MN CIP-R cases revealed ›99.8% similarity in the first 1310 nucleotides but only 90% similarity in the latter 1410 nucleotides. Phylogenetic analysis of the gyrA QRDRs from 59 *N. meningitidis*, 50 *N. gonorrhoeae*, and 19 *N. lactamica* isolates demonstrated that the sequences cluster by species and the ND/MN Cipro-R isolates grouped within the *N. lactamica* cluster. The data suggests that horizontal gene transfer (HGT) replaced the first half of *N. meningitidis* gyrA in the strain that caused the ND-MN CIP-R cases with gyrA DNA from an *N. lactamica* donor. In contrast, the CA-R isolate had the T91I mutation without evidence of HGT.
Conclusions
The emergence of ciprofloxacin-resistant *N. meningitidis* in the US through multiple introductions is concerning since CIP-R strains of *N. gonorrhoeae* spread quickly. These data suggests the mutations in the isolates described were acquired through two different mechanisms: point mutation (CA-R) and HGT between *N. meningitidis* and a CIP-R strain of *N. lactamica*, although HGT with a CIP-S *N. lactamica* followed by a point mutation cannot be ruled out. Further studies are needed to evaluate the antimicrobial resistance among commensal Neisseria spp and the potential for genetic transfer between them.

O17

Structural and biochemical analysis of penA mutations conferring penicillin resistance in *Neisseria gonorrhoeae*

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Objective
The high-level penicillin-resistant isolate FA6140 contains a penA gene encoding penicillin-binding protein 2 (PBP 2) with an insertion of an aspartic acid (D345a) and 4 mutations toward the C terminal of the protein. The goal of this study was to identify the molecular mechanisms of penicillin resistance conferred by these mutations in PBP 2.
Materials & Methods
In order to determine the relative contributions of the D345a insertion and the C terminal mutations in the penA gene from FA6140 for conferring resistance, a series of mutants of PBP 2 were prepared. In each case, the second order rates of acylation by PenG were determined, as were the MICs for penicillin after transfer of the altered penA genes into the penicillin-susceptible strain FA19. The structures of wild-type PBP 2 and of PBP 2 containing the 4 C terminal mutations (PBP 2-6140CT) were determined by X-ray crystallography.
Results
The rate of acylation for PBP 2-6140 is 10-fold lower than wild-type PBP 2. The Asp345a insertion alone lowers the rate by 5-fold but, together, the 4 C terminal mutations also lower the rate by 5-fold, thus confirming the contribution of these mutations to penicillin resistance. Of the four C-terminal mutations, P551S causes the largest decrease in the acylation rate, with the other mutations providing minor contributions. Surprisingly, the crystal structure of PBP 2-6140CT shows no significant differences when compared to the structure of wild-type PBP 2. However, thermal denaturation curves determined using circular dichroism show a lowering of thermal stability in PBP 2-6140CT, which suggests a destabilizing mechanism of antibiotic resistance. Replacement of Asp345a by Glu or Gln lowers the rate of acylation by PenG 12-fold and 4-fold, respectively, but neither insertion confers penicillin resistance to FA19. Insertion of other amino acids led to an inactive enzyme. Similarly, insertion of Gln or Lys after position 346 (346a) lowered acylation 2-fold and 5-fold respectively, but again, neither insertion conferred resistance when transformed into FA19.
Conclusion
These data suggest that, in contrast to similar investigations of PBPs in other pathogens, the molecular mechanism underlying penicillin resistance is actually very subtle in nature and involves dynamic states of the protein that are not detectable by X-ray crystallography. The apparent destabilizing effect of the mutations in PBP 2 may hinder conformational changes required for induced fit binding of penicillin or formation of the tetrahedral intermediate during acylation. The Asp345a insertion, however, exerts a very specific effect on PBP 2 and one mechanism may be disruption of a hydrogen-bonding network between the loop that contains this residue and the SxN active site motif.

O18

Development of an expanded WHO control panel of *Neisseria gonorrhoeae* for use in surveillance of gonococcal antimicrobial resistance

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Objective
Emergence and spread of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* (NG) remains a major global problem. The validity of systematic surveillance of this resistance has been enhanced by use of World Health Organization (WHO) reference cultures of NG with well-defined resistance phenotypes. However the resistance patterns represented were limited to those related to the penicillins, tetracyclines and spectinomycin. Recently, resistance to the quinolones, and a newer macrolide (azithromycin), as well as decreasing *in vitro* susceptibility to third-generation cephalosporins, including cefixime and ceftriaxone, has emerged in NG. This resistance, especially that to fluoroquinolones, has disseminated widely. The ability to monitor the frequency and spread of AMR in GC, and consequently adjust standardised treatment regimens, has been compromised by use of non-standardised methods and application of inappropriate interpretative criteria. Even when these data were reproducible in an individual setting, resistance rates were both over- and under-reported and comparability of these rates of resistance between laboratories was compromised. The International Collaboration on Gonococci (ICG) undertook to compare some more widely-used methodologies, evaluate the appropriateness of interpretive criteria, and develop, evaluate and distribute further WHO reference GC. These panel strains were to reflect contemporary AMR resistance patterns for GC and also allow direct comparisons of resistance rates.
Methods
Reference laboratories in North America (3), the United Kingdom (1) and Australia (1) supplied candidate GC. These GC were de-identified and re-distributed as batches of 'unknown' GC for testing in the participating laboratories who recorded MIC values obtained, resistance categories and complete details of test methods employed, for analysis at CDC.
Results
Although differences in MIC values obtained with the different test methods were observed, there was close agreement when method-specific criteria were used to interpret these results - for categories of 'sensitive' or 'resistant' - for the candidate GC. A selection of candidate control GC were included in the panel on the basis of required resistance profiles and suitability of GC e.g., viability in transport systems, and reproducibility of results within different laboratories and comparability of data between different test sites.
Discussion
The expanded WHO control panel is primarily for use in internal quality control and those external quality assurance activities essential to assure quality of surveillance systems for AMR in GC. These processes may also be used to validly compare data determined with different methods, and remove the need for mandating a uniform methodology that is, in any event, unlikely to be achieved. The WHO panel strains will be readily available (see www.icgngo.org) and users may select from this panel for GC with resistance patterns suitable for their needs. Users who find that their susceptibility data do not agree with those in any one of the reference procedures must undertake studies to determine appropriate interpretive criteria to ensure comparability of surveillance data. Procedures are in now place to allow inclusion of additional GC should additional resistances emerge, and most panel strains have been genotypically analysed for resistance determinants.

O19

Naturally occurring lipid A mutants in *Neisseria meningitidis* associated with reduced coagulopathy in patients with meningococcal disease

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Objectives
Recognition of lipopolysaccharide (LPS) by the TLR4/MD-2 receptor triggers the innate immune system to produce proinflammatory cytokines. *Neisseria meningitidis* has been reported to produce LPS with a hexa-acyl lipid A region, which is a highly active TLR4 agonist. Indeed, meningococcal sepsis is generally seen as the prototypical endotoxin-mediated disease. We have previously shown that insertional inactivation of the lpxL1 or lpxL2 genes required for addition of secondary acyl chains leads to reduced endotoxic activity of meningococcal LPS and whole bacteria. The possibility that such mutations might also occur naturally was suggested to us by a report showing that the group Y strain HF13 was defective in signaling through the MyD88-independent TLR4 pathway when bacteria were tested with mouse macrophages.
Methods
171 isolates from patients with meningococcal disease, representing 23 clonal complexes (cc), were obtained from the Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM). In addition the isolates of 40 patients of which we had an isolate of the blood or CSF and an isolate from the throat were included. Finally, isolates of 254 adults with meningococcal meningitis (Dutch Meningitis Cohort Study) of which clinical data were available were also included in the study. Induction by whole bacteria of cytokines IP-10 in mouse J774 cells and IL-6 in human MM6 cells was determined by ELISA.
Results
We analyzed the lipid A structure of strain HF13 by mass spectrometry and found it to be penta-acylated, suggesting a mutation in the lpxL1 or lpxL2 genes. In agreement with this, we found a frameshift mutation which had inactivated its lpxL1 gene. We next screened panels of clinical isolates for cytokine induction in mouse or human macrophage-like cells. Strains which gave clearly reduced cytokine induction were readily identified, and sequencing of their lpxL1 genes revealed mutations in all of them. Overall, these natural lpxL1 mutants were surprisingly common, occurring in ca. 13% of meningococcal disease isolates of all major serogroups. Several different mutations were found, including frameshifts in homopolymeric tracts, small deletions, insertion of IS1016 and missense mutations in highly conserved residues. Wildtype vs mutant lpxL1 alleles could also be found in isolates from different anatomical locations within a single patient. Next, we investigated the clinical correlate of these mutations in a prospective nationwide observational cohort study of 254 adults with meningococcal meningitis. Seventeen patients (7%) with meningococcal meningitis were infected with an lpxL1 mutant strain. These patients tended to be younger (median 21 yrs [IQR 19-30] vs 31 [19-51]; P=0.052) and tended to present with higher body temperature (38.9ºC [38.3-39.8] vs 38.4ºC [IQR 37.5-39.1]; P=0.052), but were significantly less likely to present with rash (5/16 [31%] vs 157/236 [67%]; P=0.006) and had higher corresponding thrombocyte counts (225 x109/L[163-279] vs 162 x109/L [123-212]; P=0.009).
Conclusions
Meningococcal lpxL1 mutants occur naturally with an unexpectedly high frequency, suggesting an important role in virulence for the resulting low-activity LPS. Patients infected by lpxL1 mutant meningococci present less frequently with rash and with higher thrombocyte counts, consistent with reduced cytokine induction and less activation of tissue-factor mediated coagulopathy.

O20

Studies of innate immune defences during Neisseria infection

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Neisseria meningitidis must overcome innate immune defences in order to establish disease. Antimicrobial peptides, complement killing, phagocytosis, and recognition by TLRs are important parts of the innate immunity. We have shown that *N. meningitidis* down-regulates the antimicrobial peptide LL-37 upon interaction with human epithelial cells. The lipooligosaccharide and the capsule helped to protect these bacteria from LL-37 killing. Interestingly, bacteria attached to human epithelial cells were more resistant to LL-37 compared to bacteria not associated with cells. In a microarray study we showed that meningococci responded to sublethal doses of LL-37 by altering transcription of surface exposed proteins.
NhhA is a meningococcal outer membrane protein that facilitates attachment to host epithelial cells and binds to the extracellular matrix proteins heparan sulphate and laminin. We have shown that NhhA is essential for bacterial colonization of the nasopharyngeal mucosa in a murine model of meningococcal disease. The protein protected bacteria from phagocytosis, mediated serum resistance, and increased protection against complement-mediated killing by preventing deposition of the membrane attack complex, which enhanced both nasal colonization and development of sepsis *in vivo*.
To further investigate innate responses we analyzed *in vivo* infection of mice deficient in TLR4, TLR2, TLR9 or MyD88. *N. meningitidis* failed to trigger disease in TLR4-/- mice as compared to control mice and TLR2-/- mice. TLR9-/- mice were more sensitive to infection by *N. meningitidis*, suggesting that TLR9 helps to decrease inflammatory responses during later stages of disease.

O21
Differential TLR2 binding of porins from pathogenic and non-pathogenic Neisseriae

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Objective
PorB porin from *N. meningitidis* has been shown to directly bind to TLR2 on the surface of transformed HEK 293 cells expressing TLR2. Furthermore, TLR1 is required for cell activation, making Nme PorB a TLR2/TLR1 ligand. A strong correlation between immune cell stimulation via TLRs and immune adjuvant activity has been proposed for several TLR ligands [i.e. CpG DNA (TLR9 ligand), Nme PorB (TLR2/1 ligand)]. We have recently demonstrated that purified porin PorB from the commensal bacterium *N. lactamica* also acts as a powerful immune adjuvant and hypothesize a potential role for TLR2 (and possibly other TLRs) on immune cell activation by Nlac PorB.
Methods, results and conclusions
Using 293 HEK cells stably transfected with various TLR constructs and using fluorescently labeled Nlac PorB, we demonstrate a specific, dose-dependent binding to 293-TLR2 cells by FACS, similarly to Nme PorB. Binding competition experiments with unlabeled Nlac PorB confirmed a specific binding of the porin to TLR2, measured as decreased cell-associated fluorescence by excess amount of inhibitor. However, when we used unlabeled Nme PorB as inhibitor, this failed to block binding of Nlac PorB to the surface of 293-TLR2 cells. This could be due to differences in co-receptor requirement or to different TLR binding site on the porins. To address the first hypothesis, we determined that Nlac PorB also binds preferentially to the TLR2/TLR1 dimer using cells transfected with both TLR2/TLR1 and TLR2/TLR6 receptors. To address the second hypothesis, we used Nlac PorB as inhibitor for Nme PorB binding and determined that Nlac

PorB could not inhibit binding of Nme PorB to 293-TLR2 cells. Interestingly, when we used Pam3CSK4, a TLR2/TLR1 ligand capable of blocking Nme PorB binding, this also failed to block Nlac PorB binding. We speculate that different binding sites on TLR2 might be engaged by Nlac PorB and Nme PorB.
As the immune adjuvant activity of Neisserial porins depends on interaction and signaling via TLRs, we examined whether binding to naive murine lymphocytes could also be determined. B cells and macrophages from C57Bl/6 WT mice and TLR2 KO mice were examined and Nme PorB was found capable of binding to the surface of cells from both WT and TLR2 KO mice. One explanation could be that, contrary to 293-HEK cells, murine B cells and macrophages normally express a whole range of TLRs, some of which might compensate for the ablation of TLR2. Nme PorB binding was inhibited by excess amounts of both Nme PorB and Nlac PorB, again suggesting that the contribution of other TLRs can not be ruled out, although TLR2 may or may not be expressed on the cell surface.

O22

A cyanobacterial LPS-antagonist inhibits cytokine production in human whole blood infected with *Neisseria meningitidis*

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Objectives
A continuing need exists for new adjunctive therapies for the treatment of meningococcal septicaemia. It is generally accepted that the progression of septicaemia closely correlates with increasing levels of circulating meningococcal lipopolysaccharide (Nm-LPS) and consequent production of cytokines, notably IL-6, TNF-alpha, IL-1beta and IL-8. The association of gram-negative LPS with the Toll-like receptor (TLR)4-MD-2 complex on myeloid cells is believed to transduce the LPS signal to activate nuclear factor kappa B (NF-kB) and trigger cytokine production. Adjunctive therapies that could neutralise the biological effects of Nm-LPS would likely improve clinical outcome but the results from clinical trials of some candidate therapies have been disappointing. Recently, a LPS-related molecule derived from the cyanobacterium Oscillatoria planktothrix FP1 and termed CyP, was reported to act as a selective TLR4-MD-2 receptor antagonist. In the current study, we used an *in vitro* whole blood model of *Neisseria meningitidis* septicaemia to evaluate the potential of CyP as an antagonist able to reduce the intravascular inflammatory response.
Methods
Whole human venous blood was treated with various concentrations of live meningococci of different serogroups, isolated outer membranes (OM) or Nm-LPS, in the presence of varying concentrations of CyP (0.01-20ug/ml). The levels of pro-inflammatory (interleukin (IL)-1alpha, IL-1beta, IL-6 and TNF-alpha) chemoattractant (MCP-1, IL-8 and RANTES), anti-inflammatory (IL-10) and growth factor related (GM-CSF) cytokine proteins were quantified by sandwich immuno-assay of plasma samples processed at 6 and 24h. Human monocyte-derived dendritic cells (mo-DC; $0.5x10^6$ cells/ml) were stimulated with Nm-LPS or *E.coli*-LPS (100ng/ml) with and without CyP (2.5-20 ug/ml) and TNF-alpha and IL-6 cytokines were measured after stimulation for 20h. Human Jurkat cells ($1x10^7$ cells/ml, expressing human MD-2), transfected to express human TLR4, were treated for 8h with Nm-LPS or E.coli-LPS(100ng/ml), with and without CyP (5-20ug/ml).The cells were harvested, lysed and reporter gene activity was measured using the Luciferase Assay System. Jurkat cells transfected with empty vector together with NF-kBLuc reporter vector were used as a mock-control.
Results
CyP (≥1ug/ml) inhibited the secretion of pro-inflammatory cytokines TNF-alpha, IL-1beta and IL-6 by ›90% ($p<0.05$) and chemokines IL-8 and MCP-1 by ˜50% ($p<0.05$), which were induced by treatment of blood with Nm-LPS (10ng/ml), OM (100ng/ml) and after infection with live meningococci (10^4-10^6 cfu) of serogroups A, B, C, W135, X and Z. CyP also efficiently inhibited cytokine production from human mo-DC

treated with Nm-LPS. Moreover, Nm-LPS only stimulated NF-kB activity in TLR4-MD2 expressing Jurkat cells and this activity was competitively inhibited by CyP (›80%; p‹0.05).

Conclusion

CyP, a LPS-like molecule derived from a cyanobacterium, inhibited cytokine production *in vitro* induced by levels of bioactive Nm-LPS that are found in patients with meningococcal septicaemia. The mechanism most likely involved competitive inhibition at the TLR4-MD2 receptor level. Moreover, cytokine inhibition was independent of meningococcal serogroup, demonstrating broad applicability. These data demonstrate that CyP is a potent antagonist of meningococcal LPS and represents a potentially new adjunctive therapy for the treatment of meningococcal septicaemia and other endotoxin-mediated inflammatory responses.

O23

Differential induction of innate immunity to *Neisseria lactamica* and *N. meningitidis* in nasopharyngeal epithelial cells

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Objective

The objective of this research was to determine how nasopharyngeal derived epithelial cells may differentially respond to challenge with pathogenic and commensal Neisseria through the induction or regulation of innate immune pathways.

Methods

An upper respiratory epithelial cell line (Detroit 562) was selected for this study due to the tropism of Neisseria for this region of the mucosal tract. Cells were challenged with commensal *Neisseria lactamica* (Nl, strain Nl4.1) and or pathogenic *Neisseria meningitdis* serogroup B (NmB, strain MC58). Bacterial adhesion and invasion was quantified by viable counting of total cell associated and gentamycin-resistant bacteria and further investigated using a selection of well defined phenotypic variants. Specificity for active invasion was demonstrated using cytocholasin D and the influence of colonisation on host ligands for bacterial adhesins and invasins were examined by confocal microscopy and flow cytometry. Downstream effects of bacterial colonisation on innate mucosal immunity were determined through Nf-κB signalling and secretion of pro-inflammatory cytokines/chemokines. Signalling was examined by Western blotting of cell lysates and cytokine profiles were determined using an inflammatory microarray and confirmed by ELISA.

Results

Our findings demonstrated that Nl was able to adhere and invade nasopharyngeal epithelium but at levels approximately one hundred fold less than NmB. The active nature of the invasion was confirmed by almost complete inhibition by cytocholasin D. The interaction with NmB was accompanied by increases in cell surface expression of pattern recognition receptors (TLR-4 and TLR-2) and the receptor for bacterial Opa (CEACAM). In contrast, culture with Nl induced no surface changes to host receptor expression but induced an upregulation of intra-cellular CEACAM. Constitutive expression of the putative pili receptor, CD46, remained unaltered by challenge with either bacterium.

Nl induced secretion of cytokines/chemokines (IL-8, IL-6, RANTES, MCP-1, GM-CSF and TNF-α) at half the level induced by NmB at the same multiplicity of infection, despite equivalent signalling through NFkB / Ik-B. Our data suggests that Nl may attenuate the NmB induced secretion of IL-8, IL-6, and TNF-α by 20- 50%. Although an absence of NmB pili markedly reduced adhesion and invasion of Detroit cells, subsequent cytokine/ chemokine secretion was unaffected. Furthermore, Nl induced cytokine/chemokine secretion was significantly enhanced by cytochalasin D treatment whilst that of NmB remained unaffected, suggesting Nl actively suppresses cytokine activation following internalisation.

Conclusion
We have demonstrated for the first time that nasopharyngeal epithelial cells differentially recognize the commensal Nl and the occasional pathogen NmB. We have ascertained that Nl weakly induces certain aspects of innate mucosal immunity but may actively suppress others, including cytokines, chemokines and host cell invasion. We hypothesize that the discrimination in the activation of innate immunity in the epithelium is central to understanding acquired immunity to the meningococcus.

O24

The Th17 axis of immunity: a new dimension in comprehending the host response to *Neisseria gonorrhoeae*

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Objectives
The newly defined Th17 cell lineage which links adaptive and innate immune responses plays a significant role in inflammatory and autoimmune disease, and in defense against certain bacterial infections (figure 1). Gonorrhea does not appear to induce an effective state of adaptive immunity against repeated infection, and the mechanisms underlying the immune and inflammatory response to *Neisseria gonorrhoeae* are incompletely understood. Symptomatic infection with *N. gonorrhoeae* is typically associated with an influx of neutrophils into the genital tract, and innate rather than specific immune defense mechanisms may be responsible for the elimination of infection. We postulate that *N. gonorrhoeae* elicits the Th17 axis of immunity, leading to the recruitment of neutrophils and other innate defense mechanisms.
Methods
To test this hypothesis we stimulated mouse spleen mononuclear cells, human monocytic THP-1 cells, or mouse bone marrow-derived dendritic cells with either whole gonococci or their outer membrane vesicles, and assayed the supernatants for cytokines by ELISA; IL-17 production by T cells was analyzed by flow cytometry. Mouse genital tract tissue was cultured *ex vivo* with N. gonorrhoeae and assayed for production of cytokines and CXC chemokines. To test the role of IL-17 in a mouse model of vaginal gonococcal infection, mice were treated with a blocking antibody to IL-17 (Amgen) or control rat IgG, and infected vaginally with N. gonorrhoeae. Duration and extent of infection was monitored by swabbing and plating, and the neutrophil influx was counted relative to total epithelial cells.
Results
Mouse spleen cells stimulated with gonococcal antigens produced IL-17, and other inflammatory cytokines associated with a Th17 response, including IL-6, TNF-α, and IL-1β. Mouse bone marrow-derived dendritic cells stimulated with gonococcal antigens produced IL-6 and IL-23, and human THP-1 cells produced IL-6, TNF-α, IL-1β, IL-23 and IL-8; these cytokine profiles are consistent with the development of a Th17 response. Vaginal explants produced IL-6 and neutrophil-attracting chemokines such as CXCL5 in response to gonococci. In mice challenged with gonococci and treated with IL-17-blocking antibody, clearance of the infection was delayed by 2-3 days compared to control mice, and these mice showed significantly diminished neutrophil influx during the course of the infection (figure 2).
Conclusions
Consistent with induction of a Th17 response, gonococci stimulated production of IL-17 and other relevant cytokines in both mouse and human cells. Genital tract tissue cultured with gonococci released CXC chemokines which are secreted by IL-17-receptor bearing cells in response to IL-17. Blockade of IL-17 *in vivo* hindered neutrophil recruitment and clearance of vaginal gonococcal colonization, indicating the relevance of IL-17-dependent responses to N. gonorrhoeae infection. We propose that stimulation of IL-17 production by *N. gonorrhoeae* is important in the elicitation of neutrophil-dependent and other innate defense mechanisms, and suggest that the Th17 axis of immunity has a crucial role in the immune-

inflammatory response to this infection. Elucidation of this pathway will represent a new dimension in comprehending the host response to gonorrhea.

Figure 1: Generation and function of Th17 cells

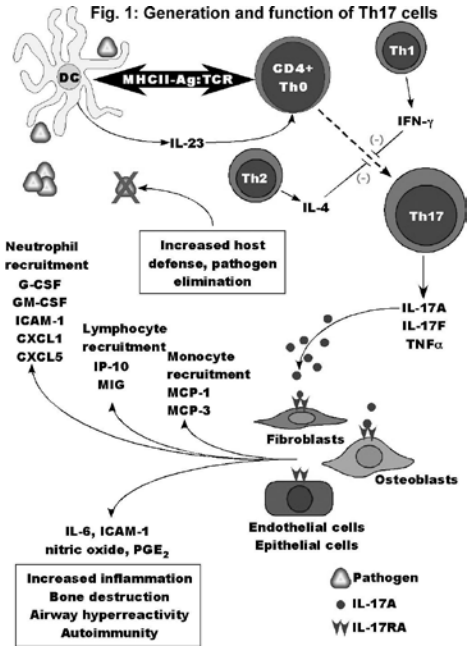
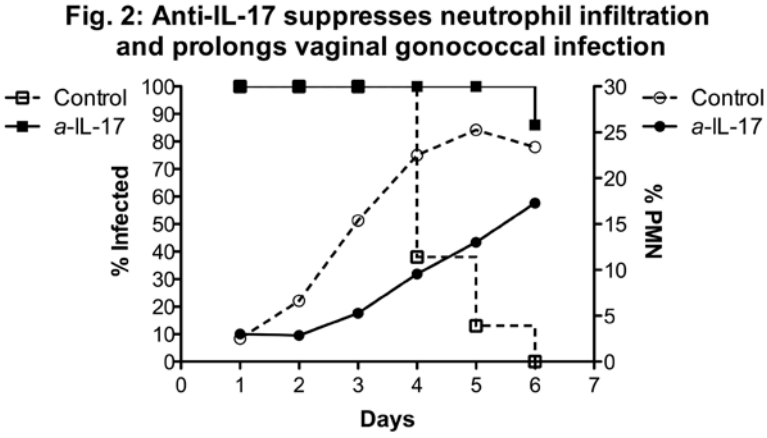


Figure 2: Gonococcal infection of anti-IL-17 treated mice



O25

Evasion of the adaptive immune system in the local mucosa during commensal colonisation of the upper respiratory tract by *Neisseria lactamica* but not *Neisseria meningitidis*

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Objectives
We have previously shown that asymptomatic colonisation of the nasopharyngeal epithelium by *Neisseria meningitidis* (Nm) is associated with the priming of an adaptive immune response. Nm is capable of colonising the upper respiratory tract (URT) for prolonged periods but it is poorly understood how Nm evades immune clearance. We have suggested that carriage may prime the induction of regulatory-activity in the mucosa. *Neisseria lactamica* (Nl) has been implicated in priming cross-protective immunity to Nm. We have therefore investigated the development of mucosal cellular immunity in response to natural carriage of Nl and its cross-reactivity with Nm to further understand how each bacteria maintains a commensal relationship with the host and its role in protection against disease.
Methods
Tonsillar mononuclear cells (TMNC) were isolated from children and adults. Antigen-specific memory T-cell and B-cell responses were quantified in response to outer membrane vesicles prepared from Nm H44/76 and Nl Y92-1009. Antigen-specific regulatory T-cell (T-reg) activity was also assessed in children by measuring proliferative responses prior to and after depletion of CD25+ cells. In addition, we assessed the production of T-cell independent immunoglobulin production in response to Nl and Nm OMV by ELISpot assay.
Results
Neither T-cell or B-cell memory was present to Nl or Nm OMV in TMNC from children ≤5 years which is the peak period of asymptomatic Nl carriage. The development of mucosal immunity to Nm corresponded to the increasing meningococcal carriage rate seen in teenagers. These results indicate that carriage of Nl does not prime a cross-reactive immune response with Nm in the mucosa. The lack of mucosal immunological memory to Nl cannot be explained by specific T-reg activity, which was also absent in young children. Interestingly, Nl OMV was found to stimulate a potent T-cell independent mitogenic response in B-cells that is absent in response to Nm OMV. This response was associated with the production of polyclonal immunoglobulin with characteristics similar to 'natural antibody'.
Conclusion
We propose that in contrast to Nm, Nl evades priming the adaptive immune system during commensal colonisation of the nasopharynx. Instead, it interacts with the innate immune system by inducing a mitogenic B-cell response. The response is associated with the production of non antigen-specific polyclonal immunoglobulin that binds to Nl OMV and a range of other antigens not contained in the outer membrane, including viral and mammalian proteins. This innate immunoglobulin may play a role in preventing invasion of the nasopharyngeal epithelium by Nl, precluding the need for an adaptive immune response and maintaining immunological ignorance. This may be an important mechanism by which Nl maintains a commensal relationship with the host. It may also cross-react with Nm and offer some protection against invasive disease independently of an adaptive immune response.

O26

Experimental challenge of adult volunteers with *Neisseria lactamica*: assessment of colonisation and immune responses

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Objectives
Neisseria lactamica is a common commensal of the upper respiratory tract of infants. There is an epidemiological relationship between colonisation by *N. lactamica* and protection of children from meningococcal disease likely due to cross protective immune responses. We have developed a human challenge model to enable characterisation of the immune response to *N. lactamica* carriage in adult volunteers.
Methods
We enrolled 200 healthy adults, screened them for Neisseria carriage and identified 128 who were not carrying Neisseria spp. at the point of entry to the study. From this group we recruited 61 Neisseria negative healthy adults, 41 received live intranasal challenge with 104 CFU of *N. lactamica* Y92-1009 and 20 received PBS control. Colonisation by the challenge strain or any Neisseria spp. was tracked by conventional and molecular identification of bacterial yields from nasopharyngeal swabs and gargles. In addition, specific mucosal and systemic antibody responses to *N. lactamica*, and induction of serum bactericidal antibody (SBA) and antibody-mediated opsonophagocytosis to UK prevalent meningococcal B strains were measured.
Results
26 out of 41(63.4%) individuals were successfully colonised with *N.lactamica*. Colonisation was intermittent in some individuals and 22 of 26 (85%) remained colonised at 12 weeks post challenge. Two controls acquired *N. meningitidis* carriage during the study period but none of the volunteers who were challenged with *N. lactamica* did so. Analysis of mucosal and serum immune responses to colonisation reveals a significant rise in anti *N. lactamica* antibodies amongst colonised individuals over the 12 week study period. Results demonstrated elevated specific *N. lactamica* IgA per ug of total IgA in colonised individuals compared to non- colonised (p=0.0039) and controls (p=0.0061). *N. lactamica* specific serum IgG responses also exhibited an elevated response in colonised individuals compared to non- colonised (p=0.0027) and controls (p=0.0028). Also, colonised and non-colonised individuals did not show significantly different baseline IgA or IgG levels. Data from a Pilot Study demonstrated 4 out of 5 colonised individuals had significant rises (›4 fold) in SBA titre against 3-4 of 6 clonally diverse strains of *Neisseria meningitidis* tested. SBA and opsonophagocytosis results from the larger study will be presented.
Conclusion
After experimental challenge of Neisseria-negative adult volunteers, the acquisition of *N. lactamica* carriage was 63.4%. Initial data confirms colonisation results in a mucosal IgA and systemic IgG response and it is likely that exposure to *N. lactamica* induces a booster response to shared specific antigens on the outer membrane of different strains of meningococci. The preliminary results support the hypothesis that colonisation by *N. lactamica* does result in cross protection against *N. meningitidis*.

O27

Defining factors that govern alternative complement pathway activation on *Neisseria meningitidis*, with an emphasis on host specificity

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Objective
Complement forms a key arm of innate immune defenses against *Neisseria meningitidis*. The alternative pathway (AP) of complement is critical for amplification of C3 deposition on bacteria. We undertook this study to better understand how the human AP is regulated on *N. meningitidis*.
Methods
Expression of capsular polysaccharide, lipooligosaccharide (LOS) sialylation and expression of factor H-binding protein (fHbp; also called GNA1870 or LP2086) modulates serum resistance of meningococci. Isogenic mutant strains were used to study the role of each of these variables in regulating the human and rabbit AP. The AP in human or baby rabbit complement was selectively activated using EGTA and 10 mM Mg2+. Human AP activation was further studied using purified human C3, factor B, factor D and properdin. AP activation was quantified by measuring C3 binding to bacteria by flow cytometry.
Results
The capsular polysaccharides of serogroups A, B, C and W-135 regulated the human AP; human AP activation was unimpeded on serogroup Y strains. Similar results were obtained when the AP was assembled using purified human AP components (C3, factor B, factor D and properdin), confirming that anticapsular antibodies were not involved. Furthermore, the O-acetylation status of the serogroup Y capsule did not influence AP activation. Rabbit complement is more bactericidal than human complement; as a control we studied activation of the rabbit AP and found that none of the five major capsular polysaccharides inhibited the rabbit AP.
In unencapsulated organisms, LOS sialylation regulated both the human and the rabbit AP; regulation was proportional to the extent of LOS sialylation. Regulation of the human AP by LOS sialic acid was only evident in the absence of fHbp-mediated fH binding.
We noted that rabbit fH did not bind to meningococcal fHbp and binding of rabbit C3 to bacteria was inhibited when human fH was added to rabbit complement. This demonstrates the importance of human fH in AP regulation on meningococci. Encapsulated serogroup Y strain, Y2220, activated the rabbit AP independent of LOS sialylation or expression of fHbp. Adding human fH to baby rabbit complement (AP alone active) resulted in inhibition of rabbit C3 binding to encapsulated Y2220; human fH-mediated inhibition of rabbit C3 binding required either LOS sialylation or expression of fHbp.
Conclusions
The serogroup A, B, C and W-135 capsules regulate human, but not rabbit AP. Inability of serogroup Y capsule to regulate the human AP may explain the greater frequency of disease with this serogroup in AP-deficient persons. LOS sialylation regulates both the human and rabbit AP. Binding of fH to meningococcal fHbp is restricted to human fH. Human fH regulates the rabbit AP on meningococci either when LOS is sialylated or fHbp is expressed. Collectively, these data shed light on mechanisms of AP activation and regulation on meningococci. Selective regulation of the human AP may provide another reason for restriction of meningococcal disease to humans. Our findings may aid development of better animal models for meningococcal disease and guide the choice of complement sources for meningococcal vaccine evaluation.

O28

Neisseria gonorrhoeae use factor H to adhere to Complement Receptor 3 on eukaryotic cells

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Objective
Gonococci bind to human complement inhibitory proteins, such as factor H (fH) and C4b-binding protein, to down-regulate the alternative and classical pathways of complement, respectively. Factor H binds to specific receptors on different cell types. Prior studies have shown that complement receptor 3 (CR3, also known as CD11b/CD18), which promotes adherence of serum opsonized bacteria, is also a ligand for fH. Primary cervical epithelial cells express CR3. We hypothesized that fH bound to gonococci could facilitate bacterial adhesion to CR3-bearing cells and thus provide another mechanism for gonococci to adhere to eukaryotic cells.
Methods
Factor H binding to CR3 transfected Chinese Hamster Ovary (CHO) cells (CHO-CR3) was measured by flow cytometry. Specificity of fH binding to CR3 was demonstrated by inhibition of this interaction with iC3b, or anti-CD11b and anti-CD18 MAbs. We also sought to identify the short consensus repeat domains (SCRs) of fH (fH contains 20 SCRs) responsible for binding to the CR3 receptor using engineered human fH/murine Fc fusion protein constructs containing contiguous fH SCR domains, SCRs 1-5, 6-10, 11-15 and 16-20. To examine fH-mediated gonococcal adherence to CHO-CR3, we used a fH binding PorB.1A-bearing strain called 252.
Results
We determined that the C-terminal region of fH spanned by SCRs 16-20, and to a lesser extent, SCRs 6-10 region, bound to CHO-CR3. Using additional fH/Fc constructs we narrowed the factor H C-terminal CR3 binding region to SCRs 18-20. Consistent with these two binding regions in fH for CR3, we demonstrated that an alternatively spliced variant of fH, called fH-like molecule-1 (FHL-1) that contains fH SCRs 1-7, and fH-related protein 1 (FHR-1) that comprises three C-terminal SCRs with ›97% homology to fH SCRs 18-20 in addition to two unique N-terminal SCRs also bound to CHO-CR3. We have shown previously that strain 252 can bind to factor H SCRs 6 and 20 and thus can bind to fH, FHL-1 and FHR-1. Full length fH, but not FHL-1 or FHR-1 enhanced association of unsialylated 252 to CHO-CR3, thus demonstrating a requirement for both CR3-binding regions in fH to facilitate bacteria-cell interactions. We hypothesize that binding of SCR 18-20 to bacteria would allow SCR 6-10 to interact with CR3, or vice versa. Sialylation of LOS abrogated fH-mediated bacteria-cell interactions. We speculate that the greater negative charge imparted by sialic acid serves to repel adherence that otherwise can be mediated by fH.
Conclusions
These results indicate a productive role between fH opsonized gonococci and CR3 on a eukaryotic cell line and may provide another means for gonococci to gain sanctuary into non-professional (non-killing) phagocytes to escape extracellular death or PMN mediated killing. PorB.1A strains that bind fH directly without a requirement for sialylation, are often absent on Gram stains of infected human secretions because Gram stains visualize extracellular organisms and those ingested by PMNs but not those internalized by epithelial cells.

O29

Laminin receptor initiates contact of neurotropic bacteria with the blood brain barrier

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Introduction
Meningitis, a devastating complication of bacteremia, is caused by a limited group of bacterial pathogens, most notably the respiratory tract pathogens: *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Given that these bacteria, through convergent evolution, adhere to and invade vascular endothelial cells through phosphorylcholine present on their surface which binds to platelet activating factor receptor (PAFr) on host cells, we hypothesized that a common molecular interaction might also underlie their tropism for the blood brain barrier (BBB) and targeting of the central nervous system.
Objective
Identify the host and bacterial receptors that mediate tropism for, and invasion of, the BBB.
Methods and results
Using affinity chromatography, co-immunoprecipitation, re-tagging, and *in vivo* imaging, the 37/67-kD laminin receptor (LR) was identified as a common receptor for all three bacteria on rodent and human brain vascular endothelial cells. Mutagenesis studies indicated the corresponding LR binding adhesins were pneumococcal CbpA, meningococcal PilQ and PorA, and OmpP2 of *H. influenzae*. Competitive binding of the bacteria, purified adhesins, LR peptides and antibodies suggested a common LR-carboxyterminal recognition site. Consistent with a shared ligand-receptor interaction, immunization with CbpA generated antibodies cross-reactive with all three pathogens and attenuated infection by these pathogens *in vivo*.
Conclusion
As with prions and certain neurotropic viruses, binding to LR contributed to bacterial tropism to the BBB and may provide a basis for generating cross-protective vaccination against meningitis.
Reference
1. Orihuela, C, Mahdavi J, Thornton J, Mann B, Wooldridge KG, Abouseada N, Oldfield NJ, Self T, Ala'Aldeen DAA, Tuomanen EI (2008). Science [Under-review]

O30

PorA strain variation causes differential processing, presentation and recognition of CD4 T cell epitopes

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Objectives
CD4 T cells are required for the induction of germinal center reactions and the generation of high affine, functional B cell responses to neisserial protein antigens. It is unknown, however, whether suboptimal cognate CD4 T cell help might contribute to poor immunogenicity of certain PorA antigens. To address this issue, we set out to characterize the murine and human PorA specific CD4 T cell response against a weakly and a strongly immunogenic PorA, and to study the influence of PorA strain variation on the efficiency of CD4 T cell epitope generation and recognition.

Methods
Balb/c and C57/Black6 mice were immunized with P1.7-2,4 or with P1.5-1,2-2 Outer Membrane Vesicle vaccines. Splenocytes as well as a T cell hybridoma obtained after vaccination, were studied for reactivity in proliferation and murine cytokine assays against recombinant PorA proteins (a gift from Wyeth vaccines, Pearl River, USA) and a series of overlapping synthetic peptides spanning the P1.7-2,4 and P1.5-1,2-2 protein sequences, respectively. PBMC, T cell lines and T cell clones obtained from healthy HLA-typed adults were tested for epitope specificity and HLA restriction using peptides and rPorA's in proliferation and human cytokine assays.
Results
A shared immunodominant CD4 T cell response against a microvariable region of PorA was identified in spleens from Balb/c and C57/Black6 mice, and only emerged after immunization with the strongly immunogenic P1.5-1,2-2. The epitope region, P1.5-1,2-2152-163, was also recognized by a Balb/c T cell hybridoma and a human CD4 T cell line, and was shown to contain a discriminating alanine at position 160 (A160). Extensive efforts to detect human T cell reactivity against the isoleucine containing P1.7-2,4 counterpart (I164) of the epitope remained unsuccessful. The CD4 T cell repertoire in C57/Black6 mice as well as in humans further contained other specificities, representing both conserved and micro-variable regions of PorA. No T cell reactivity was detected against hyper-variable PorA regions, consistent with results from epitope identification methods based on MHC-peptide isolation (1). In general, the efficiency of the P1.7-2,4 protein to stimulate specific CD4+ T cell clones after processing and presentation in antigen presenting cells was inferior to P1.5-1,2-2, not only for variable but also for conserved epitopes.
Conclusion
Residues A160 in P1.5-1,2-2 and I164 in P1.7-2,4 represent a discriminating PorA polymorphism, determining whether an immunodominant CD4 T cell response is induced or not. Also for other epitope regions including non-variable ones, P1.7-2,4 performs poorly in triggering T cells as compared to other PorA's. Collectively, these results indicate that PorA CD4 T cell immunity targets conserved as well as microvariable regions but that natural P1.7-2,4 polymorphisms, both inside and outside epitope regions, may contribute to its low level of immunogenicity.
Reference
1. van Els C et al. IPNC 2006 Poster Abstract P6.2.08

O31

Severely reduced B cell TACI expression in newborn mice plays role in impaired neonatal response against polysaccharide antigens

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Elicitation of humoral antibody response against capsular polysaccharides (PS) of *Neisseria meningitidis* is critical in protection against meningococcal bacteria. However, PSs are T cell independent type II (TI-II) antigens and they do not induce humoral immune response in newborns. Reasons for this unresponsiveness are not fully elucidated. In this study, we have shown that newborn B cells do not secrete IgG or IgA when stimulated with myeloid cytokines BAFF or APRIL. Studies in adult mice have shown that the B cell BAFF and APRIL receptor TACI is essential in the development antibody response against PS antigens. Therefore, we measured TACI protein and mRNA levels in newborn B cells, and showed that TACI is expressed at severely reduced levels compared to those of adults (figure 1A). As with adult B cells (Katsenelson, N et al. Eur. J. Immunol. 2007 37:1785-95), incubation of newborn B cells with CpG-ODN led to increased upregulation of TACI on marginal zone, follicular, and B1 cells (figure 1A and B). Coinciding with this, CpG-ODN pretreated B cells from neonates secreted significantly higher levels of IgA/IgG in response to BAFF or APRIL (figure 2A). Underscoring the role of TACI in sensitization of B cells

by CpG ODN, inclusion of blocking antibodies against TACI in CpG ODN pretreated BALB/c mouse B cells (figure 2B) or using B cells from TACI deficient mouse resulted with ablation of BAFF or APRIL induced immunoglobulin secretion. Similarly, injection of newborn mice with CpG-ODN not only led to increased TACI expression, but also rendered B cells sensitive to BAFF or APRIL mediated IgG and IgA secretion. Moreover, upregulation of TACI expression led to BAFF or APRIL mediated plasma cell development in newborn B cells. Finally, when used as an adjuvant, CpG ODN augmented the antibody response against TI-II antigens in wild type mice whereas it did not induce antibody response in TACI deficient mice. Given the pivotal role of TACI in generation of antibody responses against PS antigens, our study indicates that low TACI expression may be contributing to the susceptibility of neonates to infections with encapsulated bacteria. Also, increased expression of TACI concomitant with enhanced immunoglobulin secretion in response to BAFF and APRIL from CpG-ODN exposed cells points to a novel mechanism by which CpG-ODN may act as adjuvant.

Figure 1: The effect of CpG on newborn TACI expression

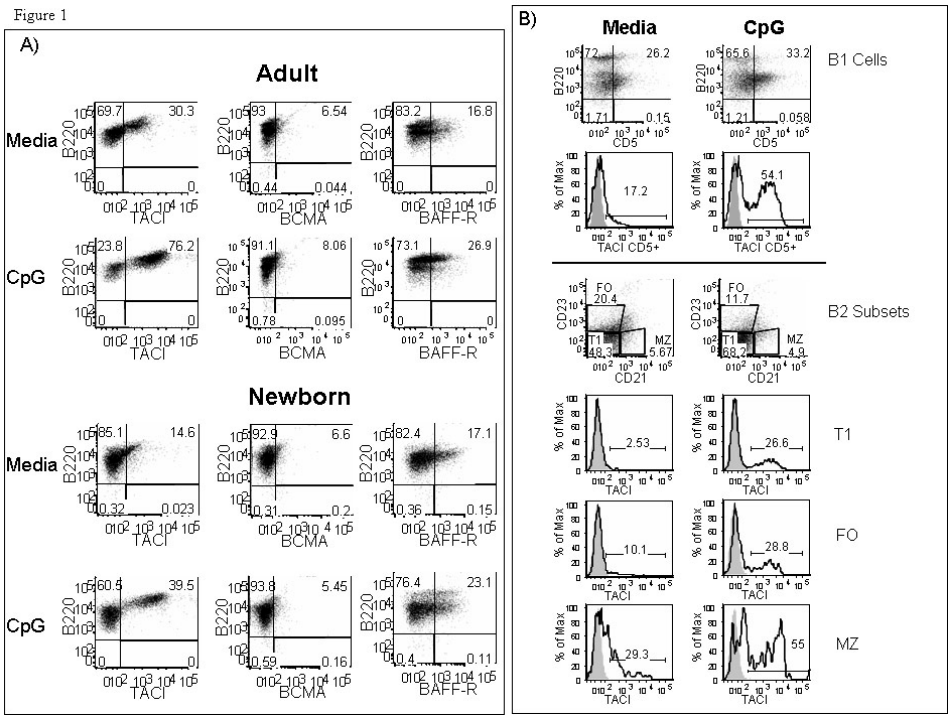
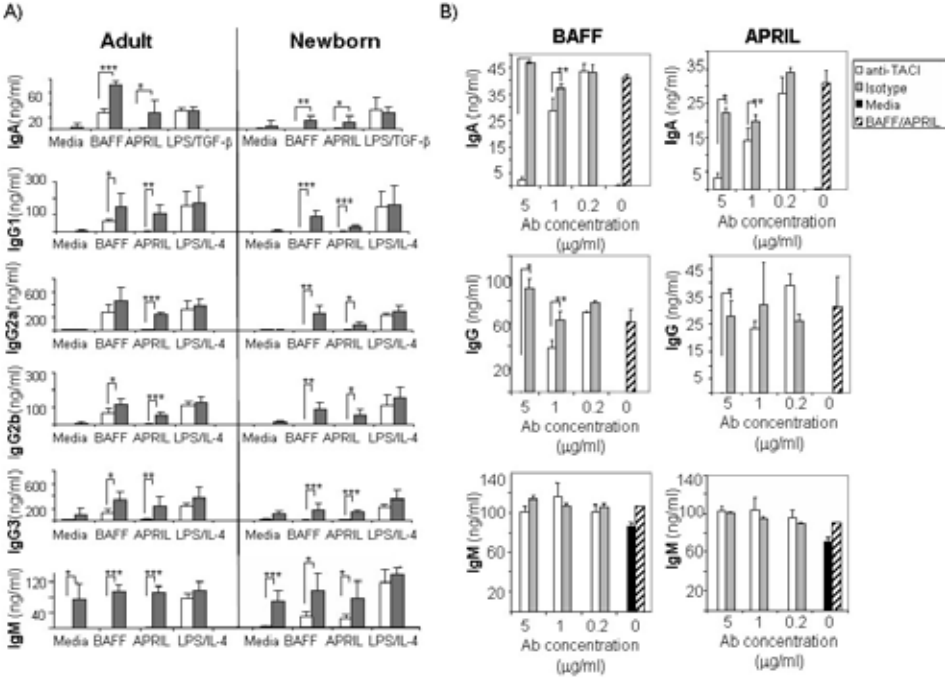


Figure 2: CpG sensitizes newborn B cells to BAFF and APRIL



O32

B cells and the maintence of naturally acquired T cell memory to *Neisseria meningitidis* following colonization

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Objectives

The importance of T cells in the generation of antigen specific B cell immunity has been well described but whether B cells reciprocate and shape T cell responses is uncertain. Our aim was to investigate the role of B cells in the generation of T cell memory to *Neisseria meningitidis* (Nm) *per se* and whether the antigen itself or the environment either mucosal or systemic had any bearing on memory T cell induction.

Methods

A disorder of human B cell maturation (X-linked agammaglobulinemia, XLA) was used to investigate the role of B cells on the induction of T cell memory to Nm and to systemic influenza vaccination. Nm and influenza specific T cell proliferation was evaluated by thymidine incorporation from peripheral blood of XLA and healthy controls. Flow cytometry was used to evaluate mucosal and systemic associated B cell activation and antigen presentation following exposure to Nm. The significance of B cell CD40 expression and T cell CD40 co-ligation was investigated in X-linked hyper IgM syndrome (X-HIM) subjects which are deficient in CD40L

Results
XLA patients displayed naïve type proliferative kinetics in response to Nm OMV that peaked on day 9 as compared with healthy controls that peak on day 5. To determine whether XLA patients were indeed deficient in Nm specific memory T cells, CD45RA+ naive cells were depleted. Following the removal of naïve cells, XLA patients had severely reduced Nm responses whilst influenza responses were still observed. We show that Nm exposure induced mucosal and systemic associated B cells to upregulate MHC class II, CD40, CD86/80 expression and that B cell antigen presentation resulted in stimulation of Nm specific T cell proliferation. When the role of CD40-CD40L was evaluated using cells from subjects with X-HIM, a similar reduction in Nm but not influenza antigen-specific T cell memory was found.
Conclusion
Our data shows that B-cell deficinet subjects had considerably reduced T cell memory to *Neisseria meningitidis* within the systemic compartment as compared to healthy controls. In contrast, there was no difference in influenza specific memory T cell responses in peripheral blood of XLA and control subjects. Together, these data suggest that B cells are involved in the induction of T cell memory to mucosal colonizing bacteria and that this interaction requires CD40-CD40 ligation. In addition, we surmise that B cells are differentially required for memory T cell induction depending upon the site of antigenic exposure.

O33

Tracking of human memory B cells specific for meningococcal serogroup C polysaccharide, induced by vaccination

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Objectives
Vaccinations should induce immunological memory to provide long term protection against infectious diseases. To explore the induction and persistence of *Neisseria meningitidis* type C polysaccharide (MenC-PS)-specific B cells in humans, blood samples were collected in infants, toddlers, children, adolescents and adults who had received one or two doses of a MenC-PS conjugated to tetanus toxoid (MenC-TT) vaccine. We tracked these cells after immunization with MenC-TT by flow cytometry using fluorochrome labeled MenC-PS.
Methods
Peripheral blood mononuclear cells (PBMC) were isolated from EDTA blood by density gradient centrifugation and stained with a cocktail of monoclonal antibodies (mAbs) in PBS/BSA/EDTA. Flow cytometric acquisition was performed on a FACS Calibur and analyzed using CELLQuest software. To determine the frequency of MenC-PS memory cells that differentiated into antibody secreting cells (AbSC) upon *in vitro* stimulation of PBMC with pokeweed mitogen, Cpg2006 and S. aureus ELISpot assay was performed. MenC-PS specific IgG and IgA AbSC enumerated. MenC-specific IgM, IgG and IgA antibodies were measured by ELISA. Frequencies of MenC-specific B cells, memory B cells, AbSC and Ab levels were compared over all age groups with one-way ANOVA and between two groups by nonparametric Mann-Whitney U test. A p-value of ‹ 0.05 was considered statistically significant.
Results
Persisting MenC-PS-specific B cells could be detected in blood up to 3 years after vaccination, which was the longest period analyzed in this study. Their median frequencies were 0.01%-0.78% of total B cells in the different vaccinated age groups. The frequencies of MenC-specific B cells and antibody levels differed significantly between the age groups. Most of the MenC-specific B cells showed a memory phenotype ($CD19^+/CD20^+/CD27^{med}$) in all age groups (N=67, median 100%, 25% percentile 87.5% and 75% percentile 100%). There ratios of MenC-specific IgG/IgM were high indicating a memory type of response. The MenC-specific memory B cell out of $CD27^{med}$ B cells correlated with total MenC-specific B-cells

(r=0.5617, *P*<0.0001), and with the frequencies of IgA^+ plus IgG^+ MenC-specific AbSC after *in vitro* stimulation (r=0.5627, *P*=0.029) across all age groups.

Conclusions

These results demonstrate that labeled MenC-PS can be used to monitor the induction and persistence of memory B cells as part of the evaluation of vaccine responses and vaccination strategies. MenC-PS specific B cells of the memory phenotype can be detected in the periphery even after administration of a single dose of conjugate vaccine to infants and young children up to at least 3 years post vaccination.

O34

Commensalism and virulence of *N. meningitidis* (Nm): prospects for prevention of Serogroup B disease by immunisation

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Serogroup B strains make a major contribution to the global morbidity and mortality caused by *Nm* diseases. Although protein conjugate vaccines against serogroups A, C, Y and W-135 have proven or potential efficacy, the structural similarities between the *Nm*B capsular antigen, a homopolymer of a2:8 linked sialic acid, and glycosylations found on human cell surface proteins have raised the possibility that use of this vaccine antigen might lead to autoimmune disease. Most commercial companies have therefore been unwilling to develop such vaccines. Thus, prevention of invasive diseases caused by *Nm*B will almost certainly be achieved through a new generation of vaccines that are not based on the *Nm*B capsular antigen. The best established of these vaccines are those based on outer membrane vesicles (OMVs). In several epidemiological settings, *Nm* OMV vaccines have demonstrated convincing efficacy. Recently, the effectiveness of an OMV vaccine in New Zealand and its ability to elicit serum bactericidal antibodies to an outer membrane PorA antigen in infants has provided important support for the concept that serum bactericidal activity is a correlate of protection against non-capsular antigens. But, there are some serious limitations to OMV vaccines. In addition to the regulatory challenges of their formulation, the major protective antigen (PorA) is highly variable (it is the basis of the sub-serotyping of *Nm*). In regions where the circulating strains causing disease are diverse and do not match the strain(s) used for the OMV vaccine, effectiveness diminishes in proportion to the amount of PorA variation of these strains. Nonetheless, several of the leading new generation vaccines are based on OMV components, including their use as vehicles for the presentation of recombinant proteins or glycolipids. Further, OMVs have adjuvant properties and may offer additional protective potential through *Nm* antigens other than PorA. The mining of information from genome sequence data has facilitated the discovery of novel vaccine antigens. A sub-set of these has been formulated into a multivalent *Nm* vaccine and phase 2 clinical trials have indicated the potential of this vaccine to prevent *Nm* disease caused by strains including, but not confined to, those of serogroup B. Also important, the analysis of many different *Nm* genomes has opened the door to a new era in our understanding of *Nm* biology. The most conspicuous feature of *Nm* genotypes, revealed by complete sequencing of several genomes, is that their gene content and organisation is characterised by sequences (coding and non-coding) that are variable, unstable and confer potential for dynamic changes in gene content and organisation. In conjunction with the high throughput sequencing that has been used for multi-locus sequence typing (MLST), genomics is providing a substantially more detailed picture of the evolutionary origins and structure of the natural population of the *Nm* species. This has profound implications for coping with a difficult challenge: how to estimate the proportion of strains that each of these new generation multivalent *Nm* vaccines can target. Importantly, because of lateral transfers of DNA from Neisseria spp. and other sources, there is not necessarily concordance between genes of the core genome (for which MLST is a proxy) and other genes, especially those encoding vaccine antigens and other surface expressed, host-interactive molecules. For multivalent vaccines, such as those developed recently to prevent diseases caused by *Nm*B strains, a challenging

problem is how to estimate strain coverage in different epidemiological settings and relate this to protective potential. Given the eclectic combinations of variant surface antigens of commensal and disease strains of *Nm*, this will prove a daunting challenge.

O35

Multivalent group B meningococcal vaccine based on Native Outer Membrane Vesicles (NOMV) has potential for providing safe, broadly protective immunity

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Objective
Development of a vaccine based on native outer membrane vesicles that will be safe and provide broad-based protection against group B strains of *N. meningitidis*
Methods
Three antigenically diverse group B strains of *N. meningitidis* were chosen and genetically modified to improve safety and to increase and stabilize the expression of desirable antigens. The vaccine strains were genetically configured to have three sets of antigens, each with potential to induce protective antibodies against a wide range of group B strains: LOS, PorA, and conserved OMPs. Each strain was modified to express a second PorA, to express a different LOS core immunotype (L3, L2, and L5), and to have increased expression of at least one conserved protein (e.g. fHbp1, fHbp2, NadA, Opc) with capacity for inducing bactericidal antibodies. To improve safety of NOMV prepared from these strains, three genes were disabled in each strain: synX, lpxL1, and lgtA. NOMV were prepared from each strain and tested for safety by cytokine release assays using whole human blood and by the rabbit pyrogen test. The NOMV were tested for immunogenicity in mice and rabbits. Bactericidal assays were performed on pooled serum using human complement and a panel of 18 different test strains. ELISA assays were performed against NOMV and several specific purified antigens.
Results
Results of rabbit pyrogen tests and tests involving the release of pro-inflammatory cytokines from whole human blood suggest that the vaccine will be safe for parenteral administration in humans. The combined NOMV vaccine induced a four-fold or greater increase in bactericidal antibodies against 16 of 18 bactericidal test strains. Analysis of the bactericidal antibody response showed that all three sets of antigens were involved in the bactericidal antibody response and that the response was not predominantly against PorA. The LOS was found to be a key component in the vaccine.
The truncated, L8-like LOS (L8-3, L8-5, L8-2) in the vaccine induced bactericidal antibodies that were able to kill wild type strains expressing predominantly full length LOS. A clinical lot of NOMV vaccine was prepared from one of the three vaccine strains, preclinical testing was done, and a phase 1 study is in progress as proof of principle to demonstrate safety and immunogenicity in humans.
Conclusions
A multivalent vaccine based on NOMV from three genetically modified strains has potential to be a safe and broadly effective vaccine against group B meningococcal disease. In this vaccine the PorAs are not the dominant antigens, but they contribute to the bactericidal antibody response along with LOS and conserved outer membrane proteins.

O36

Broad meningococcal immunity elicited by bivalent native OMV vaccine from mutants engineered to have more than one factor H binding protein variant and detoxified endotoxin

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Objectives
Promising group B recombinant protein vaccines are in development. However, to date none elicits coverage against all strains. We recently described a native (non-detergent treated) outer membrane vesicle (OMV) vaccine prepared from a mutant of strain H44/76 in which endotoxin was attenuated by inactivating the LpxL1 gene, which resulted in pentaacylated instead of hexaacylated LPS. The strain also was engineered for increased expression of factor H binding protein (fHbp) in the antigenic variant (v.) 1 group (J. Infect Dis, in press). Serum anti-fHbp antibodies elicited in mice by the mutant OMV vaccine had broad bactericidal activity against *N. meningitidis* strains expressing subvariants in the fHbp v.1 group. However, there was little activity against strains expressing fHbp in heterologous v.2 or 3 groups (~40 percent of strains). The objective of the present study was to develop an OMV-based vaccine against strains with fHbp v.1, 2 or 3.
Methods
We engineered a LpxL1 knockout (KO) mutant of strain NZ98/254 that expressed endogenous fHbp (subvariant of v.1) and heterologous fHbp v.2 (gene from 8047;average of 85% amino acid homology with fHbp v.3). We investigated immunogenicity in mice of three doses of a native OMV vaccine prepared from this mutant when given in combination with the native OMV vaccine described above from the LpxL1 KO mutant of H44/76 with over-expressed fHbp v.1.
Results
When incubated with human peripheral blood mononuclear cells, the bivalent native OMV vaccine was highly attenuated and elicited similar respective IL-1 beta, IL-6, IL-8 and TNF alpha responses as control detergent-extracted wildtype OMV vaccines. By ELISA, mice given the bivalent native mutant OMV vaccine had high serum IgG antibody responses to both fHbp v.1 and v.2 (Fig. 1). The serum bactericidal titers (GMT) measured with human complement were ›1:1000 against the homologous group B strains used to prepare the mutants. The GMTs against 7 of 8 strains tested with heterologous PorA and which expressed fHbp subvariants in the v.1, v.2 or v.3 groups ranged from ~1:100 to ›1:1000. Representative data against three of these strains are shown in Fig. 2. The bivalent native mutant OMV vaccine also elicited high bactericidal titers (GMT›1:100) against 6 of 7 invasive isolates with capsular groups other than group B, including 3 of 3 group A (Germany, 1964; and Ethiopia and Burkina, 2002-2003); 2 of 2 group W-135 (Burkina Faso, 2002 and a Hajj-related strain from France), 1 of 1 group C (U.S.), and 0 of 1 group Y (Holland). All had heterologous PorA proteins to those of the vaccine.
Conclusion
Broad protective meningococcal immunity was elicited against genetically diverse group B strains expressing fHbp in the v.1, 2 or 3 groups. The antibodies targeted non-capsular antigens and also conferred protection against strains with capsular groups other than B. Thus the OMV vaccine from the two mutants not only protects against group B strains but also could be used to augment immunity elicited by polysaccharide-protein conjugate vaccines, and may lessen the risk of emergence of strains with capsular groups not included in conjugate vaccines.

Figure 1: Serum IgG anti-fHbp titers by ELISA

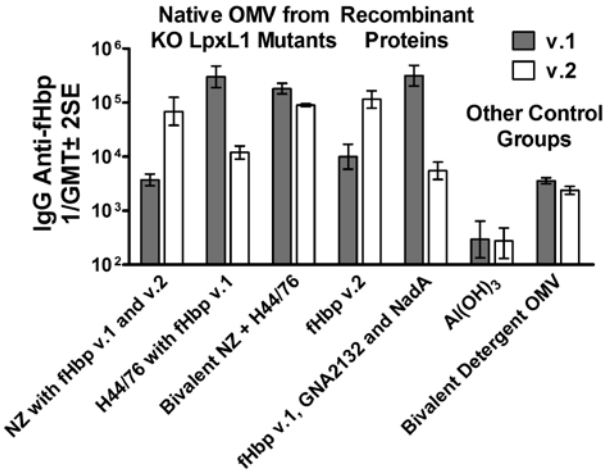
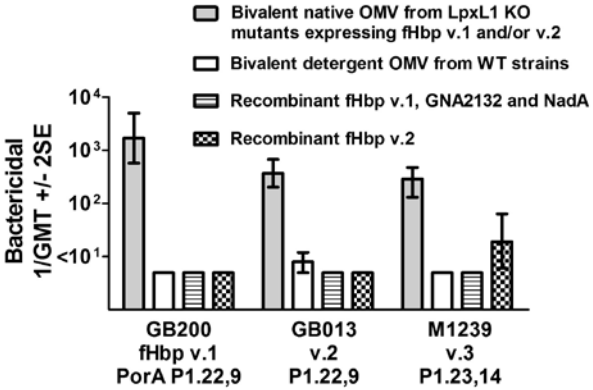


Figure 2: Serum Bactericidal, 1/GMT, human complement



O37

Genetically modified L3,7 and L2 lipooligosaccharides from *Neisseria meningitidis* serogroup B confer broad cross-bactericidal response: comparison with subunit protein approaches

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Objective

Currently available *Neisseria meningitidis* serogroup B (MenB) vaccines are based on outer membrane vesicles (OMVs) that are obtained from wild type strains and purified with the aim to decrease the lipooligosaccharide (LOS) content and hence reduce the reactogenicity of the vaccine. In children below 2 years of age, these MenB vaccines confer protection only against strains expressing homologous PorA, a

major and variable outer membrane protein. Our objective was to develop an LOS-based vaccine against MenB.

Methods

Using genetically modified porA knock-out strains expressing genetically detoxified (deletion of msbB gene) L2 and L3,7 LOS, we have developed MenB vaccines that are based on OMVs enriched in LOS.

Results

The vaccine-induced antibodies were found to be bactericidal against nearly all invasive strains irrespective of capsular serogroup and L immunotype. In addition, we have also demonstrated that LOS lacking the terminal galactose (lgtB LOS) (but not galE LOS) induce a bactericidal antibody response similar to that seen for LOS containing the full non-sialylated Lacto-N-neotetraose.

Conclusion

Our approach was compared with protein subunit approaches such as those investigating the factor H-binding protein, which demonstrated a more limited strain coverage as compared to the L2 + L3,7 OMV-LOS-based approach.

O38

On neisserial vaccine efforts: The art of war

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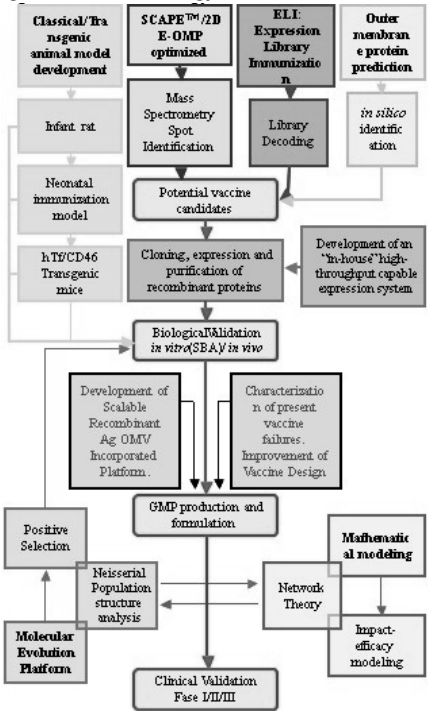
The global impact of infections due to *Neisseria meningitidis*, and the lack of a protective vaccine against serogroup B disease, has prompted the scientific community to develop a preventive universal vaccine against this deadly pathogen. Several battles have been won, particularly with the recent success of conjugate vaccines. However the quest for complete disease eradication is far from complete.
While promising recombinant vaccine candidates are under evaluation, thus putting more weapons into this host-pathogen arms race, several issues must be taken into account for the development of a successful anti-meningococcal vaccine. In this direction, the experience gathered by long term research and vaccine application performed in Cuba can give vital clues on issues such as the study of probable vaccine failures and the associated questions on host susceptibility, the rapid evolution of antigenic types amongst the circulating meningococcal population after vaccine introduction, the impact of minor OMV components on the induction of a significant immune response in humans. We have also explored new ways for the identification of vaccine candidates, for example Expression Library Immunization (ELI) and in addition, we have applied new tools from genomics, mathematical modeling and systems biology for vaccine candidate identification and other vaccine-related challenges. The current assessment of this ongoing war with Neisseria will be discussed in the context of complex systems, where the main battlefield is no longer a single host but a large and heterogeneous population of beings: all of us.

Figure 1: Global Strategy



O39

Review of proteomics as a tool in meningococcal vaccine development

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Objectives

Outer membrane vesicle (OMV) vaccines have been used to disrupt outbreaks of meningococcal disease in Norway, Cuba and New Zealand. The success of these campaigns means that OMVs are likely to form the basis of future vaccine developments. OMV vaccines present the host immune system with a complex mixture of outer membrane protein antigens, many of which remain poorly characterised in terms of their contribution to protective immunity. In addition, many of these antigens are highly variable, a number showing phase variation, or their expression is regulated in response to environmental stimuli. Manufacturing consistency is therefore an essential consideration for the producers of OMV vaccines. One dimensional SDS polyacrylamide gel electrophoresis (SDS-PAGE) has typically been used to determine the relative amounts of key antigens in an OMV by measuring the protein band intensities on a Coomassie stained gel. However, some OMV antigens were difficult to identify unequivocally using this approach. The availability of Neisseria genome sequences and improved proteomic technology have improved the definition of the OMV proteome. This presentation will review recent proteomic studies and their contribution to vaccine developments.[1]

Methods
One and two dimensional electrophoresis, mass spectrometry, differential in gel electrophoresis (DiGE) and multiplex immunodetection.
Results
Four studies have used 1D SDS-PAGE and mass spectrometry to determine the protein profiles of both lactamica and meningococcal OMVs. A further six studies have introduced an isoelectric focusing step and separated the proteins of OMVs and whole organisms by two dimensional electrophoresis proceeded by mass spectrometry. Differential in gel electrophoresis (DiGE) has been used to examine the consistency of pre-clinical and clinical batches of OMVs. It has also been used to compare OMVs derived from the same strain grown in different culture media. Combining chemiluminesent immunodetection with fluorescent dye labelling has facilitated the use of two dimensional proteome maps of the OMV to predict which proteins are antigenic.
Conclusions
Proteomic technology has provided useful tools for meningococcal vaccine development and for the development of quality control tests to ensure vaccine consistency. In conjunction with the ever increasing body of genomic data, it provides a more detailed picture of the organism and its biology.
Reference
1. Wheeler, J., C. Vipond, and I. Feavers. 2007. Exploring the proteome of meningococcal outer membrane vesicle vaccines. Proteomics-Clinical Applications 1:1198-1210.

O40

Neuraminic acid-containing polysialic acid is immunogenic and elicits antibodies that are protective against *Neisseria meningitidis*

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Objectives
The monoclonal antibodies (mAbs) SEAM 2 and 3 elicited by immunization with a vaccine containing the N-propionyl derivative of *Neisseria meningitidis* group B capsular polysaccharide (N-Pr NmBPS) are reactive with NmBPS derivatives that contain a fraction (~10%-50%) of neuraminic acid (Neu) residues. Both mAbs activate human complement protein deposition on group B bacteria and are protective in an infant rat model of meningococcal bacteremia at antibody concentrations of ~1 μg/ml. However, neither mAb mediates serum bactericidal activity (SBA) with human complement at that concentration. The purpose of this study was to evaluate the immunogenicity of protein-polysaccharide conjugate vaccines based on Neu-containing derivatives of poly alpha 2,8 N-acyl neuraminic acid (NeuPSA) and determine whether the vaccines can elicit protective antibodies against group B.
Methods
Five NeuPSA antigens were prepared from colominic acid. The fraction of Neu residues ranged from 14% to 98% and the N-acyl groups included acetyl, propionyl, and trichloroacetyl. The derivatives where conjugated to tetanus toxoid by reductive amination. The immunogenicity was evaluated in CD1 mice (10 per group), which were given three doses of vaccine containing 2 μg or 10 μg of total sialic acid with Freund's adjuvant. Antibody titers against the homologous polysaccharide and de-N-acetyl PSA were measured by ELISA after each dose. Class and subclass of antibodies elicited by the vaccines that bind to bacteria and the ability of the antibodies to activate deposition of human complement components on group B bacteria was determined by flow cytometry. Protective functional activity of antisera pools from individual groups was evaluated by SBA with human complement and passive protection in an infant rat model of meningococcal bacteremia against challenge with group B strain M986.

Results
All of the vaccines elicited anti-de-N-acetyl PSA antibody titers ›1:10,000 but variable titers against the homologous antigen and no reactivity (titer ‹1:50) with PSA that does not contain Neu. There was no significant difference between 2 μg or 10 μg doses and no increase in titer after the second dose. NeuPSA vaccine elicited antibodies binding to group B strains included IgM and IgG of all subclasses. All of the antisera were able to activate deposition of human complement on group B strains, but none were bactericidal with human complement. The lack of SBA may be related to observed hyperactivation of complement, which may result in improper insertion of the membrane attack complex in the bacterial membrane. Several of the antisera pools, however, were able to passively protect against challenge by group B bacteria in the infant rat model of meningococcal bacteremia.
Conclusion
NeuPSA antigens are immunogenic and elicit antibodies of all classes and subclasses that bind to *Neisseria meningitidis* and can protect against bacteremia in an animal model.

O41

Prediction of broad vaccine coverage for a bivalent rLP2086 based vaccine which elicits serum bactericidal activity against a diverse collection of serogroup B Meningococci

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Objective
The lack of a broadly effective vaccine for the prevention of *Neisseria meningitidis* serogroup B (MnB) disease led to the identification of outer membrane lipoprotein LP2086 as a vaccine candidate. This protein is common to all meningococci and was recently determined to be responsible for specific interactions with factor H leading to inhibition of the Alternative Complement Pathway. The LP2086 or human factor H binding protein family is comprised of two divergent branches or subfamilies (A and B). Our objective was to determine the optimal number of LP2086 lipoprotein variants that would be needed to achieve broad protection against meningococcal serogroup B disease.
Methods
Proteins were selected from each of the two subfamilies (A and B) and tested either as mono- or bivalent AlPO4 adjuvanted vaccines in rabbits. The functional activity of the rabbit immune serum was demonstrated against a small group of strains with a serum bactericidal assay that utilized human complement, and supported the initiation of clinical trials with the bivalent vaccine. Sera from rabbits and humans immunized with the bivalent vaccine were subsequently evaluated in an extensive survey of diverse serogroup B meningococcal strains. The LP2086 sequence and the level of LP2086 expression for each of these strains were determined to investigate possible associations or correlations between these variables and the bactericidal activity. FACS experiments with a broadly cross-reactive anti-LP2086 mAb were used to determine the antigen surface expression.
Results
Bivalent rLP2086 vaccine elicited broader bactericidal activity than either monovalent A subfamily or monovalent B rLP2086 vaccine against the meningococcal serogroup B strains tested. In this study 82 of 95 strains tested could be killed with the anti-bivalent rLP2086 sera. A broad range of LP2086 surface expression was observed on the meningococcal strains utilized in the serum bactericidal assay. LP2086 targeted killing of meningoccoci routinely occurred when the level of surface expression exceeded an expression threshold. Killing elicited by the bivalent vaccine in either rabbits or humans appeared to be independent of LP2086 sequence variation with each subfamily.

Conclusions
We have demonstrated both preclinically and clinically that a bivalent rLP2086 vaccine that includes a protein from each subfamily (A and B) elicits broadly functional bactericidal antibodies against a diverse collection of serogroup B meningococci. The bivalent vaccine offers an advantage over a vaccine containing only one of the two subfamilies.

O42

Distribution and variability of 5CVMB vaccine antigen genes in a panel of strains representative of global meningococcal diversity

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Objectives
The gene distribution of gna1870 (or fhbp), gna2132 and gna1994 (or nadA), which code for the main components of 5CVMB vaccine, was studied in a panel of pathogenic strains. Correspondences between the diversity of these antigens and standard typing methods were assessed. In particular, the antigen variant distribution in the meningococcal hypervirulent clonal complexes was analysed, in order to evaluate the possibility of a vaccine coverage prediction based on MLST.
Methods
The study was performed using a collection of 85 strains representative of the meningococcal variety in terms of genetical features, geographical provenience, year of isolation [1]. All strains were genetically typed by MLST [2] and serologically typed by PorA and PorB. Full-length nucleotide sequence of the genes was determined. Several approaches and algorithms were used for the interpretation of the variability.
Results
The number of alleles observed was: 34 for gna1870; 31 for gna2132 and 13 for gna1994. The observed number of alleles for all antigens was significantly lower than the number of strains, indicating the structuring of the meningococcal population analysed, and suggesting evolution under selective pressure. No correlations were found between vaccine antigen variability and serogroup diversity, area and year of isolation. Like other antigens [3, 4], sequence variants clustered with MLST clonal complexes. In agreement with the phylogenetic trees, GNA1870, GNA1994 and GNA2132 were classified into three, five and fourteen main variants families, respectively. Each variant was subdivided into peptidic subvariants. In most cases each of the sequence variants of the three vaccine components was associated with only one clonal complex, suggesting that the antigenic repertoire of each clonal complex was unique. As of the vaccine component diversity, ST-32 showed almost complete clustering in all dendrograms, and a particularly low number of vaccine antigen variants. ST-41/44 strains usually clustered well, but several variants were displayed by this clonal complex. ST-11 was the clonal complex showing most variability.
Conclusion
ST-32 was found the most homogeneous hypervirulent clonal complex. Conversely, other clonal complexes were found associated with multiple antigen variants, ST-11 being the most heterogeneous one. As a consequence, the correlations between antigenic variability and MLST are not sufficient for predicting vaccine coverage. Therefore, sequencing of the vaccine antigen genes is important for assessing antigenic variability and, consequently, for a prediction of 5CVMB vaccine potential coverage.
References
1. Giuliani MM, Adu-Bobie J, Comanducci M, et al. Proc Natl Acad Sci U S A 2006; 103:10834-9
2. http://pubmlst.org/neisseria/
3. Urwin R, Russell JE, Thompson EA, Holmes EC, Feavers IM and Maiden MC. Infect Immun 2004; 72:5955-62
4. Callaghan MJ, Jolley KA and Maiden MC. Infect Immun 2006; 74:5085-94

O43

Evaluation of recombinant Opa proteins as vaccine candidates against hyperinvasive meningococci

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Background and Objectives
Meningococcal disease cannot be comprehensively controlled due to the lack of a vaccine against meningococci expressing the serogroup B capsule. The majority of serogroup B and C disease in the past five decades was caused by a small number of hyperinvasive lineages (the ST-8, ST-11, ST-32 and ST-41/44 clonal complexes). During decades of global spread, these clonal complexes remained stably associated with limited combinations of highly immunogenic outer membrane adhesins called Opacity (Opa) proteins. The aim of this investigation was therefore to evaluate the immunogenicity in mice of Opa proteins corresponding to the combinations found in serogroup B and C-associated hyperinvasive lineages and to assess their potential as vaccine candidates.
Methods
Fourteen opa genes were cloned, expressed recombinantly in *E. coli*, refolded, and purified using affinity chromatography prior to immunisation with solubilised recombinant protein adsorbed onto aluminium hydroxide or mixed with Freunds adjuvant. Responses in pooled murine sera were detected using a Serum Bactericidal Assay (SBA) with meningococci from the same clonal complex as targets. Total serum IgG levels were assessed by whole cell ELISA against the same isolates and the specificity of the antibodies determined by Western immunoblot. Immuno-dot blot analysis was used to detect cross reactive anti-Opa antibody responses among clonal complexes which was followed by SBA to determine whether these responses were bactericidal.
Results
SDS-PAGE and MALDI-TOF mass spectroscopy were used to confirm purity and identity of purified Opa proteins. All 14 Opa proteins elicited bactericidal antibodies against at least one meningococcal isolate with SBA titres higher when the Opa proteins were mixed with Freunds adjuvant than with aluminium hydroxide. The percentage of isolates in each clonal complex killed in the SBA (defined as a titre of greater than 1:4 with at least one Opa antiserum), was 83% (ST-8 complex), 100% (ST-11 complex), 75% (ST-32 complex) and 71% (ST-41/44 complex). Serum samples that did not kill target meningococci in the SBA were shown to still contain anti-Opa antibodies by whole cell ELISA. Immuno-dot blots and subsequent SBAs showed that some anti-Opa antibody responses were cross reactive among clonal complexes. Finally, immuno-electron microscopy was performed to visualise the binding of antibodies to the surface of meningococcal isolates.
Conclusion
Immunisation with a panel of recombinant Opa proteins induces bactericidal antibody against the majority of meningococci from a collection representing the hyperinvasive lineages.

O44

Evolution of lineages and virulence in *Neisseria meningitidis*

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Objectives
Neisseria meningitidis is an example of a human commensal bacterium that occasionally causes life-threatening disease. The propensity to cause invasive disease is associated with particular meningococcal lineages that persist over many decades and during global spread. Models of lineage structure arising through purely neutral processes cannot account for the long-term persistence of these virulent lineages. This study aimed to investigate how lineages could persist in the presence of high levels of horizontal genetic exchange and how some of these could be particularly associated with disease when it plays no part in host-to-host transmission.
Methods
A modelling approach combined with molecular epidemiological data was used to test whether immune selection could explain both the persistence of multiple discrete meningococcal lineages and the association of a subset of these with invasive disease. A deterministic model of competition between lineages was created based on the novel assumption that the combinations of allelic variants of housekeeping genes that define these lineages were associated with very small differences in transmission efficiency among hosts. Further, a stochastic model of immune selection with lineage structure was developed in which strains were defined both by their combination of housekeeping genes and two variable antigenic determinants.
Results
In the lineage competition model, all possible genotypes could co-exist under low levels of competition, with the relative abundance of these variants being dependent upon their respective transmissibility. As competition increased, the genotypes with lower transmissibility declined in frequency and were no longer able to carry excess virulence as this had the effect of further reducing their transmissibility rendering them uncompetitive. With further competition, some of these genotypes dropped out and the population was dominated by only a few of the many possible combinations of housekeeping genes, each constituting a distinct lineage, with excess virulence only emerging among the more transmissible lineages which were able to tolerate the penalty.
Simulations with the immune selection model showed that, when selection was sufficiently high as to generate discrete antigenic types, lineages followed one of three distinct trajectories: i) when differences in transmissibility were low, each lineage was stably associated with an antigenic type; ii) as the differences in transmissibility among lineages increased, their relationship with antigenic type fluctuated over time, with the lineages oscillating in dominance within each antigenic type; and, iii) once the differences in transmissibility between two lineages were sufficiently large, the lineage with lower transmissibility could not be sustained in the population.
Conclusion
The patterns of variation in housekeeping genes and PorA antigen sequences from carried meningococci isolated in the Czech Republic over a twenty-five year period were consistent with housekeeping genes encoding differences in transmissibility with competition among lineages present but incomplete. These findings have general implications for the emergence of lineage structure and virulence in recombining bacterial populations.

O45

Sequence diversity of vaccine candidate LP2086 in *Neisseria meningitidis* serogoup B strains causing invasive disease

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Objective
Recombinant forms of LP2086 (also known as Factor H binding protein), a lipidated surface protein of *N. meningitidis,* are currently undergoing clinical trials as a vaccine against serogroup B meningococcal disease. The present study is an extensive survey and phylogenetic analysis of the sequence diversity of LP2086 in current invasive isolates of serogroup B *N. meningitidis.*
Methods and Results
Sequence of LP2086 was obtained from 1,837 invasive disease serogroup B isolates from the US, Europe, New Zealand and South Africa. We found 218 unique nucleotide variants of the mature fHBP gene, encoding 173 unique protein variants. Phylogenetic analysis confirms that LP2086 sequences fall into one of two subfamilies, A or B (figure 1), containing 74 and 99 unique protein sequences, respectively. Subfamily B variants are found in 70% of all strains, and subfamily A variants in 30% of strains. There is ›83% protein identity within a subfamily but only 60-75% identity between the two subfamilies. 44% of all residues are invariant among both subfamily A and B proteins. One variant, isolated twice (A62), is an apparent subfamily A-subfamily B recombinant. Minimum spanning tree analysis shows that 75% of variants, representing 96% of isolates belong to complexes within which the distance between members is ≤ 5 amino acid differences. Phylogenetic network analysis subdivides the A subfamily proteins into 4 subgroups with multiple and equally likely paths among them. Examination of the alignments further reveals that the A subfamily sequences contain one of two N-terminal domain variants and one of two C-terminal domains, which have apparently recombined to produce all 4 possible combinations. A rare third form of the N terminal domain, N3, is an apparent recombinant between N1 and N2. Although network analysis of subfamily B does not yield such obvious groupings, recombination events similar to those in the A subfamily are evident in the N-terminal domains of the subfamily B sequences.
Conclusion
Two subfamilies of LP2086 sequences were confirmed by this survey of recent invasive isolates. As serum bactericidal activity against LP2086 is known to be largely subfamily-specific, a bivalent vaccine will likely be necessary and sufficient to provide full coverage. Within families, there is heterogeneity of sequence and evidence for recombination events between families. The pattern of natural sequence variation of LP2086 suggests that the evolution of the A and B subfamilies, which split early in evolution of this protein, are now operating under different constraints, which may reflect subfamily differences in the interactions between the N- and C-terminal domains.

O46

The presence and genetic diversity of three meningococcal vaccine candidate genes, *nadA*, *fHbp* and *gna2132*, in ST-269 clonal complex isolates in England and Wales

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Objectives
The poorly immunogenic capsular polysaccharide and considerable diversity among immunodominant surface epitopes of group B meningococci (MenB) have greatly hindered the pursuit of a universal vaccine against meningococcal disease (Md). Outer membrane vesicle vaccines have proven highly successful against clonal epidemics, however, these are tailor made and exhibit moderate cross-protectivity at best. A number of proteins: factor H-binding protein (fHBP), Neisserial adhesin A (NadA) and GNA2132 have aroused considerable interest as potential universal Md vaccine components. Preliminary studies regarding surface expression, immunogenicity and cross reactivity, across several of the major invasive lineages, have been promising and a number of vaccines incorporating one or more of these components are currently under investigation. We examined the presence and genetic diversity of *fHbp*, *nadA* and *gna2132* in clinical isolates of the ST-269 clonal complex (cpx269) that has rapidly emerged to account for over 40% of MenB disease in England and Wales.
Methods
Isolates used in the study consisted of all England/Wales cpx269 clinical isolates received by the Health Protection Agency Meningococcal Reference Unit in the January of 2001 (n=15), 2005 (n=32), 2006 (n=41) and 2007 (n=25). PCR was performed on genomic DNA extracts using primers against *fHbp*, *nadA* and *gna2132*. The amplicons were then sequenced and assigned to their respective variants/s
Results
fHbp – Eleven *fHbp* variants were identified, one of which accounted for the majority of cpx269 isolates. Most of the remaining isolates harboured one of two lesser variants.
gna2132 – The majority of isolates harboured one of two closely related variants of 1284 and 1278bp respectively. These were found to share 96% identity at the amino acid level and contained several common indels. Six minor variants were also identified.
nadA - Preliminary data suggest that ‹3% of isolates harboured the *nadA* gene.
Conclusion
Though several variants of *fHbp* were accounted for, previous studies have indicated significant levels of cross-protectivity between related subvariants. *gna2132*, on the other hand, appeared to be relatively well conserved amongst these isolates. The lack of *nadA* is, perhaps, not surprising, given that only approximately 50% of *all* isolates tested are known to harbour the gene. The significance of these findings will become apparent in downstream studies including immunogenic characterisation of each variant and functional assays using post-vaccination sera on representative strains. Possible temporal trends will be confirmed/refuted as isolates from the adjoining years are characterised and MLST data become available for January 2008 isolates. The high degree of diversity across MenB isolates as a whole, precludes the likelihood that a universal vaccine would consist of a single, highly conserved MenB antigen. A combination of these antigens or their variants may, however, provide the first realistic opportunity for the development of a broadly cross-reactive vaccine.

O47

The haemoglobin receptor protein: a novel virulence determinant of *Neisseria meningitidis*

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Objectives
The haemoglobin receptor protein, HmbR, enables *N. meningitidis* to use haemoglobin (Hb) as a source of iron. Its role in invasion was investigated by analyzing the distribution of the gene in three collections of isolates containing both disease and carried meningococci dating from 1937 to 2000 and representative of all of the major serogroups and sequence types.
Methods
The isolate collections comprised: (i) 107 mainly disease-associated isolates assembled globally from 1937 to 1996 (Maiden et al PNAS 1998 95:3140-3145), (ii) 211 carried and invasive isolates forming part of a collection assembled in 1993 in the Czech Republic (Jolley KA et al J Clin Microbiol 2000 38:4492-4498) and (iii) 468 isolates obtained in England and Wales between 1999 and 2000, of which 271 were carried and 197 were disease-associated (Maiden MC et al J Infect Dis 2008, 197:737-743).
Isolates were PCR screened for the presence of the hmbR gene. The gene is located on a genetic island capable of being exchanged for multiple cassettes consisting of the putative proteins Exl2, Exl3 followed by other unidentified ORFs (Kahler CM Infect Immun 2001, 69:1687-1696). As a result, the complete genetic island was amplified and sequenced in isolates lacking the hmbR gene revealing the presence of exl2, exl3 or other ORFs.
Results
A statistically significant association between disease and the presence of the gene among the hyper-virulent clonal complexes was apparent with clonal complexes ST-4, ST-5, ST-8, ST-11, ST-18, and ST-32 exclusively hmbR positive. Few disease isolates were found lacking the gene with 11 (14%), 3 (7%) and 5 (3%) hmbR negative isolates in all three collections indicative of the significant association of the gene with virulence.
Isolates lacking hmbR were among clonal complexes ST-1, ST-22, ST-23, ST-41/44, ST-60, ST-106, ST-198, ST-549 and many sequence types currently unassigned to a clone complex. The exl2 exchangeable cassette was principally associated with serogroup Y ST-23 clonal complex isolates, while those belonging to clonal complexes ST-1, ST-106, ST-549, ST-22 and ST-60 accounted for the majority of the exl3 exchangeable cassettes found.
Conclusion
This study revealed that the hmbR gene was a virulence determinant being necessary but not sufficient for invasion. Indeed, meningococcal virulence is polygenic requiring multiple determinants including the capsule. In the present study, only 19 (6%) of the disease *N. meningitidis* isolates from all three isolate collections lacked the hmbR gene with both exl3 and exl2 strongly associated with carriage. Possession of the hmbR gene may provide an additional means of iron acquisition from haemoglobin for meningococci in the bloodstream. These data reveal the potential of hmbR in future vaccine research.

O48

Molecular characterization of invasive serogroup Y *Neisseria meningitidis* strains isolated in Latin America region

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Within the context of SIREVA II, a Latin-American and Caribbean network managed by the Pan American Health Organization focused in bacterial meningitis and pneumonia, an increase in serogroup Y invasive meningococcal cases has been notified in Colombia since 2004, raising more than 30% of the cases. Additional increases have been also notice in Argentina and Costa Rica. By contrast, group Y meningococci are only sporadically isolated in other latinamerican countries like Brazil and Chile. In order to improve the knowledge of the serogroup Y meningococcal disease in five Latin-American countries, we analyzed group Y strains received in the National Institutions of these countries over a 7 years period (2000-2006).
The Institute Carlos III of Spain, acting as Regional Reference Laboratory, processed 182 group Y isolates (62, 45, 21, 38 and 16 strains were sent by Argentina, Brazil, Chile, Colombia and Costa Rica respectively). Serotyping and serosubtyping were done by whole-cell ELISA. The genotype (porB and/or porA sequence) was done in non-typable (NT) and/or non serosubtypable (NST) strains, but also in a random sample of typable strains (162 strains). Sequence Type, clonal complex and fetA allele designation was done according to the Neisseria MLST website (http://pubmlst.org/neisseria/)
In Argentina, most strains were NT:P1.5 (59.7%); 37.8% and 24.4% isolates were NT:P1.5 and 14:NST respectively in Brazil; 38.1% showed NT:P1.5 antigenic combination in Chile; 14:NST strains were predominant in Colombia (55.3%) and finally 14:P1.10 was the most frequent combination in Costa Rica (56.3%). In general, serotype 14 appeared associated with either serosubtype P1.10 (genotyped as PorA5-2,10-12) or NST characterization (genotyped as PorA5-2,10-1). Both types of isolates belong to the CC ST-23, showed the same fetA allele F4-1, suggesting a common origin, that can not be speculated for other antigenic combinations found in this study.
Thirty four STs were found (table 1) but most of the strains (95.6%) appeared to be distributed in only 3 groups: 2 defined by clonal complexes (CC ST-23 and ST-167) and the other one by ST5770 (without CC assigned) and STs representing single or locus double variants of ST5770. Two differentiated situations were noticed: the first one which might be defined as "epidemic", observed only in countries with increase of serogroup Y cases (Argentina, Colombia and Costa Rica), with a predominant clone all over the period (CC ST-23 in Colombia and Costa Rica and CC ST-167 in Argentina); and the second one, defined as "endemic"without a dominant clone (Brazil and Chile).
In conclusion, the situation observed in Colombia but also in Costa Rica is similar to that happen in United States, particularly in the prevalence of STs belonging to the CC ST23. It is likely that the situation in Argentina is result of an independent event, with data suggesting a probable epidemic wave with a particular ST predominating. Although antigenic shift involving multiple OMP genes have been associated with increase in the incidence of serogroup Y infection, this study does not confirm this association. Further studies are necessary to evaluate the use of new conjugated vaccines in the region.

Table 1: Distribution of invasive serogroup Y meningococci by country of origin, antigenic combination and MLST definition

Antigenic Expression	Country	ST (n° of isolates) 1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total n° of Isolates (182)	Clonal Complex
2a:P1.2	Brazil									11(1)		1	11
4:P1.5	Argentina	1624(1)			1624(4)	1624(1)	1624(2)	1624(2)	1624(2)	1624(2)	1624(4)	18	167
	Brazil						6528(1)					1	None
	Chile				1624(1)							1	167
4:NST	Argentina							23(1)		23(1)		2	23
	Brazil									6533(2)		2	23
14:P1.3	Argentina										23(1)	1	23
14:P1.5	Argentina	6518(1)										1	None
	Brazil				6525(1)							1	None
	Chile				5156(1)							1	None
	Colombia							23(1)				1	23
14:P1.5,2	Brazil					23(1)	6653(1)				5770(1)	2/1	23/None
	Colombia										5024(4)	4	23
14:P1.10	Brazil										6532(1)	1	23
	Colombia									23(1)		1	23
	Costa Rica		23(1)		23(1)		23(3)	23(2)	23(2)			9	23
14:P1.12	Brazil				23(1)							1	23
14:NST	Argentina						6519(1)	23(1)				2	23
	Brazil						6527(1)	6519(1)	6527(1)	6530(1)6531(1)	6525(1)	10/1	23/None
								23 (1)		6532(1) 23(2)	6527(1)		
	Chile					23(1)			23(1)			2	23
	Colombia	6522(1)	23(1)		23(1)		23(1)	23(1)	23(8)	23(3)	23(5)	21	23
	Costa Rica					23(1)			23(1)			2	23
15:NST	Chile									1768(2)		2	None

Period not included in the study

Table 1 (continuation)

Antigenic Expression NT:P1.5	Country	ST (N° of isolates) 1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total n° of isolates (182)	Clonal Complex
	Argentina		1624(1)	1624(2)	1624(2)	1624(4)	1624(4)	1624(9)	1624(6)	1624(3)6599(1)	1624(1)	33	167
							6518(3)	6518(1)				4	None
	Brazil				5770(1)	5770(2)	5770(1)	5770(2)		5770(3)	5770(2)	11	None
							6525(1)			6525(1)		2	None
					6526(1)			3039(1)		6529(1)167(1)		4	167
	Chile					1624(1)	5770(1)	1624(1)	1624(1)	5770(1)		3/2	167/None
									6524(1)	6523(1)	6523(1)	3	None
	Colombia		6521(1)						6521(1)	6521(1)	6521(2)	5	167
										409(1)		1	41-44
NT:P1.5,2	Chile				5143(1)					23(1)		2	23
	Colombia							23(2)	23(1)			3	23
	Costa Rica	2947(3)	23(1)				6600(1)					5	23
NT:P1.5,10	Brazil										6534(1)	2	None
											6525(1)		
NT:P1.7,1	Brazil						5770(1)					1	None
NT:P1.14	Colombia					6521(1)						1	167
NT:NST	Argentina							6520(1)				1	41-44
	Brazil				5770(1)		6626(1)		844(1)		6525(1)	3/1	None/174
	Chile				23(1)		5770(1)				5770(1)	1/2	23/None
							1624(1)				1768(1)	1/1	167/None
	Colombia										23(1)	1	23

Period not included in the study

O49

Why stop MeNZB vaccination? Supporting evidence for withdrawal of epidemic strain outer membrane vesicle (OMV) vaccine in New Zealand (NZ)

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Objective

Consideration of the safety of, desirability of, and evidence for withdrawing the NZ epidemic strain OMV vaccine (MeNZB) as the epidemic subsides

Methods

Review of meningococcal disease (MD) surveillance data by region, age, ethnicity, vaccine status, and vaccine coverage in light of available MeNZB vaccine functional antibody decay data.

Results
In area of highest historical overall MD rates (peak 31/105 1997 80% epidemic strain) and highest population concentration (Auckland 2006 population 1.3 million) MD rates following MeNZB introduction (offered to 0-19 years of age 2004/05 and continued in infant vaccine schedule) continued to fall (2004,2005,2006,2007 : 10.6/105, 5.3/105, 3.5/105, 2.3/105). Cases of epidemic strain disease in ‹5 yr (2004-07) were concentrated in South Auckland (39/58, 67%) where disease rates reached a nadir in 2005 (2004:50/105; 2005:8/105) rising in 2006/07 (2006:24/105; 2007:21/105). Disease continued to be concentrated in the population groups at highest risk (0-4 yr Maori/Pacific 2005-07, 10/105, 41/105, 36/105).
Vaccine coverage (3 doses) in the mass campaign was high (all NZ 80%, South Auckland 92%) but in the follow-on period in the infant vaccine schedule with a four dose priming series the coverage was low (‹50% NZ and South Auckland). Vaccine failures were infrequent.
Conclusion
In light of the rapid decay of MeNZB vaccine functional antibody in infants and school children (Jackson C et al ICAAC Abstract G-1698/324) and low vaccine coverage for infants, 2006/07 has in effect constituted an informal MeNZB withdrawal, without resurgence of disease to date. This has led to the recommendation that MeNZB be officially withdrawn on 1 June 2008. The added utility of offering routine infant vaccination in addition to a mass campaign could be debated.

O50

Seroprevalence of antibodies against serogroup C meningococci in England in the post-vaccination era

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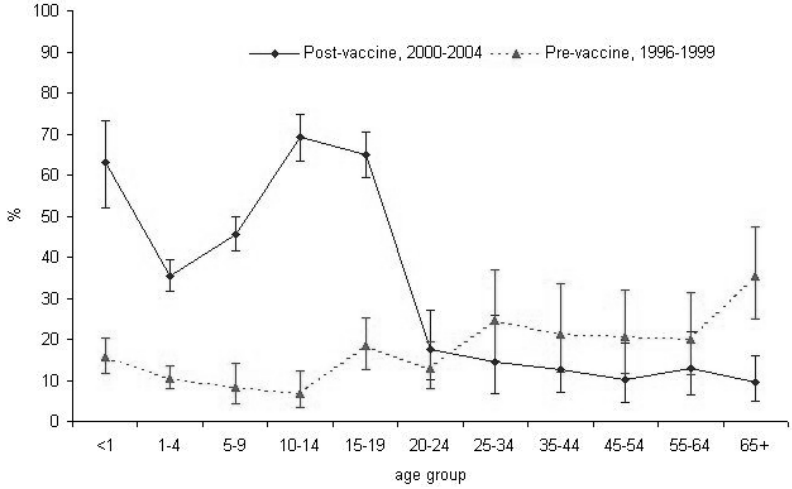
Objective
To describe the population prevalence of serogroup C serum bactericidal and IgG antibodies in England one to five years after the introduction of meningococcal serogroup C conjugate (MCC) vaccines in 1999/ 2000.
Methods
Serum samples collected between 2000 and 2004 were obtained from the Health Protection Agency Seroepidemiology Unit, which collects residual sera from participating laboratories across the country to enable serological surveillance of the National Immunisation Programme. Serum bactericidal antibody (SBA) against serogroup C strain C11 was determined for 2,396 sera using a standardised complement-mediated SBA assay with baby rabbit complement. The age-specific percentage of individuals with SBA titres ≥ 8 was calculated, together with 95% confidence intervals (CI). Results were compared with a pre-vaccine (1996-1999) seroprevalence study. In addition, serogroup-specific IgG concentrations were determined in 2664 sera using a tetraplex IgG bead assay for serogroups A, C, W-135 and Y. The age- and serogroup-specific geometric mean concentrations (GMC) for IgG and 95% CI were calculated. The year of and age at sample collection was used to infer the likely timing and schedule of MCC immunisation for each individual. In under 20 year olds all samples were taken from 2001 onwards, after the end of the MCC catch-up campaign.
Results
The percentage of individuals with SBA titres ≥ 8 was higher in the post-vaccine era for all age groups targeted for MCC vaccination (0-24 years), compared to the seroprevalence in 1996-1999 (pre-vaccine) as shown in figure 1. In the youngest children (‹1 year old), who were targeted for MCC vaccination at 2, 3, 4 months of age, 63.1% (95% CI 51.9 to 73.4%) had SBA titres ≥ 8. A higher proportion of children aged 6-11 months had protective SBA titres compared to those aged less than 6 months (p = 0.0003). In the 1-4 year age group, who would either have been offered MCC vaccine in the routine infant immunisation

schedule (˜60%) or as infants and toddlers as part of the catch-up campaign (˜40%), only 35.4% (95% CI 31.6% to 39.4%) had protective SBA titres. In children aged 10 -19 years, who received vaccine at older ages as part of the catch-up campaign, the percentage with SBA titres ≥ 8 is higher. In adults over 25 years the percentage with SBA titres ≥ 8 was lower in the post-vaccine era, although CI overlapped in all groups apart from the over 65s.
A similar age-specific pattern was observed for serogroup C IgG GMC. In contrast, GMC of IgG for serogroups W-135 and Y were low, and showed little variation by age. The serogroup A-specific IgG GMC was higher, possibly reflecting exposure to cross-reacting antigens.

Conclusions

Serological surveillance, in combination with disease surveillance, can provide important information for vaccine policy makers. The population antibody levels to serogroup C meningococci should continue to be monitored, particularly as children who received routine MCC immunisation at 2, 3 and 4 months or during early childhood as part of the catch-up campaign, mature into teenage when the risk of meningococcal disease increases.

Figure 1: Percentage (and 95% CI) of sera with serogroup C SBA titres ≥ 8 by age in the pre- and post-vaccine era



O51

Evidence of capsular switching in invasive *Neisseria meningitidis* Isolates in the pre-meningococcal conjugate vaccine era, United States, 2000-2005

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Background

A quadrivalent (A/C/W-135/Y) meningococcal conjugate vaccine (MCV4) was licensed in the United States in 2005 and recommended for all adolescents; no serogroup B vaccine is available in the U.S. *Neisseria meningitidis* has a highly plastic genome, which in response to MCV4-induced immunity could

allow for the emergence of virulent serogroup B strains that have undergone capsular switching. We examined the population structure of invasive meningococcal isolates in the U.S., 2000-2005, with a focus on apparent capsular switching, to serve as a baseline so that changes following licensure of MCV4 can be identified.

Methods

Meningococcal isolates that were collected as part of active, population-based surveillance at 10 Active Bacterial Core Surveillance (ABCs) sites throughout the United States from 2000-2005 served as the basis for this study. Isolates were characterized by multilocus sequence typing (MLST) and genetic relatedness was determined using minimum spanning tree analysis. Evidence for capsular switching in an isolate was defined as the presence of an unusual ST-serogroup combination; specifically, a genetic sequence type (ST)/clonal complex in an isolate of a serogroup not generally associated with that clonal complex and commonly associated with another serogroup. Laboratory confirmation of apparent capsular switching is ongoing by repeating the serogrouping and MLST on the same template DNA.

Results

The study occurred during a period of unusually low and declining meningococcal incidence in the United States (0.78 and 0.33 cases per 100,000 population in 2000 and 2005, respectively). There were 1,180 isolates available for study, 518 (43.9%) serogroup B, 281 (23.8%) serogroup C, 319 (27.0%) serogroup Y, and 62 (5.3%) other serogroups or nongroupable. Of serogroup B isolates, 8 (1.5%) had evidence of capsular switching: 4 were ST-103 complex (generally associated with serogroup C), 2 were ST-11 complex/ET-37 complex (serogroup C), 1 was ST-22 complex (serogroup W-135), and 1 was ST-334 complex (serogroup C). Of serogroup C isolates, 32 (11.4%) had evidence of capsular switching, all to serogroup B: 3 were ST-269 complex, 13 were ST-32 complex/ET-5 complex, 7 were ST-35 complex, 7 were ST-41/44 complex/Lineage 3, 1 was ST-461 complex, and 1 was ST-60 complex. Of serogroup Y isolates, 2 (0.6%) had undergone apparent capsular switching: both belonged to the ST-22 complex (serogroup W-135). There was substantial geographic clustering of capsular switches. For example, 7 of 13 strains with evidence of capsular switching in Oregon were the serogroup ST-32/ET-5 complex serogroup C to B "Oregon clone"switch that was previously described. This strain was also observed in 4 of 12 Minnesota isolates with evidence of capsular switching but in no other ABCs site.

Conclusions

These preliminary results suggest that, before licensure of MCV4, a substantial number of invasive meningococcal isolates had evidence of transformation events leading to capsular switching. Although the timing of the switches is unclear, substantial geographic clustering was observed. Given the lack of a serogroup B vaccine in the U.S., the circulation and spread of pathogenic serogroup B strains with STs generally associated with MCV4 serogroups, is of potential concern and requires ongoing laboratory surveillance.

O52

The effect of quadrivalent (A, C, Y, W-135) meningococcal conjugate vaccine on serogroup-specific carriage of *Neisseria meningitidis*

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Objectives

Quadrivalent (A, C, Y, W135) meningococcal polysaccharide-protein conjugate vaccine (MCV4, sanofi pasteur, Swiftwater, PA) is recommended for all adolescents in the US. Adolescents are common reservoirs for carriage and transmission of *Neisseria meningitidis.* Conjugate vaccines can interrupt acquisition of carriage and transmission of bacterial pathogens, though the impact of MCV4 on carriage is unknown.

Methods
We assessed the epidemiology and dynamics of meningococcal carriage and evaluated the effect of MCV4 on acquisition of carriage of serogroup Y *N. meningitidis* among US high school students in Georgia and Maryland. We conducted a field trial in eight schools; 4 were randomized to receive MCV4 at the start of the trial (vaccinated group), and 4 offered vaccination at the conclusion (control group). Participating students underwent pharyngeal swab for isolation of *N. meningitidis* at three time points (baseline, and approximately 6 weeks and 8 months later) during a single school year. Species and serogroup identification of all isolates were performed using standard methods. Serogroup was also identified by PCR. All isolates were further characterized using multi-locus sequence typing.
Results
A total of 3314 students were enrolled; 1636 in the vaccinated schools and 1442 in the control schools. Vaccination group and control groups were similar with respect to gender (44.3% versus 46.3% male, p=0.25) and Hispanic ethnicity (7.1% versus 5.6%, p=0.18). Vaccination group students were less likely than control group students to report smoking (9.6% versus 12.5%, p=0.01), less likely to be white (57.8% versus 63.6%, p<0.05), and more likely to be Asian (2.7% verses 1.4%, p<0.05) or other/unknown race (4.8% versus 3.2%, p<0.05). Sixteen students carried serogroup Y *N. meningitidis* at baseline; 10 (0.58%) in the vaccinated group and 6 (0.39%) in the control group (p=0.44). Baseline carriage of serogroup B was 1.0% in the vaccinated and 1.1% in the unvaccinated group. Carriage of non-groupable isolates was found in 1.9% of the vaccinated and 1.4% of the unvaccinated group students, while serogroup A, C, and W135 carriage were not found. Twenty-five students carried serogroup Y at time point 3; 13 (0.85%) in the vaccinated group and 12 (0.89%) in the control group (p=0.91). Neither group exhibited a statistically significant change in serogroup Y carriage, nor did the rate of acquisition of carriage at subsequent time points among baseline non-carriers differ significantly in either group. Nine (56%) of 16 students with serogroup Y carriage at baseline, 5 in the vaccinated group and 4 in the control group, carried serogroup Y at all three time points. All 9 carried the same sequence type at each time point; 5 ST-23 and 4 ST-183 (both ST-23 complex, Cluster A3).
Conclusion
The frequency of serogroup Y carriage observed in this study was substantially lower than observed previously in similar settings, and limited our power to detect an effect of vaccination on prevention of acquisition of carriage. A high proportion of carriers of serogroup Y had carriage of the same sequence type at each survey, suggesting a prolonged carrier state for ST-23 clonal complex serogroup Y.

O53

Caveolin recruitment prevents premature host cell entry of type IV pili producing Neisseria

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Objectives
Type IV pili (Tfp) confer the initial attachment of piliated *Neisseria gonorrhoeae* (Ngo P+) to human mucosal surfaces. This early-localized adherence precedes the formation of dense bacterial microcolonies on the surface of host cells. The initial pilus-mediated binding of Ngo P+ is characterized by localized enrichment of filamentous actin and a subset of signal transducing proteins beneath bacterial attachment sites. The same proteins have also been found to be associated with lipid rafts and caveolae. The recruitment of caveolin 1 (Cav1), the principal structural component of caveolae, to neisserial infection sites could be demonstrated in our group. We consequently aimed to investigate the role of caveolin 1 recruitment during Ngo P+ infection.

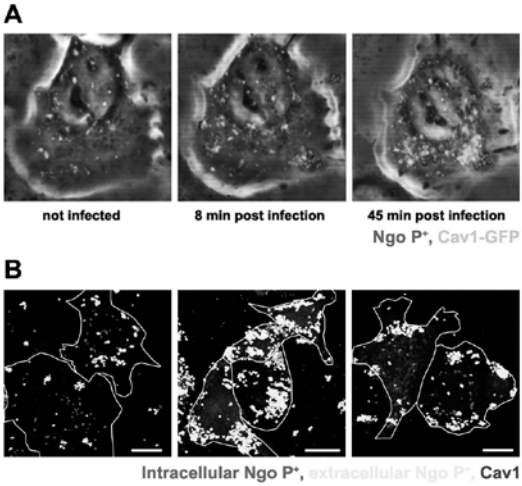
Methods and results

Confocal and electron microscopy demonstrated that attachment of piliated Ngo P+ to human epithelial cells induces recruitment of Cav1 to infection sites. Confocal live cell microscopy further revealed that this cellular response is instantly triggered even by single piliated diplococci. Invasion assays showed that down-regulation of Cav1 in the host cell leads to bacterial uptake. We consequently demonstrated by construction of the phosphorylation-defective mutant Cav1-Y14F and its expression in a Cav1-negative background that the impediment of bacterial uptake as well as the recruitment of Cav1 is dependant on tyrosine-phosphorylation of Cav1. Furthermore our results indicate an interplay of Cav1 with the actin-crosslinking protein filamin at infection sites. Conjoint with Cav1, filamin is recruited to Ngo P+ infection sites and down-regulation of filamin leads to bacterial uptake by the host cell.

Conclusion

Our results indicate that tyrosine-phosphorylation dependant Cav1 recruitment, in conjunction with F-actin and filamin, effectively prevents bacterial uptake by host cells. We conclude that this is how initial host cell binding via Tfp transiently stabilizes an extracellular bacterial state which precedes consequent secondary infection steps (e.g. host cell entry via Opa protein expression).

Figure 1



Expression of phosphorylatable Cav1 is necessary to prevent uptake of piliated *Neisseria gonorrhoeae* (Ngo P+) by host cells.

(A) Movie excerpts: Cav1-GFP (green) is recruited to Ngo P+ microcolonies as to individual attached bacteria (red) in ME-180 cells.

(B) Expression of Cav1 phosphorylation-defective mutant Cav1-14F in Cav1-negative AGS cells does not prevent bacterial uptake. AGS cells transiently expressing Cav1 or Cav1-Y14F were infected with Ngo P+ for 2 hours. Intracellular bacteria (red) are detected in AGS cells and AGS cells expressing Cav1-Y14F, but not in AGS cells expressing wildtype Cav1. Extracellular bacteria appear in yellow. Scale bars: 20 µm.

O54

A functional two-partner secretion system in *Neisseria meningitidis* contributes to adhesion

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Background and objectives
Several large exoproteins in various gram-negative bacteria are predicted to be secreted via a Two-partner secretion (TPS) system. However, relatively few of these proteins have been investigated in detail. The most extensively characterized of these exoproteins, the filamentous hemagglutinin (FHA) of *Bordetella pertussis*, is an important virulence factor of Bordetella and constitutes the main component of the pertussis vaccine. In general, FHA and related proteins, termed TpsA for two-partner-secretion-protein A, are translocated across the outer membrane via a specific transporter termed TpsB. All four published meningococcal genomes contain ORFs encoding putative TpsA (termed HrpA, for hemagglutinin-related protein A, in meningococci) and TpsB (termed HrpB) proteins as indicated by sequence similarities to known TPS systems. We have previously shown that the genes encoding HrpA and HrpB proteins are present in virtually all meningococcal clonal complexes, and that whereas the hrpB genes seem to be highly conserved, there is a high degree of C-terminal sequence variation between hrpA genes of different clonal complexes. The purpose of this study was to investigate the role of the putative HrpA and HrpB proteins in the pathogenesis of meningococcal infection.
Methods
To test whether hrpA and hrpB genes are transcribed, reverse transcription PCR was performed on total RNA isolated from a selection of meningococcal strains representing hypervirulent and non-hypervirulent clonal complexes. To further investigate the expression of meningococcal hrpA genes and to assess the role of the cognate hrpB genes, a polyclonal rabbit antiserum was raised against the recombinant HrpA protein encoded by NMB1779 of strain MC58 (serogroup B, ST-32 complex), and used in Western blot experiments. Deletion mutants of hrpA and hrpB were generated for characterisation in host cell adhesion experiments.
Results
In all strains analysed, the respective hrpA and hrpB transcripts could be detected. In addition, genes encoding HrpA and their cognate HrpB transporter proteins were shown to be organised in an operon. Furthermore, by Western blot analysis of whole cell lysates and concentrated supernatants of strain MC58 and its respective ΔhrpA and ΔhrpB deletion mutants, we provide evidence that meningococcal HrpA are expressed, translocated across the outer membrane by their cognate HrpB transporter, and mainly released into the environment. A small proportion of the secreted HrpA protein remains associated with the bacterial surface and contributes to the interaction of meningococci with human epithelial cells.
Conclusion
Neisseria meningitidis contains a functional two-partner secretion (TPS) system, which contributes to the interaction of the bacterium with host epithelial cells.

O55

Opc and its multifarious receptors identification of novel mechanisms that may operate for attachment and traversal of human endothelial cells

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Neisseria meningitidis (Nm) entry into the meninges may occur by crossing the brain endothelial cells. In addition, Nm attach to and invade endothelial cells at other sites during infection. In previous studies, Nm Opc protein was shown to utilise serum factors as bridging molecules to anchor to cell surface integrins and thus efficiently adhere to and invade primary human endothelial cells isolated from umbilical veins (Mol Microbiol, 1994, 14: 173-184 & 1995, 18: 741-754). As endothelial cells at different vascular sites may exhibit variations in their receptor expression, our current studies have compared the interactions of Nm Opc invasin with brain and dermal microvascular endothelial cell lines (HBMEC and HMEC-1). Further, since integrin modulation is known to occur on cells exposed to cytokines, both unstimulated cells and those pre-exposed to inflammatory cytokines were examined in order to assess changes in bacterial interactions that may occur during disease.

First, we examined the roles of serum factors in mediating bacterial interactions at these cellular interfaces. Previous studies on HBMEC demonstrated a role for fibronectin in cellular invasion (Unkmeir et al., Mol Microbiol, 2002, 46: 933-946), we show that serum vitronectin is a major target for Opc, and is involved in serum-dependent interactions with HBMEC; although, cellular and plasma fibronectins can also mediate adhesion. However, it is the activated/unfolded form of vitronectin that is particularly targeted. We demonstrate a binding region for Opc on vitronectin, which becomes exposed in the activated protein. We also observed that purified vitronectin can acquire conformations that can result in the protein becoming a competitive inhibitor for interactions with endothelial integrins, providing a possible explanation for apparently contradictory observations reported in the literature.

In further investigations, the use of blocking antibodies to alpha5beta1, alphavbeta3 and alphavbeta5 demonstrated involvement of the three integrins, co-operation between them and the importance of vitronectin receptors. We further show that whilst endothelial cells from the two vascular sites have distinct integrin expression profiles, the levels of their surface expression do not totally correlate with serum-dependent, Nm interactions. In addition, these endothelial cells show different responses to cytokine treatments. Changes in the levels of integrins together with their activation states were shown to determine Nm adhesion/invasion. The use of several pharmacological activators and inhibitors of downstream signalling pathways demonstrated an important role of protein kinase C in cellular invasion via the integrins. Greater infiltration of brain compared to microvascular endothelial cells was observed in some situations and has implications in disease progression.

In other studies on Opc targets, we have observed that Opc can bind to a 100kDa protein present in human cell lysates. By the use of MALDI-TOF mass spectrometry, co-precipitation and antibody inhibition, the protein was identified as alpha-actinin. Confocal imaging demonstrated that Nm co-localised with this cytoskeletal protein after cellular invasion. Significance of this interaction is under investigation. As alpha-actinin is involved in the modulation of several receptor and cytoskeletal functions, binding to it may enable meningococci to influence cellular functions to facilitate their passage across human barrier cells.

O56

Infection of human cervical epithelial cells with live *Neisseria gonorrhoeae* promotes resistance to apoptosis

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Objectives
Several microbial pathogens can modulate host-cell apoptosis, which can contribute to immune evasion. Conflicting studies have reported that infection with *Neisseria gonorrhoeae* can either inhibit or induce apoptosis. The interactions of *N. gonorrhoeae* with host cells has been studied in cancer cell lines such as HeLa cells, a cervical adenocarcinoma cell line which may not accurately reflect what is occurring *in vivo*. In addition, because *N. gonorrhoeae* infections are known to exhibit different clinical disease presentations in male versus female hosts, it is important to study the cellular interactions that occur in various host cells, including gender-specific cell types. We hypothesized that *N. gonorrhoeae* confers an anti-apoptotic effect in cell types specific to the female host, such as the cervical epithelial cell lines used in these studies, which may contribute to pathogenesis.
Methods
Human cell lines derived from the female ectocervix and endocervix were incubated with N. gonorrhoeae strains FA1090B, a derivative of FA1090 which expresses only OpaB, and F62-pEG2, a GFP-expressing derivative of F62. After infection for 24h, epithelial cells were cultured under normal or apoptotic conditions and assessed for markers of apoptotic cell death. Additionally, real-time quantative PCR was used to screen several families of genes related to apoptosis following 8h of infection, while Western blot analysis confirmed protein production at both 8h and 24h post-infection.
Results
We found that incubation of human cervical epithelial cells with *N. gonorrhoeae* strain FA1090B for 24h did not result in cell death. Subsequently, apoptosis was induced using staurosporine (STS), and *N. gonorrhoeae* infection protected these cells from STS-induced caspase activation, mitochondrial depolarization, and DNA degradation in a dose-dependent manner. The protective effect of gonococcal infection was specific to individually infected cells within a population, and dependent on live bacteria, as it was not mediated by heat-killed or antibiotic-killed organisms. Purified gonococcal porin was demonstrated to contribute to the anti-apoptotic effect. Furthermore, live *N. gonorrhoeae* induced the activation of the transcription factor NF-κB, and several genes that are known to be controlled by NF-κB were significantly upregulated, including the anti-apoptotic genes bfl-1, c-IAP-2, and c-FLIP. Importantly, heat-killed bacteria were unable to induce the same transcriptional response. We also verified that the aforementioned anti-apoptotic proteins were expressed following gonococcal infection.
Conclusions
Securing an intracellular niche through the inhibition of apoptosis has been demonstrated for various bacterial pathogens, and may be an important mechanism for microbial survival or immune evasion. Importantly, although various bacteria can inhibit cell death, the contributing mechanisms are often pathogen- and cell-type- specific. Our results indicate that
N. gonorrhoeae stimulates an anti-apoptotic response in host cells which is dependent on the interaction(s) between pathogen and host that can only be reproduced with live bacteria. Additionally, the epithelial cells used in these studies represent the first susceptible cell types encountered in the female host upon infection with *N. gonorrhoeae*. Therefore, these results indicate that *N. gonorrhoeae* may establish infection in women by inhibiting the apoptotic response to infection, potentially avoiding host defenses and allowing time for intracellular replication.

O57

Aspartyl protease complex presenilin/gamma-secretase liberates intracellular peptide signals into *N. gonorrhoeae* infected host cells by cleaving CD46 Cyt1 and Cyt2 isoforms

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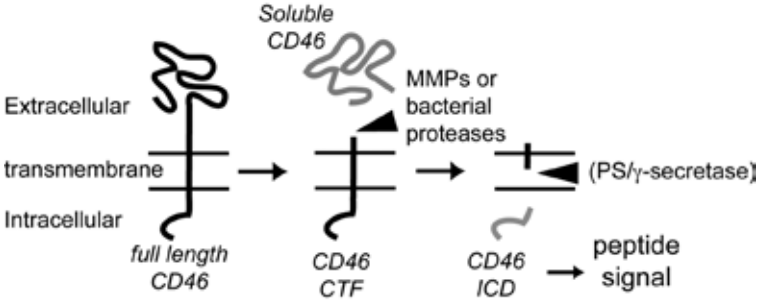
Objectives
CD46 is a multifunctional immunoregulatory protein that is best known for its role in regulating the complement cascade. It is also a target for numerous viruses and bacteria. CD46 is downregulated upon infection by several pathogens, including *N. gonorrhoeae*, i.e., infected cells have significantly reduced levels of this membrane protein. CD46 is also known to be a signaling molecule but we know little of the mechanisms by which it transmits signals. The goal of this study is to determine if CD46 downregulation by Ng involves its processing by host proteases. We focussed in particular on Presenilin/gamma-secretase because this protease complex is known to cleave Type I membrane proteins, of which CD46 is a member.
Aim 1. Establish that CD46 undergoes proteolysis in response to infection.
Aim 2. Establish the role of host cell aspartyl protease complex Presenilin/gamma-secretase in CD46 cleavage.
Methods
We have generated monoclonal antibodies to the cytoplasmic tails of the two isoforms of CD46, Cyt1 and Cyt2. We immunoprecipitated CD46 cleavage products from infected cell lyates using these tail-specific antibodies. Two approaches were used to show a role for Presenilin/gamma-secretase in CD46 cleavage: chemical inhibitors and cell lines stably expressing dominant negative Presenilins, PS1 and PS2.
Results
Ng strain MS11 (P+, Opa-nonexpressing) stimulates processing of both CD46 isoforms (CD46-cyt1 and CD46-cyt2) in several human epithelial cell lines. The cytosolic tails of both isoforms can be recovered from cell lysates by immunoprecipitation. CD46 processing is greatly enhanced by *Ng* expressing a functional PilT, the pilus retraction motor. CD46 processing is also induced by *Neisseria meningitidis* (strain 8013.6, P+, Opa-nonexpressing) and by phorbol ester treatment. Infection-induced processing is blocked by Presenilin/gamma-secretase inhibitors and in cells expressing dominant-negative Presenilin mutants.
Conclusion
We have established CD46 as a Presenilin/gamma-secretase substrate. Neisseria-induced CD46 downregulation is due at least in part to its processing by Presenilin/gamma-secretase. We hypothesize that the released cyt1 and cyt2 tails play a role in host cell signaling, like the tails of other Presenilin/gamma-Secretase substrates. Ng and Nm recruits Presenilin/gamma-secretase and several of its known substrates to the cortical plaque directly beneath adhered microcolonies. These plaques may act as a signaling platform by which proteolytic cascades liberate cytoplasmic tail signals into the infected host cell to influence infection.

Figure 1:



A model for CD46 proteolytic cleavage during Ng infection.
Host metalloproteases (MMPs) or bacterial proteases cleave the ectodomain to shed soluble CD46. The C-terminal fragment (CTF) is then cleaved by Presenilin(PS)/gamma-secretase to liberate the intracellular domain (ICD). The ICD may act as a peptide signal in the cytosol and/or nucleus. For simplicity CD46 Cyt2 and Cyt1 isoforms are depicted as one protein.

O58

Host glycoprotein Gp96 and scavenger receptor SREC interact with PorB of disseminating *Neisseria gonorrhoeae* in an epithelial invasion pathway

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Objectives
Pathogenic Neisseria express numerous variant surface proteins mediating bacterial adherence and invasion during infection. Gonococci expressing the serotype A of the major outer membrane porin PorB (PorBIA), are frequently isolated from patients with severe disseminating infections. PorBIA triggers very efficient adherence and invasion under low phosphate conditions mimicking systemic bloodstream infections. Our focus is to characterize the PorBIA-dependant invasion pathway.
Methods and results
We identified the human heat shock glycoprotein Gp96, and the scavenger receptor SREC as PorBIA-specific host cell receptors in an epithelial invasion pathway. Gonococci expressing PorBIA, but not those expressing PorB serotype B instead, bind to purified native or recombinant Gp96. Depletion of Gp96 from host cells by using RNAi technique prevented adherence, but significantly triggered gonococcal invasion. Furthermore, such invasion was blocked by chemical inhibitors of scavenger receptors, and we identified SREC as the scavenger receptor involved in PorBIA-dependant epithelial invasion. Gonococci that invade host cells by scavenger receptor SREC invade in a clathrin-dependent manner as previously shown for Listeria monocytogenes and for enteropathogenic Escherichia coli (EPEC) (Veiga et al., 2007). We could show a Clathrin recruitment as well as recruitment of other host cell proteins like actin or dynamin to the invaded gonococci. The PorBIA-dependant epithelial invasion needs only few minutes as monitored by life cell approaches. Thus it is a very fast invasion pathway. We currently study the way of the bacteria inside the host cell by co-localisation experiments with endosomes and lysosomes in more detail and identify involved signalling pathways.
Conclusion
We establish Gp96 as an anti-invasion factor and SRECs as receptors mediating host cell entry of highly

invasive disseminating gonococci. We started to understand the epithelial invasion pathways by data on a Clathrin-dependency, invasion timeframe and involved signaling.

Literature

1. Rechner C, Kühlewein C, Müller A, Schild H, Rudel T. Host glycoprotein Gp96 and scavenger receptor SREC interact with PorB of disseminating *Neisseria gonorrhoeae* in an epithelial invasion pathway. Cell Host Microbe. 2007 Dec 13;2(6):393-403.

O59

Outer membrane biogenesis in *Neisseria meningitidis*

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Objectives

Neisseria meningitidis is an excellent model organism to study outer membrane (OM) biogenesis because this bacterium can survive without the major OM component LPS (1) and because it tolerates the absence of a functional LPS transport machinery (2, 3). Thus, several genes encoding proteins involved in synthesis and transport of OM constituents have been cleanly deleted. The phenotype of such mutants is often better interpretable than depletion mutant phenotypes. Here, we report on the role of novel putative constituents of the LPS transport and the outer membrane protein (OMP) assembly machinery in *N. meningitidis*.

Methods

All studies were done with *N. meningitidis* serogroup B strain H44/76. Constructs were made using sequences flanking the gene to be inactivated, with an antibiotic resistance cassette in between. For complementation studies, the genes were placed under control of the lac promoter on a Neisseria-replicative plasmid. DNA was introduced into H44/76 by natural transformation.

Results

We inactivated the genes for the novel putative LPS transport components NMB0354, NMB0355, NMB0356, NMB1570, NMB1571 and NMB0707. Interestingly, some of these genes could only be inactivated when a complementing copy of the gene was present, showing that they are essential. Their depletion phenotypes showed similar characteristics as the phenotypes of strains lacking other LPS transport components such as MsbA and Imp, i.e. a decreased level of total LPS, indicating that these proteins indeed are involved in LPS transport. We studied the role of the periplasmic chaperones SurA and Skp in OMP assembly. Both proteins were not essential, as their respective genes could easily be inactivated. The skp mutant showed defects in porin assembly, an increased sensitivity towards several antibiotics, indicative of a compromised OM, and demonstrated enhanced OM blebbing. The surA mutant was not different from the wild-type in these features and only demonstrated a slight growth defect. NlpB and ComL are constituents of the OMP assembly machinery whose central component is the Omp85 protein. An nlpB deletion mutant was easily obtained and showed no obvious phenotype. In contrast, the comL gene could only be inactivated when a complementing copy was present. Depletion of ComL resulted in severe defects in the assembly of porins and of the Omp85 complex.

Conclusions

The identification of essential genes in *N. meningitidis* that were thought to play a role only in LPS transport, suggests that they might have an additional function. With respect to OMP assembly, we conclude that the Skp chaperone is much more critical than SurA. The ComL protein was shown to be critical for correct OMP assembly. This protein, together with Omp85, may form the core part of the OMP assembly machinery.

References

1. Steeghs et al. Nature 392:449 (1998)
2. Bos et al, PNAS 101:9417 (2004)
3. Tefsen et al. J. Biol. Chem. 280:35961(2005)

O60

Transferrin binding protein B from different pathogenic species interact with transferrin in a conserved manner

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Background
Transferrin (Tf) receptors are essential for the survival of pathogenic Neisseria. The receptor is comprised of a surface exposed lipoprotein, Tf binding protein B (TbpB), and an integral outer membrane protein, TbpA. Indirect evidence suggests that there is a conserved interaction between TbpB and transferrin in spite of the extensive sequence and antigenic variation of this surface protein. However, direct experimental evidence is currently lacking.
Objectives
The objective of this study was to compare the interaction between Tf and TbpBs from different strains and species by direct methods that could validate or refute the hypothesis regarding a conserved interaction.
Methods
Hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) experiments were initiated to provide more direct experimental information on the TbpB-Tf interaction. The procedure essentially involves three sequential steps: labeling of the proteins with heavy water (deuterium), protein digestion, and analysis of the peptides by MS to detect the heavy water label. Deuterium readily exchanges with the amide hydrogens on the surface of a protein while amide hydrogen on residues that are within the protein or involved in surface binding will be protect from HDX. Therefore, by comparing the mass spectral profiles produced when Tf is alone or in complex with TbpB, the sites of interaction can be identified. HDX-MS was employed to identify the regions on Tf that interact with the TbpB proteins from four different strains of bacteria; *Neisseria meningiditis* strains M982 and B16B6, *Haemophilus influenzae* H36, and *Moraxella catarrhalis*.
Results
The HDX-MS data revealed that there is a core group of regions on Tf that are recognized by the TbpB proteins from all four strains. In particular there were two peptides that included iron coordination residues from the C-lobe of Tf that had the most significant change in mass. These include two surface regions that are closely juxtaposed in the 'closed' iron-loaded conformation but are significantly displaced from one another in the 'open' iron-free form of the protein, which may account for the strong preference of TbpB for the iron-loaded form.
Conclusions
Our results provide direct evidence that the TbpB proteins from different bacterial strains and species interact with identical regions on Tf and offers an explanation why there is a strong preference for binding the iron-loaded form of Tf.

O61

Determination of the domain and solution structure of rLP2086, a meningococcal vaccine candidate and human factor H binding protein

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Objectives
LP2086 comprises a bicameral family of outer membrane proteins in *Neisseria meningitidis* capable of

eliciting bactericidal antibodies and more recently identified as specifically recognizing human Factor H (hFH). The ability to elicit bactericidal antibodies, which are broadly functional, makes these proteins excellent vaccine candidates. Human clinical trials are currently in progress to evaluate the potential of rLP2086 to prevent meningococcal disease. The primary objectives of our studies were to evaluate the biochemical and biophysical properties of proteins from both subfamilies, to understand the nature of the domain structure, to define the solution structure of the whole LP2086 protein and to better understand the spatial relationship of LP2086 to the outer membrane. Our secondary objective was to study the interaction of monoclonal antibodies with the N- and C-terminal domains of LP2086.

Methods

We utilized the following methodologies to study the structure and hFH binding properties of recombinant lipidated (rLP2086) and non-lipidated (rP2086) variants from both subfamilies A and B; a) Biochemical Characterization: Limited proteolysis, Circular Dichroism (CD), and Differential Scanning Calorimetry (DSC); b) Structure determination by NMR spectroscopy; c) Epitope mapping with monoclonal antibodies; d) Kinetics of hFH binding: Surface Plasmon Resonance; e) Fluorescence activated cell sorting; f) Protein and peptide ELISA.

Results

Kinetic data from surface plasmon resonance experiments indicate that the affinity of hFH for LP2086 is in the range of 24-240 nM. By limited proteolysis and DSC melting studies, LP2086 proteins appear to be composed of two domains. Protein variants from the same subfamily have very similar melting profiles. NMR spectroscopy shows that both lipidated and non-lipidated versions of LP2086 subfamily B have essentially the same structure. The N-terminal domain consists of 10 beta-strands and one short alpha-helix where six of the beta-strands form a "taco"shaped anti-parallel beta-sheet. Only the first two residues of the N-terminus are associated with the detergent micelle. The C-terminal domain consists of an eight-stranded b-barrel similar to that previously observed by others with a C-terminal fragment. The N- and C-terminal domains are connected by a structured 15 residue linker. The region immediately after the N-terminal Cys serves as a chain to link the two domains of the LP2086 proteins to the tri-Pam-Cys lipid anchor. Both N- and C-domains are recognized by monoclonal antibodies. Binding sites for monoclonal antibodies that induce bactericidal activity have been mapped to both N- and C-domains.

Conclusions

1. The LP2086 protein structure reveals a new protein fold composed of two domains constructed largely of beta-sheets.
2. A structured linker connects N- and C-domains of LP2086 and the N-terminus is chained to the lipid anchor in the membrane.
3. Both N- and C-domains are exposed and capable of interacting with antibodies both in solution and on the surface of *N.meningitidis*.

O62

Nearing success: The development of an affordable Group A meningococcal conjugate vaccine to eliminate epidemic meningitis from Africa

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Epidemic Group A meningococcal meningitis continues to be a major public health problem in Sub-Saharan Africa. Waves of epidemic Group A meningococcal meningitis occur periodically in Africa and are superimposed on high endemic rates of disease. During 2006 and 2007 Burkina Faso suffered over 45,000 cases of acute meningitis due to Group A *Neisseria meningitidis*. Over the last 20 years control measures have relied on reactive vaccination campaigns using meningococcal polysaccharide vaccines but these efforts have not eliminated these epidemics.

Objective

The Meningitis Vaccine Project (MVP) is a partnership between WHO and PATH that was established in 2001

with Gates Foundation support with the goal of eliminating epidemic meningitis through the development and widespread introduction of an affordable Group A meningococcal (MenA) conjugate vaccine.

Methods

With particular attention to vaccine target profile (elimination of meningitis epidemics), quality, cost and sustainability, MVP established and managed a novel partnership to develop a Men A conjugate vaccine using tetanus toxoid as a carrier protein that included the Serum Institute of India, Pune (manufacturer and source of tetanus toxoid); the CBER/FDA, Bethesda (conjugation method) and SynCoBioPartners, Amsterdam (source of Men A polysaccharide for initial development).

Results

Preclinical development finished in 2004 and the vaccine has been successfully tested in Phase I and Phase II and II/III clinical trials in India and Africa (1-29 years). The vaccine has been shown to be safe and highly immunogenic with serum bactericidal titers 20 times higher when compared to polysaccharide vaccine. The vaccine is expected to generate broad herd immunity. Introduction of the vaccine at public health scale is planned to begin in 2009 with large "catch up"vaccination campaigns (single dose in 1-29 year olds). For introduction in African meningitis belt countries the Men A conjugate vaccine (MenAfriVac) is priced at less than $US 0.50 per dose.

Conclusion

MVP has successfully developed and managed a "push"strategy approach for the development of an affordable new vaccine product that was of little interest to major vaccine manufacturers. Over 10 years the vaccine is estimated to prevent about 1.4 million cases of acute meningitis, over 130,000 deaths and 250,000 cases of severe disability. After wide spread introduction the Men A conjugate vaccine is expected to eliminate Group A meningococcal meningitis epidemics from Sub Saharan Africa. At a price less than $US 0.50 per dose the introduction and continued use of the vaccine in African countries is sustainable. The MVP vaccine development model is a useful paradigm that could be used to develop other products that address health care problems that are specific to low and middle income countries but hold little commercial interest for traditional vaccine manufacturers.

O63

Has MeNZBTM controlled New Zealand's epidemic?

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Objectives

MeNZBTM was introduced in mid-2004 to control a strain-specific epidemic of meningococcal B disease that began in 1991. The focus of the mass vaccine campaign was to quickly achieve high coverage. Key strategy elements were:

- control of disease incidence.
- a booster dose might be required for ‹5 year olds.
- the birth cohort would continue to receive vaccine until availability of a generic vaccine

In April 2008 a controversial announcement was made by the Ministry of Health that vaccinations with MeNZBTM would cease as from July 2008. An overview of the impact of MeNZBTM vaccine is presented.

Methods

Data used is taken from Annual Reports of the Epidemiology of Meningococcal Disease published on the NZ Ministry of Health website (www.moh.govt.nz) and from publications in the scientific literature.
Rollout of MeNZBTM was systematic, starting in July 2004 in the Northern Region of NZ where case numbers were highest, and finally implemented in the South Island during 2005. Those aged under 20 years were eligible. Except for infants, the 3-dose schedule ended in June 2006. A fourth dose was given to infants only.

Results
In 1989/90 ~50 cases of meningococcal disease were reported in New Zealand annually. From 1991 through 1996 a rapid increase in case numbers occurred with ~80% caused by the epidemic strain B:4:P1.7-2,4. Case numbers fluctuated in 1997-2000 before peaking in 2001at 650 cases (17.4/100,000). Following introduction of MeNZBTM, whereby 80% of those aged under 20 years completed three doses of the vaccine, a rapid reduction in case numbers occurred. As judged by ethnicity differences in uptake occurred. By 2007, only 47 epidemic strain cases occurred among the total of 105 cases of meningococcal disease (2.6 per 100,000 population), a rate still 1.7 times higher than the pre-epidemic rate of 1.5/100,000 and an excess of 5261 cases over the in 1989/1990 case numbers.
With over 3.1 million doses of MeNZB vaccine delivered there were no major safety issues. Considering only epidemic strain disease rates from June 2004-June 2006 and using time at risk (person-time data), a Poisson regression model calculated vaccine effectiveness as 73% (95% CI: 52-85%). Breakthrough cases occurred in all age-groups. Cases caused by non-epidemic B strain types post-vaccination with MeNZBTM occurred more frequently and earlier post-vaccination than those involving the epidemic strain (Age ‹1 yr- 6 versus 22 weeks: 1-4 yr- 38 versus 60 weeks: and 5-20yr- 43 versus 63 weeks).
Conclusion
Use of MeNZBTM to control New Zealand's epidemic of meningococcal disease has been judged as successful. Sceptics state that the epidemic was waning on its own. Based on the longevity of the Norwegian epidemic for whose population a vaccine was not delivered, it is predicted that the epidemic could have continued for a further 5-15 years causing even greater mortality and morbidity. Cuba controlled their epidemic with a strain-specific vaccine and still include their vaccine in the infant schedule. Targeted vaccines clearly have a place while debate continues around whether, or when, delivery of such a vaccine should stop.

O64

Bivalent recombinant LP2086 vaccine to provide broad protection against *Neisseria meningitidis* B disease: Immunological correlates of protection and how to assess coverage against invasive MnB strains

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Objective
Invasive meningococcal type B disease (IMD B) is rare but devastating. IMD B is responsible for 30 % of disease in the US and ›70% of all meningococcal disease in Europe. A bivalent recombinant LP2086 vaccine, also known as MnB factor H binding protein, is in development with the potential to provide broad protection against IMD B globally. Because of the rarity of MnB disease, the efficacy of this vaccine will be established using serum bactericidal antibody (SBA) responses as a surrogate for protection. Given that the vaccine target is not genetically linked to the serogroup-defining bacterial polysaccharide, an unbiased approach is needed for selecting MnB strains that will provide a true picture of the breadth of the vaccine-elicited serum bactericidal responses. We provide a proposal for such an approach, and also summarize the immune responses seen to date in the initial human trials evaluating the bivalent LP2086 vaccine.
Results and conclusion
First-in-human studies performed in young adults and adolescents have shown that the vaccine elicits SBA responses against diverse MnB clinical isolates. A dose response was observed and the response rates (percentage of vaccinees with at least a 4-fold rise in SBA titer compared to pre-vaccination levels) against multiple isolates reached 100% for the highest dosage group.
To provide the scientific basis for an unbiased selection of MnB strain for serum bactericidal analysis

of Phase III clinical trials, extensive molecular epidemiology studies of › 1200 invasive clinical isolates collected in the US, Europe and other countries were conducted. LP2086 sequences could be divided into two distinct subfamilies (A and B) that share only 60 to 75% homology. Of the isolates evaluated, 71% and 29% are members of the B and A subfamilies, respectively. Currently, PorA and MLST typing are used to epidemiologically categorize MnB clinical isolates. We determined that PorA and MLST type did not predict isolate susceptibility to killing in the SBA assay using anti-LP2086 human immune sera. We found however, that sequence and bacterial surface expression of LP2086 are predictors of strain susceptibility to killing by immune sera. We also observed extensive LP2086 sequence diversity and expression level differences within most MLST groups and conclude that MLST typing cannot be used as a basis for selecting MnB isolates to demonstrate the breadth of the anti-LP2086 immune response. We propose that an appropriate statistical approach based on the target protein's sequence and surface expression as well as the overall MnB strain diversity is required for MnB strain selection to support Phase III clinical evaluation. The details of the proposed approach, which may have general applicability to other protein-based vaccines, will be presented.

O65

The application of reverse vaccinology, Novartis MenB vaccine developed by design

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Background and aims
Invasive disease caused by *Neisseria meningitidis* serogroup B is generally not caused by a single clone but by a number of hypervirulent lineages, driving the search for conserved surface structures as vaccine candidates. One approach has been to combine uniformly conserved recombinant surface proteins, identified from the meningococcal genome. Early studies demonstrated good safety and immunogenicity profiles of the selected vaccine candidate and confirmed that the genomic approach to identify novel, potent, surface-exposed antigens able to induce bactericidal antibodies is a promising tool to develop new vaccines. The objective of the development project was to determine the right combination of antigens in order to provide broad and efficient protection against invasive meningococcal disease overtime
Methods
The immunogenicity of vaccinated infants against selected reference strains was measured by serum bactericidal assay using human complement (hSBA). Functional *in vitro* assays were used to assess the role of the vaccine antigens as virulence factors. Sequence analysis was used to evaluate vaccine antigen conservation in a panel of 85 disease causing strains representative of the meningococcal diversity.
Results
The phase 2 trials in adults and infants demonstrated satisfactory tolerability and immunogenicity. Preliminary analysis shows 90-100% (44/76-SL, ST32), (5/99, ST8) and (NZ98/274, ST41/44) hSBA ≥1:4 post 3rd dose against three serogroup B strains. The results in infants show high titres after 2nd and 3rd dose. The sequence analysis showed that the correlations between antigen variability and MLST are not sufficient to predict vaccine coverage and therefore we have developed a new method based on the presence and expression of vaccine antigens. Functional data suggest an important role for the vaccine antigens in virulence and pathogenesis.
Conclusions
Reverse vaccinology has led to the development of a generally well tolerated vaccine with the potential to provide broad coverage against invasive menB disease from early infancy. The vaccine candidate is currently being evaluated in phase 3 clinical trials.

O66

Safety and immunogenicity of a meningococcal disease vaccine based on *Neisseria lactamica* in adult volunteers

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Objectives
Immunological and epidemiological evidence suggests that carriage of *Neisseria lactamica* is involved in the development of natural immunity against meningococcal disease. We have also shown that immunisation with *N. lactamica* outer membrane vesicles can protect from lethal challenge in a mouse model of meningococcal septicaemia. Thus we have developed an OMV vaccine based on *N. lactamica* and assessed this in a phase I safety and immunogenicity in adult volunteers
Methods
A double-blind, placebo control study in adult male volunteers was carried out at the University of Sheffield Clinical trials Unit with vaccine manufactured at the HPA Centre for Emergency Preparedness and Response. 26 volunteers received three doses of OMV vaccine (25μg protein) at 0, 6 and 12 weeks. 18 of these volunteers received a fourth OMV vaccine dose 6 months later. 29 volunteers received three doses of an Alhydrogel-only placebo. Sera obtained from each volunteer before and after each dose were analysed by ELISA for total specific IgG to *N. lactamica* OMVs and to OMVs prepared from six representative UK *N. meningitidis* strains. The sera have also been analysed for serum bactericidal (SBA) and opsonophagocytosis (OPA) activity against these six strains.
Results
There were no significant differences in any safety assessment between the vaccine and placebo groups except for a greater incidence of swelling and erythema at injection site for the OMV vaccine group. Increasing titres of IgG against the vaccines OMVs were seen after each of the first three doses with a further boost response seen after the fourth dose. Significant rises in IgG cross reactive to OMV from 6 meningococcal strains was also observed following 3 doses of OMV vaccine. These titres were similar to those reported in a study of the Norwegian meningococcal OMV vaccine against heterologous meningococcal strains. Many volunteers had high pre-existing SBA activity. However, rises in geometric mean SBA titre were observed against 6 meningococcal strains and these rises were significant for 3 of the strains ($P<0.05$). However, only a modest number of individuals showed 4-fold rises against these strains. The % 4-fold rises against each strain were similar to those reported for these strains with the Norwegian meningococcal OMV vaccine against heterologous strains. Following 3 doses, the % volunteers with a significant increase in opsonophagocytic response ($P<0.05$) was higher in the OMV vaccine group than placebo group against all 6 strains, with a further rise observed following the booster dose.
Conclusion
The *N. lactamica* OMV vaccine has been shown to be safe and immunogenic, giving rises in total IgG against *N. lactamica* and a representative panel of meningococcal strains. Increases in functional antibody responses are similar to those seen with a meningococcal OMV vaccine against heterologous meningococcal strains although this is difficult to interpret in volunteers who have high levels of pre-existing immunity to these meningococcal strains.

O67

Seroprevalence of polysaccharide specific IgG and bactericidal activity to *Neisseria meningitidis* serogroup C in the pre- and post-vaccination period in the Netherlands

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Introduction
A single MenC conjugate (MenCC) vaccination was introduced in 2002 into the National Immunization Program (NIP) in the Netherlands at the age of 14 months for all newborns. In a catch-up campaign, in 2002, all persons between the age of 1 and 18 years received one dose of MenCC vaccine (vaccine coverage 94%). Currently no vaccine failures have been reported.
Aim
To determine the age specific seroprevalence of MenC polysaccharide (PS) IgG and MenC specific serum bactericidal antibodies before (period 1995/1996) and after (period 2006/2007) introduction of the MenCC vaccine.
Methods
Two representative population-based serum collections, aged 1 to 80 years, established in the pre- and post-vaccination period, were available. Using a multiplex immunoassay (MIA), MenC PS specific IgG was determined in 2886 sera from the pre-vaccination period and 5800 sera from the post-vaccination period. In addition, in a subset of sera from both serum collections MenC specific SBA titers using baby rabbit complement was determined. Results were compared with available data on meningococcal carriage and disease.
Results
In the pre-vaccination period MenC PS titers were low (GMT = 0.18 μg/ml) and remained constant in 0-10 years-old infants and children, rose during adolescence, and peaked in 25-30 years-olds (GMT = 0.56 μg/ml). In older adults the GMT maintained at a level of 0.41 μg/ml. The same trend was observed for the SBA titers; during infancy about 4% had an SBA titer ≥8, with a rise in titers during adolescence (SBA titer ≥8 around 13% in the age group of 5-9 years up to 37% in the ages 25-30 years and stabilizing at about 30% in the older age groups.
Preliminary results from the post-vaccination period indicate that MenC PS titers are equally low during the first year of life, with a rise in titers after MenCC vaccination at 14 months, but titers decline within 2 years post-vaccination. An increase in MenC PS titers is observed in the persons vaccinated in 2002 during the catch-up campaign, which were 7 to 22 years of age during serum sample collections in 2006/7. A peak in antibody response was observed at 22 years (GMT = 3.05 μg/ml). The older and unvaccinated adult groups showed very low titers (GMT = 0.10 μg/ml). SBA titers from the post-vaccination period will be presented.
Conclusions
Antibody levels were low in the pre-vaccination period, but with respect to SBA levels one third of the population showed protective levels during life as adults. MenCC vaccination introduced higher specific anti-MenC PS IgG levels compared to natural exposure in the vaccinated groups, but only older age group seem to benefit from persistence of higher specific IgG levels. However, due to mass vaccination, exposition to MenC probably stopped which lead to a decrease in titers in the unvaccinated older age groups. This may pose them at extra risk once MenC might starts re-circulating.

O68

Bystander T-cell activation promotes B-cell immunity to meningococcal capsular polysaccharide following vaccination

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Objectives
Conjugate vaccines aim to overcome the limitations of capsular polysaccharides, as T-cell independent antigens, through their conjugation to T-cell dependent carrier proteins. The Men C conjugate vaccine induces not only protective serum bactericidal activity (SBA) in all age groups, but also evidence of Men C polysaccharide (CPS) specific B-cell memory. However, following the waning of SBA, infants are no longer directly protected. Thus, the role of memory B-cells in conferring protection from invasive disease is unclear. We have investigated the activation requirements of this population and provide new understanding regarding the conditions under which the population differentiates into the plasma cells required for protection.
Methods
Healthy adults (n = 18), vaccinated with a Men C conjugate vaccine six years previously, were boosted with a CPS - tetanus toxoid (TT) conjugate vaccine (NeisVacC, Baxter). Blood was taken prior to and at time points up to six weeks following vaccination. ELISpot assays were used to enumerate CPS and TT specific plasma cells and, following their differentiation using SAC and IL-2 or other stimuli, memory B-cells. Sub-populations of cells were obtained using immunomagnetic separation techniques.
Results
Prior to booster vaccination, TT specific memory B-cells were identified in the circulation significantly more frequently than those specific for CPS. Numbers to both antigens then increased from day seven onwards. Stimulation of TT specific memory B-cells with TT effectively promoted their differentiation into plasma cells. This was dependent on the presence of CD4+ T-cells, being completely abrogated on depletion of the population. In contrast, the stimulation of CPS specific memory B-cells with CPS failed to induce their differentiation *in vitro*. We went on to investigate the role of 'bystander' T-cells on CPS specific memory B-cell differentiation. Influenza antigens, anti-CD3 antibody and TT are known to activate T-cells within our system. All three promoted the differentiation of CPS and TT specific memory B-cells, although cognate stimulation of TT specific cells was significantly more effective in this role. The differentiation induced by bystander T-cells was dependent on their direct contact with the CPS specific B-cells. Stimulation of CPS specific memory B-cells with whole killed meningococci also promoted their differentiation irrespective of the presence or absence of CPS. In this case, CD4+ T-cell depletion only partially abrogated the differentiation, indicating that, in addition to inducing bystander CD4+ T-cell help, the bacteria are also able to induce CPS specific memory B-cell differentiation through direct effects on the B-cells themselves.
Conclusion
The activation of T-cells by whole killed meningococci and other antigens, promotes the differentiation of CPS specific memory B-cells into plasma cells, although less effectively than the cognate B-cell - T-cell interaction available to protein antigens. This may influence both the duration of serological protection following vaccination and also the ability of the population to maintain protection in the absence of SBA.

O69

Immunogenicty and reactogenicity of a novel serogroup B *Neisseria meningitidis* vaccine administered from 6 months of age

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Objectives
Following the successful introduction of serogroup C meningococcal vaccines, the majority of cases of invasive meningococcal disease in Western Europe are due to serogroup B organisms (MenB). Although an outer membrane vesicle (OMV) vaccine specific to the New Zealand epidemic strain NZ98/254 has been successfully employed in that country, this vaccine induces very limited immunity against other MenB strains. A novel vaccine containing 5 recombinant proteins identified from a MenB genome analysis was administered either alone (rMenB) or together with the New Zealand OMV vaccine (rMenB + OMV). This study evaluated the immunogenicity and reactogenicity of a 3 dose course of these vaccines when administered from 6 months of age.
Methods
Sixty healthy children aged 6 to 8 months were randomised to receive rMenB or rMenB + OMV. Parents, but not staff, were blinded to the vaccine received. The vaccines were administered at enrolment (day 0), at day 60 and then at 12 months of age. Blood samples obtained at day 0 and 1 month following the 2nd and 3rd dose of vaccine were analysed for human complement serum bactericidal activity (hSBA) against 3 MenB strains (44/76 - SL, 5/99 and NZ98/254) expressing the vaccine antigens. An hSBA titre of ≥ 1:4 was used as the correlate of protection. Participants' families were supplied with diaries to record data on local and systemic reactions to the vaccines.
Results
At enrolment 30 participants were randomised to rMenB (mean age 7.0 months) and 30 participants to rMenB + OMV (mean age 7.1 months). 24 participants per group were included in the per-protocol population for the immunogenicity analysis of the complete vaccine course.
After 2 and 3 doses of rMenB or rMenB + OMV sera from all infants had an hSBA titre ≥ 1:4 against strains 5/99 and 44/76-SL. By contrast, only 9% attained an hSBA titre ≥ 1:4 against NZ98/254 following 3 doses of rMenB compared with 95% (2 doses) and 96% (3 doses) of rMenB + OMV recipients. Local and systemic reactions were similar for the two groups.
Conclusion
Both vaccines were well tolerated. rMenB + OMV vaccine is immunogenic when given as a 2 or 3 dose course from the age of 6 months against 3 strains expressing the vaccine antigens.

Notes