Objet: Porro-Tsai
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Neisseria meningitidis LPS behaves "in vivo" as a T-cell dependent antigen: implications for development of a target vaccine for prevention of bacteremia and endotoxemia

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We present a model of vaccine based on detoxified LPS conserving the supramolecular structure of micelles. Detoxification of LPS from Neisseria meningitidis group A, strain A1 (LPSA1), which lacks the lacto-N-neotetraose determinant also found on mammalian cells, has been achieved by complex formation with a synthetic anti-endotoxin decapeptide (SAEP2) binding to the lipid A moiety of LPSA1 with high affinity. In contrast to plain LPS, LPS/SAEP2 complex was inactive in releasing serum TNF in SW mice after three subcutaneous injections containing 0.5-5ug of LPS/dose, while the complex vaccine induced high titers of boostable IgG antibodies against the immunotype determinants of LPSA1, cross-reactive with group B LPS in either purified or cell-associated form. These antibodies were able to functionally fix and activate homologous and heterologous species of complement after binding to LPSA1-coated sheep erythrocytes. The purified anti-LPSA1 IgG polyclonal antibodies, significantly inhibited serum TNF production in CD1 mice intravenously challenged by homologous but not heterologous LPS. The immunogenic properties of LPS A1/SAEP2 complex, investigated by kinetic, magnitude and sub-isotype composition of the polyclonal antibodies induced, were consistent with those known for T-cell dependent antigens and were comparable to those of a glycoconjugate obtained by covalent binding of LPSA1, previously detoxified by SAEP2, to BSA working as a T-cell dependent carrier protein. These serological results have been paralleled by the capability of LPS to activate CD4+ and CD8+ T-cells "in vivo". The strategy of delivering to the immune system of mammalians non-toxic LPS expressing its supramolecular antigenic structure when in complex with SAEP2 (endotoxoid), represents a novel approach for development of a new generation of LPS-based vaccines for prophylaxis of specific Gram-negative infections leading to bacteremia and endotoxemia.

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Objet: Hobbs, Alcorn, Davis ... Cohen
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Molecular Typing of Neisseria gonorrhoeae causing Repeated Infections: Evolution of Porin during Passage within a Community.

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Thirty-three Neisseria gonorrhoeae isolates from 15 individuals infected multiple times with the same serovar were compared using por gene sequencing, opa-typing and arbitrarily-primed PCR (AP-PCR). All three molecular techniques were more discriminatory than serotyping and identified differences between some isolates belonging to the same serovar. Although there were differences among Por sequences within some serovars, 10 of 15 patients became reinfected with gonococci expressing identical Por proteins. Sequence analysis revealed evidence of horizontal genetic exchange of all or portions of por genes and point mutations in potential surface exposed regions during passage in the community.

Objet: Pollard-Levin
Date: Mon, 4 May 1998 13:52:47 +0100 (BST)
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T cell, cytokine and antibody subclass responses to meningococcal antigens following infection are independent of age

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Meningococcal disease is the leading infectious cause of death in childhood in the UK. No effective vaccine is available for Group B Meningococcal disease due to poor immunogenicity in children.
AIMS: We postulated that T helper cell function and cytokine mediated interactions between T and B lymphocytes leading to antibody production may be sub-optimal in young children. We sought to investigate immune responses to natural infection in childhood.
METHODS: OMVs from Group B meningococci and heat-killed whole bacteria were used to stimulate fresh peripheral blood mononuclear cells from 49 children ten weeks following meningococcal infection. Proliferation was measured by 3H-thymidine incorporation. Cytokine (IL-10, TNF-alpha and IFN-gamma) production in supernatants was measured by ELISA. Serum was collected from 52 children following infection and analysed for anti-meningococcal total IgG and subclass antibodies using a whole cell ELISA.
RESULTS: (1) There was no difference in T cell proliferative responses or cytokine production between cases <1 year of age and older children. (2) Anti-meningococcal class and subclass antibody levels were the same in young children as in older children
CONCLUSION: Even young children are able to mount humoral and cellular immune responses to meningococcal infection in a quantitatively and qualitatively similar manner to older children and could be expected to respond to an appropriate vaccine formulation.

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Objet: Carson-Sparling
Date: Mon, 04 May 1998 14:59:34 -0400
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Gonococcal FrpB Operon Mediates Ferric Enterobactin Utilization

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FrpB, an iron-regulated, 70 kD, Neisserial outer-membrane protein, shows sequence homology with the TonB-dependent family of receptors that transport iron into Gram-negative bacteria. Although FrpB is commonly expressed by most Neisserial strains and species, and perceived as a potential vaccine candidate for both Neisseria gonorrhoeae and N. meningitidis, its function in cell physiology was previously undefined. We now report that FrpB is a functional enterobactin receptor. N. gonorrhoeae strain FA1090 is able to utilize ferric-enterobactin as its sole iron source when supplied at a concentration of approximately 10 uM, but ferric-enterobactin growth stimulation was completely abolished when an omega cassette was inserted within frpB. FA1090 FrpB specifically bound 59Fe-enterobactin, with a Kd of approximately 3 uM. Monoclonal antibodies raised against the E. coli enterobactin receptor, FepA, recognized FrpB in western blots. Amino acid sequence comparisons revealed that residues previously implicated in ferric enterobactin binding by FepA are conserved in FrpB. In addition, downstream from frpB, four open reading frames (orfs) exist that each display sequence similarity to components of other phenolate-siderophore uptake systems. The orfs include a periplasmic siderophore binding protein homolog, and three cytoplasmic membrane protein homologs that may correspond to ABC-type transporter components. The first three of these open reading frames are transcriptionally linked to frpB, as demonstrated by RT-PCR. These data suggest that FrpB is a functional, evolutionary homolog of FepA that binds ferric enterobactin.

Objet: JELFSMUNRO
Date: Wed, 6 May 1998 14:26:05 +1000
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Carriage of ET37 Complex/ET15 in a College Setting.

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A cluster of three cases of meningococcal disease (MD) occurred in September, 1997 a college attended by adolescent day students and boarders. These cases occurred at a time when there had been several clusters of MD in the Sydney area and as a result there was heightened media and public interest. The traditional public health response is prophylaxis for household and close contacts and no throat swabbing is undertaken.
The strain involved in this cluster and other clusters in the Sydney area in 1997, was C:2a:P1.5(+/ -P1.2). This particular phenotype had been implicated in an outbreak of fourteen cases of MD the previous year and associated with ongoing hyperendemic disease within this same local government area. An increase of sporadic cases of C:2a:P1.5 during 1997 accounted for over 50% of all cases of MD in New South Wales and occurred predominantly in the 15 - 19 years age group.
The Health Department made the decision, with parental consent, to offer vaccination to everyone at the college. The question was raised: what is the frequency and distribution of this strain within the college community, given that in other clusters involving this phenotype investigators had suggested that transmission was high but carriage rates low. With the cooperation and consent of staff and parents, 1033 students and staff of the college had pharyngeal swabs taken and plated onto both selective and non selective media at the point of collection.
An overall N.meningitidis carriage rate of 8.6% (89/1033) was found. N.lactamica was found in 2.4% of those swabbed. Of the N.meningitidis strains, 13.5% were serogroup B and 14.5% serogroup C. The remainder of isolates were non groupable. Serotyping and serosubtyping of all meningococci (including non groupable isolates) revealed that 16.8% (15/89) were of a phenotype similar to that found in the three systemic cases. These isolates and for whom a phenotype was unable to be detected (eg.NG:NT:NT or B:NT:NT) were further analysed by PFGE (35 strains). Two restriction enzymes were utilised, SpeI and NheI. None of the NG:NT:NT(17strains), B:NT:NT (2 strains) or B:NT:P1.5 (1 strain) were found to have fingerprints similar to those obtained from the systemic isolates.Ten meningococci whose phenotype was similar to that of the systemic isolates yielded a fingerprint indistinguishable from that obtained from the systemic isolates. A further four differed by only one band by SpeI and were indistinguishable by NheI. One isolate was found to be distinct from the systemic and other pharyngeal isolates' fingerprints.
The availability of the phenotyping results within five days of the initial pharyngeal collection enabled the public health unit to extend the prophylaxis to the household contacts of the 15 children with a phenotype similar to that found in the three systemic cases.
The study demonstrated carriage of this strain in groups of children with no relationship to the index cases and revealed a higher carriage

rate than is described in the literature. The question as to whether prophylaxis should be extended beyond the present recommendation of close household contacts, particularly amongst a high school population, is also raised.
Jane (Jelfs)

Objet: oral presentation
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Subject : mtr regulation in Neisseria, oral presentation
First author : Rouquette
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Expression of the mtr system in Neisseria gonorrhoeae and Neisseria meningitidis is modulated by different regulatory control mechanisms.
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The mtr (multiple transferable resistance) complex in Neisseria gonorrhoeae encodes an energy-dependent efflux system, composed of MtrC-MtrD-MtrE cell envelope proteins, that exports antibacterial hydrophobic agents (HAs). The mtr system is also present in Neisseria meningitidis. However, unlike gonococci, the DNA sequence in meningococci revealed two classes of insertion sequence (IS) elements in the region that intervenes the divergent mtrR (multiple transferable resistance Regulator) and mtrCDE genes. Thus, all meningococcal strains analyzed contained a 158 bp sequence (Correia Element [CE]) previously seen elsewhere in the meningococcal and gonococcal chromosomes. A minority of strains also had an intact copy of IS1301. Neither element has been observed within this region of gonococci. RT-PCR analysis of the mtrC meningococcal gene revealed a transcript encompassing these two elements. The CE insertion creates a loop that may act as a terminator for most of the mtrCDE transcripts and consequently could constitute a new mechanism of repression of the mtr system. In addition, the mtrR sequence of three meningococcal strains revealed a non-functionnal MtrR protein. This suggests that the repression of mtrCDE by MtrR does not exist in meningococci but meningococci unlike mtrR null mutants do not express high levels of HA-resistance. We also found that the mtr system is inducible only in gonococci and this is independent of MtrR but dependent on MtrCDE. Although the mtr efflux system allows the removal of the same HAs in gonococci and meningococci, its regulation seems to be different in these pathogenic Neisserial species.
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Objet: Plante-Martin
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Characterization of the NspA protein of Neisseria gonorrhoeae

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The NspA protein was first identified in the outer membrane of Neisseria meningitidis using monoclonal antibodies (MAbs) (J.Exp. Med. 1997: 185:1173). This panel of MAbs, while reacting with all meningococcal strains tested so far, only recognized a limited number of gonococcal strains. DNA hybridization experiments demonstrated that the nspA gene is present in all gonococcal strains tested so far. Comparison of the available gonococcal and meningococcal nspA nucleotide sequences revealed a very high degree of similarities (92% identities) among these two species. The level of similarities reaches 98% identity when the two gonococcal nucleotide sequences are compared together. As it is the case for the meningococcus, the gonococcal NspA protein is highly resistant to digestion by proteolytic enzymes. MAbs specific to the gonococcal NspA were generated and used to confirm that this protein is produced by all 52 gonococcal strains tested. Radioimmunobinding assay and immunogold microscopy clearly demonstrated that the NspA protein is accessible to the antibodies at the surface of intact gonococci. These results suggest that even if the gonococcal protein closely resemble the meningococcal NspA protein, it also possesses some unique molecular and immunological characteristics. Two gonococcal NspA-specific MAbs were also shown to be bactericidal against one gonococcal strain, suggesting that antibodies directed against this protein could be implicated in host defense mechanisms against gonococcal infection.

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Objet: Källström-Jonsson
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Title: Cell signalling by the type IV pili of pathogenic Neisseria

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Pili of Neisseria gonorrhoeae and Neisseria meningitidis mediate binding of the bacteria to epithelial cells. We have identified CD46 (membrane cofactor protein, MCP) as a eucaryotic receptor for gonococcal and meningococcal pili. CD46 is an abundant transmembrane glycoprotein involved in complement regulation on host cells, and is expressed on virtually every human cell type, except erythrocytes. Antibodies directed against CD46 as well as purified recombinant CD46 block binding of pathogenic Neisseria to target cells. Piliated, but not non-piliated, bacteria adhere to CHO cells

expressing human CD46. It is likely that CD46, which is a human specific protein, determines the host specificity of the pathogenic Neisseria species. We are currently studying the role of CD46 in signal transduction and pathogenesis
We have shown that the type IV pili transduce a signal into the eucaryotic host cell. Purified adherent pili, but not pili from a non-binding mutant, trigger an increase in the cytosolic-free calcium ([Ca2+] i) in target epithelial cells, a signal known to control many cellular responses. The [Ca2+] i increase was blocked by antibodies against CD46 suggesting a role for this protein in signal transduction. Pilus-mediated attachment was inhibited by depletion of host cell intracellular Ca2+ stores, but not by removal of extracellular Ca2+. Further, kinase inhibition studies showed that pilus-mediated adherence is dependent on casein kinase II. In summary, these data reveal a novel function of the type IV pili, namely induction of signal transduction pathways in host cells.

Objet: Saunders - Moxon
Date: Mon, 11 May 1998 12:33:48 +0100
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Opc expression is controlled by variable facing and spacing mediated by alterations in the length of a promoter located homopolymeric tract.

NJ Saunders, DW Hood and ER Moxon

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Expression of Opc, a phase-variable (on +++, intermediate +, and off -) surface protein of N. meningitidis, correlates with the number of cytidines in a promoter located homopolymeric tract (HPT). Mechanisms by which repeat length might alter expression include: 1. direct, sequence dependent, interaction with the transcriptional promoter binding complex, 2. altering the distance between two promoter components which span the repeat: 'spacing', or 3. altering the relative helical presentation of promoter components on either side of the repeat: 'facing'.

Site-directed mutations of the promoter were introduced replacing the HPT with a sequence of similar melting temperature, over a range of lengths associated with altered expression of opc. These were transformed into strain MC58 and expression was determined by colony immunoblotting with MAb B306. Mutants with replacement tracts equivalent in length to those associated with both maximal (12Cs, 13Cs), and intermediate (11Cs, 14Cs) expression all exhibited strong expression of opc. Those with other lengths (8Cs, 10Cs, 15Cs, 17Cs) displayed the off phenotype. The loss of intermediate phenotypes in addition to on-off switching suggests that expression of opc is controlled in a novel fashion by both a 'spacing' effect of repeat length and also a 'facing' effect determined by the sequence of the repeat.

Using these mutant promoters as templates, the presence of large complexes in mobility shift assays (probably representing RNA polymerase) correlates with Opc expression. In addition there is a small complex unaffected by tract length. Site-directed mutagenesis of upstream sequence indicates that the additional protein may be IHF.

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Objet: Wetzler-Simpson
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T lymphocyte response to Neisseria gonorrhoeae porin (Por) in individuals with mucosal gonococcal infections.

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This study characterizes the anti-Por T cell response in patients with urogenital gonococcal disease to determine whether mucosal gonococcal infection can generate circulating T lymphocytes which are Por specific. Patients with urogenital gonococcal disease can generate a humoral response towards gonococcal outer membrane components, including Por. As Por is a protein, the anti-Por antibody response is T cell dependent. T lymphocytes from a majority of the patients examined, obtained at initial diagnosis of urogenital gonococcal infection, proliferated upon incubation with Por, as compared to minimal induced proliferation of T lymphocytes from normal volunteers. Using intracellular cytokine staining and flow cytometric analysis, we determined that a significant increase in IL-4 producing CD4+ T helper lymphocytes was seen in these patients upon incubation with Por, while no increase in IL-4 producing CD4+ T lymphocytes was observed in normal volunteers. Interestingly, the same trend was observed in CD8+ T lymphocytes from these patients. There was no measured increase in IL-2, IL-10, IL-12, IFN-gamma, and TNF-alpha production by T lymphocytes. Concomitant increases in IL-4 production in T lymphocytes that could potentially traffic to mucosal surfaces (expressing the mucosal addresin, VLA alpha4/ beta7, on their surface) upon Por incubation were also observed, but the increases were similar in T lymphocytes that were VLA alpha4/ beta7 negative. In conclusion, mucosal gonococcal disease can induce Por specific circulating T lymphocytes, with a Th2 phenotype, and a portion of these Por specific T lymphocytes can potentially traffic to mucosal surfaces.

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for information on the:
The Fifth Conference of the International Endotoxin Society
Santa Fe, New Mexico
Sept. 12-15, 1998
http://www.ies98.org/

Objet: oral presentation
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Hemoglobin utilization by Neisseria meningitidis
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Hemoglobin is the most abundant reservoir of iron in the body. Pathogenic Neisseriae have developed surface-exposed, Hb-binding receptors that enable the assimilation of Hb-iron. We have identified and characterized a TonB-dependent, highly conserved meningococcal outer membrane receptor (HmbR) that binds human Hb. HmbR belongs to a new group of TonB-dependent outer membrane receptors. These receptors are unique in that they strip iron/heme moieties from bound protein complexes and transport iron/heme into the periplasm. Using genetic and biochemical approaches we have identified functionally important receptor domains. This knowledge will enable us to understand the mechanism of Hb utilization in Gram-negative bacteria. A limited survey of 26 meningococcal strains of serogroups A, B, C, and Y identified the presence of the hmbR gene in all tested strains. However, the HmbR protein was expressed only in a small subset of these strains when tested by anti-HmbR antibodies. The Western blot data together with the nucleotide sequence analysis of hmbR non-expressing mutants suggested that the expression HmbR is under phase variation control. N. meningitidis is one of a rare pathogenic microorganisms that express more than one Hb receptor. Lewis and Dyer have identified a HpuB/A receptor pair that is involved in haptoglobin-Hb and Hb utilization. We have constructed hmbR hpuB double mutants in 26 strains of N. meningitidis in order to determine whether additional systems of Hb utilization are expressed in meningococci. Several hmbR hpuB double mutants gave rise to Hb+ revertants, suggesting that these mutants express a third Hb utilization system. An open reading frame (thbR) that encodes a potential TonB-dependent outer membrane receptor was cloned using the infomation from the nucleotide sequence of N. meningitidis A genome. An insertional inactivation of the thbR gene was conducted in N. meningitidis and N. gonorrhoae. ThbR single mutants of gonococci and meningococci did not have any phenotype. Introduction of the thbR mutation in the N. meningitidis hmbR hpuB double mutant abolished residual Hb utilization, however. We are currently condacting studies that will confirm the presence of the third Hb-utilization system in meningococci and determine the role of ThbR in this process.

Objet: retzer - schryvers
Date: Tue, 12 May 1998 12:43:15 +0000
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Studies of Transferrin-Transferrin Binding Protein Interaction

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The transferrin receptor-mediated iron acquisition pathway is utilized by human and veterinary pathogens of the Neisseriaceae. This transferrin receptor is comprised of two outer membrane proteins, transferrin binding protein A (TbpA) and transferrin binding protein B (TbpB). The transferrin (Tf) ligand from different host species exhibit a high degree of sequence identity. This has led to the hypothesis that this acquisition system is phylogenetically-related, with the receptor-ligand interaction conserved and therefore involving analogous regions on the ligands which interact with the receptor proteins. Despite these similarities, the receptor complex can demonstrate exquisite specificity in terms of host ligand binding and subsequent iron acquistion. We have investigated these apparently contrasting observations using different analytical techniques. A series of hybrid human/bovine Tfs were generated that maintain their overall tertiary structure. These chimeric proteins served as ligands in binding assays with truncated TbpB constructs from Neisseria meningitidis to localize region(s) responsible for binding. A solid-phase peptide library representing Tf was also utilized in binding studies with the TbpB constructs. We have determined that multiple sites of interaction between Tf and TbpB exist, with differing species specificity. These sites localize to both lobes of Tf as well as distinct regions of TbpB.

Objet: Jack,Turner
Date: Tue, 12 May 1998 16:31:43 +0100
De: Dominic Jack <djack@ich.ucl.ac.uk>
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Dear Sir

I would like the following to be considered for oral presentation at Neisseria98.

Thank you

Dominic Jack

Interactions of mannose-binding protein with Neisseria meningitidis serogroup B

DL Jack, NJ Klein, MW Turner

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Mannose-binding lectin (MBL) is a serum protein that has been demonstrated to activate complement independently of antibody and to enhance phagocytic cell function. Although deficiency of the protein is associated with a common opsonic defect and predisposition to infection, the role of MBL in host defence against bacterial infections such as N. meningitidis remains unclear. To address this issue we have investigated the interactions of MBL with isogenic mutants of N. meningitidis serogroup B based on a parent organism (B1940) as follows:

siaD- lacking expression of capsular polysaccharide.
cpsD- a galE mutant with truncation of the lacto-N-neotetraose of the LOS which prevented sialylation.
cps- a double mutant deficient in both capsule and LOS outer core.

Previously we have shown much higher MBL binding to and complement activation on the truncated LOS mutants or organisms with enzymatically removed sialic acid than on organisms with intact LOS. We now report that purified MBL significantly increases non-opsonic phagocytosis of the parent, cps- and cpsD- organisms by neutrophils and monocytes at 60 min in a flow cytometric assay. Furthermore, neutrophils exposed to organisms pre-treated with MBL show smaller losses of the activation marker, CD62L, compared to neutrophils exposed to untreated organisms. These results suggest that MBL alone may act as an enhancer of phagocytosis for N. meningitidis but is unlikely to constitute a major mechanism for the removal of organisms from the circulation. However, MBL may modulate the inflammatory response in favour of host survival, highlighting the possible importance of MBL in meningococcal disease.

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Objet: Neal, Nguyen-Van-Tam, Monk, O'Brien, Stuart, Ramsay
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Predicting a second case of invasive meningococcal disease in the same term amongst university students.

KR Neal 1(presenter) , JS Nguyen-Van-Tam 1, P Monk 2, SJ O'Brien 3, J Stuart 4, M Ramsay 5.

1 Division of Epidemiology Nottingham University, 2 Leicestershire Health, 3 Scottish Centre for Infection and Environmental Health, 4 CDSC - South and West, 5 CDSC - Colindale.

Introduction: There has been significant publicity concerning outbreaks of invasive meningococcal disease (IMD) among university students in the UK.

Objectives: To determine factors associated with a second case within a

university in the same term. Methods: A retrospective survey of cases of IMD notified to Consultants in Communicable Disease Control, September 1994 to April 1998.

Results: Data were available from 66 universities covering 11 university terms. A total of 239 cases were reported. Subsequent cases occurred on 63 occassions. Three major clusters were identified, 2 clusters of 6 cases (both group C) and one of 4 cases (group B). On nine occasions three cases occurred in one term. In the twelve episodes involving 3 or more cases, links were identified in seven; all of which had 2 or more cases linked to the same hall of residence complex. Independent predictors of a second case of IMD in the same term (same or indistinguishable strain) were cases in the autumn term (October - December) compared with other terms, Odds Ratio (OR) = 2.5 (95% CI 1.3 - 4.7), traditional universities compared to new universities OR = 2.9 (1.4 - 6.0) and a case in a male student, OR = 1.7 (0.9 - 3.2). No independent associations were noted with type of student accommodation, meningococcal serogroup, academic year of study or age of student.

Conclusions: Second cases of IMD were significantly associated with cases occurring in traditional universities and during the autumn term.

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Objet: Nguyen-Van-Tam, Neal, Monk, O'Brien , Stuart , Ramsay
Date: Tue, 12 May 1998 16:29:16 GMT
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Invasive meningococcal disease among university undergraduates: association with catered halls of residence.

JS Nguyen-Van-Tam (presenter) 1, KR Neal 1, P Monk 2, SJ O'Brien 3, J Stuart 4, M Ramsay 5.

1 Division of Epidemiology Nottingham University, 2 Leicestershire Health, 3 Scottish Centre for Infection and Environmental Health, 4 CDSC - South and West, 5 CDSC -Colindale.

Introduction: Cases and outbreaks of invasive meningococcal disease among in university students attract considerable media attention.

Objectives: To describe the risk of invasive meningococcal disease (IMD) among UK university students, compared with non-students of similar age.

Methods: A retrospective survey of notified cases of IMD combined with a questionnaire survey of University Accommodation Officers covering the United Kingdom, 1994/95-1996/97.

Results: University students had increased rates of IMD (9.7/100,000 (95% C.I. 8.1-11.0)) compared with non-students of similar age (3.2/100,000 (2.4-3.9)), Relative Risk (RR) = 3.0 (2.3 - 4.0), p = 10-8. Multiple regression modelling demonstrated catered hall accommodation as the key risk factor. Higher rates of disease were observed at universities providing catered hall places for ò 10% of their student population (16.2/100,000 (12.5 - 20.0)) compared with those providing places for < 10% of students (5.6/100,000 (3.7- 7.5)), RR = 2.9 (1.9 - 4.3), p = 10-7. The mean annual rates in individual universities ranged from 0 - 44.3 per 100,000 for all IMD, 0 - 20.2 per 100,000 for serogroup B disease and 0 - 24.6 per 100,000 for serogroup C disease. Although there was an increase in serogroup C disease among students from 1.4 per 100,000 in 1994/95 to 5.0 per 100,000 in 1996/97, this difference was not statistically significant. Most IMD amongst students was caused by serogroup B organisms.

Conclusions: University students have higher rates of IMD than people of the same age. This includes both group B and group C organisms. Catered halls provide significant opportunities for social mixing and transmission of meningococci.

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Rapid large increase in meningococcal carriage rates in first year University students: an explanation for the increased risk of invasive meningococcal disease seen in university students.

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Introduction: High rates of invasive meningococcal disease have been observed in first year university students.

Objectives: To determine meningococcal carriage rates in university students and identify risk factors for acquisition.

Methods: First year students at Nottingham University were swabbed during 'freshers week', 1997, and again at intervals during the autumn term. Students completed a questionnaire covering a range of social and medical factors. Pharyngeal swabs were plated onto a modified New York City medium with antibiotics and incubated at 37oC with 5% CO2. Isolates with a positive oxidase and Gonocheck tests were sent to the PHLS Meningococcal Reference Unit for further identification and characterisation.

Results: 2453 students were swabbed in the first week of term. One month later 787 students were re-swabbed and two months later 963 different students were re-swabbed. The carriage rate rose rapidly in the first week of term from 6.5% on day 1, to 11.0% on day 2, 18.4% on day 3 and 22.4% on day 4, (Chi2 for trend, p<10- 8). The carriage rate in the first week averaged 12.8%. By November this had increased to 28.0% and 31.5% in December. Multiple logistic regression analysis showed an increased risk of acquisition with frequency of visits to a hall bar, alcohol consumption and smoking. A lower risk was seen in female-only halls. No independent associations were seen with passive smoking, kissing, inhaled steroids or recent meningococcal vaccine.

Conclusions: Carriage rates increased rapidly
in the first week of term with smaller increases through the autumn term. The rapid rate of acquisition may explain the increased risk of meningococcal disease and the epidemiological features seen in university students.
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Porin induced T-cell dependent immune response to capsular polysaccharide can be abolished by blocking B/T cell costimulatory signals, in mice.

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Neisserial porins enhance the humoral immune response to poorly immunogenic antigens and induce a T-cell dependent response against capsular polysaccharide (CPS), a T-cell independent antigen. Up-regulation of B7-1 and B7-2 surface expression correlates with an increased ability of B cells to costimulate T cells through the CD28 counter-receptor. Also, the interaction of CD40L on T cells with CD40 on B cells results in isotype switching and affinity maturation. Our previous investigations have shown that neisserial porins induced B cell

proliferation, IgM secretion, up regulation of the B7-2 costimulatory ligand in mice.

A T cell-dependent response to capsular polysaccharide was elicited by a vaccine consisting of group C meningococcal CPS conjugated to class 3 neisserial porin. 4-5 week old C3HeJ mice were immunized subcutaneously at 0 and 3 weeks and CPS-specific IgG and IgM levels were monitored. Synergistic in vivo blocking of B7-1 and B7-2, resulted in complete abolition of the IgG response but did not affect the IgM response. Blocking of B7-1 alone had no effect on anti-CPS immunoglobulin levels, whereas blocking of B7-2 ligand significantly reduced the anti-CPS response. Antibodies to CD40L significantly lowered the IgG and IgM response, whereas blocking CTLA4 increased the CPS-specific IgG response 3-fold. This data supports the hypothesis that neisserial porins, even in the absence of additional adjuvants, enhance the B cell response by activating a T cell dependent pathway, and that T cell costimulatory blockade of either the CD40-CD40L or CD28-B7-1/2 pathways is capable of down regulating this response.

Objet: Pon-Jennings
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N-propionylated E. coli K92 polysaccharide-protein conjugate elicits bactericidal antibodies in mice against group C but not group B N. meningitidis

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Escherichia coli K92 polysaccharide-tetanus toxoid conjugates (K92P-TT) have been proposed as potential bivalent vaccines against group B meningococci (GBM) and group C meningococci (GCM) (1). However, while they elicit good immune responses to the group C meningococcal polysaccharide (GCMP), that elicited to the the group B meningococcal polysaccharide (GBMP) is not satisfactory. On the evidence that N-propionylated (NPr) GBMP-protein conjugates elicit bactericidal antibodies against GBM (2), we attempted to conserve the bivalent vaccine approach by utilizing an NPrK92P-TT conjugate.

When injected in mice together with adjuvant (RIBI), the resultant antisera contained predominantly GCMP- and NPrGCMP-specific antibodies of which only the former were bactericidal. The smaller group B response was dominated by NPrGBMP-specific antibodies with only minimal quantities of GBMP-specific antibodies being detected. Surprisingly, despite the presence of NPrGBMP-specific antibodies, which have been previously demonstrated to be bactericidal for GBM (2), the antisera was not bactericidal for GBM.

This can be explained by the fact that the NPrK92P cannot adopt the same conformational epitope which has been demonstrated to be a requirement for the generation of NPrGBMP-specific bactericidal antibodies (2). It has been proposed that the conformational epitope is located on extended helical segments of the NPrGBMP and the inability of NPrK92P to adopt this unique extended helical epitope was confirmed by NMR spectroscopic analysis.

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Objet: Russell-Hook
Date: Tue, 12 May 98 15:38:21 EDT
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Analysis of mucosal and circulating antibody and cytokine responses in gonorrhea: evidence for immune response evasion by Neisseria gonorrhoeae

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Although Neisseria gonorrhoeae is quintessentially a mucosal pathogen, mucosal immune responses against gonococci have not received in-depth quantitative analysis. Studies of the immunocompetent cell populations in the human female cervix and male urethra reveal that both represent effector sites of the common mucosal immune system, yet the predominant immunoglobulin isotype in both female and male genital secretions is IgG of uncertain origin; secretory IgA is also present at lower concentrations. Using sensitive methods of evaluating even modest antibody responses in female and male secretions, we have begun to make a more precise analysis than has hitherto been possible. Sequential quantitative determinations of IgM, IgG, IgA1, and IgA2 antibody responses against homologous isolates of N. gonorrhoeae, and of inhibitory antibodies against gonococcal IgA1 protease, in the genital secretions and serum of gonorrhea patients suggest that gonococci possess the ability to avoid inducing effective immune responses. This interpretation is supported by the finding that women infected in the rectum, which contains organized mucosal inductive lymphoid follicles (unlike the genital tract), do not show significantly greater antibody responses. Furthermore, inflammatory cytokine responses in female secretions are also minimal, though circulating cytokine levels may be greatly elevated in some women who are co-infected with other STD pathogens. Although the extensive variety of gonococcal antigens undoubtedly enables N. gonorrhoeae to evade host immune responses, our results leave open the possibility that the induction of strong genital tract antibody responses to appropriate shared gonococcal antigens by applying new advances in mucosal immunization may have protective value.

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CD66 Antigens Expressed on ME180 and RA-HL60 Cells Determine the Interaction with Neisseria gonorrhoeae

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One of the most frequently used cell lines in studies of Neisseria gonorrhoeae is the ME180 human cervical cell line. We first showed that OpaA and OpaI E. coli were able to adhere to ME180 cells. However, OpaI mediated interaction could not be inhibited with heparin. These results indicated that there are two distinct interactions for attachment to a single epithelial cell line promoted by Opa proteins. Studies have shown that the interaction of the OpaA GC with epithelial cells involves binding to heparan sulfate attached to syndecan receptors while other Opa proteins interact with CD66 antigens. Thus, we speculated that some CD66 antigens might be expressed on this cell line. We were able to obtain the PCR products of (CD66c) and CEA (CD66e) from cDNA library of ME180. DNA sequence confirmed these PCR products. We further observed the expression of 180-kDa CEA protein in ME180 by Western blotting. That ME180 cells expressed BGP, NCA and CEA on their cell surface was demonstrated by flow cytometric analysis. Finally, the QSC-BEADs with quantification program was used to define the absolute number of each CD66 antigen expression. The expression rates of absolute number of each CD66 antigen are BGP > CEA > NCA on the surface of ME180. The interactions of ME180 with OpaI proteins were inhibited by the combination of anti-BGP, NCA and CEA antibodies. These results demonstrated that interaction of ME180 cells with Opa+ gonococci is due to the expression of CD66 antigens on this cell line.

Study of opsonin-independent interaction of Opa+ bacteria with neutrophils has depended on freshly isolated human blood cells, since a permanent neutrophil-like cell line model has not been developed. Human myeloid leukemia cell line HL60 cells have offered a unique model to examine the expression and regulation of these antigens on myeloid cells. This cell line after treatment with dimethylsulfoxide (DMSO) or retinoic acid (RA) differentiates into granulocyte-like cells, but no attempts have been made to examine whether RA treated HL60 could interact with Opa+ bacteria. Our results were that RA treated but not the DMSO treated or untreated HL60 cells phagocytosed the OpaI E. coli. To characterize RA treated HL60 cells, a cDNA library of this cell line was constructed. A 280 bp PCR product from All-CD66 primer has been detected and this PCR product confirmed by DNA sequence is from CD66 family. We further showed that RA treated HL60 cells expressed the CD66a (BGP) antigens, the level of CD66a surface expression was correlated with the Interaction of Opa+ bacteria in HL60 cells, and the interaction of RA treated HL60 cells with Opa+ bacteria is inhibited by anti CD66 antibodies.

Objet: HEYDERMAN-KLEIN
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PLEASE ACKNOWLEDGE RECEIPT OF THIS MESSAGE:

Endotoxin priming of the neutrophil response to Neisseria Meningitidis and the inhibition of adhesion molecule expression and phagocytosis by recombinant bactericidal/ permeability-increasing protein (rBPI-21)

RS Heyderman, CA Ison, M Peakman, M Levin, NJ Klein

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In severe meningococcal sepsis, polymorphonuclear neutrophil (PMN) activation enhances microbial clearance but also contributes to the vascular damage and multi-organ failure associated with the disease (1). A whole blood model of meningococcal bacteraemia using defined strains of Neisseria meningitidis was employed to examine the PMN changes that follow invasion (1,2). In this model we observe loss of PMN L-selectin and upregulation of the beta-2-integrin subunit CD11b, followed by opsonophagocytosis. PMN priming with either Escherichia coli lipopolysaccharide (LPS) or N-formyl-l-methionyl-l-leucyl-l-phenylalanine (fMLP) resulted in enhancement of PMN L-selectin shedding in response to N. meningitidis serogroups B and C (1.5-4 fold). Phagocytosis of these strains was also augmented following PMN priming (2-3 fold) which did not result in enhanced bacterial killing. Blockade of the lipid A moiety of meningococcal LPS with either recombinant BPI-21, the N-terminal fragment of the naturally occurring cationic protein (3,4), or with polymyxin-B sulphate (PMB) resulted in partial inhibition of the PMN activation and phagocytosis response to N. meningitidis. The effect of rBPI21 and PMB was reversed by excess E. coli LPS or fMLP. We propose that either release of soluble cell wall components or direct pathogen-host cell collision primes the PMN for an exaggerated activation and phagocytosis response. rBPI has considerable potential for interrupting this process. It remains to be seen whether ongoing control trials of BPI-21 will substantiate the efficacy of this molecule in preliminary clinical studies of meningococcal septicaemia (4).

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Objet: Abstract to Neisseria 98, Nice France, 1-6 November 1998
Date: Wed, 13 May 1998 12:38:51 +0200
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I hereby send the following abstract to be considered for oral presentation at the Neisseria 98 meeting in Nice, France.

ABSTRACT

TITLE:
Humoral Antibody Response in Pharyngeal Carriers of Neisseria meningitidis

AUTHOR:
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OBJECTIVE:
To investigate whether pharyngeal carriage of meningococci is associated to the presence and acquisition to the induction of meningococcal antibodies.

MATERIAL AND METODS:
Male recruits (n=1069) were followed for 3 months after entrance to military service. Pharyngeal swabs for culture of Neisseria meningitidis were collected on days 0, 15, 30, 60 and 90 and blood samples for examination of meningococcal antibodies in Meningococcal AntibodyTest (MAT) and ELISA (Anti-capsular antibodies) were drawn on days 7, 15, 60 and 90.

RESULTS:
At entrance, 22% of the recruits had detectable meningococcal antibodies as determined by MAT. Carriers had 4-5 times as frequent antibodies as non-carriers. Acquisition of meningococci was observed in 200 recruits and 53% had a significant increase in MAT-titre. At entrance 10% had IgM and 25% IgG antibodies directed towards the serogroup C capsular polysaccharide; high titres were clearly associated to carriage and a significant increase in titre to acquisition of serogroup C meningococci. In contrast, 90% had low IgM titres directed towards the serogroup B polysaccharide; this was not associated to current carriage of serogroup B meningococci.

CONCLUSION:
Meningococcal antibodies were present in most young male adult pharyngeal carriers of meningococci. These antibodies were induced upon acquisition of meningococci and the antibody level was comparable to that observed in patients during the convalescent phase of meningococcal disease. The

anti-C immune reponse upon carriage of serogroup C meningococci was also comparable to that seen in postvaccinees. Asymptomatic meningococcal carriage might thus induce production of protective meningococcal antibodies.

Yours sincerely,

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Impact of the preventive vaccination on the control of meningitis epidemics.

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Face to the meningitis epidemics, the WHO control strategy in meningitis belt is based on the early detection of cases and consists in insure the treatment of cases and the mass vaccination of the population targets. Epidemics that occurred these last four years have shown the limit of such a strategy.
We have analyzed bacteriological data of the CERMES that receives all cerebrospinal fluids (CSF) of the Hospital of Niamey, from 1981 to 1996. Information on anti-meningococcal vaccinations in Niamey have been gathered for the period 1978-1996.
The incidence of the meningitis A increased in 1984 to 1986, then after a progressive reduction until 1993-94, strongly increased during the 1994-95 epidemic. The vaccine coverage was very irregular. High before 1988, it gradually decreased between 1988 and 1994. Thus, it is possible to oppose the weak impact in Niamey of the 1984-86 epidemics (< 200 cases per 100 000) to the high vaccine coverage during the preceding years (about 50%) and, reciprocally, the dramatic epidemic explosion in 1994-95 (about 350 cases per 100 000) while the vaccine coverage was less than 10% during the preceding years).
The appearance of epidemics at Niamey seemed linked to previous vaccination campaigns and vaccine coverage rates. Waiting the conjugate vaccine whose immunological superiority no longer makes doubt, the regular utilization of the polysaccharidic vaccine in the course of preventive vaccination campaigns would reduce significantly the number of deceased by meningitis in the meningitis belt.

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Modification of lipid A biosynthesis in Neisseria meningitidis

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With the aim of making outer membrane vaccines containing less toxic LPS, we have investigated the possibility of modifying lipid A

biosynthesis in Neisseria meningitidis. The LpxA protein catalyses the addition of the O-linked 3-OH fatty acid to UDP-GlcNAc, which is the first step in the lipid A biosynthesis pathway. While attempting allelic replacement of the meningococcal lpxA gene, we discovered that a knockout mutation in this gene results in a viable but LPS-deficient mutant. The evidence for the absence of LPS consisted of (i) no bands visible in Tricine-SDS-PAGE of whole cell lysates after silver-staining for LPS, (ii) no binding of LPS-specific mAbs in whole cell ELISA, (iii) no endotoxin activity with a cell suspension in a chromogenic Limulus assay, and (iv) absence of the lipid A-specific 3-OH C12 and 3-OH C14 fatty acids. No differences between mutant and wildtype were observed in expression of the major outer membrane proteins. The ultrastructure of the outer membrane was not visibly altered when examined by electron microscopy. Analysis of phospholipids showed that PE molecules with shorter-chain fatty acids have most likely replaced the missing LPS. In another approach to lipid A modification, we used the available gonococcal genome sequences to identify two different homologues of the htrB/msbB genes, which in E.coli are required for addition of the acyloxyacyl fatty acids. Knockout mutations for both genes were constructed in N.meningitidis. For one of the genes, this was only possible if combined with a mutation leading to a truncated LPS oligosaccharide chain such as a galE mutant. For both mutants, LPS with a reduced amount of laurate was found. Data showing that the two mutants differ in both structure and biological activity will be presented.

Objet: Delvig-Robinson
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B-and T-cell epitope structure of the serotype 15 PorB protein as a component of the Norwegian meningococcal serogroup B OMV vaccine.
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Human B-and T-cell epitopes were mapped on the serotype 15 PorB protein (strain 44/76), as recognised after immunisation with the Norwegian OMV vaccine, or after systemic meningococcal disease (SMD). An immunodominant B-cell epitope (17-30) was located to the VR1 region corresponding to the putative loop 1; the relevant synthetic peptide was recognised by 74% sera obtained 6 weeks after the third dose of the OMV vaccine, and by 14% sera from SMD patients. We purified PorB-specific IgG antibodies which proved to promote strong opsonophagocytosis measured as respiratory burst response of human neutrophils and internalisation of opsonised FITC-labelled meningococci. The data indicate that PorB protein is an immunogenic component of the Norwegian OMV vaccine, and that PorB elicits antibodies against a single immunodominant epitope. Evidence was obtained that PorB-specific IgG antibodies account for 30-57% of the bulk serum opsonic activity against heat-killed bacteria and therefore may contribute to the opsonophagocytic route of pathogen clearance.
Mapping human T-cell epitopes revealed multiple epitopes spanning the entire PorB sequence in conserved hydrophobic transmembrane regions (i.e. 163-180) and surface exposed loop 1 (19-36, overlapping with the dominant B-cell epitope). In addition, three murine T-cell epitopes (55-72/I-Af,s, 163-180/I-Ad, and 226-261/H2d,f,s) were located to the highly conserved transmembrane regions of the Neisserial porin family, thus not overlapping

with the antibody-binding sites. Altogether, T-cell epitopes identified on the PorB protein, particularly those presented by several MHC class II molecules, could have important implications for the development of meningococcal vaccines.

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Immunogenicity of an N.meningitidis LPS-deficient mutant: Influence of adjuvants on the immune response

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Inactivation of the lpxA gene in N.meningitidis leads to loss of LPS. The immunogenicity of outer membrane complexes (OMCs) or heat-inactivated bacteria of such a mutant derived from strain H44/76 was studied. The immune response against the major outer membrane proteins (OMPs) was poor compared to the immune response elicited by wildtype immunogens. However, addition of external H44/76 lipopolysaccharide (LPS) to mutant OMCs, but not to heat-inactivated bacteria, entirely restored the immune response. By using an LPS-deficient mutant it may be possible to substitute a less toxic compound as adjuvant in meningococcal outer membrane vaccines. Therefore, a broad panel of adjuvants was tested for their potential to enhance the immunogenicity of LPS-deficient OMCs. AlPO4, R.sphaeroides LPS, monophosphoryl lipid A (MPL) and alkaline-hydrolysed meningococcal LPS showed weak adjuvant activity inducing significantly lower immune response compared with wildtype LPS. Strong adjuvant activity similar to wildtype LPS was found among others, with E.coli LPS, meningococcal icsB and rfaC LPS, QuilA and meningococcal htrB LPS, containing penta-acylated lipid A. The isotype distribution of the antibodies differed markedly among the antisera elicited with weak and those elicited with strong adjuvants. IgG1 antibodies were predominantly found in antisera elicited with the less active adjuvants whereas strong adjuvants also induced high IgG2a and IgG2b antibody titers in addition to IgG1. IgG2a and IgG2b antibodies are thought to be protective which was consistent with the bactericidal activity found in the antisera having this antibody isotype profile. Furthermore, the toxicity of the LPS adjuvants was compared in TNF- and LAL assays. These experiments showed reduced toxicity of htrB LPS, which makes it an interesting adjuvant. This study demonstrates that the immunogenicity of meningococcal LPS-deficient OMCs can be restored by using less toxic adjuvants which opens up new avenues for vaccine development against

meningococcal disease.

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Mapping of functional and immunoreactive regions of meningococcal TbpA.

Pajon, R.(1), Gorringe, A.(2), Borrow R.(3), and Silva, R (1).
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Pathogenic Neisseria express two transferrin binding proteins (TbpA and TbpB) which are involved in the uptake of iron from human transferrin (hTf)1,2. TbpA, is thought to be a conserved integral outer membrane protein which forms a gated channel for the passage of iron (3). In this study we have characterised the immunoreactive and functional regions of TbpA, using two approaches: (i) The whole tbpA gene was cloned and expressed in E. coli as a fusion protein. The purified TbpA protein was refolded in vitro with 7 out of 60 different folding conditions, as tested by hTf binding, and was also incorporated into vesicles of dipalmitoyl-phosphatidylcholine and cholesterol. Antisera were then raised by subcutaneous administration of TbpA-containing vesicles, and detergent-folded protein into mice. (ii) The peptide spot synthesis approach, previously described by Frank (4), was used to scan the whole B385 TbpA sequence. Overlapping TbpA peptides (18-mer long), with a sequence shift of 10 amino acids between consecutive peptides, were synthesized on two cellulose sheets, with 44 spotted peptides on each. Their ability to bind mouse, rabbit and human sera, recombinant TbpB, and hTf, as ligands, was determined in duplicate.

This study has revealed important features of TbpA, including the existence of well isolated transferrin binding regions throughout the whole TbpA sequence, and the characterization of different TbpB-binding regions at the TbpA N-terminal half. The preliminary characterization of target regions, of mice, rabbit, and human response in convalescent meningococcal disease sera will be presented.

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4.Frank, R. Tetrahedron, 1992; 48:9217-9232.

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Objet: abstract
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Distribution and characterisation of the opc genes in Neisseria meningitidis and Neisseria gonorrhoeae
P. Zhu, G. Morelli, M. Achtman Max-Planck Institut fuer molekulare Genetik, Ihnestr.73, D-14195 Berlin, Germany

Former data indicated that the opc gene encoding the Opc invasin protein was only present in some meningococci. We have now sequenced the 12 Kb region surrounding opc from 2 meningococci and 2 gonococci and examined the presence of the opc gene in a large variety of strains by hybridization and PCR. The results show that 20/20 gonococci possess an opc-like gene and that meningococci of the ET-37 complex and the A4 cluster possess a deletion at that position. This gene will henceforth be called opcA because a second opc-like pseudo-gene (opcB) is located at a different location in the chromosome of both gonococci and meningococci.
The OpcA protein has the same 2-dimensional structure in meningococci and gonococci but the highly basic surface-exposed loops differ completely in sequence. These differences might represent different specificities for adhesion and invasion. Transcription of opcA in gonococci was demonstrated by Northern hybridisation and by primer extension. The promoter region is totally different from the promoter region in meningococci and does not have any oligonucleotide repeats.

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Genetic exchange in nature
Martin Schencker, Bodo Linz, Mark Achtman
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The 30 kb region spanning tbpB and opaA was sequenced from serogroup A, subgroup IV-
1 and serogroup C ET-37 complex meningococci isolated from The Gambia and Mali, respectively. The region contains various housekeeping genes and repetitive DNA immediately flanking the tbp operon and opaA. Gene fragments from two of these housekeeping genes, potF and amiA, were chosen for detailed analysis as well as from
tbpB and opaA. These 4 gene fragments were sequenced from 100 subgroup IV-1

serogroup A meningococci isolated during and after an epidemic in The Gambia in the
1980's and from 100 ET-37 complex serogroup C
meningococci isolated during endemic disease in Mali in the early 1990's. The tbpB gene
and the amiA gene varied due to import of 5 - 11 kb pieces of foreign DNA in a few of the
subgroup IV-1 bacteria.

In an attempt to find the donors, the tbpB gene fragment was
also sequenced from over 100 strains of other serogroups and species isolated from the
nasopharynx of healthy individuals in these 2 countries. Potential donors were found
among strains of N. lactamica and N. cinerea isolated from the same countries. Similarly,
pairs of N. lactamica and N. cinerea were found with identical sequences. These results
indicate that DNA exchange among N. meningitidis, N. lactamica and N. cinerea is
frequent during nasopharyngeal colonization.

best regards
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Evolution of an intergenic region in the neisseriae
Peixuan Zhu, Giovanna Morelli and Mark Achtman
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Several kb downstream of the opc gene is an intergenic region between trpE and purK
which contains the dcmD-dcrD restriction modification gene pair in N. gonorrhoeae and
an open reading frame (ORF) of unknown function in most N. meningitidis. Analysis of this
intergenic region in diverse neisseriae revealed an astounding degree of evolutionary
variation. 3 distinct ORFs, called orfA, B and C, each with diverse sequence variants, were
found in different neisserial species and 3 sequence variants of dcmD-dcrD (94 - 99%
identical) were also found. This is the first time that closely-related sequence variants of an
R/M system have been described.

10/10 gonococci possessed one variant of the R/M. Whereas 105 N. meningitidis of diverse MLST STs possessed orfA, STs 25 and ST26 possessed a 2nd variant of the R/M.

Half of the N. cinerea tested possessed orfA and the remainder possessed a third R/M
variant . Sequencing rRNA from these bacteria revealed that the bacteria possessing the
R/M pair were clustered together, independently of species, and apart from bacteria
possessing an ORF. Similarly, the housekeeping genes flanking the intergenic region
clustered in different groups depending on whether the bacteria possessed an ORF or an
R/M gene pair in that region.

orfA was found in N. meningitidis, N. cinerea, N. lactamica and N flava. orfC was found in
N. lactamica and N. flavescens. orfB was found in N. elongata, N. mucosa and N. denitrificans.

The correlation between rRNA sequences and the intergenic region could be explained if
the currently recognized species represented polyphyletic descent followed by
homogenization through transformation. In accordance, STs 25 and 26 differ at 2-3 of the 6
housekeeping genes used in MLST from all other known meningococci and might have
also inherited these genes from an ancestor unrelated to that of other meningococci.

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Objet: Abstract submission
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Dear Sir:

This abstract is being submitted with the understanding that its contents will be held in confidence until the abstracts are distributed (As I understand it, this restriction does not limit peer review). Please also let me know the anticipated distribution data of the published abstract book as soon as possible.

Abstract

Immunogenicity of a combination of serogroup C conjugate vaccine and an outer membrane-protein based serogroup B vaccine for prevention of Neisseria meningitidis (Nm) disease.
D. M. Granoff, I. Aaberge, B. Haneberg, J. Holst and H. Raff
Chiron Vaccines, Emeryville, and Children's Hospital Oakland Research Institute, Oakland, California, USA; and the National Institute of Public Health (NIPH), Oslo, Norway.

Serogroup B and C strains of Nm together account for the majority

of invasive disease in Europe and the US. The NIPH NmB vaccine consists of partially purified outer membrane proteins from strain 44/76 (B15:PI 7,16:L3,7,9), presented as proteoliposomic vesicles adsorbed to aluminum hydroxide (alum). This vaccine is safe, elicits strain-specific immunity in children and adults, and is efficacious in preventing NmB disease in adolescents. The Chiron NmC conjugate (conj.) vaccine consists of oligosaccharides conjugated to the carrier protein, CRM197, a non-toxic diphtheria toxin, and is adsorbed to alum. This vaccine also is safe, elicits high titers of serum bactericidal antibody in infants vaccinated as young as 2 and 3 months of age, and induces immunologic B cell memory to the unconjugated NmC polysaccharide.

We investigated the immunogenicity of each of these vaccines given alone, or in combination, in guinea pigs. We also investigated whether MF59, an adjuvant consisting of a micro-fluidized emulsion of squalene in water, augments serum antibody responses to the combination vaccine. To date, MF59 has been evaluated in more 6000 humans (ranging in age from birth to >100 ys), and has been shown to be safe and to augment serum antibody responses to a variety of investigational vaccines.

Groups of guinea pigs (N=15 animals) were assigned to receive one of the following vaccines: Group 1: NmC-Conj/alum; Group 2: NmB/alum; Group 3: NmC-Conj/NmB/alum; Group 4: NmC polysaccharide/NmB/alum; and Group 5: NmC-Conj/NmB/MF59. An additional group of control animals (Group 6, N=5) received alum alone. Each animal received two injections, IM, separated by 28 days. Serum samples were obtained prior to each injection, and 18 days post 2nd injection and assayed for IgG anticapsular antibody concentrations to NmC by ELISA, and for complement-mediated bactericidal titers to NmC, and NmB strain 44/76. Respective geo. mean NmC IgG anticapsular antibody concentrations (units/ml) post-1 and post-2 were as follows: Group 1: 20, 155; Group 2: <1, <1; Group 3: 10, 71; Group 4: <1, 1.5; Group 5: 15, 426; and Group 6 <1, <1. Bactericidal titers were assayed in pooled sera from each group.

		NmC(1/titer)			NmB (1/titer)		
Group	Vaccine	Pre	Post-1	Post-2	Pre	Post-1	Post-2
1	NmC-Conj/alum	<5	80	>3375	<5	<5	<5
2	NmB/alum	<5	<5	15	<5	15	800
3	NmC-Conj/NmB/alum	<5	25	2000	<5	25	5000
4	NmC polysaccharide NmB/alum	<5	<5	30	<5	25	1500
5	NmC-Conj/NmB/MF59	<5	50	>3375	<5	25	4000
6	Alum (control)	<5	<5	<5	<5	<5	<5

In guinea pigs, the NmC conj/NmB vesicle combination vaccine is immunogenic for each of the components and elicits high titers of serum bactericidal antibody. This combination merits further evaluation in humans.

Objet: Mayer - Stephens
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DNA sequence based subtyping of _N. meningitidis_ using genes encoding basic metabolic enzymes
Mayer, L.W., Tondella, M.L.C., Sacchi, C.T., Polavarapu, R.G., Popovic, T., Reeves, M.W., Rosenstein, N.E., Perkins, B.A., and Stephens, D.S.

Meningitis and Special Pathogens Branch, Centers for Disease Control and Prevention, Emory University, and VA Medical Center, Atlanta, GA, USA
DNA sequence subtyping systems offer many advantages, the most important of which may be interlaboratory comparison. We have developed a typing system using the sequences of some of the genes encoding basic metabolic enzymes used for typing by multilocus enzyme electrophoresis (MEE) to relate sequence based typing to the dataset of MEE and epidemiological information. We can also use the relative amount of genetic diversity estimated by MEE to select which genes will be appropriate for short- or long- term comparisons. These genes should have less selective pressure on them compared to genes for outer surface molecules. PCR primers were designed to amplify a portion of the coding regions. These products were purified and both strands sequenced usually with internal primers. The genes, their symbols, MEE abbreviations, and the length of the sequenced portion are: aspartate transaminase, _aspC_, GOT, 515 bp; carbamylate kinase, _car_, CBK, 817 bp; isocitrate dehydrogenase, _icdA_, IDH, 444 bp; malic enzyme, _mae_, ME, 371 bp; and shikimate dehydrogenase, _aroE_, SKD, 415 bp. The quality of correlation between MEE and sequence based typing varied with each gene. For group C strains from Texas and ET-5 group B strains from Oregon the best agreement was using SKD. ME also clustered the ET-5 strains and IDH clustered the TX group C strains well, but several disagreements were also observed. Sequence based typing of meningococci provides a useful system comparable but not identical to MEE or other typing methods.

Objet: Klee - Achtman
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Distribution of 8 genetic islands in different strains of Neisseria meningitidis.

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Eight chromosomal regions identified by Tinsley and Nassif, 1996 as being present in serogroup A, subgroup IV-1 strain Z2491 and absent in Neisseria gonorrhoeae were sequenced. The regions consist of DNA inserts of 2 - 40 kb inserted into intergenic regions of the meningococcal genome. One region of 21 kb (hmwp) encodes several open reading frames, including a high molecular weight protein with homology to filamentous hemagglutinin of Bordetella pertussis. Another region of 40 kb, probably a prophage, is very similar to a prophage in Haemophilus influenzae and also contains several unknown ORFs. The other 6 regions (2 - 6 kb) encode proteins with homology to siderophore receptors, and leukotoxin secretion proteins, which might be virulence factors, as well as other proteins whose pathogenic potential is unknown. The presence of the regions and the individual ORFs was tested by PCR and dot blots in 10 meningococcal MLST-representatives. The prophage is integrated at the same position in epidemic serogroup A strains but lacking at that position in other bacteria which, however, possess several of the prophage ORFs at other locations. The hmwp region varies from 13 - 26 kb in different strains. The ORFs from 5 of the smaller regions were present in all meningococci.

_____
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Objet: Joseph P. Dillard
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A pathogenicity island in Neisseria gonorrhoeae
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We are characterizing a region of the gonococcal chromosome that has the characteristics of a pathogenicity island. Southern blotting experiments indicated that the region is only present in some strains of N. gonorrhoeae. Two thirds of the gonococcal strains we have surveyed contain the island. A comparison of a strain containing the island to one lacking it suggests it occupies about 60-90 kb of the chromosome. Examination of the DNA sequence indicated that the region was acquired by horizontal transfer. The G+C content was found to be significantly lower than that of the gonococcal chromosome overall (42 vs. 50%), and the region is deficient in gonococcal DNA uptake sequences. The island contains a serum resistance locus. Other genes in the region are involved in genetic transformation. Two of these open reading frames encode proteins homologous to those involved in conjugation of the E. coli F-plasmid. We speculate that the region encodes a secretion system which transports DNA for transformation and may transport other factors important for virulence. We are currently determining if the island is found in isolates associated with particular manifestation of gonococcal infection.

Objet: JELFSCAUGANT
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Global study of a new variant of the ET-37 complex of Neisseria meningitidis.
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4. WHO Collaborating Centre for Reference and Research on Meningococci, Oslo, Norway.

During the early 1990s, a new variant of meningococci belonging to the ET-37 complex was associated with extensive outbreaks of meningococcal disease. This new variant was first detected in Canada and recently has been associated with ongoing disease in many developed countries. This new variant is referred to as ET-37 complex/ET15 and the phenotypes most

commonly associated with the new variant is C:2a:P1.5,2, C:2a:P1.2 or C:2a:P1.5.
Of greatest concern, is the tendency of this variant to be associated with high attack rates amongst the 15 - 19 years age group, increased clustering of cases, particularly community cases and increased morbidity and mortality rates. This picture has generally not been found with meningococcal disease associated with other strains belonging to the ET-37 complex.
Seventy five meningococcal isolates referred to the WHO Collaborating Centre for Reference and Research on Meningococci, Oslo were examined. These 75 isolates were from ten countries and spanned several years and in addition, a control group of thirteen "old" ET-37 complex strains (including a 1917 strain of meningococci) and the reference strains H44/76 and B1940 were included.
The above strains were subjected to restriction digestion using two rare cutting restriction enzymes, SpeI and NheI and the digested chromosomal DNA separated by pulsed field gel electrophoresis. Amongst the 75 ET-37 complex/ET 15 strains 48 SpeI PFGE fingerprint patterns were observed and statistical analyses suggested that there was a single introduction and subsequent dissemination of this new variant into each country. PFGE fingerprinting by both enzymes revealed a high degree of banding similarities amongst all ET-37complex/ET15 variant strains including those from geographically distant countries. The PFGE fingerprints obtained were distinct from those of the thirteen "old" ET-37 complex isolates.
Additionally, Southern hybridisation was also performed on all of these isolates and the fingerprints hybridised with chemiluminescent labelled probes prepared from PCR products for the following genes: porA, porB, penA, dhps, IgA, pilQ, Class I pilin, recA, opa and CtrA.
Results suggest that the new variant is genetically distinct from the "old" ET-37 complex strains and that numerous genetic changes have occurred in addition to the mutation within the housekeeping gene that has permitted differentiation of this variant.

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TO THE SCIENTIFIC COMITTEE:
ELEVENTH INTERNATIONAL PATHOGENIC NEISSERIA MEETING, NICE

Please, consider for oral presentation the following abstract:

DEVELOPMENT OF AN EXPERIMENTAL MENINGOCOCCAL B:4:P1.4 OUTER MEMBRANE VESICLE VACCINE AND EVALUATION IN A MOUSE MODEL
E. Rosenqvist, N. Bjæring Hansen, E. A. Høiby, D.A. Caugant, J. Holst, E. Namork and L.O. Frøholm
Department of Vaccinology and Bacteriology, National Institute of Public Health,
P.O. Box 4404 Torshov, Oslo, Norway.

An outer membrane vesicle (OMV) vaccine, based on a B:15:P1.7,16 Neisseria meningitidis strain has been developed in Norway ("MenB-Folkehelsa"). In a clinical trial among teen agers the vaccine was shown to give significant protection against systemic group B meningococcal disease. However, the serum bactericidal activity (SBA) of vaccinee sera was dependent on the target strain in the assay, indicating that in other epidemiological situations the vaccine would probably be less efficacious.

We have now prepared experimental OMV vaccines from other epidemiologicaly relevant strains by the same technology as "MenB-Folkehelsa". Two of the new vaccine strains were systemic isolates from New Zealand (B:4:P1.4) and one was from Norway (B:8:P1.4). All belonged to the lineage III clone. The vaccine strains expressed different immunotypes. Combination of these vaccines with "MenB-Folkehelsa", were also prepared. The vaccines were characterized chemically, physically and immunologically. All vaccines were highly immunogenic in mice, and induced bactericidal antibodies. However, whereas cross-reactive antibodies were detected by ELISA and immunoblotting, only low levels of SBA to heterologous strains were observed with the vaccines based on only one OMV type. With combined vaccine preparations, the level of cross-reactive SBA was significantly increased, indicating that "combination vaccines" is a possible strategy to confer protection against a broader range of group B meningococcal strains.

13. May 1998

Sincerely

Einar Rosenqvist, Ph.D
Senior Scientist, NIPH

Objet: Pettersson-Tommassen
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The meningococcal lactoferrin receptor
Annika Pettersson1, Thorsten Prinz1, Peter van der Ley2, Jan T. Poolman3, and Jan Tommassen1
1Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. 2National Institute of Public Health and Environmental Protection, P.O Box 1, 3720 BA Bilthoven, The Netherlands. 3SmithKline Beecham Biological s.a., Rue de l'Institut 89, 1330 Rixensart, Belgium.

Pathogenic Neisseriae are able to obtain iron directly from transferrin, lactoferrin and haem- containing compounds, for which specific receptors in the outer membrane are produced under iron limitation. The lactoferrin receptor consists of two proteins, LbpA and LbpB. The structural genes for these proteins are organized in an operon, which is flanked by long direct repeats and contains a binding-site for the Fur repressor in the promoter region. LbpA is an integral outer membrane protein and shows homology to TonB-dependent siderophore receptors in E. coli. A topology model, according to which the protein traverses the membrane 26 times in a beta-sheet conformation, was verified in whole-cell ELISAs by using antisera against synthetic peptides corresponding to the exposed loops.
The LbpB protein is a lipoprotein, which binds lactoferrin on blots. The LbpB sequence contains two long stretches of negatively charged residues, which could be important for lactoferrin binding. Isogenic mutants lacking LbpA or LbpB showed reduced ability to bind lactoferrin. Plate feeding assays with lactoferrin as the sole iron source showed that the lbpB mutant was still able to use lactoferrin, whereas the lbpA mutant was not. A model for iron acquisition via lactoferrin is proposed.
The LbpB protein is an interesting vaccine candidate because it is

probably entirely exposed to the cell surface and anchored to the membrane only via its lipid moiety. To gain insight in the variability of the LbpB protein, the lbpB genes of five strains were sequenced. The sequences were about 70% identical to each other. The immunogenicity of the protein is currently being investigated in animals and humans.

Objet: Samuelsson-Hansen
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Do prophylactic measures against meningococcal disease in Denmark fulfil the purpose?

Susanne Samuelsson, Elisabet T. Hansen

Neisseria Department and Department of Epidemiology, Statens Serum Institut, Copenhagen, Denmark.

The guidelines for prevention of meningococcal disease aim to prevent the devel-opment of serious illness in secondary cases, to reduce the risk of occurrence of secondary cases and to protect against serogroup A and C disease. The preventive measures include information, chemoprophylaxis and vaccination to those with an increased risk of disease.

Objective:
- To assess to which extent recommended prophylactic measures are received and followed by the target groups.
- To describe the occurrence and characteristics of clusters of meningococcal dise-ase.

Study design:
- During October 1995 - April 1997 172 telephone interviews of an adult in the household of notified cases were carried out and 634 questionnaires were delive-red to parents to contacts in schools/daycare institutions.
- 75 clusters registered in the Notification System for Communicable Diseases 1980-1996 were analysed.

Results:
Information about early signs and symptoms of illness reached only about 40% of the households. Almost 100% of the parents to contacts in institutions were in-formed. This difference depends on whether they received written information. In all 90% of household-like contacts received chemoprophylaxis. Attack-rate was significantly higher for those who did not receive chemoprophylaxis than for tho-se who did. The vaccination rate for contacts to serogroup C cases was 59%. Secondary cases accounted for 2.2% of notified cases. Case fatality rate was sig-nificantly higher for primary than for secondary cases. The relevance of the actual indication for vaccination of contacts after a single case due to serogroup C will be discussed.

neiss98.doc

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Encoding: x-uuencode

Objet: Lehmann - Guttormsen and Halstensen
Date: Thu, 14 May 1998 10:32:45 +0000
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Bergen, May 14.th 1998

To the Scientific Committee,
Neisseria 98

Enclosed please find TWO abstracts submitted for consideration for oral presentation(-s) at Neisseria 98 in Nice:

€ ABSTRACT I. "Patient opsonins recognize meningococcal outer membrane class 1 and class 3 proteins"

€ ABSTRACT II. "Patient opsonins recognize meningococcal transferrin binding protein A+B complexes"

To dissect which of the numerous meningococcal outer membrane components that mediate human opsonophagocytosis responses, a new functional assay reflecting antigen-specific opsonin-dependent phagocytosis and oxidative burst activities was employed in both studies. Abstracts I and II briefly introduce the method, and demonstrate opsonophagocytic responses against variuos outer membrane components during meningococcal disease.

An ALTERNATIVE approach for an oral presentation,- depending on the input from the scientific commitee, could be to present a summary of the two studies as ONE talk, presenting the method more thoroughly and the main results obtained with both the outer membrane vesicles, class 1 and 3 proteins, and the transferrin binding proteins.

The abstracts follow below.

Yours sincerely,

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ABSTRACT 1.

Patient opsonins recognize meningococcal outer membrane class 1 and class 3 proteins

A.K. Lehmann1, A. Halstensen1, I. Aaberge2 , J. Holst2, S. Sørnes1, L.M. Wetzler3, H.-K. Guttormsen 4

Medical Department B, University of Bergen, Bergen, Norway1, Department of Vaccinology, National Institiute of Public Health, Oslo, Norway2 , The Maxwell Finland Laboratory for Infectious Diseases, Boston Medical Center, Boston University School of Medicine,Boston3, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston4

Human serum opsonins produced against meningococcal outer membrane components during meningococcal disease were quantified by flow cytometry

(1) using sera from 40 patients infected with different Neisseria meningitidis strains. Outer membrane vesicles (OMV) and class 1 and class 3 proteins (porins) purified from the same strain (44/76) were adsorbed to fluorescent latex beads, opsonized with individual patient sera and exposed to human leukocytes.

Increasing amounts of serum opsonins against both OMV and the two porins were detected during disease, as reflected by leukocyte phagocytosis products (PP; percentage phagocytes times mean number of beads per phagocyte). The PPclass 1 and PPclass3 during disease correlated closely with the PPOMV values. The PPclass3 were higher than the PPclass 1 values; median (range) of 754 (17-1057) and 107 (4-458), respectively ($p<0.001$).The oxidative burst corresponded closely with the PP values. No differences were found between anti-porin serum opsonic activities of patients infected with strains of homologous or heterologous serotype or serosubtype to those of the target antigens.

Anti-class 3 IgG levels were higher than anti-class 1 IgG levels (ELISA). The patient anti-OMV and anti-porin IgG titers correlated with the opsonic activities in disease sera ($rs=0.50$, $rs=0.71$, $rs=0.63$; $p=0.01$ for all anti-OMV, anti-class 1 and anti-class 3 IgG versus PP values).

We conclude that human serum opsonins produced in response to meningococcal disease recognize meningococcal OMV, class 1 and class 3 proteins in functional assays, and that both phagocytosis and intracellular oxidative burst microbicidal activities are initiated by anti-porin opsonins. The class 3 protein appeared more immunogenic than the class 1. The anti-porin opsonins recognize epitopes other than those employed in the current serotyping schemes.

Reference

1. Lehmann A.K., A. Halstensen, J. Holst, and C.-F. Bassøe. Functional assays for evaluation of serogroup B meningococcal structures as mediators of human opsonophagocytosis. J. Immunol. Meth. 1997;200:55-68.

ABSTRACT II.

Patient opsonins recognize meningococcal transferrin binding protein A+B complexes

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Human serum opsonins produced against meningococcal transferrin binding protein A+B complexes (TbpA+B) during meningococcal disease were quantified by flow cytometry, using TbpA+B-coated latex beads in a recently developed opsonin-dependent phagocytosis and oxidative burst assay (1). Sera were obtained on admission to hospital and during the convalescence from 40 patients infected by a variety of Neisseria meningitidis strains. TbpA+B purified from two strains (K454 and B16B6, expressing 85kDa and 68 kDa TbpB, respectively) were adsorbed to fluorescent latex beads, opsonized with individual patient sera and exposed to freshly obtained human leukocytes.

Increasing amounts of opsonins were detected against TbpA+B from both strains during meningococcal disease, as reflected by leukocyte phagocytosis products (PP; percentage phagocytes times mean number of beads per phagocyte). The PPB16B6 were higher than the PPK454 values ($p<0.0001$ both with acute and convalescent sera),but significant correlations were found

between PPK454 and PP B16B6 values. Phagocytosis and oxidative burst responses corresponded closely (rs=0.90 and rs=0.99; p=0.01, for anti-K454 and anti-B16B6 TbpA+B activities in convalescent sera). Phagocyte ingestion of opsonized TbpA+B-beads and intracellular oxidative burst activities were visualized by confocal laser scanning microscopy.

The patient IgG responses measured by ELISA were also higher against TbpA+B from strain B16B6 than from strain K454 ($p<0.001$), and correlated with PP values (rs=0.37, and rs=0.33; p=0.05, for anti-B16B6 and anti-K454TbpA+B IgG versus PP values using convalescent sera).

We conclude that human serum opsonins produced in response to meningococcal disease recognize meningococcal TbpA+B complexes in functional assays, and that both phagocytosis and intracellular oxidative burst microbicidal activities are initiated by anti-TbpA+B opsonins. TbpA+B from strain B16B6 was the most immunogenic in both ELISA and functional assays, but the results demonstrate opsonin cross-reactivity against TbpA+B from the two strains in human sera.

Reference

1. Lehmann A.K., Halstensen A., Holst J., and Bassøe C.-F. Functional assays for evaluation of serogroup B meningococcal structures as mediators of human opsonophagocytosis. J. Immunol. Meth. 1997;200:55-68.

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A gonococcal porA pseudogene: implications for understanding the evolution and pathogenicity of Neisseria
gonorrhoeae.

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All members of the genus Neisseria, which includes the important human pathogens Neisseria meningitidis and
Neisseria gonorrhoeae, express at least one member of a family of related porins. Exceptionally, N. meningitidis
expresses two porins including the most divergent Neisseria porin, the meningococcal serosubtyping antigen PorA,

which was thought to be unique to this species. Unexpectedly, a porA gene was identified in four unrelated gonococcal
isolates. Both the gonococcal and meningococcal porA loci were adjacent to a locus encoding a gene homologous to the
Escherichia coli greA gene, although the IS1106 element downstream of porA in at least some meningococci was
absent in the gonococcal isolates examined. Mutations in the promoter region, together with frame shift mutations in the
coding region, of the porA gene in each of the four gonococci examined indicated that it was likely to be a pseudogene.
Determination of the nucleotide sequence of the porA pseudogene directly from urethral exudate confirms that the
gonococcus does not express a PorA protein during infection and that the mutations are unlikely to be a consequence of
the subculture of isolates. It is proposed that this gene was selectively inactivated upon divergence of gonococci and
meningococci, implying that, while advantageous during colonisation of the upper respiratory tract, this protein has no
function in, or hinders, colonisation of the urogenital tract. No porA homologues were detected in other Neisseria
species.

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Most antibodies to PorB and Rmp do not bind to viable meningococci, but bind strongly to ethanol-killed bacteria

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MAbs against PorA, Opc and LPS are regularly highly bactericidal, whereas most MAbs against PorB, Rmp and Lip (H.8) are not bactericidal. The mechanism behind this lack of activity has not been revealed. We will present data indicating that these antigens are not significantly exposed on viable meningococci.

The binding of antibodies to viable and ethanol-killed meningococci were examined by flow cytometry. This method renders the surface structures of the meningococci relatively undisturbed, compared to denaturing methods like immunoblottting, colony-blotting, and also ELISA. A panel of mouse MAbs and absorbed human sera were tested for binding to different meningococci.

All MAbs against PorA, both anti-P1.7 and anti-P1.16, as well as anti-Opc, anti-LPS and anti-capsule, displayed strong binding to viable bacteria.

Surprisingly, most MAbs against the PorB antigen bound very weakly to viable meningococci. Only one MAb (anti-serotype 15) showed a significant, but variable binding (day-to-day variations). The binding was considerably higher against a mutant expressing short-chain LPS. A

corresponding, low binding against viable bacteria was observed with serotype 4 MAbs. Conversely, both serotype 15 and serotype 4 MAbs bound strongly to killed bacteria. Human sera absorbed with a PorB-lacking mutant, did neither bind significantly to viable bacteria, but they bound strongly to killed bacteria.

Similarly, MAbs against Lip and Rmp also proved negative in binding to viable bacteria, while they reacted strongly in immunoblotting.

These experiments probably explains why many MAbs (e.g. anti-PorB) do not demonstrate any bactericidal activity: they simply do not bind to viable bacteria because the epitopes are not properly expressed on the bacterial surface.

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aase-michaelsen.doc

Name: aase-michaelsen.doc
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Objet: Rouppe van der Voort -van Alphen-Poolman
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Immunogenicity studies with a genetically constructed multivalent PorA and a wild type meningococcal group B outer membrane vesicle vaccine in infant cynomolgus monkeys

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Abstract
Two meningococcal outer membrane vesicle vaccines were compared for their immunogenicity in infant cynomolgus monkeys. A Norwegian vesicle vaccine was prepared from a wild type case-isolate, termed H44/76 (B:15:-P1.7-,16). In our laboratory, two recombi-nant vaccine strains derived from H44/76 were constructed, expressing three different PorAs each. From these tailor-made triple PorA strains a Dutch hexavalent PorA vesicle vaccine was prepared. The Norwegian vaccine contained all four classes of major outer membrane proteins (OMP) and wild-type L3/L8 lipopolysaccharide (LPS). The Dutch vaccine consisted mainly of class 1 OMPs (90%), class 4 OMP and truncated GalE LPS. Groups of three infant monkeys were immunized with a human vaccine dose at the age of 1*, 2* and 4* months. Two monkeys of each

group received a fourth dose at the age of 11 months. The antibody responses were analyzed by ELISA and bactericidal assay. In ELISA, both vaccines were immunogenic and led to booster responses, particularly after the fourth immunisation. Genetically constructed or well characterised wild type meningococcal strains were used as targets in the bactericidal assay to study the specificity of the induced bactericidal activity. The Norwegian vesicle vaccine mostly induced sero-subtype P1.7,16 specific bacterici-dal antibodies, although some serotype 15-specific bactericidal antibodies were induced as well. The Dutch vesicle vaccine induced bactericidal antibodies against all six PorA sero-subtypes included in the vaccine, although differences in titre were observed.

Objet: Bennett, Williams, Srivastava, and Rest
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Note: we are unable to underline in this e-mail system. Items that should be underlined are between *text*.

Title: Interactions of intracellular gonococci with host cytoplasmic proteins.

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Abstract: Using the yeast two-hybrid system, we found 5 host proteins that interact with Opa P from gonococcal strain F62SF. Of these 5 Opa-interacting proteins (OIPs), two were homologous to known host proteins: one being pyruvate kinase (PK) and the other, thyroid hormone receptor-interacting protein (Trip6), a recently described cytoskeleton-associated protein. Initial demonstration of PK interactions with gonococci has been published by Williams, et al. (Mol. Micro. 27:171-186). Ongoing work has expanded on these observations. (1) PK and Trip6 interactions with gonococci is not specific for OpaP. As shown with the yeast two-hybrid system, PK and Trip6 can interact with several different Opa proteins from gonococcal strain FA1090. (2) We have used the two-hybrid system to dissect which of the 3 individual and specialized LIM domains of Trip6 interact with Opa proteins. (3) Antibodies to Trip6 have been generated and used as a probe to verify the cytoplasmic localization of this protein. Preliminary results show that recombinant Trip6 binds to Opa expressing gonococci in vitro. In addition, intracellular gonococci stain brightly with anti-Trip6 antibodies, indicating that the bacteria may be capable of directly interacting with Trip6 within host cells. These are exciting first glimpses at the intracellular lifestyle of the gonococcus in human cells.

Objet: oral presentation
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Dear Organization,

We hereby would like to submitt our presentation "Characterization including sequence determination of b-lactamase genes in two Neisseria meningitidis plasmids" for your consideration concerning oral presentation at the Eleventh International Pathogenic Neisseria Conference 1-6 November 1998.
Presentation person is Anders Bäckman, adress is as stated in the abstract.

Tel: 46-19 151520, Fax: 46-19 127416,

Characterization including sequence determination of b-lactamase genes in two Neisseria meningitidis plasmids.

Anders Bäckman 1, Paula Orvelid 1, Julio A Vazquez 2, Ola Sköld 3 and Per Olcén 1.
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SE-701 85 Örebro, Sweden, 2 Servicio de Bacteriologíca, Centro Nacional de Microbiologíca, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid, Spain, 3 Department of Pharmaceutical Bioscience, Biomedical Center, Uppsala University, Uppsala, Sweden.

The aim of the present study was to identify and characterize the plasmids (5.1 kb) with genes for b-lactamase found in two N. meningitidis strains, isolated in Spain (1), by DNA sequencing and comparison to known types of plasmids and b-lactamase genes.

The b-lactamase producing N. meningitidis strain MC9690-129 (9917) was isolated from blood of a patient with meningitis and MC9690-130 (9919) from the throat of a contact to the patient. Both strains were serogroup B:4:P1.15. (1).
Antibiograms for the N. meningitidis strains were performed with the E-test (Biodisk, Sweden) and MIC (mg/L) were almost identical for the two strains: penicillin G 1.0, penicillin V 3.0, ampicillin 2-3, piperacillin 0.032, oxacillin 24, piperacillin-tazo *0.016, imipenem 0.19, cefuroxime 0.38, ceftriaxone <0.002, ceftazidime 0.023, ciprofloxacin 0.004, rifampicin 0.0047-0.008, chloramphenicol 0.5-0.75, sulfadiazine R and trimethoprim-sulfadiazine R.
Plasmid DNA was prepared from the MC strains (Wizard, Promega). Restriction enzyme digestions (Xba I, Pvu II, Bam HI and Hinf I) showed that the MC plasmids were nearly identical to N. gonorrhoeae plasmid pJD5, called African (2), of 5.1 kb.
The MC plasmids were then digested with restriction enzymes (Xba I, Pvu II) and cloned into M13mp18/19, E. coli JM105 (Pharmacia Biotech, Sweden). The DNA was cycle sequenced on the GeneAmp PCR System 9600 (Perkin-Elmer) with ABI PRISMTM BigDyeTM Terminator Cycle Sequencing and analyzed on an ABI PRISMR 310 Genetic Analyzer (Perkin-Elmer) with the Data Utility software. The b-lactamase gene and the surrounding regions (2.5 kb) were determined and compared to registered international databases.

The genes correspond to a TEM-1 B variant (3) and the surrounding sequences are almost identical to the N. gonorrhoeae plasmid pJD5 (African) but with identified differences, the background of which can be discussed.

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Isolation of a strain of b-lactamase producing Neisseria meningitidis in Spain. Eur J Clin Microbiol Infect Dis 15:181-182, 1996
2. Dillon, J-A R, Yeung K-H. b-lactamase plasmids and chromosomally mediated antibiotic resistance in pathogenic Neisseria species. Clin Microbiol Rev 2:125-133, 1989
3. Canica, M M M, Lu C Y, Krishnamoorthy R, Paul G C. Molecular diversity and evolution of blaTEM genes encoding b-lactamases resistant to clavulanic acid in clinical E. coli. J Mol Evol 44:57-65, 1997

Objet: Seifert-Serkin
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Specialized DNA recombinations mediate gonococcal pilin antigenic variation.

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Antigenic variation of the gonococcal (Gc) pilus is characterized by the high frequency, unidirectional transfer of a short portion of pilS sequences into pilE. Complementation of a Gc recA mutant with the E. coli (Ec) recA gene increases pilin variation 10-fold. In contrast, the Ec RecA catalyzes Gc DNA transformation at normal levels but does not mediate DNA repair at all. We have also shown that the RecA protein must interact with the Gc RecF-like pathway of homologous recombination for pilin variation, but uses the Gc RecBCD pathway of homologous recombination for DNA transformation. Finally, we have demonstrated that circular DNA intermediates carrying pilE/pilS hybrids can be isolated using genetic means, and production of these circular intermediates is RecA-independent. The pilS sequences linked to pilE in the hybrid are preferentially transferred into pilE during DNA transformation at a frequency 100-fold higher than general transformation. Moreover, the recombination with pilE is RecA-dependent. Therefore, circular intermediates explain all of the observed properties of the pilin variation system, and both homologous recombination and other unknown factors are involved in their formation and integration at pilE. While the homologous recombination reactions used to transfer pilS sequences into pilE are fundamentally different from those used for general recombination, they rely on the RecF-like pathway of homologous recombination.

Objet: Ram-Rice
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The role of complement factor H in determining gonococcal serum-resistance

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Factor H (fH), a key complement regulator, binds polyanions, as well as

certain microbes to enhance pathogenicity. Here, we identify two distinct fH receptors on N. gonorrhoeae. Recently, we demonstrated fH binding to the sialylated lactosamine LOS structure (obtained by growth in media containing CMP-NANA), converting the organism to a complement non-activator surface (J Exp Med,1998:187;5:743-52). Gonococcal surface sialic acid bound fH through a previously undefined polyanion binding region within short consensus repeat domains 16-20 in fH. Non-sialylatable stably serum-resistant gonococcal strains also bind fH. The fH receptor has been shown to be Por1A using isogenic gonococcal strains differing only in their porin molecule. fH binding results in the inactivation of most surface-bound C3b to iC3b, and converts serum-sensitive gonococci to resistance. Using gonococcal strains with hybrid Por1A/B molecules, as well as synthetic peptides corresponding to exposed porin loop regions, Por1A loop 5 was identified as the fH binding region. Using the loop 5 peptide in an inhibition assay we diverted binding of fH from serum-resistant organisms to the peptide which resulted in increased serum-sensitivity of gonococci in a bactericidal assay.
A third gonococcal binding site that results in serum-resistance and involves fH has been observed with strain 398078 (phenotypically serum-sensitive). Neuraminidase treatment results in fH binding and confers serum-resistance, suggesting endogenous gonococcal sialylation. The sialic acid residue is possibly configured in a manner that does not bind fH; desialylation exposes a fH-binding target. Thus, fH binds to a number of gonococcal structures whose final common pathway results in serum-resistance.

Objet: Duensing - van Putten
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Recruitment of Adhesive Matrix Proteins by Pathogenic Neisseria is Mediated by Sulfated Polysaccharides and Directs Cell Tropism

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The pathogenic Neisseria species possess a complex genetic machinery that drives surface antigen variation and directs the invasive behavior of this pathogen towards various cell types. Here we report that OpaA+ gonococci can functionally extend their repertoire of surface variation by recruiting host factors such as vitronectin and fibronectin, and utilize these molecules to direct their cell tropism. Binding assays, zero length crosslinking experiments, and ligand blotting of isolated Opa proteins demonstrated that sulfated polysaccharides were key players in this recruitment by forming molecular bridges between OpaA and the matrix molecules. This was further confirmed by the observation that precoating the bacteria with sulfated polysaccharides was required to establish efficient fibronectin and vitronectin binding and to direct efficient invasion of proteoglycan deficient epithelial cells. This indicated that soluble polysaccharides could substitute for cell surface glycosaminoglycans in the internalization process. Experiments with fibronectin fragments, RGD peptides, and anti-integrin antibodies demonstrated integrin dependent invasion of CHO and HEp-2 epithelial cells with vitronectin and fibronectin coated gonococci, respectively. On the basis of these results, we propose a novel form of functional gonococcal surface variation in which externally derived sulfated polysaccharides bind to the surface of gonococci and mediate binding of

specific cell matrix molecules, which in turn dictate cell tropism and stimulate bacterial invasion through disitnct integrin receptors on mammalian cells.

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Abstract for oral presentation herewith.

Chemoprophylaxis for meningococcal disease: does it work?

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Antibiotics are usually recommended for contacts of a case of meningococcal disease to reduce the risk of further cases. Such a policy should be based on assessments of the risk of disease, the reduction of risk due to intervention, and the costs of intervention. A review of the published evidence was undertaken.

Studies have consistently shown a raised relative risk of further cases in household contacts, around 500-1200 if chemoprophylaxis is not given. Outside the household, a lower but raised risk in military, educational, other institutional and social settings has been shown.

In household contacts two approaches to prophylaxis are used: treatment of incubating infection with penicillin, or eradication of carriage with rifampicin or ciprofloxacin. Reduction of risk has never been demonstrated by controlled trials but the data suggest that 50% of further cases are preventable by either approach.

In closed communities of military recruits, trials of mass chemoprophylaxis have demonstrated clear reductions in incidence following intervention. In other settings, reduction of risk has not been clearly shown. Uncertain benefit from wide scale administration of antibiotics must be set against potential side effects, development of antibiotic resistance, and eradication of non-pathogenic immunising strains. Of relevance to some educational settings is that young children have low rates of Neisseria meningitidis carriage and high rates of Neisseria lactamica carriage. When cases occur in nurseries or kindergartens, it may be more effective to give antibiotics to teachers and parents. This approach is justifiably different from that advocated in control of Haemophilus influenzae disease.

(Text word count 250)

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Meningococcal PorA Class 1 Proteins Exist in Nature as a Heterotrimeric Porin with PorB Proteins
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Neisseria meningitidis contains at least three major outer membrane proteins (i.e., class 1 , 2 and 3), all of which exhibit porin-like activity. We have isolated and purified each of these three porin proteins from meningococci in order to study their individual biochemical, structural, and functional characteristics. PorB proteins, i.e. class 2 and class 3 proteins, can be isolated in relatively pure form from organisms naturally lacking in expression of PorA class 1. However, naturally occurring meningococci lacking expression of PorA have not been found to date. A genetically mutated meningococci, lacking PorB expression, was used to obtain purified PorA porins. We have found evidence that although meningococcal porins may be expressed and refolded as homotrimers, the availability of other porins with which they may coexist in vivo in the outer membrane may favor assembly as heterotrimeric structures. Supporting our assumption, PorA class 1 protein obtained by purification from mutant meningococci lacking PorB expression, form highly SDS stable, protease resistant homotrimeric structures which further associate to higher order complexes. These properties are not characteristic of PorA class 1 proteins found in wild type meningococci which take on the SDS sensitivity of the co-expressed PorB protein which in the case of PorB class 3 is highly SDS sensitive. PorA in wild type organisms are also highly sensitive to proteolysis. In addition, wild type meningococcal strains which co-express PorA class 1 and PorB class 3 proteins lack the ability of absorbing polyclonal antibodies generated against homotrimeric forms of PorA class 1 protein. Finally, reconstitution experiments employing unfolding/refolding techniques provide the requisite fundamental basis for the preferential heterotrimeric assembly. These results reveal that although significant contributions in the elucidation of porin structures have been derived from studies of highly purified porin homotrimers, attention should be focused on the structural characterization of heterocomplexes which seems to be the more native structure for meningococcal PorA proteins.

Objet: Trees-Knapp
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Mutations in GyrA and ParC Genes of Neisseria gonorrhoeae Isolated in the Far East and the United States

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Mutations in gyrA and parC of 234 gonococcal strains isolated in the Far East and the United States and exhibiting clinically significant ciprofloxacin resistance (CipR) or intermediate ciprofloxacin resistance (CipI), were characterized by PCR/restriction enzyme analysis and sequencing. Isolates were also characterized by auxotype/serotype (A/S) classification, antimicrobial susceptibility patterns, and plasmid profile. A number of GyrA/ParC mutation patterns were identified among

CipI/CipR isolates from Republic of Philippines, Cambodia, Thailand, and Ohio, U.S.A. The most prevalent mutation pattern among CipR isolates was GyrA-91,95/ParC-Asp-86 (91,95/Asp-86). In addition, five isolates exhibited ParC mutation patterns previously undescribed in the gonococcus; three contained double mutations at amino acids Ser-87 and Glu-91, one contained double muations at Gly-85 and Arg-116, and one contained a single mutation at Arg-116. Characterization of 91,95/Asp-86 containing isolates showed that they belonged to a number of A/S classes, penicillin/tetracycline resistance phenotypes, and B-lactamase plasmid profiles. These results strongly suggest that the continuing emergence of ciprofloxacin resistant gonococci is not due to expansion of a single or a few strains but is due to numerous factors such as spread of existing strains, importation of new strains, and possibly de novo development of ciprofloxacin resistance in susceptible strains. The results also reinforce previous observations that the number of GyrA and ParC mutations present in a strain correlates with the ciprofloxacin MIC, in that isolates which contained double mutations in both gyrA and parC had MICs as high as 64.0 ug/ml of ciprofloxacin.

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Genetic polymorphisms and replacement of the hemoglobin receptor locus (hmbR) with a novel transferrin binding protein allele in Neisseria meningitidis.

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Among different strains of meningococci and gonococci, some regions of the genome are highly polymorphic and these polymorphisms may influence virulence. The hemoglobin receptor, encoded by hmbR, is one of four known surface receptors found in pathogenic neisseriae and is required for the sequestration of complexed iron from human tissues. In one serogroup A, twelve serogroup B, sixteen serogroup C meningococcal strains, and in three gonococcal strains, we found that hmbR was located between orfA, a homologue of the E. coli paraquat-induced stress gene, pqiA, and col, which encodes a protein with 63% identity/ 75% similarity to a putative E. coli collagenase. The hmbR to orfA region was 84% identical between meningococci and gonococci, but contained different arrangements of IS1016 and neisserial repeat elements. The intergenic region between hmbR and col was very polymorphic and contained neisseria repeat elements; a 500bp open reading frame, orfU, flanked by 33 bp inverted repeats; and a 1.28 kb sequence (40% G+C content), found in different combinations in meningococci and gonococci. Serogroup Y meningococcal strains (19 of 21) contained a novel polymorphism where hmbR was eliminated from the genome and the locus was completely replaced by a 1.1 kb open reading frame, orfB. OrfB contained a 94 aa motif which was 35% identical/52% similar to a region common to a group of neisserial transferrin binding proteins (TbpB). However, orfB is half the size of other known tbpB alleles. In this region, genetic polymorphisms were associated with replacement of the virulence determinant, hmbR.

Objet: Fusco - Schulz
Date: Thu, 14 May 1998 14:50:00 -0400
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Comparison of group B meningococcal conjugate vaccines in adult and infant rhesus monkeys: rPorB versus tetanus toxoid as protein carrier

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1 North American Vaccine, Inc., Beltsville, MD, USA; 2 Pasteur Mérieux Connaught, Lyon, France

A novel group B meningococcal conjugate vaccine, consisting of N-propionylated group B meningococcal polysaccharide (NPr-GBMP) conjugated to a recombinant class 3 porin (rPorB), was compared with NPr-GBMP conjugated to tetanus toxoid (TT) for immunogenicity in both adult and infant rhesus monkeys. Groups of 8 adult monkeys were given 3 intramuscular (IM) injections at 6-week intervals for both conjugate formulations, both unconjugated mixture controls (NPr-GBMP + protein), an rPorB control, and a sham control. Groups of 4 infant monkeys (3 months old) were also given 3 IM injections, but at 4-week intervals, for each conjugate, an NPr-GBMP + rPorB mixture control, and a sham control. Sera were obtained immediately before and 2-6 weeks after each injection. Controls showed no significant polysaccharide-specific mean response. Regardless of age, both conjugate vaccines resulted in highly significant NPr-GBMP-specific IgG, IgM, and bactericidal (BC) responses (i.e., >10-fold higher geometric mean titers) after the first injection. IgG booster responses showed additional increases (above initial >50-fold rises) that were, for rPorB and TT conjugates respectively, 13-fold and 6-fold in adults, and >4-fold and 3-fold in infants. BC titers were not significantly different in adults, but the rPorB conjugate consistently and significantly outperformed the TT conjugate at most time points, with greatest significance in infants (e.g., ~1 order of magnitude greater antibody and BC mean titers after last injection). These results suggest that the rPorB conjugate is a superior candidate for a human vaccine that would cover a major meningococcal serogroup for which no licensed vaccine exists.

Objet: Shih, Kahler, Carlson, Rahman, Coleman, Stephens
Date: Thu, 14 May 1998 17:46:00 -0400
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The ice-2 locus in Neisseria meningitidis: a polycistronic operon required for biosynthesis of lipooligosaccharide inner core and membrane phospholipids.

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We have identified and characterized a novel polycistronic operon, ice-2 (inner core extension, locus 2), in Neisseria meningitidis which encodes three enzymes involved in the biosynthesis of the meningococcal cell membrane components lipooligosaccharide (LOS) and phospholipid. Based on its

truncated LOS phenotype, mutant 469 was isolated from a panel of Tn916 insertion mutants in N. meningitidis serogroup B strain NMB. Mutant 469 exhibited a very truncated LOS of 2.9 kDa on tricine SDS-PAGE and electrospray mass spectrometry of this LOS structure confirmed that it was composed of two Kdo moeities and variably phosphorylated lipid A.

Sequencing of chromosomal DNA flanking the Tn916 insertion in mutant 469 revealed that the transposon insertion disrupted the expression of three co-transcribed genes, as demonstrated by RT-PCR of the wild type strain. orfA encoded a 188 aa protein containing a Walker box ATP binding motif common to the ABC transporter family. Specific polar and non-polar orfA mutations in NMB reproduced the truncated LOS phenotype of mutant 469. nlaB encoded a 248 aa enzyme that is one of a family of meningococcal lysophosphatidic acid acyltransferases required to produce phosphatidic acid, a critical intermediate in phospholipid biosynthesis. orfC encodes a 132 aa protein with homology to rfbO of Vibrio cholerae O1 and may be involved in synthesis of a glycerol sugar intermediate in LOS biosynthesis. The unusual juxtaposition of genes involved in LOS inner core biosynthesis alongside genes involved in phospholipid assembly in the ice operons represents a intriguing paradigm in meningococcal cell membrane assembly.

Objet: Bos-Belland
Date: Thu, 14 May 1998 18:31:24 -0400
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CD66 Receptor Specificity Exhibited by Neisserial Opa variants is controlled by Protein determinants in CD66 N-Domains

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Neisseria gonorrhoeae strain MS11 is able to express eleven different opacity (Opa) proteins on its outer surface. A number of these Opa proteins have been shown to function as adhesins through binding of CD66 receptors present on human cells. CD66 antigens, or carcinoembryonic antigen (CEA) family members, comprise a family of glycoproteins belonging to the immunoglobulin superfamily. Opa variants recognize this class of receptors in a differential manner such that certain Opa variants recognize up to 4 different CD66 receptors (CD66a, c, d and e) while others recognize only two (CD66a and e), or none. We explored the basis for this receptor tropism in the present study. Our data show that glycoforms of CD66e and deglycosylated CD66e are recognized by gonococci in a Opa-specific manner. Binding by Opa variants of recombinant N-terminal domains of CD66 receptors expressed in Escherichia coli reflected the adherence specificities of Opa variants to HeLa cells expressing native CD66 molecules. These data indicate that recognition of CD66 receptors by Opa variants is mediated by the protein backbone of the CD66 N-domains. Furthermore, by using chimeric constructs between different CD66 N-domains we identified distinct binding regions on the CD66e-N-domain for specific groups of Opa variants, suggesting that the

differential recognition of CD66 receptors by Opa variants is dictated by the presence of specific binding regions on the N domain of the receptor.

Objet: Nowicki - Nowicki
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EXPERIMENTAL TRANSMISSION OF NEISSERIA GONORRHOEAE FROM PREGNANT RAT TO THE FETUS. Stella Nowicki, D.D.S.1,2, Raj Selvarangan, VD 2, Garland Anderson, M.D.1, Bogdan Nowicki, M.D, Ph.D.1,2, Dept. of Ob/Gyn1 & Microbiology and Immunology2, The University of Texas Medical Branch, 301 University Boulevard, Galveston TX 77555-1062, USA.

It is not understandable why pregnancy among patients with gonorrhea is associated with 40% increased risks for disseminated gonococcal infection (DGI). DGI during pregnancy is a risk factor for fetus mortality and morbidity.

The purpose of this investigation was to evaluate whether N. gonorrhoeae may be transmitted from pregnant mother to the fetus in utero and evaluate whether C1q may affect transmission.

Sprague-Dawley rats in day 20 of pregnancy were infected by intraperitoneal inoculation (i.p.) with three different N. gonorrhoeae strains originating from patients with pelvic inflammatory disease (PID), DGI or local infection (LI). Each group was divided into two subgroups consisting of those inoculated with 5x107 N. gonorrhoeae pretreated with 80(g/mL C1q or BSA in control.

Blood samples were collected from pregnant rats, newborns or fetuses and cultured. Quantitation of bacterial infection was evaluated by counting colony-forming units (CFU) and calculated per mL of blood.

This report demonstrates that N. gonorrhoeae associated with PID and DGI but not with LI were able to spread from pregnant rats to the fetuses and developed DGI. These two strains possess 344 bp DNA fragment of sac-4 conferring C1q dependent resistance to human and newborn rat serum in vitro and vivo. However, the mean counts per ml of blood were significantly higher with DGI than PID strain.

We propose that C1q facilitate DGI during pregnancy and that C1q dependent transmission of gonococcal infection from mother to rat fetus may be relevant for evaluation of pathogenesis of fetal and neonatal bacteremia and associated complications.

Objet: Nowicki - Nowicki
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RELATIONSHIP BETWEEN CHLAMYDIAL COINFECTION AND C1Q DEPENDENT SERUM RESISTANCE OF NEISSERIA GONORRHOEAE. Stella Nowicki, D.D.S.1,2, Audrey Hart, B.A.1, Garland D. Anderson, M.D., Cindy Peyton1, Buffy Turner1 and Bogdan Nowicki, M.D., Ph.D. 1,2, Dept. of Ob/Gyn1 & Microbiology and Immunology2, The University of Texas Medical Branch, 301 University

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Neisseria gonorrhoeae and chlamydia trachomatis (CT) are two of the most frequent microorganisms associated with pelvic inflammatory disease (PID).

It is not clear if mixed N. gonorrhoeae-CT infection is a random event or is associated with unique virulence factors of N. gonorrhoeae strains. Recently, we identified new virulence factor specific for gonococcal PID strains, the C1q dependent serum resistance that is conferred by sac-4 region (S. Nowicki et al. Infect. Immun. June 1997;65:2094-2099).

Here we evaluated the prevalence of C1q dependent serum resistance and sac-4 in N. gonorrhoeae strains isolated from patients with mixed N. gonorrhoeae-CT infection.

DNA was isolated by standard method from 28 strains of N. gonorrhoeae (14 with mixed N. gonorrhoeae-CT infection and 14 with N. gonorrhoeae infection only). PCR with primares for detection of sac-4 in N. gonorrhoeae genome was used.

In 10 of 14 (71%) N. gonorrhoeae strains isolated from patients with mixed N. gonorrhoeae-CT and in one of 14 (7%) N. gonorrhoeae strain isolated from patients with gonococcal infection only, sac-4 was detected by PCR. Correlation between presence of sac-4, patients' gender and PID is also analyzed.

High frequency of sac-4 positive N. gonorrhoeae in patients with mixed N. gonorrhoeae-CT infection appear to be the first report suggesting a non random association between specific virulence factor of N. gonorrhoeae and CT infection. These may suggest that C1q that contribute to N. gonorrhoeae virulence may also facilitate environment for chlamydial coinfection in human genital tract. We consider a hypothesis that gonococcal infection with sac-4 positive GC increases the risk for development of mixed infection with Chlamydia trachomatis.

Objet: kaczmarski-fox
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Optimising laboratory ascertainment of meningococcal disease using non culture case confirmation - the impact of a national service for serodiagnosis, PCR and improved latex agglutination methods

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The national reference laboratory for England and Wales has developed and evaluated methods for non culture case confirmation of meningococcal infections. This has been done to facilitate case ascertainment during enhanced surveillance of disease activity in anticipation of novel vaccines for prevention of serogroup C infection becoming available within the next 2-3 years.

Serodiagnosis has been developed to identify the serogroup in B and C cases, which comprise 95% patients, however the time taken for

reactivity to become stably manifest means that a result is seldom available within a timescale required for public health measures to be instituted.

PCR based tests which provide rapid case confirmation have been introduced to overcome this. These are now widely utilized and about 8,000 specimens from 6,000 patients a year are being investigated. The high workload has necessitated the employment of automation using the Applied Biosystems fluorescence based system (TaqMan). A highly sensitive and specific screen confirms the diagnosis and identifies specimens to submit to a less sensitive but epidemiologically more useful asssay which identifies and distinguishes serogroup B and C infections.

In the first 9 months of use, the current PCR strategy confirmed an additional 640 cases over and above the 1150 diagnosed by culture. The serogroup was identified for 390 (60%) of these additional cases. The role of serodiagnosis has diminished considerably however this served to confirm a further 90 cases. Work has also been done examining the effect of improving the performance of latex agglutination tests and some additional cases are identified.

The overall effect of applying non-culture methods has been to push the laboratory confirmed patient total up close to that of notified cases and has enhanced the appreciation of the current period of hyperendemic disease activity in Endland and Wales.

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Continuation of meningococcal disease epidemic in New Zealand.

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A progressive increase in attack rates for meningococcal disease has occurred in New Zealand since 1991. This has taken the rate from an average of 1.5/100,000 (n=53 cases) in the years 1989 and 1990 to 17/100,000 (n=615 cases) in 1997. In 1998, the numbers are continuing to increase. One particular strain, B:4:P1.4, has been responsible, almost totally, for the increase in numbers of cases occurring. In 1997, isolates with the P1.4 PorA antigen accounted for 86% (236/275) of all cases of serogroup B disease. With increasing use of antibiotics, the number of cases with a clinically compatible illness but negative cultures has increased. PCR testing of CSF, blood, or tissue aspirates from cases showed that 29 of 35 (83%) non-culture-proven PCR-positive

cases had subtype P1.4 porA DNA present in their specimens.

Age-specific rates for infants (<1 year) were 210/100,000 in 1997 with rates in Pacific Islands infants of 980/100,000 and Maori infants of 283/100,000. Highest rates are located in the north of New Zealand where infants currently have greater than 1 in 100 chance of acquiring meningococcal disease. Overall case-fatality rate has been around 5%. In 1997, only 20 out of 411 notifications (4.9%) were recorded as possible secondary cases.

Objet: van Putten/Duensing
Date: Thu, 14 May 1998 18:12:04 -0600
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Gonococcal invasion of epithelial cells driven by the P.IA porin.
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The neisserial porin P.I is a GTP binding protein that forms a voltage-gated channel that translocates into mammalian cell membranes, and modulates host cell signaling events. Here we report that P.I confers invasion of Neisseria gonorrhoeae into Chang epithelial cells and that this event is controlled by GTP as well as other phosphorus containing compounds. Bacterial invasion was observed only for strains carring the P.IA subtype of porin, which is typically associated with the development of disseminated neisserial disease, and did not require Opa proteins, previously recognized as gonococcal invasins. Allelic replacement studies showed that the bacterial invasiveness co-transferred with the P.IA gene. Mutation of the P.I associated protein Rmp did not alter the invasive properties. Cross-linking of labelled GTP to the porin revealed more efficient GTP binding to the P.IA than P.IB porin subtype. GTP binding was inhibited by an excess of unlabelled GTP, ATP, GDP as well as inorganic phosphate, but not by UTP or beta glycerophosphate, fully in line with the respective invasion-inhibitory activities observed for these compounds. The P.IA mediated cellular invasion may explain to the more invasive behavior of P.IA strains in the natural infection and may broaden the basis for the development of a P.I based gonococcal vaccine.
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Objet: Robinson Stuart Cartwright Abramson
Date: Fri, 15 May 1998 10:30:47 +1000
De: Priscilla Robinson <robinspr@cryptic.rch.unimelb.edu.au>
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Antibiotic prescriptions and the carriage of Neisseria meningitidis and Neisseria lactamica

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Background, Aims and Methods
Two studies preceded this work - (i) a community study of N.meningitidis and N.lactamica carriage (the Stonehouse study); and (ii) a study of erythromycin prescribing patterns in towns with high and low rates of meningococcal meningitis.

This study investigated the hypothesis that N. meningitidis carriers were more likely to have been prescribed antibiotics, particularly erythromycin, prior to demonstrated carrier status. All available general practitioner records for the study population of 380 people of N. meningitidis, N.lactamica, and a control population, were examined for references to prescription of antibiotics by any medical practitioner in the three years preceding the Stonehouse study (November 1986).

Results
After re-matching available records (319, 84%), 40 sets of N. meningitidis carriers and their controls and 36 N.lactamica carriers and controls (232, 61%) remained. Overall 47% N. meningitidis were prescribed antibiotics compared with 46% of controls. Although prescribed infrequently, erythromycin was significantly more likely to have been prescribed to N. meningitidis carriers than controls (Goodness-of-fit p=0.029).

N.lactamica carriers were much less likely to have been prescribed antibiotics compared with controls (40%:60%; OR 0.67, 0.43-1.03; p=.096), erythromycin significantly less (Goodness-of-fit p=0.014)

Conclusions
Antibiotic prescriptions (particularly erythromycin) may have been an important factor in the outbreak of meningococcal disease local to this study. Prescribing antibiotics appears to interrupt the carriage of potentially protective N.lactamica in children, the group most vulnerable to pathogenic N.meningitidis infections.

NB: The original web site instructions gave a word limit of 250 words. This is 229, but it will be impossible to edit further and get agreement from all my colleagues.
Also, my email dies not allow an underline. The presenting author is expected to be Priscilla Robinson.

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Some demographic characteristics of close contacts of cases of meningococcal disease.

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2 Infectious Disease Unit, Health Protection, Department of Human Services, Victoria, Australia

Background, Aims and Methods
Whilst a few groups have investigated risk factors for meningococcal disease, little attention has been paid to the characteristics of close contacts of cases. It is not known how well close contacts reflect known characteristics of carriers of meningococci.

Two community controls were identified for each of 90 cases. Information was collected on all people with whom the subject recalled more than casual contact during the two weeks prior to onset of case illness. As a part of an interview protocol information was collected regarding gender, age, relationship, degree of physical contact, smoking status including marijuana and other inhaled substance use (including use of joints and bongs) of all nominated contacts. A 'carrier likelihood score' based on some of these characteristics has been developed.

Results
Methods of analysis will be discussed, composite risk ratios determined, and the main epidemiological similarities and differences between the contacts of cases and controls will be compared. Analysis will be undertaken to determine whether the characteristics of contacts of cases more closely mimic the characteristics of meningococcal carriers compared with the contacts of controls.

Conclusions
Meningococcal disease in developed countries is rare, and although frightening for cases and their families is unlikely for most people. Preventive strategies using a population approach based on case-contact profiles may provide a useful way of personalising and publicising potential risky and protective behaviours.

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Are the risk factors for meningococcal disease in Victoria, Australia similar to those described elsewhere?

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Background, Aims and Methods

There have been few case-control studies of risk-factors for meningococcal disease, and none in Australia. The aim of this study was thus to investigate the role of local risk-factors for meningococcal disease described elsewhere, in particular exposure to smokers, smoke, dusts, and prior influenza, for this disease in Australia.

A case-control study of all cases notified to the Infectious Diseases unit (the Victorian Department of Human Services) in 1997 has been undertaken. Two community controls per case were recruited. All contacts and interviews were completed by the first author.

Results
Of the 91 eligible cases 90 were recruited to the study. Of original identified controls approached, three refused and were replaced.

Initial results suggest that the risk of disease is higher in rural compared with urban areas, and that some groups, particularly people of Maori or Pacific Island origin have a higher rate of disease compared with other migrant (European or South East Asian origin) or indigenous (Aboriginal) peoples. Exposures with increased risks appear to be similar to those described elsewhere, however mechanisms of exposure are not necessarily co-terminus with those highlighted in other studies.

Conclusions
We have identified groups of people who are at increased risk of meningococcal disease. Strategies developed in other countries may be partly appropriate as preventive strategies.

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Presenting author: Priscilla Robinson

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Objet: Tappero - Perkins
Date: Thu, 14 May 1998 22:19:52 -0400
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Serum Bactericidal Activity Against Homologous and Heterologous Strains Elicited by Two Outer Membrane Protein Serogroup B Meningococcal Vaccines Among Infants, Children, and Adults

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1Centers for Disease Control and Prevention, USA; 2Hospital Roberto del Río, Chile; 3Centro para Vacunas en Desarrollo-Chile; 4Instituto de Salud Publica, Chile; 5National Institute of Public Health, Norway; 6Organización Panamericana de la Salud, Chile, 7Universidad de Chile; 8Laboratory for Vaccine Development and Immune Mechanisms, National Institute for Public Health and Environmental Protection, The Netherlands.

Two efficacious serogroup B outer membrane protein (OMP) meningococcal vaccines have been developed by the Finlay Institute (FI) in Cuba and the National Institute of Public Health (NIPH) in Norway. Vaccine efficacy, however, has not been demonstrated in persons <4. In 1993, Chile, had an epidemic caused by clonal, serogroup B Neisseria meningitidis; 60% of cases occurred among children <5.

We evaluated serum bactericidal activity (SBA) to several N. meningitidis strains as a correlate for vaccine efficacy in a randomized, double-blind, controlled trial among Chilean infants (<1; n=187), children (2-4; n=183), and adults (17-30; n=173). Participants received 3 doses of either the FI or NIPH meningococcal vaccine, or a control vaccine, 2 months apart. Blood samples were obtained before doses 1 and 3, and 4-6 weeks following dose 3. Response was defined as a >4-fold rise in SBA titer compared with prevaccination titer.

Children and adult recipients of either meningococcal vaccine were more likely than control recipients to be responders to the heterologous Chilean epidemic strain (both $p<0.05$ vs. control). However, among infants there was no significant difference in response rate to the heterologous Chilean strain among those vaccinated with either the FI (10%), NIPH (12%) or control vaccine (6%). Against homologous vaccine type-strains, the 3-dose regimen response rate was >67% for children and adults (all $p<0.001$ vs. control), and surprisingly, >90% among infants. Subsequent SBA against 7 isogenic homologous target strains identified class 1 OMP as the immunodominant antigen.

Among infants and children, neither vaccine conferred protection against the heterologous Chilean strain. However, for all age groups, both vaccines elicited high SBA against its homologous vaccine type-strain; class 1 OMP accounted for this immunogenicity. The development of multivalent, class 1 OMP vaccines could lead to the control of both endemic and epidemic serogroup B meningococcal disease.

Objet: Tappero - Perkins
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Serum Bactericidal Activity Against Homologous and Heterologous Strains Elicited by Two Outer Membrane Protein Serogroup B Meningococcal Vaccines Among Infants, Children, and Adults

JW Tappero1, R Lagos2,3, AM Ballesteros4, B Plikaytis1, D Williams1, J Dykes1, LL Gheesling1,G Carlone1, EA Høiby5, J Holst5, H Nøkleby5, E Rosenqvist5,J Vega6, J Garcia4, P Herrera7, JT Poolman8, BA Perkins1

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Among infants and children, neither vaccine conferred protection against the heterologous Chilean strain. However, for all age groups, both vaccines elicited high SBA against its homologous vaccine type-strain; class 1 OMP accounted for this immunogenicity. The development of multivalent, class 1 OMP vaccines could lead to the control of both endemic and epidemic serogroup B meningococcal disease.

Objet: Tappero - Perkins
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Serum Bactericidal Activity Against Homologous and Heterologous Strains Elicited by Two Outer Membrane Protein Serogroup B Meningococcal Vaccines Among Infants, Children, and Adults

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Two efficacious serogroup B outer membrane protein (OMP) meningococcal vaccines have been developed by the Finlay Institute (FI) in Cuba and the National Institute of Public Health (NIPH) in Norway. Vaccine efficacy, however, has not been demonstrated in persons <4. In 1993, Chile, had an epidemic caused by clonal, serogroup B Neisseria meningitidis; 60% of cases occurred among children <5.

We evaluated serum bactericidal activity (SBA) to several N. meningitidis strains as a correlate for vaccine efficacy in a randomized, double-blind, controlled trial among Chilean infants (<1; n=187), children (2-4; n=183), and adults (17-30; n=173). Participants received 3 doses of either the FI or NIPH meningococcal vaccine, or a control vaccine, 2 months apart. Blood samples were obtained before

doses 1 and 3, and 4-6 weeks following dose 3. Response was defined as a >4-fold rise in SBA titer compared with prevaccination titer.

Children and adult recipients of either meningococcal vaccine were more likely than control recipients to be responders to the heterologous Chilean epidemic strain (both $p<0.05$ vs. control). However, among infants there was no significant difference in response rate to the heterologous Chilean strain among those vaccinated with either the FI (10%), NIPH (12%) or control vaccine (6%). Against homologous vaccine type-strains, the 3-dose regimen response rate was >67% for children and adults (all $p<0.001$ vs. control), and surprisingly, >90% among infants. Subsequent SBA against 7 isogenic homologous target strains identified class 1 OMP as the immunodominant antigen.

Among infants and children, neither vaccine conferred protection against the heterologous Chilean strain. However, for all age groups, both vaccines elicited high SBA against its homologous vaccine type-strain; class 1 OMP accounted for this immunogenicity. The development of multivalent, class 1 OMP vaccines could lead to the control of both endemic and epidemic serogroup B meningococcal disease.

Objet: abstract for the 11th International Pathogenic Neisserial Conference
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Characterization of a unique carbohydrate epitope recognized by a bactericidal MAb 2C7
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The lipooligosaccharides (LOSs) of Neisseria gonorrhoeae are important antigenic and immunogenic outer membrane glycolipids. Antibody against LOS mediates complement activation with resultant bactericidal and opsonophagocytic activity. Although these properties may make LOS an excellent candidate vaccine antigen, recent studies has indicated that some gonococcal LOS are antigenically similar to human glycosphingolipids(2) and that this antigenic similarity is due to the presence of identical oligosaccharide (OS) structures (3)
Although gonococci may mimic the host carbohydrate epitope, LOSs are immunogenic and could be potentially utilized as vaccines. We previously identified a gonococcal LOS epitope, recognized by MAb 2C7, that is widely expressed in vivo, elicits a bactericidal and opsonic immune response in natural and experimental infection. MAb 2C7 does not share identity with previously identified cross-reactive human glycosphingolipid structures (4), and its epitope could be such a safe site which would not evoke autoimmune reactions.
Previous work indicated that MAb 2C7 recognizes the OS moiety of the gonococcal LOS molecule(3), however, the structure of its carbohydrate epitope is unknown. In the present study, we analyzed the OS structure of WG LOS recognized by MAb 2C7 by using chemical, enzymatic, MS, and 2D NMR methods. We determined the structure of WG OS and have found that the epitope is expressed even on gonococcal LOS which carries structurally and antigenically identical to the host carbohydrate epitope.

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4.Gulati, S., McQuillen, D. P., Mandrell, R. E., Jani, D. B., and Rice, P. A.J.Infect.Dis.174 (1996)1223-1237.

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Presenter: Julia Griffith

Epidemiological Monitoring of Pathogenic Strains of Neisseria meningitidis in Victoria, Australia, 1992 - 1997

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2. Clinical Epidemiology and Biostatistics Unit, University of Melbourne, Victoria, Australia
3. Infectious Diseases Unit, Health Protection, Department of Human Services, Victoria, Australia

Background, Aims and Methods
We describe the use of pulsed field gel electrophoresis (PFGE), together with serogrouping, serotyping/subtyping and antibiotic susceptibility patterns, in monitoring the epidemiology of local outbreaks of meningococcal disease.

Results
Between 1992 and 1997, 483 known cases of meningococcal disease occurred in Victoria. Of the 338 culture-positive cases, 315 isolates were submitted to MDU. Serogrouping showed that B-strains increased from 39% in 1992 to 70-80% in
1996-1997 (51% nt:nst), whereas C-strains declined from 56% in 1992 to 16% in 1997 (three nt:nst). All strains were sensitive to penicillin, rifampicin, ceftriaxone and ciprofloxacin. The highest penicillin MIC of 0.5 ug/ml was in a single C-strain in 1995.

Serotyping/subtyping was also performed on 282 isolates. The temporal pattern
of the three most common serotype/subtypes clearly illustrated the emergence in
1993 of B.4:P1.4 strains (including B.4:nst and B.nt:P1.4), since responsible for 47 geographically dispersed infections and 24% of local B-strains. The decline of B.2b:P1.10 and C.2a:P1.2 strains over this time can also be seen. To date, PFGE patterns indicate that B.4:P1.4 isolates are heterogeneous.

Conclusions

B.4:P1.4 and related strains have emerged as the most commonly identified pathogenic strain of meningococcal disease in Victoria. PFGE patterns indicate that this serotype/subtype is heterogeneous. In contrast, a single genotypic strain has been responsible for a major outbreak of disease in New Zealand. PFGE and serotyping/subtyping together have been useful in monitoring the emergence of outbreak strains of Neisseria meningitidis in Victoria.

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Presenter: Priscilla Robinson
Nb. Please ignore previous email from robinspr@cryptic.rch.unimelb concerning this abstract (10am version server error)

Are the risk factors for meningococcal disease in Victoria, Australia similar to those described elsewhere?

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1 Clinical Epidemiology and Biostatistics, University of Melbourne, Victoria, Australia
2 Microbiological Diagnostic Unit, University of Melbourne, Victoria, Australia
3 Infectious Disease Unit, Health Protection, Department of Human Services, Victoria, Australia

Background, Aims and Methods
There have been few case-control studies of risk-factors for meningococcal disease, and none in Australia. The aim of this study was to investigate the role of risk-factors (described elsewhere) for meningococcal disease in particular exposure to smokers, smoke, dusts, and prior influenza, for this disease in Victoria, Australia.

A case-control study of all cases notified to the Infectious Diseases unit (the
Victorian Department of Human Services) in 1997 has been undertaken. Two community controls per case were recruited. All contacts and interviews were completed by the first author.

Results
Of the 91 eligible cases 90 were recruited to the study. Of original identified
controls approached, three refused and were replaced.

Initial results suggest that the risk of disease is higher in rural compared with urban areas, and that some groups, particularly people of Maori or

Pacific
Island origin have a higher rate of disease compared with other migrant (European or South East Asian origin) or indigenous (Aboriginal) peoples. Exposures with increased risks appear to be similar to those described elsewhere, however mechanisms of exposure are not necessarily co-terminus with those highlighted in other studies.

Conclusions
We have identified groups of people who are at increased risk of meningococcal disease. Strategies developed in other countries may be partly appropriate as preventive strategies.

Objet: Brudastov Y.A., D.G.Deryabin, N.R.Akhunova
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Neisseria gonorrhoeae and Staphylococcus aureus. Similarity in opsonization but differences in antiopsonic properties of extracellular products.
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Research Institute of Cellular and Intracellular Symbiosis, POB 1492, 460001, Orenburg, Russia
The data obtained from 14 clinical isolates of Neisseria gonorrhoeae (NG) and 12 - Staphylococcus aureus (SA) is analyzed.
The effect of treatment by normal human serum (NHS) and R1, R3, R5 (reagents which were deficient in C1, C3 and C5) on the ability of bacteria to stimulate the respiratory burst of neutrophils (RBN, chemiluminescence technique) and on the hydrophobicity (partitioning in aqueos two-phase system) were studied. This phenomenon was examined both under the influence and in the absence of bacterial extracellular products (broth culture supernatants, BCS). There in BCSs were used in two regimens: a) "preincubation" procedure, where BCS was incubated with serum and reagents before opsonization; b) "postincubation" procedure allowed contact of BCS with bacteria just had been opsonized in normal conditions.
RESULTS: 1. Hydrophobicity and RBN stimulation were increased when bacteria had been opsonized by NHS or R1, R3, R5. For the reagents a maximum of this effects was found for R5 (both pathways of complement activation), while minimum - for R3. There was characterless analogy in changes of hydrophobicity and RBN stimulation measured for NG and SA. 2. We found that the BCSs of NG and SA were able to diminish the increases of hydrophobicity and RBN mediated by opsonization. This antiopsonic properties of NG BCSs were realized mainly under conditions of "preincubation" procedure while SA BCSs were found to be most antiopsonic if used under "postincubation" procedure.
Relevance of obtained data to general strategies of NG and SA survival in host is considered.

Objet: Deryabin D.G., Akhunova N.R., Brudastov Y.A.
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Role of the Anticomplementary activity of N.gonorrhoeae in interaction with cellular and non cellular host factors
Deryabin D.G., Akhunova N.R., Brudastov Y.A.

Research Institute of Cellular and Intracellular Symbiosis 460000, Russia, Orenburg, Pionerskaya str., 11
1) The ability of extracellular products of Neisseria gonorrhoeae (NG) to inactivate the complement system (anticomplementary activity, ACA) were studied.
2) The original method of ACA detection consist in measuring of suppressing a complement-depending hemolysis by bacterial culture supernatant. The value of ACA calculated from the results of this method was divisible CH50.
3) ACA has been detected in 100% of the investigated cultures of NG, its values varying from 1.9 to 20.9 anti-CH50 per ml of supernatant. The average level of ACA amounted to 11.5+2.4 anti-CH50.
4) It is shown that ACA results from the relatively specific inactivation of C1 and C3 components.
5) A preincubation of NG's extracellular products with serum complement causes a sharp decrease in bactericidal effects. There was detected a positive correlation between the level of ACA in supernatants of NG and increase of their following survivalence in serum.
6) It was established that ACA has antiopsonic effect manifested in decrease of neutrophils chemiluminescence when its induced NG cells opsonised by serum preliminary incubated with bacterial supernatants.
7) A relation between the ACA of NG and forms of gonococcal infections has been established. The high level of ACA was detected mainly in the cases of chronic and recurring forms of this disease.
8) A detailed study revealed local reduction of complement activity in vivo (cervical probes) correlated with ACA level of NG. As a result of this studies some conclusions about system "complement - anticomplementary activity" were made. Conclusion: ACA is an additional mechanism which allows NG to escape host defense. From the results it is concluded that ACA is a factor of NG persistence in the host.

Objet: Toropainen-Käyhty
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Murine antibodies raised against PorA of Neisseria meningitidis show reduced protective activity in vivo against B:15:P1.7,16 subtype variants

Toropainen M,1 Saarinen L,1 van der Ley P,2 Rouppe van der Voort EM,2 Sarvas M,1 Käyhty H1

National Public Health Institute, Helsinki, Finland,1 National Institute of Public Health and the Environment, Bilthoven, The Netherlands2

Infant rat protection model was used to evaluate the protective activity of murine monoclonal (mAb) and polyclonal anti-PorA specific antibodies. Saline was used as negative and mAb735 to group B capsular polysaccharide as positive control. As challenge, four isogenic PorA variants (designated a to d, Infect. Immun. 1996:64;2745-51) from reference strain H44/76 (B:15:P1.7,16) were used: one wildtype strain (PAYYTKDTNNNLTL, P1.16a), two variants having a single amino acid change in loop 4 within the predicted P1.16 epitope (PAYYTKNTNNNLTL, P1.16b and PAYYTKHTNNNLTL, P1.16d), and one having an amino acid deletion outside the epitope (PAY-TKDTNNNLTL, P1.16c). A challenge dose of 105 cfu/pup was given i.p. 1-2 hours after the i.p. injection of antibodies, and the development of bacteremia and meningitis was assessed by culturing blood

and CSF samples taken six hours after challenge.

The anti-P1.7 mAb MN14C11.6, a reference mAb for serosubtype P1.7 epitope located in predicted loop 1 (VR1), was equally protective against all loop 4 variants. The three anti-P1.16 specific mAbs tested (MN5C11G, MN12H2 and 62-D12) are reference antibodies for serosubtype P1.16 epitope (TKDTNNN) located in loop 4 (VR2). They all protected animals completely against the P1.16a, variably against the P1.16b and P1.16c but not against the P1.16d variant. Similarly, two murine sera, taken after immunization with Finnish BacP1.7,16 (PorA produced in Bacillus subtilis) vaccine, protected animals completely against the P1.16a, variably against the P1.16b and P1.16c but not against the P1.16d variant. The results suggest that some PorA variants may escape protection raised by wildtype PorA based vaccines.

Objet: Jones - Heckels
Date: Fri, 15 May 1998 09:52:37 +0100
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Dear Colleagues

I am attaching an abstract of a presentation which we would like to submit for consideration as an oral presentation for the Neisseria 98 meeting.
The presentation would be given by my colleague Dr. Graeme Jones. As he does not have ready access to Email, if there are any problems or for general correspondence please address any Email to me.

Thanks for your consideration

Regards

John Heckels

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Specificity of the immune response to Neisseria meningitidis following colonisation in military recruits and during an outbreak of meningococcal infection in university students

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1Public Health Laboratory, Southampton, UK; 2Molecular Microbiology, University of Southampton Medical School, UK

The development of natural immunity to meningococcal infection and the contribution that individual antigens make in the immune response to meningococcal colonisation are poorly understood. We have therefore studied meningococcal carriage and the immune response to colonisation in two groups of young adults; a cohort of military recruits

undergoing basic training and in students during the largest outbreak of meningococcal disease in a UK university.

In military recruits, subtyping by determination of the class 1 protein sequence clearly differentiated between strains and demonstrated the dynamics of carriage and transmission. Expression of class 1 protein by each strain remained stable during prolonged carriage by different individuals. Following colonisation, a marked increase in Western blot reactivity developed to the homologous class 1 protein, together with a lesser increase to other OMPs. This was associated with a marked increase in serum bactericidal activity, which was specific for the subtype of the acquired strain. Subjects colonised by multiple strains showed evidence of sequential subtype specific bactericidal immune responses, directed against the class 1 protein of each strain acquired.

Following an outbreak of meningocccal infection in university students , similar techniques have been used to study carriage and the immune response to outbreak and carriage isolates. Isolates were obtained from infected individuals and unaffected students from whom pre and post-outbreak sera were available. The data obtained in these studies on the specificity of the immune response to infection and carriage have important implications for the design of vaccines for the prevention of meningococcal disease.

Objet: Christodoulides - Heckels
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Dear Colleagues

I am attaching an abstract of a presentation which we would like to submit for consideration as an oral presentation for the Neisseria 98 meeting.
The presentation would be given by my colleague Dr. Myron Christodoulides.
You can contact him directly at mc4@soton.ac.uk but we would prefer that if
there are any problems or for general correspondence please address any
Email to me and I will pass it on to him..

Thanks for your consideration

Regards

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Interactions of pathogenic neisseria with host cells derived from

normal human tissues.

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Recent studies have begun to identify at the molecular level potential mechanisms involved in the pathogenesis of human infections caused by pathogenic neisseria. However such studies have often been carried out either with cells which are transformed or not derived from the normal target tissue. In order to study the interactions between pathogenic Neisseria and human cells we have developed new model systems which exploit surgical specimens of relevant fresh normal human tissue.

Interaction of gonococci with cells of the female genital tract has been studied using human endometrial cells isolated and grown in culture. To facilitate these studies the gene encoding expression of the green fluorescent protein from Aequoria victoria has been cloned into gonococci. This resulted in viable gonococci which were constitutively fluorescent and could be observed by confocal microscopy in studies of cell attachment and invasion.

The molecular mechanisms of interaction of meningococci with human brain are poorly understood. We have therefore developed in vitro models using fresh human brain tissue and have shown that meningococci attach specifically to the meninges, and are not found in association with cerebral cortex or with vessels. Further studies on attachment and invasion have therefore been carried out with cultures of leptomeningeal cells.

These model systems which utilise fresh human tissues have been used to investigate the role of bacterial surface ligands in attachment and invasion of the host cells, and in the release of cytokines and chemokines responsible for the acute inflammatory response characteristic of infections with the pathogenic Neisseria.

Objet: Van den Dobbelsteen - van Alphen
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RIVM hexavalent PorA vesicle vaccine induced antibodies are bactericidal to wild type isolates

G. van den Dobbelsteen(superscript: 1), H. van Dijken(superscript: 1), B. Kuipers(superscript: 1), H. Rümke(superscript: 2)and L. van Alphen (superscript: 1)

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The RIVM hexavalent vesicle vaccine containing 6 PorAs (P1.7,16; P1.5.2; P1.19,15; P1.5(superscript: c),10; P1.12.13; P1.h7.4) was used in a clinical phase 2 trial carried out in toddlers (2-3 years) and schoolchildren (7-8 years) in the Netherlands. All children were immunized

3 times (0-2-8 months). The bactericidal activity (SBA) of antibodies against six isogenic PorA strains of H44/76 was determined using 10% human complement as an exogenous complement source. SBA were found against all 6 isogenic strains. Percentages of children with at least 4-fold increase in SBA were against P1.7,16=45%; P1.5.2=92%; P1.19,15=25%; P1.5(superscript: c),10=97%; P1.12.13=63% and P1.h7.4=30%. Positive sera against the isogenic strains were also analyzed for the ability to kill wild type isolates from the Netherlands and New Zealand. The SBA against the wild type isolates were comparable to the isogenic strains. The SBA was found to be PorA specific.
To define the contribution of loops 1 and 4 of PorA in the development of bactericidal anti-PorA antibodies, we used a set of isogenic class 1 OMP loop-deficient strains of H44/76 lacking the predicted loop 1 or 4 or both loop 1 and 4 of class 1 OMP of sero-subtype P1.5(superscript: c),10.
The bactericidal antibodies were mainly directed against loop 4. Previous studies (Rouppe v/d Voort Infect. Immun.,1996:64:2745, 1997:65:5184) have shown that for PorA P1.7,16 bactericidal antibodies were mainly induced by loop 1.
The results indicate that immunizations with RIVM hexavalent PorA vesicle vaccine induce protective bactericidal antibodies. This bactericidal activity is directed against PorA and epitope specificity (loop 1 or 4) is depending on sero-subtype.

Objet: van den Dobbelsteen - van Alphen
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Isotype distribution of antibodies induced in toddlers and schoolchildren after immunization with RIVM hexavalent PorA vesicle vaccine

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A clinical phase 2 trial with the RIVM hexavalent vesicle vaccine containing 6 PorAs with different sero-subtypes have been carried out in toddlers (2-3 years) and schoolchildren (7-8 years) in the Netherlands. All children were immunized 3 times (0-2-8 months). Sera were analyzed for isotype distribution in ELISA using isotype specific (IgG1,2,3,4) conjugates. ELISA plates were coated with whole cells of isogenic strains P1.5(superscript: c),10 or P1.12,13 or P1.h7,4. These strains are class 3 and 4 negative and have low expression of class 5 proteins.
P1.5(superscript: c),10 and P1.12,13 were more immunogenic in children compared to P1.h7,4. Irrespective of the used PorA, IgG total titers found after 3 immunizations were slightly higher in toddlers (n=44) compared to schoolchildren (n=46). After vaccination, IgG1 antibodies dominated the response followed by IgG3 and low levels of IgG2. No IgG4 was detected.
Although the immunogenicity of the 3 porA differs, the isotype distribution was similar for all 3 tested PorAs. The percentage of IgG3 was higher in toddlers compared to schoolchildren.
Elisa titers were compared with bactericidal titers against the 3 isogenic strains and no correlation was observed between the bactericidal titers and the levels of total IgG antibodies or any of the isotype specific titers.

In children, the RIVM hexavalent PorA vesicle vaccine is capable of inducing antibodies of the IgG1 and IgG3 isotypes which are considered to be most important for protection against disease.

Objet: Kaczmarski-Fox
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Optimising laboratory ascertainment of meningococcal disease using non-culture case confirmation - the impact of a national service for serodiagnosis, PCR and improved latex agglutination methods

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The national reference laboratory for England and Wales has developed and evaluated methods for non-culture case confirmation of meningococcal infections. This has been done to facilitate case ascertainment during enhanced surveillance of disease activity in anticipation of conjugate vaccines for prevention of serogroup C infection becoming available within the next 2-3 years.

Serodiagnosis has been developed to identify the serogroup in B and C cases, which comprise 95% of patients, however this requires testing of both acute and convalescent sera delaying confirmation beyond the timescale required for public health measures to be implemented.

PCR based tests which provide rapid case confirmation have been introduced to overcome this. These are now widely utilized and approximately 8,000 specimens from 6,000 patients a year are being investigated. The high workload has necessitated automation using the Applied Biosystems fluorescence based system (TaqMan). A highly sensitive and specific PCR screen confirms the diagnosis and identifies specimens to submit to a less sensitive but epidemiologically more useful asssay which identifies and distinguishes serogroup B and C infections.

In the first 9 months of use, the current PCR strategy confirmed an additional 640 cases over and above the 1150 diagnosed by culture. The serogroup was identified for 390 (60%) of these additional cases. The role of serodiagnosis has diminished considerably but has still served to confirm a further 90 cases. An evaluation of an enhanced methodology of latex antigen detection has been performed in which some additional cases were identified.

The overall effect of applying non-culture methods has been to raise the laboratory confirmed patient total closer to that of notified cases and has enhanced the appreciation of the current period of hyperendemic disease activity in England and Wales.

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Objet: Vogel/Frosch
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Role of capsule and LPS for meningococcal serum resistance

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Sialic acids on cellular surfaces protect cells against the lytic activities of the complement system. LPS sialylation in gonococci contributes to serum resistance, probably by increased binding of the inhibitory factor H (1). Serogroup B and C meningococci express LPS accessable to sialylation, however, sialic acids also appear as homopolymers of the capsule. We used bactericidal assays and the infant rat model of meningococcal infection to analyse the impact of the polysialic acid capsule and the LPS sialylation on meningococcal serum resistance. Isogenic mutants of the strain B1940 (B:NT:P1.3,6,15) either lacking the capsule expression (siaD mutant) or exhibiting a truncated LPS (galE mutant) were susceptible to normal human serum and avirulent (2). An a-2,3-sialyltransferase (lst) mutant of this strain was serum resistant, suggesting that in encapsulated meningococci the lacto-N-neotetraose, and not its sialylated variant, is protective against the lytic effects of the complement system (3). C3b binding to meningococci was promoted both by the AP and the CP and occured predominantly on mutants with truncated LPS, irrespective of the capsule (2). In order to rule out strain or serogroup specific effects, isogenic lst and galE mutants of two other strains (MC58, B:15:P17,16b; 2120, C:NT:P1.2,5) were also examined yielding comparable results: the lst mutants were only marginally less serum resistant than the parent wildtype strain. We conclude that LPS sialylation of serogroup B or C meningococci is of minor importance for the meningococcal serum resistance when compared to the lacto-N-neotetraose and the capsule.

1. Ram et al.1998. J. Exp. Med. 187:743-752.
2. Vogel et al. 1997. Infect. Immun. 65:4022-4029.
3. Vogel et al. 1997. Med. Microbiol. Immunol. 186:159-166.

Objet: Achtman-Spratt
Date: Fri, 15 May 1998 11:05:21 +0100
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Fusing epidemiology and population biology: the role of sequence typing.

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The complexities of meningococcal population biology and antigenic variation hinder epidemiological typing of these bacteria. For example, Serotyping and serosubtyping are frequently incomplete and often misleading concerning the genetic relationships of isolates. Multilocus enzyme electrophoresis (MLEE) provides information on genetic relationships and population biology, but is not suitable for the routine comparison of isolates between laboratories.

Advances in nucleotide sequence technology, and reductions in its cost, provide alternative routes for bacterial typing. A consortium of our laboratories has developed multi locus sequence typing (MLST), which uses the same approach, of analysing multiple housekeeping loci, as MLEE but assigns alleles by nucleotide sequencing. The higher resolution of the technique, typically distinguishing >20 alleles per locus, allows fewer loci to be used for isolate characterisation: seven MLST loci can distinguish >4 hundred million sequence types. A preliminary study of 107 meningococci showed excellent congruence with MLEE assignments and proved the feasibility and effectiveness of this approach. As sequence data permit unambiguous and rapid comparison of strains electronically, we have established a web site for this purpose.

Sequence typing data are directly usable in phylogenetic and evolutionary
studies, permitting routine epidemiology to be integrated with theoretical analyses with advantages for both fields. Our laboratories have extended MLST in the following ways: investigations of meningococcal disease outbreaks; an analysis of 90 serogroup A meningococci; studies of the dhps gene, providing information on drug resistance; sequencing of porA and porB antigen genes, providing information on antigenic variation; and evolutionary analyses.

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Objet: Bart - Van der Ende
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Hyperendemic lineage III Neisseria meningitidis strains contain a

restriction-modification system that is absent in non-lineage III strains.

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Neisseria meningitidis is a highly sexual bacterium, due to its natural transformability, resulting in a non-clonal population structure. Since the early 1980's, however, hyperendemic meningococcal disease in the Netherlands is caused by strains from one genetic cluster, lineage III. Apparently, these strains either diversify at a lower rate than other strains, or have the ability to cause disease more effectively, or have a combination of these characteristics. We hypothesize that both of these phenomena are the result of a genetic trait. The aim of this study was to identify the chromosomal DNA differences between lineage III strains and non-hyperendemic strains. DNA fragments that are specific for lineage III meningococci were isolated by representational difference analysis. Four of the DNA fragments are located in a single 1.8 kb locus in the chromosome of lineage III isolates. The locus contains three open reading frames, flanked by both inverted and direct repeats. The open reading frames encode a putative restriction modification system, consisting of a restriction endonuclease, a methyltransferase, and a regulatory protein, respectively. The average G + C content of the locus is only 33%, in contrast to the flanking regions with a G + C content of >50%, as usual for neisserial sequences. The methyltransferase gene was cloned and expressed in Escherichia coli. Restriction enzyme digestion experiments allowed identification of the specific modification site. We conclude that lineage III strains have acquired a foreign specific restriction modification system, which may contribute to the clonal structure of the lineage III complex.

Objet: Oral presentation Neisseria98- Malvar, Farjas
Date: Fri, 15 May 1998 12:20:31 +0200
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EVALUATION OF A MASS INMUNIZATION CAMPAIGN IN THE CONTROL OF SEROGROUP C MENINGOCOCCAL DISEASE

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During 1996, 394 suspected cases of meningoccocal disease (MD) were detected in Galicia (Spain) (crude rate=14.8 cases per 100,000 inhabitants). In 35 of these cases serogroup B N. meningitidis (SB) was isolated (crude rate=1,4), and in 172 serogroup C N. meningitidis (SC) (crude rate= 6.3), almost entirely corresponding to phenotype C:2b:P1.2,5. 113 of those 172 were between 2- 20 years old (specific age rate=17.4).

To deal with this situation, a vaccination campaign took place between 9/12/96 and 31/1/97, targeted at 548,995 residents aged between 18 months and 19 years, reaching an overall coverage of 86%, which was higher than 98%

in children aged 3-13 years.

During 1997, 283 MD suspected cases were detected (crude rate =10.4). In 40 of these cases SB was isolated (crude rate=1.4), and SC in 90 (crude rate= 3.3). 30 SC cases (8 vaccinated and 22 unvaccinated) were between 2- 20 years old (specific age rate=4.6).

Using only the SCMD cases, the direct effectiviness was estimated as 96.5% (CI95%: 93.2-98.2) in the 2-5 years age group, 99.4% (CI95%:98.6-99.8) in the 6-13 years age group, and 94.1% (CI95%:73.3-98.7) in the 14-19 years age group. Adding to this calculation the vaccinated suspected cases, we estimated the minimum direct effectiviness in these age groups as 70.0% (CI95%:43.2-84.2), 74.3% (CI95%:33.7-90.0) and the 70.7% (CI95%:16.2-89.7), respectively.

We also estimated the number of prevented cases, which was 354 when only the SCMD cases were used. If we add the vaccinated suspected cases, the number falls to 261.

Objet: Oral presentation Neisseria98- Fernandez-Gestal
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CARRIER RATES OF NEISSERIA MENINGITIDIS C:2b:P1.2,5 AND ITS RELATIONSHIP WITH MENINGOCOCCAL DISEASE

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The introduction of strain C:2b:P1.2,5 in Galicia (Spain) caused a dramatic increase in the incidence of meningococal disease. This fact led to the implementation of an intensive A+C vaccination campaign of the population between 18 months and 19 years. During this campaing we studied the prevalence of carriage in two different areas with, respectivally high and low incidenceof meningococcal disease.

The study, using a two-stage sample design with stratification of the first-stage units, was carried out among 9829 subjects. The sample size was calculated with an absolute error of +/- 0.3% in high incidence and +/-0.1% in low incidence, and an estimated prevalence of the strain C:2b:P1.2.5 of 1% in high incidence and 0.2% in low incidence. Inmediately before the vaccination, nasopharyngeal samples were taken and plated onto Thayer-Martin plates, incubated and finally sent to the Neisseria Reference Laboratory (Madrid-Majadahonda) for analysis.

The prevalence of the strain C:2b:P1.2,5 was 0.58% (IC 95% : 0.29 – 0.88) in the high incidence area and 0.41% (0.00 – 1.04) in the low incidence area, and that of serogroup C 1.33% (0.80 – 1.80) and 0.89% (0.09 – 1.69) respectively. The prevalence of N. Meningitidis was almost the same in both areas (8%). Carriers of the strain were not found in the 2-4 age group, that most affected by the disease. The rates of N. meningitidis B was significantly higher than serogroup C. No was differences between areas by age group or sex.

Objet: Ison, Levin
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Whole blood assay for assessment of immune responses to meningococci.

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The whole blood assay (WBA), an ex-vivo model of meningococcal bacteriaemia, that assesses the complete bactericidal activity of blood, has been compared with the serum bactericidal assay (SBA), which measures antibody-mediated complement lysis. We have investigated naturally infected and uninfected children and have measured activity using the patients infecting strain and two reference strains, serogroup B (MC58, B:15:P1.7,16) and C (NCTC 8554, C:NT:P1.5).

In 26 serogroup C infected children both the WBA and SBA were found to be sensitive methods for measuring bactericidal activity against the infecting and reference serogroup C (NCTC 8554) strains. However, more children showed activity against the serogroup B reference strain (MC58)in the WBA (12/26, 46%) than in the SBA (4/26, 15%).

In 28 serogroup B infected children bactericidal activity against the infecting strain was detected by both assays although SBA titres were lower compared to C patients (median, 16 vs 512). The WBA was more sensitive than the SBA in detecting bactericidal activity to both reference strains: B (71% vs 32%) and C (61% vs 14%). WB killing was detected even in children less than one year old. Lower levels of bactericidal activity were detected in control children.

In conclusion, the WBA is a more sensitive marker of immune response in patients previously infected with serogroup B strains, and detects a response which is a cross-reactive to both the infecting and other serogroup B strains.

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Meningococcal transferrin binding proteins: Characterisation of transferrin binding and vaccine potential.

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Meningococcal transferrin binding proteins (TbpA and TbpB) are required for the acquisition of transferrin-bound iron. As iron uptake is a vital step in pathogenicity, Tbps are being considered as candidate antigens for vaccines against serogroup B meningococcal disease. Studies are now in progress to investigate, at the molecular level, the nature of the specific interaction of the Tbps with human transferrin (hTf) and to explore the vaccine potential of recombinant TbpA and TbpB.

Gel filtration and surface plasmon resonance (SPR) have been used to determine the nature of purified TbpA+B, TbpA and TbpB in solution and their transferrin-binding properties. TbpA exists as a dimer in solution while co-purified TbpA+B comprises a TbpA dimer complexed with a single molecule of TbpB. The complex is able to bind up to two molecules of hTf. Unlike TbpA, TbpB binds iron-saturated in preference to apo hTf and the TbpA+B complex shows intermediate discrimination. SPR analysis of the binding indicates that TbpA and TbpB recognise distinct sites on hTf.

SPR analysis using human-bovine chimeric transferrins indicates that the primary recognition site for TbpA is located within the C-lobe of hTf. TbpB has at least two distinct hTf-binding regions, one in the hTf C-lobe and a secondary, lower affinity site, in the interdomain bridging region (residues 245-355). Tbps incorporated into liposomes have been used in a separate SPR study of hTf-Tbp interactions and data will be presented on the effect of synthetic hTf peptides on these interactions.

Our SPR studies have allowed the development of a more sophisticated model of the meningococcal transferrin receptor in which TbpB acts to orientate hTf over the dual pores of the putative TbpA dimer. This arrangement may potentiate uptake of iron from either the C- or N-lobe binding sites of hTf.

The protective potential of recombinant (r) TbpA and TbpB against lethal meningococcal challenge in mice has been determined and the recombinant Tbps have been found to be as protective as native Tbps. Data will be presented on protection by rTbpB alone or rTbpB+rTbpA against a range of meningococcal strains.

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Objet: Hopman-van derEnde
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RS3 sequences involved in recombination events leading to deletions in porA upstream sequences and of porA in Neisseria meningitidis

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PorA is candidate to be a constituent in a vaccine against meningococcal infection. However, in the strain collection of the Netherlands Reference Laboratory for Bacterial Meningitis we encountered porA- N. meningitis isolates from patients with meningitis. To test the hypothesis that the porA deletion was caused by site specific recombination we analyzed the upstream and downstream sequences of porA from 3 (H44/76, H355 and 860183) meningococci strains. The region upstream of the porA promoter appeared to be repetitive. It is flanked, in direct orientation, by neisserial repeats (Correia FF et al, J Biol Chem 1988; 263:12194-98). In strain 860183, the region between these repeats exists of 3 repeats of 517 bp, containing 6 inverted repeats with RS3 core sequences (ATTCCC-N8-GGGAAT) (Haas R, and Meyer TF, Cell 1986; 44:107-15) and 3 RS3 core sequences (ATTCCC). The 517 bp repeats are followed by another but truncated repeat. The porA upstream region of the other 2 strains showed deletions, probably caused by recombination between RS3 core sequences. The sequences of the porA downstream region of H44/76 and H355 containing the IS1106 element, were identical to that of NmF207 (Knight AI et al, Mol Microbiol 1992; 6:1565-73), but strain 860183 had only 2 instead of 4 DR2 repeats downstream of IS1106. A 116 bp part of the 517 bp upstream repeat shows homology with the DR2 repeat. Sequence analysis of the porA- variants indicated that the deletion of porA occurred via recombination between these regions. Presumably, RS3 core sequences are involved in this recombination event.

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Objet: MacLennan-Greenwood
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Meningococcal Serogroup C Conjugate Vaccination In Infancy Induces Persistent Immunological Memory

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Oxford Vaccine Group, Oxford, UK1, MRC The Gambia2, CDC, Atlanta3, London School of Hygiene and Tropical Medicine, UK4

Aims: To assess persistence of antibody and immunologic memory 5 years after vaccination with the Chiron meningococcal A/C conjugate vaccine (MenConj).
Methods: Gambian children were randomised in infancy to receive 1, 2 or 3 doses of MenConj, boosted at 2 years with MenConj, meningococcal A/C polysaccharide (MPS) or inactivated polio vaccine (IPV), and assessed at 5 years of age. 39 previously unvaccinated age matched controls were also recruited. Serum was obtained for bactericidal assay to N.meningitidis serogroup C before and 10 days after a booster containing 10 micrograms MPS.
Results: The serogroup C serum bactericidal geometric mean reciprocal titre and 95% CI before (pre) and 10 days after (post) the MPS booster at 5 years are tabled. The results for 1, 2, or 3 doses of conjugate in infancy are grouped together. Post boost titres were inversely correlated with the number of doses of MenConj in infancy (analysis of trend $p<0.001$)

	Booster at 2 years MenConj (n=52)	MPS (n=48)	Controls IPV (n=76)	(n=39)
Pre	67.5 (41.8,109.1)	35.4 (20.1, 62.4)	13.3 (9.1, 19.4)	11.6 (6.5, 20.7)
Post	3420 (2122, 5512)	426 (231, 785)	2790 (1826, 4262)	485 (295, 799)

Conclusions: 1) Long term immunologic memory is induced after 1, 2, or 3 doses of MenConj in infancy. 2) Increased antibody titres were demonstrated 3 years after a MenConj booster. 3) An MPS booster after MenConj in infancy results in a loss of memory. 4) A single dose of MenConj in infancy, without a booster, gives the highest response at 5 years.

Objet: Käyhty - Greenwood
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Salivary antibodies after revaccination with meningococcal A/C polysaccharide vaccine following repeated immunisation during early childhood

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5. London School of Hygiene and Tropical Medicine, London UK

Aim: To assess the mucosal response to meningococcal polysaccharide (MPS) vaccination in children previously vaccinated with meningococcal vaccines and previously unvaccinated controls.
Methods: Gambian children randomised to receive 1, 2, or 3 doses of meningococcal A/C conjugate vaccine (MC, n=173) or 2 doses of MPS (n=41) in infancy, and either MC, MPS, or no meningococcal vaccine at 2 years

of age were assessed at age 5 years. 25 children aged 4 years vaccinated with MPS at 2 years and 64 age matched controls who had never received a meningococcal vaccine were also investigated aged 4-5 years. Saliva and serum samples were collected before and 10 days after an MPS booster. Antibodies to group A (MenA) and C (MenC) PS were determined by EIA.
Results and Conclusions: MPS vaccination resulted in a rise in salivary IgG, IgA and sIg. IgG anti-MenA and -MenC was found pre vaccination in 0-20% and 5-27%, and post vaccination in 30-82% and 50-86%, respectively. Salivary IgG anti-MenA and MenC correlated (r=0.54, 0.76, respectively) with serum concentrations suggesting a serum origin. IgA anti-MenA and -MenC was found pre vaccination in 21-47% and 4-47%, respectively. The majority of children had a rise in IgA after vaccination with MPS; 73-100% being positive for anti-MenA and 38-92% for anti-MenC. Salivary IgA and sIg correlated (r=0.93, 0.89, respectively) suggesting that IgA in saliva is secretory and locally produced. The controls had similar IgA responses to previously vaccinated groups suggesting that previous vaccination with MC or MPS did not result in mucosal memory.

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Opsonophagocytic and protective capacity of murine monoclonal antibodies to N- Propionyl group B meningococcal polysaccharide (N-Pr MenB PS) of different fine specificity

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2. National Public Health Institute, Helsinki, Finland,
3. Chiron Vaccines, Emeryville, and Children's Hospital Oakland Research Institute, Calif., USA.

Mabs were produced from mice immunized with N-Pr MenB oligosaccharide - tetanus toxoid conjugate Mabs (J. Immunol 1998;160:5028). 14 were selected that showed complement-mediated bactericidal activity (BCA) when tested with either rabbit (n=5), or with human or rabbit complement (n=9), and were further characterized for opsonophagocytic activity (OPA), and protection in the infant rat passive protection (IRPP) model. Of the 14 Mabs, 1 was IgG2a, 7 were IgG2b and 6 were IgG3. Two different groups of Mabs could be distinguished by epitope specificity: 8 recognizing N-Pr MenB PS and native N-acetylated (N-Ac) MenB PS, and 6 reacting only with N-Pr-MenB PS. 5 Mabs showed no cross-reactivity with human polysialic acid (PSA). 8 of the 14 Mabs had OPA (5 when tested with rabbit complement only, and 3 with rabbit or human complement). Mabs that recognized N-Pr-MenB only tended to have OPA less frequently (2/6) than Mabs that recognized both N-Pr-MenB Ps and N-Ac-MenB PS (6/8). 12 of the 14 Mabs were tested in the IRPP model; 2 ug/pup gave protection in 9 cases. Of these, 7 had showed both BCA and OPA, and two were only bactericidal. Protection in rats was not related to which complement source was positive in the OPA or BCA assays; or to the epitope or isotype specificity. However. all three Mabs with no protective activity in rats had BCA but not OPA. Thus, BCA alone, or OPA alone, is not sufficient for predicting protection in this model. The Mabs with no detectable cross-reactivity to PSA, but with OPA, and

protective activity in infant rats, may be useful for the identification of mimetics capable of eliciting protective but not auto-antibodies.

Objet: Høiby - Rosenqvist
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To the Organizing Committee of The Eleventh International Pathogenic Neisseria Meeting:

Please consider for oral presentation the following abstract. It could be considered in connection with the abstract submitted by J Fuglesang et. al:

Cross-reactive serum bactericidal activities after vaccination of teenagers with three doses of the Norwegian group B outer membrane vesicle vaccine
E.A. Høiby, N. Bjæring-Hansen, D.A. Caugant, E Wedege, E. Rosenqvist

National Institute of Public Health, P. O. Box 4404 Torshov, N-0403 Oslo, Norway.

A vaccine against systemic group B meningococcal disease based on outer membrane vesicles from a B:15:P1.7,16 meningococcal strain has been produced at NIPH in Norway. The vaccine was shown to give a significant protection (57%) in Norwegian teenagers when given as two doses six weeks apart. Serum bactericidal activity seems to correlate with observed protection rates. In a new clinical phase 2 trial among Norwegian teenagers (13-14 years old), the vaccine was administered as a three-dose regimen: the two first doses at 6 weeks' interval, the third ten months later.

Cross-reactive bactericidal antibodies against various other Neisseria meningitidis strains have been studied after two and three vaccine doses in representative subsets of sera from the vaccinees (10 and 34 vaccinees). The results demonstrated that three vaccine doses induced significant increases in serum bactericidal activity (SBA) titers against heterologous meningococcal strains when compared to two doses. However, the SBA responses against some of the heterologous strains still remained unsatisfactory and indicated that combinations of different OMVs might be considered.

Strains studied in serum bactericidal assays with subsets of sera from Norwegian teenagers

Strain	Origin	Clone	Group: type: subtype
44/76-SL	Norway	ET-5	B:15:P1.7,16
			Vaccine strain
N31/95	Norway	ET-5	B:4:P1.19,15
CH539	Chile	ET-5	B:15:P1.3,(7)
MC50	England	ET-32,33	C:21:P1.21,16
M467/90	Iceland	ET-32,33	B:21:P1.21,16
N138/95	Norway	N-18	B:4:P1.18,25
N144/95	Norway	Lineage III	B:4:P1.4,(7):L8
G1963	Austria	Lineage III	B:4:P.1.4,(7):L3,7,9
NZ95/46	New-Zealand	Lineage III	B:4:P1.4,(7):L3,7,9
NZ92/18	New-Zealand	Lineage III	B:4:P1.4,(7):L8
Mk83/94	Mali	Clone III	A:4/21:P1.9,20

Objet: Fuglesang - Rosenqvist
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To the Scientific Committee: Eleventh International Pathogenic Neisseria Conference.

Please, consider the following abstract for oral presentation.

We would request the Scientific Committee to let this presentation be the first part of a double presentation with the paper by Høiby et al. ("Cross-reactive serum bactericidal .....") as the second part, followed by a discussion of both papers.

Jan E. Fuglesang, M.D., Senior Medical Officer

"Increased and longer-lasting immune responses to the Norwegian meningococcal group B OMV vaccine in teenagers with a three-dose compared to a two-dose regimen."

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National Institute of Public Health, P.O.Box 4404 Torshov, N-0403 Oslo, Norway

The Norwegian outer membrane vesicle vaccine against group B meningococcal disease is based on a representative epidemic Neisseria meningitidis B:15:P1.7,16 strain. In a double-blind, placebo-controlled protection trial among 172,000 teenagers in 1988-91, two vaccine doses, administered 6 weeks apart, gave 57% protection against serogroup B disease during an observation period of 29 months. Based on the absence of cases among vaccinees during the first 10 months of the study, the effect of a third vaccine dose given 10 months after the second dose was examined. A total of 370 teenagers were included in the study. Meningococcal A + C polysaccharide vaccine was given to 1/6 of the volunteers as a control.

Blood samples, drawn at different times during the 22 months study period, were analysed in a serum bactericidal assay with the vaccine strain 44/76-SL.

Per cent responders
(>=4-fold increase in bactericidal titers)

Time after vaccination	2. dose	3. dose
6 weeks	78%	98%
1 year	28%	81%

These responses were also reflected in geometric mean titers. Thus, compared to two doses, three doses resulted in a significantly higher number of responders and in higher antibody levels. The number of vaccinees that maintained high antibody levels one year after the third dose was particularly promising. Our findings suggest that a higher number of vaccinees will be protected, and that protection will last substantially longer with a 3-dose regimen.

Objet: oral abstract
Date: Fri, 15 May 1998 13:37:33 +0000
De: ds64@umailsrv0.umd.edu
A: oral.neisseria@necker.fr
Copies à: parice@bu.edu, ecg@rockvax.rockefeller.edu, bandoa@rockvax.rockefeller.edu

Please find below an updated version of a previously submitted abstract.

Thank you.

Dan Stein

Current understanding of lipooligosaccharide biosynthesis in the Neisseriaceae.

Daniel C. Stein+, Asesh Banerjee*, Rong Wang+, Sacha Uljohn+, Peter Rice§, and Emil C. Gotschlich*. *Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University; +Laboratory of Mass spectrometry, The Rockefeller University, 1230 York Ave., New York, NY 10021; §The Maxwell Finland Laboratory for Infectious Diseases, Boston Medical Center, Boston, MA 02118; and +Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742.

+presenting author

Neisserial lipooligosaccharide (LOS) consists of a core of two heptoses and two KDOs. Attached to this core are three polysaccharide branches: alpha; beta; and gamma. We have previously described the genes needed for expression of the gonococcal alpha chain (lgtA-E), and defined the mechanism by which the composition of the sugar units on this chain can vary. We have determined that the gonococcus also possesses a two gene cluster where one of the gene products adds the first glucose onto the alpha chain (lgtF), and the other adds N-acetylglucosamine as the gamma chain (lgtH). The lgtA-E gene cluster can become abbreviated, either through a recombination between lgtB and lgtE, or lgtA and lgtD. Cells that possess this recombination are restricted in the types of LOS structures that they may express. We have cloned the gene needed to initiate synthesis of the beta-chain (lgtG). When this gene produces a functional protein it contains a string of 11 cytosines. Changes in the number of cytosines (+1 or -1) produces strains that fail to elongate the beta-chain. We have further shown that a functional lgtE gene is needed for the completion of the lactosyl moiety on the beta-chain. A molecular genetic approach, applied in conjunction with biochemistry and Mass spectrometry, clearly attribute the reactivity of Mab2C7 to the presence of the lactosyl moiety found on the beta-chain. We have also demonstrated that in the absence of this lactosyl group, a phosphoethanolamine takes its place to generate the antigenic epitope recognized by Mab 2-1-L8. We have identified strains that are capable of elongating the gamma chain. These strains add a galactose onto N-acetylglucosamine, and the resulting structure reacts with Mab1B2. Using Southern hybridization or PCR experiments, we have shown that while lgtA-G can be found in the gonococcus or the meningococcus, their presence in "non-pathogenic" Neisseria spp. is less conserved, with most strains lacking these genes. In strains that possess these genes, the polyguanine tracts that allow for the variable expression of these genes have been lost. These data provide further evidence for the importance of LOS antigenic variation in Neisserial diseases.

Objet: Liu - Rest
Date: Fri, 15 May 1998 09:44:13 -0400
De: Rick Rest <restr@auhs.edu>

Société: MCP-Hahnemann School of Medicine
A: oral.neisseria@necker.fr, zyw@uscom.com

Functional Regulation of Sialyltransferase Gene Expression in Pathogenic Neisseria

SHI V. LIU*, YAO-BIN LIU, RICHARD F. REST.
Allegheny Univ. of the Health Sciences, 2900 Queen Lane
Philadelphia, PA 19129, U.S.A.

Lipooligosaccharide sialylation plays multifaceted roles in neisserial pathogenesis. To study how sialylation is regulated and why sialylation varies in pathogenic Neisseria, we studied transcriptional regulation of the sialyltransferase gene, lst. We found significant differences in the lst upstream region (lst-up) between Neisseria meningitidis (Nm) and Neisseria gonorrhoeae (Ng) and within species. To study the effects of these lst-up variations on lst expression, fusions between various lst-ups and lacZ were constructed and transformed into neisserial chromosomes or maintained in plasmids of Escherichia coli (Ec). We found that transformants containing Ng lst-up consistently express higher b-galactosidase (b-Gal) activity than do transformants containing Nm lst-up. Also the differences between Ng and Nm lst-up-controlled b-Gal activities are greater when fusions are expressed in Neisseria than when expressed in Ec (4~7 fold vs. 1~2 fold). These results suggest that lst-up differences between Nm and Ng play a major role in determining lst expression and Neisseria-specific trans acting factors may contribute to differential lst expression. In addition, we observed that Nm is more capable than Ng to down-regulate lst expression and sialyltransferase activity when growth conditions are changed from broth to plate. These results suggest that a rapid and reversible transcriptional regulation of lst expression may provide a mechanism for regulating sialyltransferase activity during Neisseria-host cell interactions. Ongoing side-by-side comparative genomic studies between Nm and Ng in their lst expression regulation will be combined with cell invasion assays to better understand the molecular and cell biology of regulation of sialylation during neisserial infection and disease.

* Underline feature is not available for this email application so I used this symbol to indicating the presenting author.

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Objet: Haneberg - Aaberge
Date: Fri, 15 May 1998 15:55:05 +0200
De: Bjørn Haneberg <haneberg@online.no>@online.no
A: oral.neisseria@necker.fr

Intranasal immunizations with group B outer membrane vesicle (OMV) vaccines: dosages, immunogenicity and booster responses.

B. Haneberg, H. Bakke, P.N. Huynh, I.L. Haugen, J. Holst and I.S. Aaberge.

Department of Vaccinology, National Institute of Public Health, N-0403 Oslo, and Department of Microbiology, Institute of Pharmacy, University of Oslo, Norway.

Administration of non-proliferating vaccines directly onto mucosal surfaces may induce local mucosal as well as systemic immune responses, provided that a mucosal adjuvant is also given. We have demonstrated that OMVs made from group B meningococci are strongly immunogenic in humans when given intranasally, even without an additional mucosal adjuvant. The present study was undertaken to refine and simplify the immunization schedule, which hitherto consisted of four doses at weekly intervals, and to explore the possibility of inducing booster responses.

In one part of the study, we immunized 4 groups of 8 mice each intranasally with either four weekly doses of 25 or 250 µg OMVs, or with single doses of 100 or 1000 µg. The immunizations were repeated two months after start of the experiment. In another part of the study, we immunized 6 groups of mice either intranasally with four weekly doses of 25 µg OMVs, or subcutaneously with a single dose of 25 µg OMVs plus Al-hydroxide as adjuvant. Four of these latter groups were pre-immunized two months earlier with either of the two immunization regimes. IgA antibodies to OMVs in saliva and feces, and IgG antibodies in sera, were analyzed by ELISA.

Primary nasal immunizations with four weekly doses led to higher antibody responses, in both sera and secretions, than one single large dose equivalent to the sum of four individual doses. Moreover, the responses in serum and feces to 4 x 25 µg OMVs were even better than 1 x 1000 µg. Secondary nasal immunizations led to marked booster effects which was also better after repeated small nasal doses than after one single large dose. Similar marked booster responses in secretions and sera to nasal immunizations were observed after subcutaneous priming. We found no evidence that nasal priming led to induction of tolerance upon later nasal or subcutaneous immunizations.

In conclusion, it appears that non-proliferating nasal vaccines can be part of immunization schedules also incorporating parenteral immunizations. In order to optimize the effect of such nasal vaccines, they should probably be given in repeated doses, or be formulated in a way to increase the time of contact with the mucosal surfaces.

Objet: Dixon-Klein
Date: Fri, 15 May 1998 14:58:17 +0100
De: Garth Dixon <G.Dixon@ich.ucl.ac.uk>
A: oral.neisseria@necker.fr

The Influence of Bacterial Structure on Endothelial Cell Adhesion Molecule Expression by Neisseria meningitidis.

Garth L.J. Dixon, Robert S. Heyderman, Ulrich Vogel, Matthias Frosch, and Nigel J. Klein.

Address: Immunobiology Unit, Institute of Child Health, London

Endothelial activation is considered to be a critical factor in the pathogenesis of severe meningococcal disease. Isogenic mutants of Neisseria meningitidis B1940 were used to examine the influence of encapsulation and lipooligosaccharide structure on the degree and pattern of endothelial activation. The expression of cell adhesion molecules E-selectin, ICAM-1 and VCAM-1 in response to bacteria and purified endotoxin were measured. Exposure of endothelium to the mutants siaD- (unencapsulated, LOS sialylated), cps- (unencapsulated, LOS unsialylated) and cpsD- (encapsulated, LOS unsialylated) and the Wild type organism (encapsulated,

LOS sialylated) caused a time and dose dependent increase in expression of cell adhesion molecules. Much higher levels were seen with the cps- and siaD- mutants than the cpsD- mutants and parent organism, which was highly significant (P>0.001). The cps- and siaD- mutants have previously been shown to bind more avidly to the endothelium. Interestingly, the pattern of cell adhesion molecule expression with purified endotoxin and the unencapsulated organisms was different, with these organisms causing more E-selectin expression than endotoxin. In contrast, endotoxin induced greater expression of ICAM-1. These findings indicate that the ability of organisms to adhere to vascular endothelium can influence the nature and degree of endothelial activation. Differences in the patterns of adhesion molecule expression may depend on as yet undefined bacterial factors.

Sessions topic choice: 1). Neisseria interactions with cellular and non cellular host factors 2). Vaccine and Immunobiology

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Objet: bash-frasch
Date: Fri, 15 May 1998 10:17:43 -0400
De: "Margaret C. Bash MD" <mbash@helix.nih.gov>
A: "'oral.neisseria@necker.fr'" <oral.neisseria@necker.fr>

Antigenic Characteristics and Immunogenicity of Synthetic Peptide Vaccines for the Pathogenic Neisseria: Neisseria meningitidis and Neisseria gonorrhoeae.
Margaret C. Bash, Freyja Lynn, Margaret McGowen, Dorothea Thompson, Gregory Glenn, Carl E. Frasch
Division of Bacterial Products, Center for Biologics Evaluation and Research, FDA, Bethesda, Maryland USA

The porins of the pathogenic Neisseria are considered potential vaccine candidates. Anti-porin bactericidal antibodies are directed against conformational epitopes of type specific surface exposed loops (variable regions). To characterize immune responses to individual loops of N. meningitidis PorB and N. gonorrhoeae PI, we have constructed cyclic, lipid tailed peptide loops incorporated into liposomes. Mouse immunization studies with peptides corresponding to loop 1 of serotypes 4 (VR1-4) and 15 (VR1-15) of N. meningitidis have been completed. Immunization with two N. gonorrhoeae peptides from MS-11 are underway. The VR1-4 peptide loop binds both type 4 monoclonal antibody and polyclonal rabbit antisera raised to a hybrid type 4/type 15 strain suggesting that cyclization approximates the native protein conformation. Immunization with 10 ug of peptide and 10 ug lipid A in liposomes resulted in antibodies only to the peptide. Immunization with 25 ug of either VR1-4 or VR1-15 peptide with 0 to 40 ug per dose of lipid A stimulated antibodies that bind to the native PorB in outer membrane. Individual responses post dose 2 and post dose 3 suggests superiority of the low density lipid A preparations (10ug - 14 ug per dose). Bactericidal assays will be done.

Objet: Jennings-Moxon
Date: Sat, 16 May 1998 00:25:57 +1000
De: Michael Jennings <jennings@biosci.uq.edu.au>
A: oral.neisseria@necker.fr

Dear Neisseria98 Committee Members,

Please find enclosed my abstract for oral presentation at the upcoming Neisseria meeting.

TITLE: "Genetics of biosynthesis of the pilin linked trisaccharide structure of Neisseria meningitidis".

Michael P. Jennings1*, Mumtaz Virji2‡, Debbie Evans2, Virginia Foster2, Yogitha N. Srikhanta1, Peter Power1, Liana Steeghs3, Peter van der Ley3, E. Richard Moxon4.

1 Department of Microbiology, The University of Queensland, Brisbane, Queensland, 4072, Australia.
2 School of Animal and Microbial Sciences, University of Reading, Reading, RG6 6AJ, UK.
3 National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
4 Molecular Infectious Diseases Group, Department of Paediatrics, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England.
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The pili of Neisseria meningitidis is a key virulence factor, being the major adhesin of this capsulate organism which contributes to specificity for the human host. Recently it has been reported that meningococcal pili are post-translationally modified by addition of an O-linked trisaccharide, Gal(beta1-4)Gal(alpha1-3)2,4-diacetimido-2,4,6-trideoxyhexose. Using a set of random genomic sequences from Neisseria meningitidis strain MC58, we have investigated a series of novel genes which are homologous to genes involved in oligosaccharide biosynthesis, and therefore candidates genes for the biosynthesis of the pilin trisaccharide. To investigate a potential role for these genes in pili glycosylation, insertional mutants were constructed and transferred to the chromosome, and the effect on pili glycosylation assessed. One of these genes, here designated pglA, was homologous a family of glycosyltransferases. PglA mutants showed no alteration in the phenotype of LPS as judged by gel migration and the binding of monoclonal antibodies. In contrast, decreased gel migration of the pilin subunit molecules from pglA mutants was observed. This migration was similar to that of pilin from galE mutants of same strains, supporting the notion that pglA is involved in the biosynthesis of the pilin linked trisaccharide structure. The pglA mutation, like the galE mutation reported previously, had no effect on pilus-mediated adhesion to human epithelial or endothelial cells. Pilin from pglA mutants were unable to bind to monospecific antisera recognising the Gal(beta1-4)Gal structure, suggesting that PglA is a glycosyltransferase involved in the addition of galactose of the trisaccharide substituent of pilin.

Yours sincerely

Mike Jennings

=-

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==========

Objet: abstract Neisseria98
Date: Fri, 15 May 1998 16:32:37 +0200
De: Hans Rumke <Hans.Rumke@rivm.nl>
A: oral.neisseria@necker.fr

To Dr Xavier Nassif, Inserm U411, Faculté de Médecine Necker-Enfants Malades, Paris, France

Dear Dr Nassif,

Please find here an abstract for oral presentation at the Neisseria98 conference.
Title: Clinical studies with RIVM hexavalent meningococcal PorA vesicle vaccine

Hans C. Rümke, Jerry Labadie, Germie van den Dobbelsteen, Loek van Alphen and the study
teams of PHL Gloucestershire, UK (Prof Keith A.V. Cartwright) and Sophia Children's
Hospital, Rotterdam, Netherlands (Dr R. De Groot)

RIVM (Netherlands Institute of Public Health and the Environment)
Laboratory for Clinical Vaccine Research
PO Box 1, 3720 BA Bilthoven, Netherlands

RIVM hexavalent vaccine was made using vesicles of two meningococcal strains that each expressed three different class 1 outer membrane protein (PorA) through homologous DNA recombination and exchange.
Immunogenicity (serum bactericidal activity; SBA) and adverse reactions were investigated in three trials: in infants (Gloucestershire, UK), toddlers and school children (Rotterdam, Netherlands). Infants were vaccinated at age 2-3-4 and 15 months, the older children at time 0-2-8 months. SBA tests were performed at PHL Manchester, UK and RIVM, Bilthoven. Rates and severity of the observed side effects were acceptable. Systemic reactions did not appear more frequently than in the control group. Local reactions were mild.
The vaccine was moderately immunogenic in infants, toddlers and school children. The SBA builds slowly, and four doses of vaccine are required in infants, inducing an at least fourfould rise in SBA to the 6 strains in >90% of infants. Responses in older children (30-95% after 3 doses) were found lower than in infants, possibly due to interlaboratory differences. Some of the PorA components of the hexavalent vaccine, including the P1.4 PorA, were observed to be weaker immunogens, while one PorA per trivalent vesicle appeared to dominate over the other two.
Persistence of immunity after vaccination is under study. Two lines of future research by RIVM will be highlighted: plans for an intervention study to combat an epidemic in New Zealand with a monovalent P1.4 vesicle

vaccine, and the further development of an improved hexavalent vaccine for use in European countries.

We look forward to your approval of our abstract for an oral presentation at the Conference, and wish you and your team a nice sprit in the organisation.

Kind regards,

Dr Hans C. Rümke, pediatrician - epidemiologist

RIVM (Netherlands Institute of Public Health and the Environment)
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Objet: Feil, Spratt
Date: Fri, 15 May 1998 15:47:49 BST
De: E.J.Feil@sussex.ac.uk (Edward Feil)
A: oral.neisseria@necker.fr

Micro-evolution of clones of N. meningitidis is predominantly driven by recombination rather than mutation

Edward J. Feil Brian G. Spratt

Scool of Biological Sciences,
University of Sussex,
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(from 10th July)
Wellcome Trust Centre for the Epidemiology of Infectious Disease,
Department of Zoology,
University of Oxford, UK
Oxford OX1 3PS

The rate and evolutionary impact of recombination within different microbial species is a matter of continuing debate, although the ease with which large volumes of nucleotide sequence data can now be routinely generated is at last shedding light on this most fundamental of evolutionary questions. Recently, a large nucleotide sequence data set for N. meningitidis has been generated as part of the development of a Multi-Locus Sequence Typing project (MLST) which incorporated 7 housekeeping loci. These data represent all of the major hyper-virulent clonal lineages from serogroups A, B and C previously identified through Multilocus Enzyme Electrophoresis (MLEE). Most of the isolates belonging to a particular clonal group share identical sequence types, that is to say they have identical sequences at all the loci examined. However, a few isolates show non-congruencies from the clonal consensus at 1 or 2 loci and these variants are assumed to have arisen recently (i.e. since the clonal group emerged). An analysis of the nature of these non-congruencies allows us to deduce whether they have arisen primarily though mutation or recombination, and it appears that the main force behind the diversification of these

clonal lineages is emphatically recombination. A conservative estimate of the ratio of the per nucleotide rate of change derived from recombination compared to point mutation is 300:1. This analysis therefore confirms previous suggestions that recombination has a much more significant impact on the micro-evolution of N. meningitidis than mutation has.

Objet: Qvarnström Swedberg
Date: Fri, 15 May 1998 16:48:27 +0200
De: "Yvonne Qvarnstrom" <Yvonne.Qvarnstrom@farmbio.uu.se>
A: oral.neisseria@necker.fr

Adaptation to sulfonamide resistance in Neisseria meningitidis may have required compensatory changes to retain enzyme function. Kinetic analysis of dihydropteroate synthases from Neisseria meningitidis .

Yvonne Qvarnström and Göte Swedberg
Department of Pharmaceutical Biosciences, Division of Microbiology, Faculty of Pharmacy, Uppsala University
Postal address; Box 581, Biomedicum, S-751 23 Uppsala, Sweden

The enzyme dihydropteroate synthase (DHPS), of the folate biosynthesis pathway, is the target for sulfonamide drugs. This enzyme is essential for survival of bacteria and thus a good target for inhibition, but a problem is that mutational changes may lead to resistance to the inhibitor.

In Neisseria meningitidis, clinical resistance to sulfonamides is mediated by alterations of the chromosomally encoded DHPS. One type of resistant DHPS differs substantially from sensitive enzymes. It also has an apparent insertion of two additional amino acids (Ser-Gly).

A deletion of this Ser-Gly insertion led to a sensitive enzyme. However, the Km for the substrate para-aminobenzoic acid (p-AB) was raised tenfold, suggesting that compensatory changes have been accumulated. Several independent resistant isolates have changes at position 68 compared to sensitive isolates. Changing position 68 back to wild-type sequence after removal of the Ser-Gly led to a lowering of the Km to almost the same level as in the original resistant strain.

The Km for the other substrate pteridinepyrophosphate was affected in a different way. The original resistant isolate showed a very high value, which was reduced to wild type levels when the Ser-Gly insertion was removed.

Changing position 68 in a sensitive enzyme to the amino acid found in the resistant isolate, led to a raise in Km for p-AB while not affecting the Km for pteridine. The Ser-Gly insertion was also introduced in the same enzyme and the effect of this insertion is presently under study.

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Objet: Richmond-Cartwright
Date: Fri, 15 May 1998 15:55:00 +0100
De: Manchester Public Health Laboratory <manphl@manphl.demon.co.uk>
Répondre à: ray <ray@manphl.demon.co.uk>

A: oral.neisseria@necker.fr

Dear Sir/Madam,

Could you please replace an abstract (entitled the same) submitted earlier today by same authors with the following. I am very sorry about any confusion this may cause.

Reactogenicity, immunogenicity and priming of two different strengths of a meningococcal serogroup C conjugate vaccine in UK infants.

Richmond PC (1), Miller E(1), Borrow R (presenting author)(2), Clark S(2), Sadler F(2), Fox AJ(2), Begg N(1), Morris R (3), Cartwright KAV(3)

(1) Immunisation Division, CDSC PHLS, 61, Colindale Avenue, London, NW9 5EQ, UK.
(2) Public Health Laboratory, Withington Hospital, Manchester, M20 2LR, UK.
(3) Public Health Laboratory, Gloucestershire Royal Hospital, Gloucester, GL1 3NN, UK.

The safety, immunogenicity and priming capacity of two different strengths (2 versus 10ug) of a Wyeth-Lederle meningococcal serogroup C polysaccharide-CRM197 conjugate vaccine was assessed in two cohorts, each of 57 UK infants. Immunisation was at 2, 3 and 4 months of age with 25 and 24 infants of the 2 and 10ug cohorts receiving a booster dose of plain serogroup A/C polysaccharide vaccine at 15 to 23 months of age. The vaccine proved safe with local reactions significantly ($p < .001$) less common than at the site of the DTP/Hib immunisation. One infant developed an urticarial rash had received a 0.5ml booster dose of the polysaccharide vaccine. Significantly higher ($p < .01$) rises in both Ig and bactericidal titres were noted for both strengths after the first and second doses. This increase was less marked after the third dose. Antibody levels decreased significantly ($p < .001$) between 5 and 14 months of age but a booster response was demonstrated. Significantly higher ($p < .01$) Ig levels were observed after the second and third doses of vaccine for the 10ug (GMTs of 15.7 and 17.6ug/ml) than the 2ug strengths (GMTs of 7.8 and 10ug/ml) though no significant difference was observed in bactericidal titres (GMTs of 766.3 and 1011 versus 553.8 and 1102.9 respectively). However, significantly ($p < .01$) higher Ig levels were observed for the 2ug cohort after boosting (GMTs 19.0 versus 12.1ug/ml) which was reflected in higher bactericidal titres (GMTs 581 versus 256). Thus both strengths of this vaccine were well tolerated, immunogenic and induced memory in UK infants.

--
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Objet: Dempsey, Cannon

Date: Fri, 15 May 1998 11:35:12 -0400
De: "Janne G. Cannon" <jgc@med.unc.edu>
A: oral.neisseria@necker.fr

Mismatch Repair Influences Phase Variation of Surface Proteins in Neisseria gonorrhoeae.
Jo Ann Fanney Dempsey (presenter), Diana B. Johannsen, Peter Leone, and Janne G. Cannon, Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Genomes of the pathogenic Neisseria appear to undergo frequent rearrangement and recombination, with expression of genes encoding some surface components varying via recombination or slipped-strand mispairing (SSM). These events might be influenced by DNA repair, especially mismatch repair (MMR). We demonstrated that the gonococcus has a functional MMR system, in part by constructing a mutS mutant of strain FA1090 in which MMR is inactivated and mutation rates are elevated.

The rate of phase variation of the hpuA gene (hemoglobin-haptoglobin utilization), which is accomplished by changes in the number of residues in a run of G's within the gene, was increased ten-fold in FA1090mutS compared to FA1090, confirming a possible role of MMR in regulating SSM. Phase variation of Opa was unaltered in the mutant, perhaps because MMR does not function on loops of 5 bp formed by the CTCTT repeats.

In E. coli, the signal for strand discrimination in MMR is Dam methylation; Dam- mutants have increased mutational frequencies. Gonococcal strains can be Dam+ or Dam-, and it has been speculated that Dam phenotype might show phase variation. We showed that strains that are Dam- in vitro are also Dam- in vivo, and that Dam methylation is not the signal for strand discrimination in gonococcal MMR. In FA1090 and other Dam- strains, there is a 0.9 kb segment containing a gene similar to dpnC of S. pneumoniae replacing over half of dam; dpnC encodes the DpnI restriction endonuclease that would digest Dam-methlated DNA if the two genes co-existed.

Objet: S.D. Gray-Owen and T.F. Meyer (fwd)
Date: Fri, 15 May 1998 18:02:33 +0200 (MET DST)
De: nassif@citi2.fr (Xavier Nassif)
A: oral.neisseria@necker.fr

Forwarded message:
>From scott.gray-owen@tuebingen.mpg.de Fri May 15 14:45 MET 1998
Message-ID: <355C382F.4021BE04@tuebingen.mpg.de>
Date: Fri, 15 May 1998 14:42:23 +0200
From: Scott Gray-Owen <scott.gray-owen@tuebingen.mpg.de>
X-Mailer: Mozilla 4.03 [en] (Win95; I)
MIME-Version: 1.0
To: Neisseria98 <neisseria98@ceylan.necker.fr>
Subject: S.D. Gray-Owen and T.F. Meyer
References: <v01530500b179aa3480d6@[194.254.89.10]>
X-MIME-Autoconverted: from 8bit to quoted-printable by mailer1.tuebingen.mpg.de id OAA23972
Content-Transfer-Encoding: 8bit
X-MIME-Autoconverted: from quoted-printable to 8bit by bisance.citi2.fr id OAA27911
Content-Type: text/plain; charset=iso-8859-1
Content-Length: 2186

The outcome of Neisserial Opa-mediated binding depends upon target cell type

and its expressed CD66 receptor repetoire.

Scott D. Gray-Owen(1), Christoph Dehio(1), Andreas Popp(1), Jun Wang(1), Petra Munzner(1), Christof Hauck(1), Erich Gulbins(2), Fritz Grunert(3), Wolfgang Zimmerman(3) and Thomas F. Meyer(1,4).

(1) Max-Planck-Institut fuer Biologie, Abteilung Infektionsbiologie, Spemannstrasse 34, 72076 Tuebingen, Germany; (2) Physiologisches Institut, Universitaet Tuebingen, Gmelinstrasse 5, 72070 Tuebingen, (3) Universitaet Freiburg, Immunbiologisches Institut, Stefan-Meier-Strasse 8, 79104 Freiburg, and (4) Max-Planck-Institut fuer Infektionsbiologie, Abteilung Molekulare Biologie, Monbijoustrasse 2, 10117 Berlin, Germany.

CD66 receptors are expressed by a wide range of host tissues, and their expression can be upregulated by proinflammatory cytokines such as TNFalpha which predominate during neisserial disease. Neisserial Opa binding to CD66 receptors is a protein-protein interaction involving b-strand C of the N-terminal domain, and predicted differences in domain structure between individual CD66 proteins does influence the specificity of these interactions. Gonococcal CD66-mediated engulfment by phagocytic cells involves the concomitant activation of Src-family kinases and the inactivation of SHP-1 phosphatase, with a subsequent activation of Rac1, PAK and the stress-activated protein kinase JNK. In polarised T84 epithelial cells, Opa-mediated binding to apically-expressed CD66 receptors results in the transcytotic passage of bacteria to the basolateral surface without disruption of the monolayer's barrier function. Both of these cell types do, however, express multiple CD66 family members. Although bacterial engulfment following Opa-mediated binding to stably-transfected cells can be mediated by four different CD66 receptors, uptake via each of these proteins occurs via distinct signalling events. Transfected cells expressing a series of recombinant constructs containing mutations in the cytoplasmic domains of CD66a and CD66d have thus been constructed and their influence on bacterial uptake mediated by these two receptors assessed.

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Xavier Nassif

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Objet: Re: Neisseria meeting - Abstract submission (fwd)
Date: Fri, 15 May 1998 18:06:47 +0200 (MET DST)
De: nassif@citi2.fr (Xavier Nassif)
A: oral.neisseria@necker.fr

Forwarded message:
> From coleja@bcmsrv4.bham.ac.uk Fri May 15 15:55 MET 1998
> From: "Jeff Cole" <coleja@bcmsrv4.bham.ac.uk>
> Organization: The University of Birmingham
> To: neisseria98@ceylan.necker.fr (Neisseria98)
> Date: Fri, 15 May 1998 14:46:13 GMT
> MIME-Version: 1.0
> Content-transfer-encoding: 7BIT
> Subject: Re: Neisseria meeting - Abstract submission

> Reply-to: j.a.cole@bham.ac.uk
> X-Confirm-Reading-To: j.a.cole@bham.ac.uk
> X-pmrqc: 1
> Return-receipt-to: j.a.cole@bham.ac.uk
> Priority: normal
> In-reply-to: <v01530500b1809202ed24@[194.254.89.10]>
> X-mailer: Pegasus Mail for Windows (v2.53/R1)
> Message-ID: <ECA0252673@bcmsrv4.bham.ac.uk>
> Content-Type: text/plain; charset=US-ASCII
> Content-Length: 7845
>
>
> Identification of an FNR homologue essential for gonococcal adaptation
> from aerobic to anaerobic or oxygen-limited growth
>
> Sarah Lissenden1, Tess Regan1, Helen Crooke1 Jean Cardinale2, Tracy
> Schipp2, Virginia Clark2, Harry Smith1 and Jeff Cole1
>
> 1 School of Biochemistry, University of Birmingham, Birmingham B15
> 2TT, UK and 2 Department of Microbiology, University of Rochester,
> Rochester, NY 14642, USA.
>
> Gonococci can grow aerobically with glucose, lactate or pyruvate as
> the major source of carbon and energy, but otherwise are limited in
> their ability to adapt to other growth environments. The few examples
> of transcription factors which activate gonococcal gene expression in
> response to environmental signals include PilA-PilB and RegF, which
> regulate pilin synthesis. Gonococci can also grow anaerobically using
> nitrite as a terminal electron acceptor, nitrite being reduced by an
> anaerobically inducible nitrite reductase encoded by aniA.
>
> In Escherichia coli, synthesis of two alternative nitrite reductases
> is absolutely dependent upon the functional transcription factor, FNR
> (the fumarate and nitrate reductase regulator protein) which is also
> essential for the expression of more than twenty operons encoding
> proteins involved in the synthesis or assembly of anaerobically
> expressed electron transfer components. Analysis of the gonococcal
> genome sequence database revealed the presence of a gene encoding a
> protein predicted to be 40 % similar in amino acid sequence to the E.
> coli FNR protein. This gene has been cloned into E. coli,
> insertionally inactivated and the mutated gene has been transformed
> into various gonococcal strains. The transformants are totally
> defective for anaerobic growth and are less able than the parental
> strain to adapt to oxygen-limited growth in the presence of nitrite.
> Interruption of the gonococcal fnr gene results in loss of
> transcription from the aniA promoter, and inability to express nitrite
> reductase activity during oxygen-limited growth. Experiments to
> demonstrate that the gonococcal fnr mutant is pleiotropically
> defective in the expression of other anaerobically induced genes will
> be reported.
>
>
> Second contribution:
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>
> Evidence for the involvement of a two-component regulatory system in
> anaerobic growth and nitrite reduction by gonococci.
>
> Tess Regan1, Sarah Lissenden1, Helen Crooke1, Jean Cardinale2, Tracey
> Schipp2, Virginia Clark2, Harry Smith1 and Jeff Cole1
>

> 1 School of Biochemistry, University of Birmingham, Birmingham B15
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> Rochester, NY 14642, USA.
>
> Gonococci have a limited capacity to adapt to anaerobic growth by
> using nitrite as a terminal electron acceptor. The nitrite reductase
> encoded by the aniA gene is not synthesised during aerobic growth:
> AniA synthesis is partially induced during anaerobic growth in the
> absence of nitrite, but is optimally expressed only during anaerobic
> growth in the presence of nitrite.
>
> In Escherichia coli, there are two biochemically and genetically
> independent pathways for nitrite reduction, both of which are
> regulated by multiple environmental signals. Two, two-component
> regulatory systems, NarX-NarL and NarQ-NarP, integrate signals
> resulting from the presence of nitrate or nitrite in the environment
> and, in partership wth the transcription factor, FNR, induce or
> repress nitrite reductase synthesis according to the energy
> requirements of the bacteria. Analysis of the gonococcal genome
> sequence database revealed the presence of a gene encoding a protein
> predicted to be 56 and 54 % similar in amino acid sequence to the E.
> coli NarL and NarP proteins, respectively. This gene has been cloned
> into E. coli, insertionally inactivated and the mutated gene has been
> transformed into various gonococcal strains. The transformants are
> less able than the parental strain to: (1) adapt to anaerobic growth;
> (2) initiate transcription from the aniA promoter in response to the
> presence of nitrite, or (3) catalyse nitrite reduction during growth
> in anaerobic or oxygen-limited media. Mutation of the gonococcal narL
> gene also results in decreased ability to express other anaerobically
> induced proteins. As far as we are aware, this is only the second
> example of a two-component regulatory system involved in the
> regulation of transcription of environmentally regulated genes in
> neisseria.
>
>
> Third contribution:
>
> Lactate causes profound changes in gonococci including enhanced
> lipopolysaccharide synthesis during short-term incubation in media
> containing glucose
> Lihui Gao1, Nicholas J. Parsons1, Alan Curry2, Jeff A. Cole1, and
> Harry Smith3* 1School of Biochemistry, and 3the Medical School,
> University of Birmingham, Birmingham B15 2TT, U.K., 2Public Health
> Laboratory, Withington Hospital, Manchester M20 8LR, U.K.
>
>
>   Gonococci (strain BS4(agar)), just emerging from lag-phase during
>   1-1.5 h incubation with 5 *M or 50 *M sodium lactate in a defined
>   medium containing glucose (28 mM) showed, as in previous studies, an
>   enhanced capacity for their lipopolysaccharide (LPS) to be
>   sialylated by cytidine 5'-monophospho-N-acetyl neuraminic acid.
>   However, the sialyltransferase content of the lactate treated
>   gonococci was not greater than that of control organisms and there
>   were no differences between them in the nature and number of LPS
>   components seen after tricine-sodium dodecyl-polyacrylamide
>   electrophoresis and silver staining. The total LPS content of the
>   lactate treated gonococci, measured by assay of
>   2-keto-3-deoxyoctonate, was 10-20% above that of control organisms.
>   This provided a simple possible explanation for lactate enhancement;
>   more sialyl receptors becoming available during an overall
>   stimulation of LPS synthesis. The protein content of the lactate

> treated gonococci was higher than that of controls indicating
> stimulation of protein synthesis. Some samples of lactate treated
> gonococci showed an increase in pentose content suggesting greater
> ribosome production. Electron microscopy showed that hair-like
> external appendages on the control organisms were largely absent
> from the lactate treated gonococci. When the concentration of
> glucose and lactate in the medium was adjusted to values more like
> those occurring in vivo (glucose 5 mM alone and with either 1 mM or
> 10 mM lactate) and gonococcal multiplication occurred during the
> short incubation (1-1.5 h), lactate again induced greater contents
> of LPS, protein and pentose. Clearly, lactate has a profound effect
> on gonococci growing in a synthetic medium containing glucose. The
> increased content of LPS, a gonococcal virulence determinant, is
> important. It should be a constant feature of gonococci growing in
> their natural habitat, human urogenital tissues, where lactate is
> ever-present with glucose
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> **
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Objet: Richmond P, Miller E
Date: Fri, 15 May 1998 17:18:03 +0100
De: "CDSC - Richmond, Peter" <PRichmon@phls.co.uk>
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An investigation of the immunogenicity and reactogenicity of three
meningococcal serogroup C conjugate vaccines administered as a single
dose in UK toddlers

Richmond PC (1), Cartwright KAV (2), Borrow R (3), Morris R (2), Clark S
(3), Burrage M (4), Thornton C (4), Kaczmarski E (3), Miller E (1).

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Introduction of meningococcal C conjugate (MCC) vaccines would have greater impact on morbidity and mortality if introduction to the infant schedule is accompanied by a mass catch-up immunisation programme. An essential question is whether a single dose of MCC vaccine for children is adequate for long term immunity. We are evaluating the response to a single dose of MCC vaccine, antibody persistence and response to meningococcal AC polysaccharide booster vaccine 6 months later. A total of 225 children, 12-17 months of age will be randomised to receive one of three candidate MCC vaccines, two containing CRM197, and one tetanus toxoid. Antibody responses are being measured by ELISA and serum bactericidal assay using standardised protocols.
As of April 98, 162 children had received a single dose of MCC vaccine at the same time as routine MMR vaccine. The MCC vaccine has been well tolerated with no severe vaccine attributable adverse events. Immunogenicity data on 69 subjects has shown that MCC vaccine induced high levels of anti-capsular IgG antibody rising from 0.15 ug/ml (95% CI 0.12 - 0.2) to 19.2 ug/ml (95% CI 14.4 - 25.5). 68/69 children had levels >2ug/ml. Differences in antibody responses were seen between MCC vaccines although groups were small.
MCC conjugate vaccines are immunogenic and well tolerated in UK toddlers. Confirmation of the adequacy for priming of a single dose in this age group would allow a single dose strategy to be used in a mass immunisation campaign for all ages above 12 months.

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Objet: De Gaspari
Date: Fri, 15 May 1998 17:08:23 GMT
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A new combined vaccine against Neisseria meningitidis B
EN. De Gaspari
Immunology Section ,Adolfo Lutz Institute, São Paulo-SP-Brazil
Although there is good evidence that locally produced antibodies are more effective at preventing respiratory infections, most vaccines are given parenterally, which stimulates systemic immune responses, including serum antibodies but induce mucosal immune responses poorly.This probably explains why parenteral vaccines are only partly effective in preventing respiratory diseases. It is therefore apparent that novel strategies are required to achieve effective defence. Production of a meningococcal vaccine capable of generating long-lasting immunity in all age groups is still a high priority worldwide.The combined vaccine using native outer membrane vesicle (NOMV) of Brazil epidemic strain B:4:P1.15 was determined in mice. Mice developed serum bactericidal antibodies as well as high levels of specific and cross reactive serum IgA and IgG as determined by ELISA using whole cells and NOMV of different Brazil strains.Western blot analyses of

the mice sera showed IgG antibodies responses to the class 1 and 5 , meanwhile a strong cross reactivity could be observed with the peptides of 80kDa ,70kDa, 50kDa and 14kDa in the strains B:6:P1.6; B:14:NT; B:NT:P1.9; B:17:P1.14; B:19:P1.15 and B:8:P1.6 . With the the help of a fluorescence-activated cell sorter (FACS), established the feasibility of measuring antibody with surface -exposed epitopes on whole bacteria . FACS analysis ,using 8C7Br1 monoclonal antibody and intact bacteria showed that the 50kDa epitope is expressed on the bacteria surface.The peptide recently sequenced confirmed that this protein has never been described previously. The combined vaccine no produced antibodies directed against class 4, that have been reported to block the bactericidal activity of other antibodies. The kinetics of IgA and IgG stimulated by the vaccine reflect the presence and persistence of immunological memory at B cell level. The reactivity with proteins that are highly conserved, expressed at the surface of intact bacterial cells and that it cam elicit the production of bactericidal antibodies greatly emphasize its potential use in a broad range vaccine. The protection of upper or lower respiratory tracts were investigated. This protection correlated with the presence of antibacterial IgA and IgG antibodies. Thus in agreement with other studies protection using combined vaccine correlated with "local immunity".The importance of the specific sites stimulation of the immune system were analysed.

Objet: Rosenstein-Perkins
Date: Fri, 15 May 1998 13:40:00 -0400
De: "Rosenstein, Nancy" <nar5@cdc.gov>
A: "'Neisseria Conference'" <oral.neisseria@necker.fr>
Copies à: "Rosenstein, Nancy" <nar5@cdc.gov>

Evaluation of Control of Meningococcal Disease Epidemics in Sub-Saharan Africa.
Nancy E. Rosenstein, J.A.D. Leake, G. Armstrong, C. Woods, C. Tetteh, S.O. Sackey, S. Bugri, B.A. Perkins, and the Africa Meningitis Working Group
Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, NCID, CDC, Atlanta, GA; Ministry of Health (MOH), Ghana; Emerging and Other Communicable Diseases, WHO, Geneva

Severe epidemics of serogroup A meningococcal disease occur periodically in sub-Saharan Africa. In 1996, the largest recorded meningitis epidemic in sub-Saharan Africa occurred, causing 152,813 cases and 15,783 deaths. In 1997, 61,301 cases of meningococcal disease were reported, and between January and April 1998, 7,595 cases of meningococcal meningitis have been reported.

The currently available meningococcal polysaccharide A/C vaccines provides protection in adults and children >4 years of age but does not elicit long-term protection in young children. Vaccine efficacy among adults during epidemics has been estimated at 85-90%. WHO guidelines recommend monitoring of weekly district-level meningitis rates using a threshold rate (15 cases/100,000 for 2 consecutive weeks) to trigger mass meningococcal vaccine campaigns. We evaluated sensitivity and specificity of the threshold as well as the operational impact and feasibility of this strategy.

To evaluate the WHO epidemic threshold strategy, we used 1997 district-level surveillance data from seven countries and 177 districts. A total of 44,670 cases were reported, and 60 districts (34%) crossed the WHO threshold rate. The sensitivity of the threshold was 92%, specificity 98%, and positive predictive value 97%.

In 1996, Ghana experienced a meningococcal disease epidemic with 18,703 cases. Cases continued to occur despite mass vaccination

campaigns with 70% coverage. We used surveillance data and vaccination distribution records to evaluate the impact of vaccination campaigns. We estimated that 5,438 cases and 304 deaths were prevented by vaccination. If vaccination campaigns had occurred immediately following crossing of the threshold, 16,296 cases and 995 deaths would have been prevented.

Objet: Charalambous and Feavers
Date: Fri, 15 May 1998 20:01:04 +0100
De: Ian Feavers <ifeavers@nibsc.ac.uk>
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Characterisation of consensus peptides that cross-react with anti-Neisseria meningitidis LOS 3,7,9 monoclonal antibodies.

Bambos M. Charalambous (1) and Ian M. Feavers (2)
(1) Dept. of Biochemistry & Molecular Biology, Royal Free Hospital School of Medicine, Rowland Hill St, LONDON NW3 2PF UK.
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Serogroup B meningococcus immunotype LOS3,7,9 is responsible for >50% of cases seen in Europe. The polysaccharide capsule and outer membrane lipo-oligosaccharide (LOS) are major virulence factors in this organism, which reduce immunogenicity and mimic human cell-surface structures. Polysaccharides are not good vaccine candidates since they only induce a T-independent response, which is poorly developed in infants and where the incidence of disease is typically greatest. However, mice immunised with peptides, based on CDR loops of anti-idiotypic meningococcal C polysaccharide antibodies, can protect against lethal challenge of meningococcus (M.A.J. Westerink, et al Proc. Natl. Acad.Sci. USA, 92, 4021-25).
The binding kinetics and affinities of two Neisseria meningitidis (Nm)LOS3,7,9 monoclonal antibodies have been determined by surface plasmon resonance. In addition we are investigating the use of NmLOS3,7,9 monoclonal antibodies to isolate idiotypically cross-reactive peptides to study protein-carbohydrate and protein-protein interactions, as well as their potential as vaccines. Interestingly, these antibodies have several LOS epitopes which resemble amino acids. We have obtained several consensus peptides from phage-display panning, and are determining their cross-reactivity and binding affinities to the NmLOS3,7,9 monoclonal antibodies, as well as testing their immunogenicity in animal models. The binding kinetics and affinity of the most prevalent consensus peptides have been determined. Immunogenicity trials with Diptheria Toxoid conjugated peptide are showing elevated serum IgG levels towards both consensus peptide and LOS3,7,9. It remains to validate these findings and extend the immunogenicity studies.

Objet: Dehio-Meyer
Date: Fri, 15 May 1998 21:07:47 +0200 (MET DST)
De: Christoph Dehio <christoph.dehio@tuebingen.mpg.de>
A: oral.neisseria@necker.fr

Alternative pathways of heparan sulfate proteoglycan-dependent internalisation of Neisseria gonorrhoeae by epithelial and endothelial cells.

Christoph Dehio(1), Michaela Dehio(1), Elke Freissler(1), Oscar G. Gomez-Duarte(1), Heike Grassme(1), Erich Gulbins(2), Guido David(3) and Thomas F. Meyer(1,4).

(1) Max-Planck-Institut fuer Biologie, Abteilung Infektionsbiologie, Spemannstrasse 34, 72076 Tuebingen, Germany; (2) Physiologisches Institut, Universitaet Tuebingen, Gmelinstrasse 5, 72070 Tuebingen, Germany, (3) Center for Human Genetics, University of Leuven, Leuven, Belgium and (4) Max-Planck-Institut fuer Infektionsbiologie, Abteilung Molekulare Biologie, Monbijoustrasse 2, 10117 Berlin, Germany.

Heparan sulfate proteoglycan (HSPG)-dependent internalization of Opa50-expressing gonococci occurs by at least two alternative mechanisms. Invasion into Chang conjunctiva cells depends on the activation of phosphatidylcholine-dependent phospholipase C and acidic sphingomyelinase which results in the generation of the second messengers diacylglycerol and ceramide, respectively. In other epithelial cell lines, e.g. HeLa, this signaling pathway appears to be less prominent and bacterial entry is only poor. However, invasion is triggered in the presence of the extracellular matrix protein vitronectin (VN) which binds specifically to Opa50-expressing gonococci and stimulates bacterial uptake in an alphaV integrin-dependent manner. This signaling process also appears to require an activation of protein kinase C. A specific role for HSPG-ligation in the two different Opa50-dependent bacterial uptake mechanisms represented by Chang cells and HeLa cells was confirmed using latex beads coated with antibodies directed against HSPGs as a model system.

Syndecans are HSPGs with a transmembranous core protein. Over-expression of syndecan-4, a syndecan widely expressed in epithelia, in HeLa cells increases gonococcal internalization, while over-expression of a mutant form carrying a deletion of the cytoplasmic domain instead diminishes bacterial uptake. Hence, syndecan-4 seems to play a major role in mediating bacterial uptake and the cytoplasmic domain appears to be critical for this process.

Due to the ubiquitous expression of HSPGs on eukaryotic cells, Opa-mediated interactions with HSPGs are not limited to epithelial cells. Interestingly, Opa50-expressing bacteria bind strongly to endothelial cells, while uptake is stimulated both by VN and the extracellular matrix protein fibronectin by potentially alternative pathways.

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Objet: Fox-Knapp
Date: Fri, 15 May 1998 15:26:59 -0400
De: kim_fox@mail.enr.state.nc.us (Kim Fox)
A: oral.neisseria@necker.fr
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Gonorrhea in Men who Have Sex with Men (MSM): United States, 1992-1997
Fox KK, Levine WC, and Knapp JS for the GISP Investigators' Group

Centers for Diseases Control and Prevention, Atlanta, GA

[Address of presenting author (I cannot underline in this email system):
Kimberley K. Fox, MD, MPH
HIV/STD Prevention and Care Section
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Affiliation of presenting author is still CDC, Atlanta, GA]

Objective: Evaluate trends in gonococcal infection in MSM.
Methods: In the Gonococcal Isolate Surveillance Project (GISP), isolates and case information were collected from 26 urban STD clinics. MSM cases were compared to heterosexual cases, and 1994-97 MSM cases to earlier MSM cases.
Results: Of 25,493 cases, the proportion that were MSM increased from 4.7% in 1992-93 to 11.2% in 1997 (through June) ($p<0.001$). Compared to heterosexuals, MSM were older (median 30 vs. 25 years, $p<0.001$), more likely to be white (59.4% vs. 7.4%, $p<0.001$) and to have had gonorrhea previously (52.6% vs. 46.2%, $p<0.001$), though fewer had had gonorrhea in the past year (15.4% vs. 19.1%, $p<0.001$). MSM isolates were less likely to produce penicillinase (1.2% vs. 6.7%, $p<0.001$), but more likely to have chromosomal tetracycline resistance (TetR) (15.2% vs. 8.4%, $p<0.001$) or erythromycin MIC>1.0 ug/ml (7.3% vs. 2.3%, $p<0.001$). MSM with gonorrhea in 1994-97 were older than those with gonorrhea in 1992-93 (median 31 vs. 29 years, $p=0.01$) and a higher proportion had had gonorrhea in the previous year (16.9% vs. 11.7%, $p=0.01$). A higher percentage of MSM isolates during 1994-97 compared to 1992-93 had TetR (17.4% vs. 9.8%, $p=0.001$), chromosomal tetracycline and penicillin resistance (CMRNG) (7.7% vs. 3.0%, $p=0.001$), or erythromycin MIC>1.0 ug/ml (9.0% vs. 2.3%, $p<0.001$).
Conclusions: In a large group of U.S. STD clinics, MSM account for an increasing proportion of gonorrhea cases. Repeat infection and strains with high erythromycin MICs are increasingly common. These trends are alarming given recent data indicating that gonorrhea facilitates HIV transmission.

Objet: Popovic-Perkins
Date: Fri, 15 May 1998 15:36:00 -0400
De: "Popovic, Tanja" <txp1@cdc.gov>
A: "11th Int'l Path Neisseria Conf" <oral.neisseria@necker.fr>
Copies à: "Popovic, Tanja" <txp1@cdc.gov>

Dear Sir/Madam:

Here is the abstract to be considered for the oral presentation at the 11th International Pathogenic Neisseria Conference to be held in Nice, November 1-6, 1998. Presenting author: Tanja Popovic.

Popovic, T., Ajello, G.W., Reeves, M.W., Reiss, J.A., Kim, C., Tondella, M.L.C., Schmink, S.E., Mayer, L.W., Rosenstein, N.E., and Perkins, B.A.

Analysis of outbreak-associated and sporadic _Neisseria meningitidis_ serogroup C isolates by five molecular subtyping methods

Centers for Disease Control and Prevention, Atlanta, GA, USA

The number of serogroup C meningococcal disease (SCMD) outbreaks in the U.S. has increased since 1990, while the endemic rate of meningococcal disease has remained stable at 1-1.5/100,000; SCMD outbreaks are usually caused by strains identified as closely related by subtyping methods, such as multilocus enzyme electrophoresis (MEE). However, MEE cannot always distinguish outbreak-associated strains from those causing sporadic disease. In the fall of 1993, an outbreak of SCMD occurred in Grayson County, Texas: 14 county residents aged 2-29 years were affected. All outbreak-associated isolates, as well as the background endemic strains, were of ET24 or of a few ETs closely related to ET24. In this study we assayed twelve of the outbreak-associated isolates, ten epidemiologically unrelated _Neisseria meningitidis_ serogroup C (SC) strains from Texas (1994-1996), and six sporadic SC strains from throughout the U.S. by five subtyping methods: MEE, pulsed field gel electrophoresis (_Nhe_I), ribotyping (_Cla_I, _Sma_I, _Xho_I), random amplified polymorphic DNA assay (6 primers), and serosubtyping. SC strains from throughout the U.S. differed from each other and from the Texas strains. Outbreak-associated and sporadic Texas isolates were not readily distinguishable, suggesting that in some instances differentiation of outbreak-associated and sporadic strains circulating in the same geographic area may not be possible with the currently available subtyping methods. A study to evaluate the usefulness of variable-number, tandem-repeats size-typing and DNA sequence based typing in outbreaks like this one is underway. The development of outbreaks may depend on as yet unidentified microbiological factors as well as epidemiological risk factors.

Sincerely yours,

Tanja Popovic, M.D., Ph.D.
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Objet: wakarchuk-richards
Date: Fri, 15 May 1998 15:42:00 -0400
De: "Rondeau, Guylaine" <Guylaine.Rondeau@nrc.ca>
A: "'Neisseria 98'" <oral.neisseria@necker.fr>
Copies à: "Richards, Jim" <Jim.Richards@nrc.ca>,
"Rondeau, Guylaine"
<Guylaine.Rondeau@nrc.ca>

Please note that Dr. Jim Richards would like to be considered for an oral presentation for the 11th International Pathogenic Neisseria Conference to

be held in Nice from November 1-6, 1998. As requested, you will find below the abstract of the work he would like to present.
Considering today is the deadline for submission of abstracts, I would appreciate it if you could confirm receipt and acceptance of this abstract submission by return e-mail. Thanks.
Guylaine Rondeau
(on behalf of Dr. Jim Richards)

Structural requirements for sialic acid expression on lipopolysaccharides of Neisseria meningitidis.

W. Wakarchuk, M. Gilbert, A. Martin, N.-H. Khieu, J.-R. Brisson, P. Thibault, and J.C. Richards

Institute for Biological Sciences, National Research Council of Canada
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Recent cloning of the lipopolysaccharide (LPS) alpha-2,3-sialyltransferase from Neisseria meningitidis immunotype L3 (1) has permitted examination of other immunotypes for this structural gene (lst). We have now identified the gene in immunotype L1 and L8 strains. Expression of lst genes was deduced using a sensitive enzyme assay and the alpha-2,3-sialyltransferase specificities of the enzymes were confirmed by NMR spectroscopy. We have also demonstrated that this sialyltransferase can use alpha- and beta-linked terminal galactose residues as acceptors (2). Mass spectrometric analysis of LPS from the three immunotypes revealed substitution of the terminal D-galactose of lacto-N-neotetraose (immunotype L3) and the pK antigen, i.e. alpha-D-Gal-1,4-beta-D-Gal-1,4-beta-D-Glc, (immunotype L1) by sialic acid residues, but not of the lactose side chains of immunotype L8. We have demonstrated that sialic acid occurs at the O6 position of the terminal alpha-D-galactopyranosyl residue of the pK trisaccharide chain of immunotype L1 LPS (3). Molecular modeling of the oligosaccharide regions of the LPS molecules suggests that steric effects from an inner-core phosphoethanolamine moiety precludes or modifies enzyme recognition of the terminal D-galactose of lactose (immunotype L8) or the pK trisaccharide (immunotype L1); only when the oligosaccharide chain contains four sugars (immunotype L3) does this enzyme mediate alpha-2,3-sialylation. This was tested using our enzymic assay with core oligosaccharides in which the phosphoethanolamine residues were removed. This study demonstrates the power of the combined application of molecular genetics, enzymology and structure in the understanding of the molecular requirements for virulence expression in N. meningitidis LPS.

(1) Gilbert, M., Watson, D.C., Cunningham, A.-M., Jennings, M.P., Young, N.M., and Wakarchuk, W. (1996). J. Biol. Chem. 271: 28271-28276.

(2) Gilbert, M., Cunningham, A.-M., Watson, D.C., Martin, A., Richards, J.C., and Wakarchuk, W. (1997). Eur. J. Biochem. 249: 287-194.

(3) Wakarchuk, W., Gilbert, M., Martin, A., Wu, Y., Brisson, J.-R., Thibault, P., and Richards, J.C. (1998). Eur. J. Biochem. (In Press).

Objet: Dillon, Francis, Ramirez, Bernatchez and Salimnia
Date: Fri, 15 May 1998 14:03:23 -0700 (PDT)
De: "Dr. J.R. Dillon" <jdillon@uottawa.ca>
A: oral.neisseria@necker.fr

Please find below an abstract for oral presentation at the Neisseria 98 Conference in Nice, France. I have also attached this abstract which was

typed in WordPerfect 7.0.

Expression and Localization of Cell Division Genes from Neisseria gonorrhoeae

J.R. Dillon*, F.M. Francis, S.M. Ramirez, S. Bernatchez, and H. Salimnia
Department of Biochemistry, Microbiology and Immunology. University of Ottawa, Ottawa, Canada

We have identified three clusters of cell division genes in Neisseria gonorrhoeae which differ in gene order, content and organization from homologues in other bacteria. The expression of some genes was influenced by environmental conditions and the localization of the gonococcal FtsZ has been studied in Escherichia coli. The mur-fts cluster contains at least 13 genes; it is not transcribed as a single transcript as in E. coli, but contains four paired Neisserial uptake sequences which act as functional terminators as shown by RT-PCR. The min cluster contains five genes (rpoA, rplQ, minC, minD, and minE) which are co-transcribed, while ftsE and ftsX are also co- transcribed and encode a possible ABC transporter whose function is unknown. Promoters upstream of ftsZ and other genes were identified by primer extension and some were used predominantly under anaerobic growth conditions. Fragments containing the promoters of ftsE and ftsZ were cloned upstream of a promoterless lacZ in pLES94 and b-galactosidase expression in a gonococcal background increased in the presence of isoleucine and urea and under anaerobic conditions. In addition, Northern hybridizations showed that ftsE, ftsX and ftsZ are expressed differently under aerobic and anaerobic conditions. Gonococcal FtsZ-GFP fusions showed that the gonococcal FtsZ does not co-localize to the division site in E. coli, but forms several different localization patterns. We are presently studying localization of FtsZ and Min proteins in a gonococcal background. Transformation of E. coli with plasmid constructs containing minCDE caused minicell formation while insertion mutants, which were shown to be polar, resulted in normal morphologies.

Dr. Jo-Anne R. Dillon
Professor and Interim Chair
Department of Biochemistry, Microbiology and Immunology
and
Director
WHO/PAHO Co-ordinating Centre for Gonococcal Antimicrobial Susceptibility in the Americas

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---

Objet: Cannon, Cohen
Date: Fri, 15 May 1998 17:39:14 -0400
De: "Janne G. Cannon" <jgc@med.unc.edu>
A: oral.neisseria@necker.fr

Studies with the Human Challenge Model of Gonococcal Infection: Population Dynamics of Gonococci During Experimental Infection and Infectivity of Isogenic Mutants.

Janne G. Cannon (presenter), Diana Johannsen, J. David Hobbs, Noah Hoffman, Jo Ann Fanney Dempsey, David Johnston, Hakan Koyman, and Myron S. Cohen.
Departments of Microbiology and Immunology and of Medicine, University of

North Carolina School of Medicine, Chapel Hill, NC, USA

Studies of N. gonorrhoeae pathogenesis and identification of virulence factors of this organism have been hampered by the fact that the gonococcus is so well-adapted to human hosts that there are few appropriate animal models. One approach to studying gonococcal virulence is to carry out experimental infection of human male volunteers. Experimental infection in men is safe, and reproduces the clinical features of natural gonorrhea. We have used the human challenge model to study antigenic variation of surface components such as Opa proteins and pilin during early infection. These experiments have shown that there are multiple selective bottlenecks in the bacterial population during early infection, with selection for new variants expressing different surface traits. In one subject, there were two different population bottlenecks in only four days of infection. In other experiments, we constructed isogenic mutants of strain FA1090 that were unable to express potential virulence factors, including IgA protease, sialyltransferase, and pilin. The IgA protease-negative mutant and the sialyltransferase-negative mutant were unaffected in their ability to cause infection, whereas the pilin-negative mutant colonized the urethras of volunteers but elicited little of the characteristic inflammatory response and urethral discharge. These results reinforce the conclusion that gonococcal virulence is multifactorial, and that there is probably considerable redundancy of critical functions. The results also show that the human challenge model is appropriate for discriminating among strains that are compromised to different extents in their infectivity, and that the model will be useful for further studies of gonococcal pathogenesis.

Objet: Oral Presentation on Neisserial surface structures at Neisseria98
Date: Fri, 15 May 1998 16:54:20 -0500
De: "Phillip E. Klebba" <peklebba@chemdept.chem.ou.edu>
A: oral.neisseria@necker.fr

Dr. Xavier Nassif

Dear Dr. Nassif,

Fred Sparling mentioned that you might be interested in hearing about the biochemistry of iron-transporting ligand-gated porins at the upcoming Neisseria98, so I've prepared an abstract.

Regards, PEK

Structural, Functional, and Evolutionary Relationships among Ligand-Gated Porins: Implications on Iron Transport by Pathogenic Neisseria. Phillip E. Klebba, University of Oklahoma, Norman OK, 73019.

In the cell envelopes of Gram-negative bacteria specialized outer membrane
(OM) proteins transport metals that are essential to procaryotic metabolism. Most such systems are dedicated to iron acquisition, which is an important determinant of bacterial pathogenesis. In several ways these metal transporters differ from the general and specific porins that create open channels for diffusion of small molecules into the cell. First, they avidly bind the molecules that they internalize within loops on the cell surface: the dissociation constants of these binding interactions range from 10-6 to 10-9 M. Next, although OM metal transporters are porins in that they contain aqueous transmembrane channels presumably formed by a $-barrel, their pores are normally closed by the ligand-binding loops.

After ligand binding, the uptake reactions that transfer molecules into the cell apparently open the closed channels, ergo the designation "ligand-gated porins." Finally, these transporting actions require energy and the participation of another cell envelope protein, TonB. These requirements are enigmatic, because the uptake occurs across the OM, while evidence suggests that TonB is anchored in the inner membrane.

Although many bacteria elaborate siderophores to scavenge iron in the environment, the pathogenic Neisseria do not, and instead obtain iron from host proteins like lactoferrin and transferrin. In spite of this fundamental mechanistic discrepancy, Neisserial iron transporters apparently also fall into the family of ligand-gated porins: they manifest significant sequence homologies and require energy and the actions of TonB to internalize the iron that they appropriate from host proteins. These differences and similarities will be analyzed in light of the new structural and functional information that has emerged about the ligand-gated porins of Escherichia coli, especially regarding the evolutionary adaptations in the Neisserial proteins that allowed the abandonment of siderophore biosynthetic systems.

Phillip E. Klebba, Ph. D Tel: 405-325-4969
Assoc. Prof. Chem. & Biochem. Fax: 405-325-6111
University of Oklahoma
Norman, OK 73019

Objet: Giardina, Apicella
Date: Fri, 15 May 1998 18:18:39 -0500 (CDT)
De: "Peter C. Giardina, Ph.D." <pgiardin@blue.weeg.uiowa.edu>
A: oral.neisseria@necker.fr

Neisseria-encoded porins influence actin dynamics, in vitro.

Peter C. Giardina1, Kuo-Kuang Wen2, Richard Williams3, David Lubaroff3, Milan S. Blake4, Peter A. Rubenstein2 and Michael A. Apicella1

1Department of Microbiology, 2Department of Biochemistry, and 3Department of Urology, University of Iowa, Iowa City, IA 52242 and 4North American Vaccine, Beltsville, MA 20705-4223

Reports from our lab and others, have described Neisseria-induced host cell cytoskeletal reorganization in primary epithelial cells and in immortalized cell lines. Transient focal actin recruitment is accompanied by microvilli extension from the surface of the infected cell which engage the adherent bacteria. Purified Neisseria-encoded porins alter neutrophil actin dynamics, similar to the affects of E. coli porin on renal tubular epithelium. To study this porin-actin interaction in more detail in vitro, we have employed biochemical techniques to analyze these dynamics. Actin co-polymerization light scattering analysis showed that both gonococcal porin and meningococcal PorA and PorB enhance actin polymerization by nucleating the polymerization process. Native polyacrylamide gel electrophoresis showed that a G-actin/porin complex formed under low ionic strength conditions which may serve as the actin nucleation seed for polymerization. Experiments using fluorescently labeled actin monomers and vaccine quality porin preparations suggested that porin induced the formation of oligomeric actin. This observation supports the hypothesis that porin and actin are forming a discrete complex, thus overcoming the unfavorable kinetics of actin nucleation. Furthermore, kinetic analysis using labeled actin suggests that porin and actin binding is saturable at a ratio of approximately 2 actin monomers to 1 porin monomer. Transmission electron microscopy analysis of co-polymerization experiments showed that porin alters actin filament structure. SDS-PAGE analysis of low speed

co-sedimentation pellets and supernatants confirmed that porin bound actin filaments. Previous investigators have demonstrated that Neisseria porins translocate into eukaryotic membranes during invasion. Our results suggest that these translocated Neisseria-encoded porins are involved in host cell actin reorganization during infection.

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http://www.geocities.com/CapeCanaveral/Lab/1690/
****

Objet: Koomey - van Putten
Date: Fri, 15 May 1998 20:46:51 -0400
De: Mike Koomey <mkoomey@umich.edu>
A: oral.neisseria@necker.fr

Insights into gonococcal pilus biogenesis and pilus -associated phenotypes revealed by studies of pilT, a twitching motility gene

Michael Koomey (1), Matthew Wolfgang (1) and Jos van Putten (2)

(1) Dept. of Microbiology and Immunology, Univ. of Michigan Medical School, Ann Arbor, Michigan 48109; (2) Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, NIH, Hamilton, Montana 59840

We have studied gonococcal pilus biogenesis in order to gain insights into the molecular basis for pilus-associated phenotypes (competence, adherence, twitching motility). Using genetically defined mutants, correlations have been established between pilus expression and these properties but the basis for these associations remains unclear. The precise functions served by most the biogenesis factors are also unknown.

PilT is a member of a family of cytoplasmic NTP-binding proteins engaged in membrane trafficking of macromolecules which is required for twitching motility and DNA uptake during transformation. It is however dispensable for the expression of morphologically and biochemically normal pili. We found that mutants lacking both PilT and particular biogenesis components have unique phenotypes absent in strains carrying only single mutations. For example, the absolute biogenesis defect in pilC mutants can be completely suppressed by loss of function mutations in pilT. Other combinations of pilT and biogenesis gene mutations have unique properties which have made it possible to 1) identify at least two discreet steps in biogenesis (assembly and extrusion), 2) assign the step at which biogenesis factors act and 3) propose a highly testable hypothesis as to how PilT influences the biogenesis pathway and functions in twitching motility and other pilus-associated phenotypes.

Objet: Tønjum
Date: Fri, 15 May 1998 21:16:11 -0400
De: Tone Tonjum <tone.tonjum@rh.uio.no>
A: oral.neisseria@necker.fr

Structure and function of a meningococcal secretin required for pilus

biogenesis

T. Tønjum

Institute of Microbiology, National Hospital, Oslo, Norway

Secretins are a large family of proteins associated with membrane translocation of macromolecular complexes and a subset of this family, termed PilQ proteins, are required for type IV pilus biogenesis. Meningococcal PilQ is unique among secretins because of its abundance in the outer membrane and its N-terminally located polymorphic region containing repetitive elements. To address the potential function of the polymorphic region, Mc strains were constructed so as to express chimeric PilQ molecules in which this domain was replaced by the corresponding but unrelated region of the Pseudomonas aeruginosa (Pa) PilQ protein. The findings suggest that the polymorphic region of PilQ influences Mc pilus expression quantitatively, but not qualitatively. The permissiveness for structural alterations in this region while retaining function is distinct from other secretins.

The new genetic techniques developed in the hybrid studies have allowed us to construct more defined mutants to further address the roles of the domains within PilQ. In particular, studies focusing on multimerization of the complex and its membrane localization are being pursued. This work is critical to understanding how PilQ functions in pilus biogenesis and what other components it might interact with during the process.

Objet: Wolfgang - Koomey
Date: Fri, 15 May 1998 22:15:28 -0400 (EDT)
De: Matthew C Wolfgang <matwolfy@umich.edu>
A: oral.neisseria@necker.fr

Identification of gonococcal prepilin-like molecules that influence type IV pilus biogenesis and pilus-associated phenotypes

Matthew Wolfgang(1), Steve Dunham(1), Jos van Putten(2) and Mike Koomey(1)

(1) Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, 48103
(2) Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT 59840

Type IV pili (Tfp) are filamentous appendages composed primarily of a single subunit termed pilin. Prepilin-like molecules have a highly conserved amino-terminus, and are processed at a specific cleavage site to produce mature pilin. We have identified two gonococcal prepilin-like molecule, designated ComP and PilH, based on their high degree of identity to the amino-terminus of pilin and the presence of conserved prepilin cleavage sites. comP mutants express fully functional Tfp but are deficient in natural transformation, a pilus associated phenotype. The defect in comP mutants is at the level of DNA uptake into a DNAse-resistant state, identical to the defect in non-piliated mutants. This represents the first class of mutants which are incapable of DNA uptake but show no alteration in other Tfp associated phenotypes. pilH mutants show reduced levels of piliation but retain pilus-associated phenotypes. Current data indicate that PilH is required for efficient pilus assembly, potentially by acting as a pilin chaperone. Based on their relatedness to pilin, it is likely that these molecules are capable of forming polymeric structures. As such, one or both may be minor components of the pilus filament.

Objet: abstract
Date: Mon, 18 May 1998 09:31:43 +0100
De: Rachel Urwin <rachel.urwin@zoology.oxford.ac.uk>
A: oral.neisseria@necker.fr

Please find attached an abstract submitted for consideration for an oral presentation at Neisseria98. I would be grateful if you would consider the abstract despite the fact that it is now late. I did send it last week, but I received a message from Xavier Nassif this morning informing me that it had been sent to the wrong address.

Many thanks
Rachel Urwin

MOLECULAR EVOLUTIONARY ANALYSIS OF NEISSERIA MENINGITIDIS

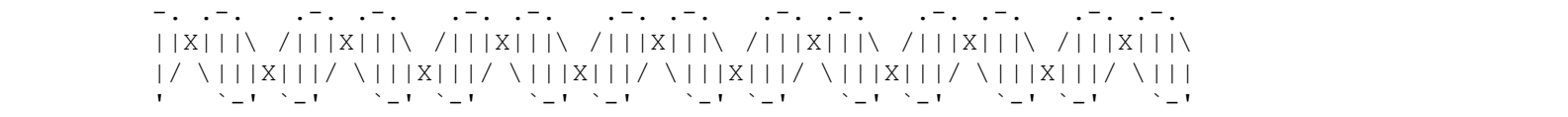
R. Urwin, E. C. Holmes, M. C. J. Maiden.

The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS. UK.

Although the bacterium Neisseria meningitidis causes serious disease worldwide, the organism frequently exists in a harmless, commensal relationship with the human host. It is therefore possible that colonisation by multiple strains presents the opportunity for these naturally transformable organisms to exchange genetic material by recombination, thereby producing strains with different phenotypic properties, including virulence.

To identify the evolutionary mechanisms that determine the population structure of N. meningitidis, nucleotide sequences from multiple loci were obtained from diverse invasive and non-invasive meningococcal strains. Included in the analysis were conserved 'housekeeping' genes previously used for MLEE typing of meningococci and also for Multi Locus Sequence Typing of N. meningitidis, and the porB gene, which encodes a major surface antigen and epidemiological marker. Split decomposition analyses of these sequences showed that relationships between alleles comprise a network of interconnected branches rather than a tree-like phylogeny. Calculation of the ratio of synonymous and nonsynonymous substitutions support the hypothesis that there is directional selection for amino acid change in the surface exposed regions of PorB, but in the housekeeping genes, most amino acid substitutions are removed by purifying selection.

These data suggest that recombination is abundant and shapes meningococcal
population structure at both highly conserved and variable loci. It is proposed that additional variation identified in surface antigens is affected by the duration of carriage of different meningococcal strains: in particular the rapid evolutionary change associated with serogroup B meningococci is the result of long carriage rates and prolonged exposure to the host immune response.



rachel.urwin@zoology.oxford.ac.uk

Objet: de Vries, van Putten (Oral presentation neisseria 98)
Date: Tue, 19 May 1998 13:44:25 +0200
De: fp de Vries <fpdevries@wxs.nl>
A: oral.neisseria@necker.fr

Human neutrophil defensins promote internalization of pathogenic neisseria by epithelial cells, regardless of opacity protein expression or HSPG host cell receptors

Frits P. de Vries, Jacob Dankert and Jos P.M. van Putten.

Dep. of Med. Microbiology
University of Amsterdam/AMC
15 Meibergdreef 1005 AZ Amsterdam
The Netherlands

PMN granules contain a variety of microbicidal proteins and proteases, that can be released onto mucosal surfaces. We noticed that small amounts of unfractionated PMN granule extracts abolished Opa/Opc dependent interactions of meningococci with epithelial cells, by destroying HSPG receptors. Considering the large number of known pathogens, that can exploit HSPG receptors, this may be a rationale for the extracellular protease release by stimulated PMN's.

Increasing amounts of granular extract, stimulated the interaction between epithelial cells and otherwise non-adherent, non-invasive meningococci, that lacked pili, Opc and/or Opa. The active components in the extracts were identified as defensins (HNP1-3). Capsule, but not LPS sialylation blocked HNP mediated interactions. Several gonococcal strains tested, showed strongly increased invasion in the presence of purified HNP1-3 (2-10 ug/ml). No increase was observed with commensal neisseria, or several other tested bacteria. Reduced, alkylated defensins lost their infection-stimulating activity.

Cytochalasin prevented defensin stimulated invasion. Phalloidin-FITC staining showed typical actin recruitments at sites of bacterial entry. Similar events were observed for Opc positive meningococci and Opa expressing gonococci, which utilize host cell proteoglycans as receptors. Heparitinase or trypsin treatment of host cells had no effect on defensin mediated uptake, while Opa/Opc mediated entry was effectively blocked. The PC-PLC inhibitor D609 had no effect on defensin mediated uptake in Chang cells, while effective against Opc meningococci. Thus, defensins appear to stimulate neisserial interaction with epithelial cells via an actin-dependent mechanism that involves a separate class of protease resistant receptors and a different signaling pathway than the known adhesins Opc and Opa.

Objet: Mueller-Rudel
Date: Tue, 19 May 1998 18:05:59 +0100
De: Anne Mueller <mueller_a@mpiib-berlin.mpg.de>
A: oral.neisseria@necker.fr

Neisserial Porin (PorB) causes rapid Calcium-Influx and induces Apoptosis in Target Cells by the Activation of Cysteine Proteases

Anne Mueller*, Dirk Guenther, Frank Duex, Michael Naumann, Thomas F. Meyer and Thomas Rudel

Max-Planck-Insitut fuer Infektionsbiologie, Abteilung Molekulare Biologie,
Monbijoustr. 2, 10437 Berlin, Germany

The porin (PorB) of Neisseria gonorrhoeae is a fascinating bacterial factor because of its ability to translocate from the outer bacterial membrane into host cell membranes, where it modulates such essential infection processes as invasion, phagosome maturation and oxidative burst. Here we report that pathogenic Neisseria strains induce programmed cell death after prolonged infection of epithelial cells. The underlying mechanism we propose includes translocation of the porin, transient increase in cytosolic Ca2+ and subsequent activation of the Ca2+ dependent protease calpain as well as proteases of the caspase family. The activity of these enzymes was monitored as cleavage of the specific substrates poly (ADP-ribose) polymerase and a-fodrin. Peptide inhibitors of the proteases prevent substrate breakdown as well as the biochemical and morphological features typical of apoptosis, i.e. membrane blebbing, phosphatidylserine exposure, chromatin condensation and segmentation of the nucleus. Our data provide hints that the neisserial porin, which shares structural and functional homologies with the mitochondrial voltage-dependent anion channels (VDAC) especially in its ATP binding properties, represents an ancient form of the permeability transition pore, the putative central regulator of apoptosis. Therefore we propose that on the basis of the serial endosymbiont theory very early intracellular bacteria not only brought aerobic metabolism to the primitive eucaryotic cell but also the basic machinery for the evolution of apoptosis.

Objet: Campagne-Chippaux
Date: Thu, 21 May 1998 08:11:53 +0200
De: Jean-Phillippe Chippaux <chippaux@niamey.orstom.ne>
A: oral.neisseria@necker.fr

Impact of the preventive vaccination on the control of meningitis epidemics.

Campagne G., Djibo S. and Chippaux J.-P.
CERMES, B.P. 10887, Niamey, NIGER

Face to the meningitis epidemics, the WHO control strategy in meningitis belt is based on the early detection of cases and consists in insure the treatment of cases and the mass vaccination of the population targets. Epidemics that occurred these last four years have shown the limit of such a strategy.
We have analyzed bacteriological data of the CERMES that receives all cerebrospinal fluids (CSF) of the Hospital of Niamey, from 1981 to 1996. Information on anti-meningococcal vaccinations in Niamey have been gathered for the period 1978-1996.
The incidence of the meningitis A increased in 1984 to 1986, then after a progressive reduction until 1993-94, strongly increased during the 1994-95 epidemic. The vaccine coverage was very irregular. High before 1988, it gradually decreased between 1988 and 1994. Thus, it is possible to oppose the weak impact in Niamey of the 1984-86 epidemics (< 200 cases per 100 000) to the high vaccine coverage during the preceding years (about 50%) and, reciprocally, the dramatic epidemic explosion in 1994-95 (about 350 cases per 100 000) while the vaccine coverage was less than 10% during the preceding years).
The appearance of epidemics at Niamey seemed linked to previous vaccination campaigns and vaccine coverage rates. Waiting the conjugate vaccine whose immunological superiority no longer makes doubt, the regular utilization of the polysaccharidic vaccine in the course of preventive vaccination campaigns would reduce significantly the number of deceased by meningitis in

the meningitis belt.

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Jean-Philippe Chippaux
OCCGE - CERMES
BP 10887
Niamey - Niger
Tél. (227)75 20 45
Fax (227)75 31 80

Objet: Popovic - Perkins
Date: Fri, 22 May 1998 12:07:15 +0200
De: neisseria98@necker.fr (Neisseria98)
A: oral.neisseria@necker.fr

>From: "Popovic, Tanja" <txp1@cdc.gov>
>To: neisseria98@ceylan.necker.fr
>Cc: "Popovic, Tanja" <txp1@cdc.gov>
>Subject: Popovic - Perkins
>Date: Fri, 15 May 1998 15:21:00 -0400
>Importance: high
>X-Priority: 1
>MIME-Version: 1.0
>
>Dear Sir/Madam:
>
>Here is the abstract to be considered for the oral presentation at the
>11th International Pathogenic Neisseria Conference to be held in Nice,
>November 1-6, 1998. Presenting author: Tanja Popovic.
>
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>Popovic, T., Ajello, G.W., Reeves, M.W., Reiss, J.A., Kim, C., Tondella,
>M.L.C., Schmink, S.E., Mayer, L.W., Rosenstein, N.E., and Perkins, B.A.
>
>Analysis of outbreak-associated and sporadic _Neisseria meningitidis_
>serogroup C isolates by five molecular subtyping methods
>
>Centers for Disease Control and Prevention, Atlanta, GA, USA
>
>The number of serogroup C meningococcal disease (SCMD) outbreaks in the
>U.S. has increased since 1990, while the endemic rate of meningococcal
>disease has remained stable at 1-1.5/100,000; SCMD outbreaks are usually
>caused by strains identified as closely related by subtyping methods,
>such as multilocus enzyme electrophoresis (MEE). However, MEE cannot
>always distinguish outbreak-associated strains from those causing
>sporadic disease. In the fall of 1993, an outbreak of SCMD occurred in
>Grayson County, Texas: 14 county residents aged 2-29 years were
>affected. All outbreak-associated isolates, as well as the background
>endemic strains, were of ET24 or of a few ETs closely related to ET24.
>In this study we assayed twelve of the outbreak-associated isolates, ten
>epidemiologically unrelated _Neisseria meningitidis_ serogroup C (SC)
>strains from Texas (1994-1996), and six sporadic SC strains from
>throughout the U.S. by five subtyping methods: MEE, pulsed field gel
>electrophoresis (_Nhe_I), ribotyping (_Cla_I, _Sma_I, _Xho_I), random
>amplified polymorphic DNA assay (6 primers), and serosubtyping. SC
>strains from throughout the U.S. differed from each other and from the

>Texas strains. Outbreak-associated and sporadic Texas isolates were not
>readily distinguishable, suggesting that in some instances
>differentiation of outbreak-associated and sporadic strains circulating
>in the same geographic area may not be possible with the currently
>available subtyping methods. A study to evaluate the usefulness of
>variable-number, tandem-repeats size-typing and DNA sequence based
>typing in outbreaks like this one is underway. The development of
>outbreaks may depend on as yet unidentified microbiological factors as
>well as epidemiological risk factors.
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>---
>Sincerely yours,
>
>Tanja Popovic, M.D., Ph.D.
>Chief, Bacterial Meningitis Laboratory
>Meningitis and Special Pathogens Branch
>Division of Bacterial and Mycotic Diseases
>National Center for Infectious Diseases
>Centers for Disease Control and Prevention
>MS CO2, Building 5, Room 346
>1600 Clifton Road
>Atlanta, GA 30333
>Phone: 404 639-1730
>Fax: 404 639-3123
>
>
>
> ----------
>From: neisseria98@necker.fr
>To: neisseria-part@infobiogen.fr
>Subject: Neisseria meeting - Abstract submission
>Date: Saturday, May 09, 1998 2:42AM
>
>The organizing committee of the next Neisseria meeting to be held in
>Nice
>in November 1998 thanks everyone who has submitted an abstract and would
>like to recall that the deadline for submission of an abstract, if you
>wish
>to be considered for an oral presentation, is Friday May 15 1998.
>
>A summary of the work to be presented should be sent by e-mail to the
>following address :
>   oral.neisseria@necker.fr
>
>The e-mail should be presented as follows
>   subjectfield of e-mail last names of the first author - last
>name
>of the last author
>The abstract should be sent as a plain body not as an attached file with
>the following
>   The title
>   The authors (underline the presenting authors' name)
>   The authors' address
>   The text (not to exceed 200 words)
>
>All information regarding the meeting is available on either of the two
>web

>sites.
>
>http:// ceylan.necker.fr/neisseria/welcome-uk.html
>http:// pasteur.fr/Conf/Neisseria98/welcome-uk.html
>
>
>We are looking forward to seeing you and to a very exciting meeting.
>
>The Organizing Committee

Objet: Meninge B vaccine - B. Danve - M.J. Quentin Millet
Date: Tue, 26 May 1998 16:40:00 +0200
De: "Lespinasse, Chantal - MLE" <CLespinasse@fr.pmc-vacc.com>
A: "Nassif X." <oral.neisseria@necker.fr>

Safety and immunogenicity of a Neisseria meningitidis group B transferrin binding protein vaccine in adults

B. Danve1, M. Cadoz1, L. Lissolo1, E. Boutry1, F. Guinet1, D. Speck1, X. Nassif2 and
M.-J. Quentin-Millet1

1 - Pasteur Mérieux Connaught, Lyon, France;2 - Hôpital Necker, Paris, France

Iron plays a vital role in bacterial metabolism; it is crucial for in vivo multiplication and also implied in the regulation of expression of several genes involved in metabolism and virulence. Many iron regulated proteins have been identified for Neisseira meningitidis including receptors specific for lactoferrin, hemoglobin and transferrin. The transferrin binding protein (Tbp) is a surface exposed lipoprotein made of two subunits TbpA and TbpB produced in small quantities by Neisseria meningitidis grown under iron restricted conditions. We have shown that Tbp complex and TbpB subunit elicited high bactericidal titers in laboratory animals and protected them against a lethal challenge with an homologous strain. These results suggest that TbpB could be used as a vaccine candidate. The gene of TbpB was cloned and the recombinant lipoprotein purified from E. coli showed the same biological properties in animals as the native protein. To further validate this approach, GMP material was prepared and a phase I study was conducted in France on eleven 18 to 40 years old volunteers. They received a 25 µg dose of a recombinant TbpB B16B6 vaccine by the intramuscular route on days 0, 28 and 180. Blood samples were taken on days 0, 28, 56, 180 and 208 and analyzed for specific antibody response by Elisa and for bactericidal and opsonophagocytic activities. Preliminary results showed that the vaccine was safe and immunogenic. The complete serological analyses are in progress and the results will be presented.

Objet: Ayala-So
Date: Wed, 27 May 1998 12:48:57 -0700
De: Magdalene So <somaggie@ohsu.edu>
A: oral.neisseria@necker.fr

File: Ayala-So
Abstract Title: Alteration of Lysosomes by the Pathogenic Neisseriae
Patricia Ayala, Sylvia Hopper, Lan Lin and Magdalene So
Dept. of Molecular Microbiology and Immunology, L220
Oregon Health Sciences University

3181 SW Sam Jackson Park Rd
Portland, OR 97201-3098

We are interested in understanding how the pathogenic Neisseriae avoid lysosome killing within cells. Our studies indicate that the IgA1 protease plays a major role in the ability of meningococci and gonococci to survive within epithelial cells, as iga- mutants are defective in intracellular replication. The IgA1 protease is also responsible for altering the lysosomes of infected cells. The protease cleaves LAMP1, a major lysosomal glycoprotein, thereby accelerating its degradation and reducing its steady state levels in infected cells. LAMP1 is an integral membrane protein which has been hypothesized to play a role in protecting the lysosomal membrane from the hydrolytic activity of its resident enzymes. If so, then a reduction in LAMP1 levels in a cell is likely to result in changes to the lysosomes. Consistent with this hypothesis are our observations that the levels of other lysosomal markers are also reduced in infected cultures. These markers are not the direct targets of the IgA1 protease, nevertheless, their levels are indirectly affected by the enzyme. Thus, IgA1 protease-mediated LAMP1 degradation leads to multiple changes in lysosomes and these changes are likely to directly promote Neisseria intracellular survival.

Objet: Competitive speakers selection
Date: Fri, 29 May 1998 13:44:04 +0200
De: "Thomas F. Meyer" <thomas.meyer@tuebingen.mpg.de>
Répondre à: sekretariat.infbio@tuebingen.mpg.de
Société: MPI-Biologie Abt. Infektionsbiologie
A: oral.neisseria@necker.fr

Dear Xavier, thank you for your kind E-mail reply. Following is an abstract by Thomas Schwan to be considered for the competitive speakers selection. Best regards, Thomas

Identification and characterisation of genomic loci of both pathogenic Neisseria species mediating independent recognition of glycolipids and binding to human epithelial cells.

E. THOMAS SCHWAN, Sandra Eickernjäger, Eckhard Fischer, Jürgen Maier, Paul A. Manning, Thomas Rudel, Ina Scheuerpflug, and Thomas F. Meyer

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Adherence of the pathogenic Neisseria species, and the elaboration of exquisitely tuned tissue tropisms, have evolved by the development of an intricate molecular interplay between both neisserial surface structures and host factors. Molecular dissection of these processes will lead to better understanding of the onset of disease and possibly highlight directions of pharmacologic intervention.
We have cloned novel genomic loci of both pathogenic Neisseria species conferring tissue and species specific adherence to an otherwise non-adherent E. coli strain. Experiments focussing on the nature of cellular host receptors identified certain glycolipids known to be recognized by gonococci. N. gonorrhoeae has been shown to bind to a subset of lactose-containing glycolipids present on human epithelial cells (Stromberg et al., 1988; Paruchuri et al., 1990). Separation of the original genomic clone gave rise to different subclones expressing adherence either to epithelial cells in tissue culture or to specific glycolipids in TLC overlay assays. Each binding activity could be assigned to individual open reading frames.

Thus, we have identified two new families of neisserial adhesins located in close vicinity on both genomes. One gene family confers binding to epithelial cells by interaction with a currently unknown host receptor. The second group of adhesins specifically targets host-derived glycolipids.

Objet:
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>
>Monsieur,
>je vous remercie de bien vouloir considéré cette abstract pour une
>présentation
>orale à la conférence sur les Neisseria qui se déroulera à Nice en Novembre 98.
>
>Francois Belanger
>
>TITLE : Weekly incidence thresholds for the detection of meningitis
>epidemics in
>Northern Togo.
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>Keywords: Meningitis epidemic, threshold, sub-Saharan Africa
>
>
>Introduction :
>Strategies for the control of meningitis epidemics in Africa are based upon
>early reactive mass vaccination. The weekly incidence (WI) threshold of
>15
>cases/100,000 inhabitants (averaged over 2 weeks) has been widely used to
>detect
>meningitis outbreaks. Different thresholds are studied to assess their
>usefulness
>in epidemic control in Northern Togo.
>
>Population and methods :
>Meningitis cases recorded from 1990 to 1996 in Northern Togo (500,000
>inhabitants) were reviewed. WIs were calculated for different area.
>Sensitivity,
>specificity, and positive and negative predictive values of different
>thresholds
>were assessed to estimate their
>ability to detect a subsequent outbreak. The number of potentially prevented
>cases was calculated.
>
>Results :
>Outbreaks occurred in 1 district in 1995-96 and in all 4 districts in 1996-97.
>At the district level, a WI of 7 cases/100,000 inhabitants during 1 week was

>an excellent predictor of an outbreak. At the sub-district level, WI was a poor
>predictor, but a weekly absolute number of new cases exceeding 7 had a high PPV
>(72%). Using the 7/100,000 threshold at district level instead of 15/100,000
>averaged on 2 weeks increased the time elapsed between threshold and peak
>by 2.4
>weeks. Combining information at
>district and sub-district level increased the time available for
>intervention by
>4 weeks.Varying the threshold used to initiate a vaccination campaign, between
>62% and 67% of the cases could have been prevented.
>
>Discussion :
>>From 1990 to 1996 in Northern Togo, an incidence of 7 cases/100,000
>inhabitants/week was an excellent predictor of meningitis epidemics and,
>combined
>with an early vaccination campaign, might have prevented 67% of cases. Similar
>studies should be undertaken in other countries of the African meningitis
>belt.
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