Old Herborn University Seminar Monograph 17:
Possibilities for active and passive vaccination against opportunistic infections.
Editors: Peter J. Heidt, Richard I. Walker, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 181-200 (2004).

NEISSERIA MENINGITIDIS, *NEISSERIA LACTAMICA* AND *MORAXELLA CATARRHALIS* SHARE CROSS-REACTIVE CARBOHYDRATE ANTIGENS*

JAN M. BRAUN[1,2], JOSEF BEUTH[1], C. CAROLINE BLACKWELL[3], SONJA GIERSEN[2], PAUL G. HIGGINS[4], GEORGINA TZANAKAKI[5], HEIKE UNVERHAU[1], and DONALD M. WEIR[6]

[1]Institute for Scientific Evaluation of Naturopathy, University of Cologne, Germany; [2]Meningitis-Vaccine, Cologne, Germany; [3]Hunter Immunology Laboratories, Newcastle, Australia; [4]Institute of Medical Microbiology, Immunology and Hygiene, University of Cologne, Germany; [5]National Meningitis Reference Laboratory, National School of Public Health, Athens, Greece; [6]University of Edinburgh Medical School, Edinburgh, Scotland

SUMMARY

Carriage of commensal bacteria species is associated with the development of natural immunity to meningococcal disease, with lipo-oligosaccharides (LOS) of meningococci being one of the main virulence factors associated with severity of meningococcal disease. Meningococcal reference strains and isolates from the commensal species *Neisseria lactamica* and *Moraxella catarrhalis* were assessed for the presence of cross-reactive glycoconjugate antigens. Binding of human blood group antibodies of the P and Ii system to meningococcal immunotype reference strains were in accordance with the presence of known LOS carbohydrate structures. Binding studies with meningococcal immunotyping antibodies and blood group phenotyping antibodies to *N. lactamica* strains from different European countries showed, that a greater number of isolates obtained from native Greek and Scottish adults and children bound anti-meningococcal L(3,7,9) immunotyping ($p<0.001$), pK ($p=0.035$) and paragloboside ($p<0.001$) blood group typing antibodies compared to isolates obtained from children of Russian immigrants in Greece. A greater number of *M. catarrhalis* strains isolated from children in Scotland bound anti-L(3,7,9) antibodies (38.2%) compared to strains isolated from adults (22.2%) ($p=0.017$). These findings provide evidence that blood group like glycoconjugate antigens found on the commensal species *Neisseria lactamica* and *Moraxella catarrhalis* might be involved in the development of natural immunity to meningococcal endotoxins during childhood, and might be exploited as anti-meningococcal vaccine candidates.

---

*: Reprinted with permission from: Vaccine 22, 898-908 (2004). All references should be made to the original article.

INTRODUCTION

Evidence of common oligosaccharide structures for meningococci and *N. lactamica* (NL) or meningococci and *Moraxella catarrhalis* (MC) has been reported to share oligosaccharide antigens with some carbohydrate structures found on human tissues including paragloboside, P, P1, p^K, and Ii blood group antigens (*Mandrell* et al., 1988). The expression of blood group related LOS on meningococcal carrier strains and outbreak strains differs greatly. While disease is mainly associated with meningococcal immunotype L(3,7,9) showing homology with the paragloboside antigen (a precursor of the human nP1 blood group antigen), carrier strains isolated in Britain were found to express LOS immunotypes similar to the p^K and ceramide-dihexocide blood group antigens L1 and L8, respectively (*Jones* et al., 1992). There has been no systematic screening of commensal NL or MC isolates from different regions of Europe with the immunotype antibodies used to classify NM immunotypes or antibodies to human blood group antigens.

Thirteen major LOS types were identified for *N. meningitidis* using polyclonal and monoclonal antibodies by passive haemagglutination inhibition techniques and whole cell ELISA (*Abdillahi* and *Poolman*, 1988; *Scholten* et al., 1994). The majority of meningococcal isolates express one or more of the immunotypes L1-L12, while nontypable and L13 immunotypes are rare. The twelve major LOS types have a relative molecular weight ranging from 3.15 to 7.1 kDa. The oligosaccharide chain, also referred to as the α-chain or variable LOS region one (R1), is composed of the saccharides glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc). Sialylated forms contain the terminal saccharide *N*-acetylneuramic acid (NeuNAc) is added to terminal galactose residues by endogenous or exogenous sialyl transferases. Abbreviations for core moieties are used as follows: glycero-D-manno-heptopyranoside (Hep or heptose); phospho-ethanolamine (PEA); 2-keto-3-deoxyoctulosonic (KDO). The complete structures of immunotypes L10 – L13 are not elucidated, but there is evidence that L10 contains the paragloboside residue and L11 shows some homology with L1. The PEA residue of immunotype L2 can be expressed in two forms that undergo phase variation: The PEA on the G3 region can be linked in (1→6) or (1→7) conformation. The PEA can be replaced by a hydrogen (H) atom. The PEA residue of immunotypes L4 and L6 express both PEA (1→6) and (1→7) linkages. The expression of meningococcal immunotypes is associated with serogroups. Immunotypes L8, L9, L10, L11 and L12 are found on group A strains, while serogroup B and C meningococci express immunotypes L1 – L8 (*Scholten* et al., 1994; *Dell* et al., 1990; *Gu* et al., 1992; *Jennings* et al., 1983; *Kim* et al., 1994; *Kogan* et al., 1997; *Michon* et al., 1990; *Pavliak* et al., 1993; *Plested* et al., 1999; *Rahman* et al., 1998a; *Wakarchuk* et al., 1998)

Immunotypes and pathogenicity

LOS immunotype expression is thought to be linked to the pathogenicity of the organism. Immunotypes L(3,7,9) are isolated predominantly from patients with invasive meningococcal disease. Other immunotypes are found predominantly among carrier strains. Immunotypes L3, L7 and L9 are thought to be similar in their immunochemical structures with immunotype L3 being sialylated by endogenous sialyl transferases. Immunotypes L3 and L7 are found on

serogroup B and C meningococci and they have similar G2 core components, PEA (1→3) HepII. Immunotype L9 is expressed on group A strains (*Jones* et al., 1992; *Plested* et al., 1999).

The presence of the sialylated phenotype on invasive meningococci is associated with resistance to complement-mediated killing by masking the terminal galactose with NeuNAc. This mechanism is thought to reduce the recognition of the epitope by anti-LOS antibodies directed against the non-sialylated epitopes. Free or membrane bound sialyl-L(3,7,9) also upregulates neutrophil activation markers and results in increased injury of epithelial cell lines. Sialyl L(3,7,9) phenotypes can evade the complement mediated bacteriolysis cascade and reduces complement and anti-LOS antibody mediated phagocytosis by professional phagocytes (*Mandrell* et al., 1991,1993; *Mandrell* and *Apicella*, 1993; *Hammerschmidt* et al., 1994; *McLeod Griffiss* et al., 2000).

Expression of major and minor immunotypes by *N. meningitidis*

Meningococci are able to express more than one immunotype. Isolates from patients with meningococcal disease in the Netherlands (1989-1990) showed different immunotype combinations (*Scholten* et al., 1994).

1. Group A meningococci L9 (54%), L9,8 (8%), L10 (24%), L10,11 (8%) and non-typable (NT) (8%).
2. Group B meningococci L1 (1%), L1,8 (11%), L2 (10%), L3,7 (36%), L3,7,1 (4%), L3,7,1,8 (2%), L3,7,8 (28%), L4 (4%), and L8 (5%).
3. Group C meningococci L1,8 (2%), L2 (30%), L3,7 (37%), L3,7,1 (1%), L3,7,1,8 (3%), L3,7,8 (7%), L4 (15%), L8 (3%), and NT (3%).

The expression of multiple immunotypes within a meningococcal population is thought to allow the organism to diversify its antigenic structure, evading selective pressure of the host's immune system. Sialylation and the expression of paragloboside gene cluster IgtABE are the main phase variable phenotypes known (*Jennings* et al., 1999).

The expression of meningococcal immunotypes undergoes phase variation due to *in vitro* growth conditions. The variability of meningococcal phenotypes and LOS expression depends on the growth rate and phase, as well as the presence of exogenous sialyl transferases (*Berrington* et al., 2002).

LOS based vaccines

The most common LOS immunotype associated with disease is L(3,7,9) found in both group B and C outbreak strains of meningococci in Europe and America, and group A in sub-Sahara-Africa (*Varaine* et al., 1997; *Booy* and *Kroll*, 1998; *Fonkoua*, 2002). Meningococcal LOS is closely associated with the severity and fatality of disease. This is mainly due to its involvement in inducing large amounts of pro-inflammatory cytokines in a CD14 dependent mechanism. Anti-meningococcal LOS antibodies are not only bactericidal, but also opsonising in nature, resulting in the phagocytosis of invading bacteria and LOS containing blebs by human monocytes. Normal human serum of adults usually contains antibodies against meningococcal LOS, suggesting its important role in development of natural immunity to meningococcal disease (*Braun* et al., 2002). Several anti-meningococcal LOS vaccine candidates are currently under investigation (*Katial* et al., 2002; *Saunders* et al., 1999).

P-related blood group system

Carbohydrate antigens are widely distributed on human blood cells and tissues. Their expression is facilitated through glycosyl-transferases during the post-translational modification of

Table 1: Blood group antigens of the P and Ii system

Blood group	α chain moiety
CDH	Galβ (1→4) Glcβ
P globoside	GalNAcβ (1→3) Galα (1→4) Galβ (1→4) Glcβ
p^k, CD77	Galα (1→4) Galβ (1→4) Glcβ
P1	Galα (1→4) Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ
Paragloboside	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ
i a	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ
i b	*S* - Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ
I a αβ, I b β	Galβ (1→4) GlcNAcβ (1→3; 1→6) Galβ (1→4) GlcNAcβ (1→4) Galβ (1→4) Glcβ
I c α	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→6) GalNAcβ
I c β	Galβ (1→4) GlcNAcβ (1→6) GalNAcβ
I d α,β	Galβ (1→4) GlcNAcβ (1→6) GalNAcβ

CDH, ceramide-di-hexocide; I, adult; i, foetal; S, sialyc acid; the glucose is linked to the membrane anchored ceramide (Glcβ (1→1) Ceramide)

proteins (glycoproteins) or linkage to ceramide (N-linked fatty acyl sphingosine). The ABO blood group antigens can be expressed either as glycoproteins or as ceramide glycolipids. The P-blood group system is thought to be expressed in glycolipid form on red blood cells and other tissues (*Prokop* and *Uhlenbruck*, 1965; *Race* and *Sanger*, 1975; *Hakomori* and *Kannagi*, .1986; *Bailly* et al., 1992; *Brown* et al., 1993) It consists of a single oligosaccharide chain linked to a membrane-anchored ceramide (Table 1). One member of the P-system, the globotriaosylceramide (p^K or CD77) is associated with the differentiation and maturation of human B cells (*Butch* and *Nahm*, 1992) and B-cell Burkitt lymphomas (*Wiels* et al., 1981). While other members of the P-system are readily expressed on human red blood cells, the expression of p^K is relatively rare (*Marcus* et al., 1976). It is thought that oligosaccharides with a terminal galactose residue can be found in sialylated or non-sialylated forms due to the sialyl-transferases found in human serum, an enzyme that is also associated with the sialylation of meningococcal LOS (*Wakarchuk* et al., 1998; *Mandrell* et al., 1991,1993; *Mandrell* and *Apicella*, 1993; *Hammerschmidt* et al., 1994; *McLeod Griffiss* et al., 2000).

Ii-blood group system

Similar to the p^K antigen, Ii determinants are associated with developmental maturation in humans. Although, p^K might be expressed in children and adults, i-antigens are found in foetal tissue but rarely in children or adults. I-antigen expression coincides with the loss of i-blood group moieties (*Marsh* and *Jenkins*, 1960; *Marsh*, 1961; *Wiener*, 1973). The i-determinant has a single chain oligosaccharide structure linked to a membrane anchored ceramide, while the I-blood group antigens consist of a branched structure at the third terminal saccharide. Ia and Ib are glycolipids linked to ceramide, while the carbohydrate antigens Ic and Id form the glycosyl structure of glycoproteins linked to the amino acids serine and threonine.

Structural homology of *N. meningitidis* LOS with blood group antigens

The oligosaccharide moiety of the α-chain of NM LOS shares structural homology with some human blood group antigens (Table 2), and these

Table 2: Oligosaccharide and core structures of meningococcal LOS immunotypes

LOS type	Terminal oligosaccharide α chain oligomer of the G1 region	Core		
		[A]	G2	G3
L1	Galα (1→4) Galβ (1→4) Glcβ	-	PEA (1→3)	H
L2	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	-	Glcα (1→3)	PEA H, (1→6), (1→7)
L3	S (2→3) Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	-	PEA (1→3)	H
L4	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	-	H (→3)	PEA (1→6), (1→7)
L5	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	Glcβ (1→4)	Glcα (1→3)	H
L6	GalNAcβ (1→3) Galα (1→4) Glcβ	-	H(→3)	PEA (1→6), (1→7)
L7	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	-	PEA (1→3)	H
L8	Galβ (1→4) Glcβ	-	PEA (1→3)	H
L9	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	-	PEA (n.e.)	n.e.
L10	Galβ (1→4) GlcNAcβ (1→3) Galβ (1→4) Glcβ	n.e.	PEA (n.e.)	n.e.
L11	Galα (1→4) Galβ (1→4) Glcβ	n.e.	PEA (n.e.)	n.e.
L12	n.e.	n.e.	PEA (n.e.)	n.e.
L13	n.e.	n.e.	n.e.	n.e.

Abbreviations: Gal, galactose; GlcNAc, N-acetylglucosamine; Glc, glucose; S, sialyc acid; Hep, heptose (glycero-D-manno-heptopyranoside); PEA, phospho-ethanolamine; H, hydrogen; [A], Glcβ (1→4) insertion of the α chain; n.e., not elucidated.

Table 3: Primary structure of α and β chains of MC LOS of MC immunotypes A, B and C

LOS type	Chain	Terminal oligosaccharides (variable regions)	Homology with human oligosaccharides	Homology with meningococcal immunotypes
A	α	Galα (1→4) Galβ (1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
	β	GlcNAcα(1→2) Glcβ		
B6	α	Glcα (1→2) Glcβ		
	β	Glcβ		
B7	α	Glcα (1→2) Glcβ		
	β	Glcα (1→2) Glcβ		
B8	α	Glcα (1→2) Glcβ		
	β	Galβ(1→4) Glcα (1→2) Glcβ	Dihexoceramide	L8
B9	α	Galβ(1→4) Glcα (1→2) Glcβ	Dihexoceramide	L8
	β	Galβ(1→4) Glcα (1→2) Glcβ	Dihexoceramide	L8
B10	α	Galα (1→4) Galβ(1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
	β	Galβ(1→4) Glcα (1→2) Glcβ	Dihexoceramide	L8
B11	α	Galα (1→4) Galβ(1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
	β	Galα (1→4) Galβ(1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
C8	α	Glcα (1→2) Glcβ		
	β	Galβ(1→4) GlcNAcα(1→2) Glcβ	Paragloboside	L2, L(3,7,9), L5
C10	α	Galα (1→4) Galβ(1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
	β	Galβ(1→4) GlcNAcα(1→2) Glcβ	Paragloboside	L2, L(3,7,9), L5
C11	α	Galα (1→4) Galβ(1→4) Glcα (1→2) Glcβ	p^K blood group	L1, L11
	β	Galα (1→4) Galβ(1→4) GlcNAcα(1→2) Glcβ	P1 blood group	

Abbreviations: Gal, galactose; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine ; Glc, glucose.

structures have been identified in the LOS of several isolates of NM and *N. gonorrhoea* (*Mandrell* et al., 1988; *Kim* et al., 1989). The G1 region of L1 and L11 meningococcal LOS immunotypes show identical terminal oligosaccharide residues of ceramide trihexocide, Galα (1→4) Galβ (1→4) Glcβ, identical to the human p^k blood group antigen (CD77) (*Griffiss* et al., 1987a,b).

The lacto-N-neotetranose residue, Galβ (1→4) GlcNAcβ (1→3) Galβ, a 4.5 kilodalton LOS component of immunotypes L2, L(3,7,9), L4 and L5 is identical to paragloboside with different distributions in NM, *N. gonorrhoea* and NL (*Kim* et al., 1989). Additionally, paragloboside, a precursor of P1 blood group antigen found in 75% of Caucasians, is the terminal structure of the human I-erythrocyte antigen and the embryonic i-antigen (*Mandrell* et al., 1988; *Hakomori* and *Kannagi*, 1986; *Tsai* and *Civin*, 1991). It is also an epitope homologous to type 14 pneumococcal polysaccharide capsules (*Siddiqui* and *Hakomori*, 1973). Immunotype L6 shares its two terminal sugars, Galβ (1→4) GlcNAcβ, with the P blood group antigen, and L8 shares its terminal disaccharide with ceramide dihexocide, Galβ (1→4) Glcβ, the common precursor of the P blood group system and steroid receptors.

Structural homology of *M. catarrhalis* LOS with blood group antigens

The oligosaccharide moieties of the α-or ß-chains of *M. catarrhalis* LOS shares structural homology with some human blood (Table 3). Serological typing of MC is based upon its LOS. All serotypes of MC have LOS of a similar molecular weight, about 5.5 kDa (*Holme* et al., 1999). This differs greatly from immunotypes found in meningococci and *N. gonorrhoeae, which* have variable sizes of LOS, 4.1-5.0 kDa and 3-5 kDa, respectively (*Schneider* et al., 1984).

There are three major LOS types (A, B, and C) found in approximately 95% of all MC isolates identified in 61%, 29%, and 5% of isolates, respectively (*Rahman* et al., 1997,1998a,b; *Rahman* and *Holme*, 1996; *Vaneechoutte* et al., 1990a,b; *Edebrink* et al., 1994,1995, 1996; *Masoud* et al., 1994). Lipid A is anchored in the outer membrane of the bacterial envelope linked to KDO-I that is linked to KDO-II. A Glcβ (3→1) Glc moiety is linked to (1→5) KDO-I forming the backbone of all known MC immunotypes. The LOS β-chain is linked to the 4^{th} carbon, the α-chain to the 6^{th} carbon of the glucose (1→5) KDO-1 residue. Both chains are variable in length and oligosaccharide composition that determines the MC immunotypes. In contrast to the LOS of meningococci, heptose is not present in the LOS of MC (*Holme* et al., 1999). The major difference between the immunotypes of MC is that groups A and C contain GlcNAcα within its β-chain, while group B contains Glcα in its place. Several authors have reported some structural and antigenetic homology or similarity between commensal bacteria, or *Neisseria* and *Moraxella* species (*Rahman* et al., 1998b; *Jonsson* et al., 1992,1993,1994).

OBJECTIVES

The aim of this study was to test the hypothesis that LOS found on the commensal species *N. lactamica* and *M. catarrhalis* share cross-reactive antigens with meningococcal immunotypes and human blood group antigens that might

induce protective immunity against meningococcal disease. The objectives of this study were: 1) to assess *N. lactamica* and *M. catarrhalis* isolates for binding of antibodies to blood group antigens and NM immunotype antigens and 2) to compare binding of these antibodies by commensal isolates from different geographical regions of Europe and the age groups.

MATERIAL AND METHODS

Bacterial strains

Standard meningococcal immunotype strains L1-L12 were obtained from Dr. W. D. Zollinger, Washington D.C. *N. lactamica* isolates were obtained from our culture collection or colleagues in different regions of Europe: Dr. P. Krizova, National Reference Laboratory for Meningococcal Disease, Prague, Czech Republic; Dr. K. Jónsdóttir, University Hospital, Reykjavik, Iceland; Dr. G. Tzanakaki, National Meningococcal Reference Laboratory, National School of Public Health, Athens, Greece; Dr. S. Clarke, Scottish Meningococcal and Pneumococcal Reference Laboratory (SMPRL), Glasgow, Scotland. *M. catarrhalis* clinical isolates from our culture collection were kindly provided by Dr. El-Ahmer. None of the NL or MC isolates were agglutinated by standard meningococcal capsular serogroup reagents.

Bacterial growth conditions

Cultures were grown overnight at 37ºC on human blood agar (HBA) containing: lysed erythrocytes concentrates (100 ml) obtained from the blood transfusion services, University of Cologne; special peptone (23 g) (Difco); corn starch (1 g) (Sigma); NaCl (4.5 g) (Sigma); D-glucose (1 g) (Sigma); technical grade agar (10 g) (OXOID); K_2HPO_4 (4 g) and KH_2PO_4 (1 g); 900 ml of distilled water.

Antibodies

Primary antibodies used to detect expression of meningococcal immunotype antigens were as follows: L1 (17-1-L1), L(3,7,9) (12C10), L8 (6E7-10) and L10 (14-1-L10) (all mouse IgG) were provided by W. Zollinger, Walter Reid Army Institute for Research, Washington D.C. Antibodies directed against human blood group antigens were used as follows: paragloboside (mouse IgM) (1B12-1B7, ATCC); P1 (mouse IgM) (Z202) and Ii (human IgG) (Z248) (both Diagnostic Scotland, Edinburgh, UK); P (rabbit IgG) (A0302 118, DAKO); pK (rat IgM) (MCA579, Serotec). The following horseraddish peroxidase (HRP) conjugated secondary antibodies were used: protein A (*S. aureus*) (Sigma); anti mouse IgM μ chain (goat) (Sigma); anti-rabbit IgG γ chain (rat) (Biosource); and anti rat IgM μ chain (mouse) (Biosource).

Whole cell ELISA (WCE)

WCE was used to screen for binding of antibodies to blood group and immunotype antigens on bacteria based on previously published methods for the detection of antigens on meningococci (*Abdillahi* and *Poolman*, 1988; *Scholten* et al., 1994). Bacteria were grown overnight on HBA, harvested in sterile filtered 0.5% (v/v) buffered formalin and washed twice in sterile filtered phosphate buffered saline (PBS). The cell suspension was adjusted to a final concentration of 10^{10} bacteria ml^{-1} which correlated to an absorption of OD_{546}=0.600. The cell suspension (100 μl) was distributed into sterile flat bottom 96 well PVC microtitre plates (Greiner) and allowed to dry overnight

at 56°C. The coated plates were kept at room temperature (RT) for up to three months.

Assay

The coated plates were washed 3 times with washing solution containing Tween 80 (0.25 ml) (Sigma) in 1 litre of tap water, aspirated, and blocked for 15 min with 50 µl CT buffer containing casein (3 g) (Sigma) and Tween 80 (0.11 ml) dissolved in 1 litre of distilled water at 37°C. The CT buffer was removed and 50 µl of primary antibodies diluted in CT buffer were added to the appropriate wells. The plates were incubated at 37°C for 30 min in a moist chamber. The samples were washed three times in washing solution. The peroxidase-conjugated secondary antibody diluted in CT buffer (100 µl) was added to each appropriate well and incubated for 30 min at 37°C in a moist chamber. The wells were washed three times with washing solution (100 µl), and 100 µl of freshly prepared 3,3',5,5' tetramethyl-benzidine (TMB) (Sigma) substrate diluted 1 in 5 in distilled water was added to the appropriate wells. The plates were incubated at room temperature in the dark for 15 min. The peroxidase activity was stopped by adding 50 µl of sulphuric acid (2N) to each well. The absorption of each well was measured at an optical density of 450 nm with a reference filter at 630 nm using a 96 well plate reader (Dynex MRX II) and analysed with the Dynex Revelation software for PCs .Two separate batches of bacteria were assessed in duplicate in three independent experiments. Each ELISA plate contained a negative control for non-specific binding of the HRP-conjugated secondary antibody tested for each individual strain. This sample was used to set the negative value against which the samples incubated with the primary and secondary antibody was judged as negative or positive. An increase in the absorbance of more than 0.5 in the reading for the test compared to the negative control was considered to be positive for binding of the antibody (+). Values below 0.5 were scored as negative (-) (*Scholten* et al., 1994).

Every ELISA plate contained a positive control, reported to express the immunotype antigens, immunotype reference strains L1, L3, L7, L8 and L10. Quality control between plates was assessed by adding 5 µl of the HRP-conjugated secondary antibody and TMB substrate (100µl) to an empty well. Variability in absorbance between plates was less than 0.100.

Statistical analysis

Binding of meningococcal immunotype or blood group antibodies were expressed as positive (1) or negative (0) scores. Statistical analysis was performed with the software package SPSS version 10.0.7a for Macintosh (SPSS Inc.). Differences in WCE scores across groups with two factor models were examined using Chi-Square tests (Fisher's exact test, $F\chi^2$) and symmetric interval-by-interval measures (Pearson's regression, *PR*), and groups with more than two factor models were examined using Chi-Square tests (Pearson's two sided Chi-Square test, $P\chi^2$) and symmetric interval by interval measures (Pearson's regression one-sided test, *PR*). P values of <0.05 were considered statistically significant.

Table 4: Whole cell ELISA to detect binding of antibodies to blood group antigens by meningococcal immunotype reference strains

	Blood group				Immunotype				
Reference strain	P	P1	p^K	Paragloboside	I	L1	L(3,7,9)	L8	L10
L1	+	-	+	-	-	+	-	+	-
L2	+	-	-	+	+	-	+	-	-
L3	+	-	+	-	+	-	+	+	-
L4	-	-	-	+	-	-	-	-	-
L5	-	-	-	+	+	-	+	-	-
L6	+	+	-	-	-	-	-	-	-
L7	+	+	+	+	+	-	+	+	-
L8	+	+	+	+	+	-	+	+	-
L9	-	-	+	+	+	-	+	+	-
L10	-	-	+	+	+	-	-	+	+
L11	+	-	+	-	+	-	-	+	-
L12	-	-	-	+	-	-	-	-	+

Data: detection of antigens; + binding of typing antibodies; -, absence of antibody binding.

RESULTS

Binding of blood group and immunotype antibodies to meningococcal immunotype strains

Blood group antibodies

The immunotype reference strains bound the following blood group antibodies: L1 reacted with P and p^K; L2 with P, paragloboside and Ii; L3 with P, p^K, and Ii; L4 with paragloboside; L5 with paragloboside and Ii; L6 with P1; L7 and L8 reacted with all blood group antibodies used; L9 with P1, p^K, paragloboside and Ii; L10 with p^K, paragloboside and Ii; L11 P, p^K and Ii; L12 reacted only with paragloboside (Table 4). The detection of anti-I antibodies to meningococcal reference strains using either HRP-conjugated protein A or HRP-conjugated anti-human IgG was identical.

Meningococcal immunotype antibodies

The immunotype reference strains bound the following anti-LOS antibodies: L1 reacted with L1 and L8; L2 with L(3,7,9); L3 with L(3,7,9) and L8; L4 with none; L5 with L(3,7,9); L6 did not bind any of the antibodies used; L7 reacted with L(3,7,9) and L8; L8 with L8 and L(3,7,9); L9 with L(3,7,9) and L8; L10 with L10 and L8; L11 with L8; and L12 reacted with L10 (Table 4).

Detection of blood group or immunotype antigens on NL from different sources

The binding of antibodies to blood group antigens and LOS immunotypes to NL isolates from the Czech Republic (n=4), from children (n=2) and adults (n=10) in Scotland, from Russian immigrant children in Greece (n=27), and from native Greek children (n=34), juveniles and adults (n=38) (*Kremastinou* et al., 1999a,b) were assessed by WCE in three independent experiments. The binding of blood group antibodies (Table 5) and meningococcal immunotype antibodies (Tables 6, 7, and 8) are summarised by country and age group.

Table 5: Binding of antibodies to human blood group antigens by commensal *N. lactamica* isolates obtained from Czech, Icelandic and Scottisch childrenand adults, and Russian immigrant children in Greece

Origin	Age(years)	n	P	P1	pK	Paragloboside	Ii	no binding
Czech Rep	≤13	4	1 (25.0)	2 (50.0)	2 (50.0)	4 (100)	4 (100)	0 (0.0)
Scotland	>0	12	1 (8.3)	2 (16.7)	8 (66.7)	8 (66.6)	7 (58.3)	6 (50.0)
Russia	≤13	27	10 (37)	4 (14.8)	8 (29.6)	1 (3.7)	8 (29.6)	0 (0.0)
Iceland	≤13	1	1 (100)	1 (100)	0 (0.0)	1 (100)	1 (100)	0 (0.0)

Data: number and percentage (%) of isolates binding the human blood group phenotyping antibodies; n.t.: not tested; n.s., not significant.

Significantly fewer NL isolates from Russian immigrant children in Greece expressed p^K (p=0.035) and paragloboside (p<0.001) antigens compared to isolates from Scotland. There was no significant difference in the expression of P, P1 or Ii antigens between the samples from different countries tested. Meningococcal immunotypes L(3,7,9) (p<0.001) and the L(3,7,9) positive L8 negative phenotype (L379+L8-) (p<0.001) were expressed by fewer NL isolates obtained from Russian immigrant children in Greece compared to isolates obtained from Scottish, Czech or from native Greek adults or children (Table 7).

The majority of NL isolates obtained from children (60.6%) and adult (82.2%) carriers expressed antigens cross-reactive with meningococcal immunotype L(3,7,9). There was no significant difference in the binding of meningococcal immunotyping antibodies between strains isolated from juveniles (age 14 to 19) compared to isolates obtained from adult carriers (age ≥20 years). These two populations were consequently grouped together as adults (age ≥14 years). Significantly more strains isolated from adults bound antibodies against meningococcal immunotype L(3,7,9) (p=0.014) and phenotype L(3,7,9) positive L8 negative (p=0.009) compared to isolates obtained from children carriers.

Binding of antibodies to blood group and L(3,7,9) antigens by *M. catarrhalis* isolates from Scotland

The binding of blood group antibodies against P, P1, p^K, paragloboside, I (n=126) to isolates obtained from children (n=27) and adults (n=99) was measured by WCE. The binding of meningococcal immunotyping antibodies L1, L(3,7,9) and L10 to isolates of children (n=87) and adults (n=99) was measured.

Most clinical isolates of MC bound one or more antibodies to the blood group antigens tested (Table 9). Significantly more strains isolated from adults bound antibodies to P1 (p=0.024), paragloboside (p=0.034) and Ii (p=0.022) compared to isolates obtained from children. Binding of antibodies to P, p^K or strains that did not bind any of the blood groups tested did not differ significantly between isolates obtained from adults or children.

Most clinical isolates of MC obtained from children (n=89) bound one or more meningococcal immunotyping antibodies. The majority of *M. catarrhalis* strains isolated from children (71.9%) bound immunotyping L1 antibodies, while L8 antigens were detected on a small number of isolates (5.6%). Significantly more strains isolated from children (38.2%) bound antibodies to L(3,7,9) compared to isolates obtained from adults (n=99) (22.2%) (P=0.017).

Table 6: Binding of meningococcal immunotyping antibodies by commensal *N. lactamica* isolates obtained from Czech, Icelandic, Greek and Scottisch children and adults, Russian immigrant children in Greece

Origin	Age (years)	n	L1	L(3,7,9)	L8	L(3,7,9)+ L8+	L(3,7,9)+ L8-	L(3,7,9)- L8+	L(3,7,9)- L8-
Czech Rep	≤13	4	1 (25.0)	4 (100)	2 (25.0)	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)
Scotland	≤13	2	0 (0.0)	2 (100.0)	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)
Scotland	≥14	10	1 (10.0)	7 (70.0)	2 (20.0)	2 (20.0)	5 (50.0)	0 (0.0)	3 (30.0)
Iceland	≤13	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Russia	≤13	27	4 (14.8)	9 (33.3)	4 (14.8)	4 (14.8)	5 (18.5)	0 (0.0)	18 (66.7)
Greece	≤13	34	12 (35.3)	25 (73.5)	3 (0.8)	3 (0.8)	22 (64.7)	0 (0.0)	9 (26.5)
Greece	≥14	38	10 (26.3)	32 (84.2)	4 (10.5)	4 (10.5)	28 (73.7)	0 (0.0)	6 (15.8)

Data: number and percentage (%) of isolates binding the human blood group phenotyping antibodies.

Table 7: Binding of meningococcal immunotyping antibodies by commensal *N. lactamica* isolates by region

Origin	n	L1	L(3,7,9)	L8	L(3,7,9)+ L8+	L(3,7,9)+ L8-	L(3,7,9)- L8+	L(3,7,9)- L8-
Czech Rep	4	1 (25.0)	4 (100.0)	2 (50.0)	2 (50.0)	2 (50.0)	0 (0.0)	0 (0.0)
Scotland	12	1 (8.3)	9 (75.0)	2 (16.7)	2 (16.7)	7 (58.3)	0 (0.0)	3 (25.0)
Russia	27	4 (14.8)	9 (33.3)	4 (14.8)	4 (14.8)	5 (18.5)	0 (0.0)	18 (66.7)
Greece	72	22 (30.6)	57 (79.2)	7 (9.7)	7 (9.7)	50 (69.4)	0 (0.0)	15 (20.8)

Data: number and percentage (%) of isolates binding the human blood group phenotyping antibodies; n.t.: not tested; n.s., not significant.

Table 8: Binding of meningococcal immunotyping antibodies by commensal *N. lactamica* isolates by age

Age (years)	n	L1	L(3,7,9)	L8	L(3,7,9)+ L8+	L(3,7,9)+ L8-	L(3,7,9)- L8+	L(3,7,9)- L8-
≤13	71	16 (22.5)	43 (60.6)	10 (14.1)	10 (14.1)	33 (46.5)	0 (0.0)	28 (39.4)
≥14	45	12 (26.7)	37 (82.2)	5 (11.1)	5 (11.1)	32 (71.1)	0 (0.0)	8 (17.8)

Data: number and percentage (%) of isolates binding the human blood group phenotyping antibodies; n.t.: not tested; n.s., not significant.

Table 9: Binding of antibodies to blood group antigens by clinical *M. catarrhalis* isolates obtained from Scottisch children and adults

Origin	Age (years)	n	P	P1	pK	Paragloboside	Ii	no binding
Scotland	≤13	27	1 (3.7)	2 (7.4)	20 (74.1)	1 (3.7)	1 (3.7)	6 (22.2)
Scotland	≥14	99	15 (15.2)	28 (28.3)	60 (60.6)	21 (21.2)	23 (23.2)	26 (26.1)

Data: number and percentage (%) of isolates binding the human blood group phenotyping antibodies; n.t.: not tested; n.s., not significant.

DISCUSSION

Binding of antibodies to blood group and meningococcal immunotype antigens by meningococci in relation to previous studies

All immunotype antibodies used bound to strains previously reported to express these antibodies (*Scholten* et al., 1994). The anti-paragloboside antibody bound to all immunotype reference strains reported to express this antigen as its terminal moiety. The anti-paragloboside antibody did not bind to the sialylated paragloboside moiety of immunotype L3. In addition, immunotype L8 that bound anti-paragloboside IgM co-expresses immunotype L(3,7,9). Immunotype L12 bound anti-paragloboside, an antigen found on immunotype L10. The antibody against immunotype L(3,7,9), an antigen that contains the paragloboside moiety, did not bind to immunotypes L4, or L10 that bound anti-paragloboside antibodies. These findings suggest that the L(3,7,9) antibody recognises an epitope that is either not accessible in these immunotypes, or that the L(3,7,9) antibody is directed against an epitope other than or in addition of the oligosaccharide moiety. This epitope might be present in the core structure of the L(3,7,9) antigen, or it might include a combination of core and paragloboside structure.

The binding of blood group antibodies corresponds to the presence of these antigens within the published structures of meningococcal LOS. These findings provide evidence that antibodies used for blood group typing can detect similar antigens on meningococci. Antibodies found in human serum directed against blood group antigens might also cross-react with meningococcal oligosaccharide moieties, for example anti-I antibodies found in human serum. Their possible biological functions in relation to complement dependent bactericidal activity, ability to neutralise the toxicity of meningococcal LOS, or ability to opsonise meningococci and commensal species has not been reported.

Binding of antibodies to blood group and meningococcal immunotype antigens by NL isolates from different countries in Europe

Several authors have investigated carriage rates of meningococci and NL within normal populations in the USA (*Gold* et al., 1978), Norway (*Holten* et al., 1978), Nigeria (*Blakebrough* et al., 1982), Spain (*Saez-Nieto* et al., 1985), England (*Cartwright* et al., 1987; *Coen* et al., 2000), Faroer Islands (*Olsen* et al., 1991), Wales (*Davies* et al., 1996), Greece (*Kremastinou* et al., 1999a,b), and New Zealand (*Simmons* et al., 2000). There has, however, been no systematic survey of antigens on NL cross-reactive with those on meningococci. All of these surveys investigating carriage of NL reported, that carriage rates of NL were higher in young children (12-65%) compared to young adults (2-5%). Carriage of NL were found to exceed those for carriage of meningococci within the younger age groups by up to 6 to 1 and the two species are not isolated from the same individual (G. Tzanakaki, personal communication). This demonstrates that NL is a commensal found world-wide in young children, and its association with the development of natural immunity to meningococcal disease appears to be of great importance in many communities.

The significant differences in the binding of antibodies to carbohydrate antigens by strains isolated from carriers from different European regions indicate that regional phenotypic differences of NL isolates might contribute to the development of different herd im-

munities. The majority of NL isolates from children and adults tested, expressed the cross-reactive L(3,7,9) meningococcal immunotype associated with virulence in pathogenic meningococci, suggesting that this phenotype on commensal species might be a major antigen involved in the development of natural immunity to meningococcal disease. This could lead to greater susceptibility to meningococcal disease in some populations, for example, those in which there is a low proportion of commensal strains expressing the L(3,7,9) or L8 epitopes. Little is known about the LOS immunotypes of meningococcal isolates from Eastern Europe, the Americas, Australia or African countries.

Binding of antibodies to blood group and L(3,7,9) antigens by *M. catarrhalis* obtained from adults and children

The majority of MC isolates (73.8%) bound one or more antibodies to blood group antigens or L(3,7,9) immunotype. Antibodies to the blood group p^K (63.5%) were bound by most of the isolates, and a large proportion of isolates obtained from children (38.2%) and adults (22.2%) bound the monoclonal meningococcal immunotyping antibody to L(3,7,9). These findings support our hypothesis, that carriage of MC might induce protective immunity against meningococcal disease. Most of these isolates were from adults with respiratory tract infections. Strains isolated from children with otitis media showed a higher proportion of binding of L(3,7,9) antibodies to those MC isolates compared to isolates causing disease in adults. Similar studies with MC isolates from children from different geographical regions and ethnic groups with respiratory or ear infections and carrier isolates need to be carried out. Little is known about natural antibodies induced by MC cross-reactive to meningococci or NL. Naturally occurring IgG2 antibodies that bound to whole cells of MC were detected in children older than 5 years (*Goldblatt* et al., 1990). The development of natural immunity to MC is thought to be facilitated by glycoconjugates (*Murphy* and *Bartos*, 1989). Carriage rates of MC are higher in early childhood compared to NL and meningococcal carriage rates combined (*Vaneechoutte* et al., 1990a,b; *Faden* et al., 1991; *Harrison* et al., 1999), and consecutive carriage of genetically and phenotypically different MC strains is common among young children (*Rahman* et al., 1998b; *Faden* et al., 1994).

The presence of antibodies to MC in the serum of older children, the frequent carriage rate of multiple strains by children, and its cross-reactivity with some of meningococcal antigens provides evidence that MC might be involved in the development of natural immunity to meningococcal disease as described by *Goldschneider*, *Gotschlich* and *Artenstein* (1969a,b). The higher frequency of carriage of MC compared to NL and meningococci further suggests that MC might play an important role in the development of antibodies that protect against meningococcal disease.

CONCLUSIONS

The commensal species *N. lactamica* and *M. catarrhalis* express antigens that bound antibodies used for meningococcal immunotyping and typing of human blood group antigens. Significant differences in the binding of antibodies to carbohydrate antigens were observed among *N. lactamica* strains isolated

from children and adult carriers from different European regions. This indicates that regional phenotypic differences of *N. lactamica* isolates might contribute to the development of different herd immunities that could lead to greater susceptibility to meningococcal disease in some of these populations. Differences in the expression of carbohydrate antigens between isolates of *M. catarrhalis* obtained from children and adults, the presence of anti-MC antibodies in older children, its frequent presence in the pharyngeal cavities of children, and apparent high levels of strains with L(3,7,9) epitope isolated from children with otitis media provide evidence that MC might be involved in the development of natural immunity to meningococcal LOS. The presence of glycoconjugate antigens on commensal bacteria might be exploited as anti-meningococcal vaccine candidates.

ACKNOWLEDGEMENTS

The work was supported by grants from The Meningitis Association of Scotland, Meningitis Köln, and the Institute for Scientific Evaluation of Naturopathy, University of Cologne

LITERATURE

Abdillahi, H. and Poolman, J.T.: Typing of group-B *Neisseria meningitidis* with monoclonal antibodies in the whole-cell ELISA. J. Med. Microbiol. 26, 177-180 (1988).

Bailly, P., Piller, F., Gillard, B., Veyrieres, A., Marcus, D., and Cartron, J.P.: Biosynthesis of the blood group Pk and P1 antigens by human kidney microsomes. Carbohydr. Res. 228, 277-287 (1992).

Berrington, A.W., Tan, Y.C., Srikhanta, Y., Kuipers, B., van der Ley, P., Peak, I.R., and Jennings, M.P.: Phase variation in meningococcal lipooligosaccharide biosynthesis genes. FEMS Immunol. Med. Microbiol. 34, 267-275 (2002).

Blakebrough, I.S., Greenwood, B.M., Whittle, H.C., Bradley, A.K., and Gilles, H.M.: The epidemiology of infections due to *Neisseria meningitidis* and *Neisseria lactamica* in a northern Nigerian community. J. Infect. Dis. 146, 626-637 (1982).

Booy, R. and Kroll, J.S.: Bacterial meningitis and meningococcal infection. Curr. Opin. Pediatr. 10, 13-18 (1998).

Braun, J.M., Blackwell, C.C., Poxton, I.R., Ahmer, O.E., Gordon, A.E., Madani, O.M., Weir, D.M., Giersen, S., and Beuth, J.: Proinflammatory responses to lipooligosaccharide of *Neisseria meningitidis* immunotype strains in relation to virulence and disease. J. Infect. Dis. 185, 1431-1438 (2002).

Brown, K.E., Anderson, S.M., and Young, N.S.: Erythrocyte P antigen: Cellular receptor for B19 parvovirus. Science 262: 114-117 (1993).

Butch, A.W. and Nahm, M.H.: Functional properties of human germinal centre B cells. Cell. Immunol. 140, 331-344 (1992).

Cartwright, K.A., Stuart, J.M., Jones, D.M., and Noah, N.D.: The Stonehouse survey: Nasopharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol. Infect. 99, 591-601 (1987).

Coen, P.G., Cartwright, K., and Stuart, J.: Mathematical modelling of infection and disease due to *Neisseria meningitidis* and *Neisseria lactamica*. Int. J. Epidemiol. 29, 180-188 (2000).

Davies, A.L., O'Flanagan, D., Salmon, R.L., and Coleman, T.J.: Risk factors for *Neisseria meningitidis* carriage in a school during a community outbreak of meningococcal infection. Epidemiol. Infect. 117, 259-266 (1996).

Dell, A., Azadi, P., Tiller, P., Thomas-Oates, J., Jennings, H.J., Beurret, M., and

Michon, F.: Analysis of oligosaccharide epitopes of meningococcal lipopolysaccharides by fast-atom-bombardment mass spectrometry. Carbohydr. Res. 200, 59-76 (1990).

Edebrink, P., Jansson, P.E., Rahman, M.M., Widmalm, G., Holme, T., Rahman, M., and Weintraub, A.: Structural studies of the O-polysaccharide from the lipopolysaccharide of *Moraxella* (*Branhamella*) *catarrhalis* serotype A (strain ATCC 25238). Carbohydr. Res. 257, 269-284 (1994).

Edebrink, P., Jansson, P.E., Rahman, M.M., Widmalm, G., Holme, T., and Rahman, M.: Structural studies of the O-antigen oligosaccharides from two strains of *Moraxella catarrhalis* serotype C. Carbohydr. Res. 266, 237-261 (1995).

Edebrink, P., Jansson, P.E., Widmalm, G., Holme, T., and Rahman, M.: The structures of oligosaccharides isolated from the lipopolysaccharide of *Moraxella catarrhalis* serotype B, strain CCUG 3292. Carbohydr. Res. 295, 127-146 (1996).

Faden, H., Waz, M.J., Bernstein, J.M., Brodsky, L., Stanievich, J., and Ogra, P.L.: Nasopharyngeal flora in the first three years of life in normal and otitis-prone children. Ann. Otol. Rhinol. Laryngol. 100, 612-615 (1991).

Faden, H., Hong, J.J., and Pahade, N.: Immune response to *Moraxella catarrhalis* in children with otitis media: Opsonophagocytosis with antigen-coated latex beads. Ann. Otol. Rhinol. Laryngol. 103, 522-524 (1994).

Fonkoua, M.C.: Meningococcal meningitis in Africa. Am. J. Nurs. 102, 17 (2002).

Gold, R., Goldschneider, I., Lepow, M.L., Draper, T.F., and Randolph, M.: Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J. Infect. Dis. 137, 112-121 (1978).

Goldblatt, D., Turner, M.W., and Levinsky, R.J.: *Branhamella catarrhalis*: Antigenic determinants and the development of the IgG subclass response in childhood. J. Infect. Dis. 162, 1128-1135 (1990).

Goldschneider, I., Gotschlich, E.C., and Artensen, M.S.: Human immunity to meningococcus. I. The role of humoral antibodies. J. Exp. Med. 129, 1307-1326 (1969a).

Goldschneider, I., Gotschlich, E.C., and Artensen, M.S.: Human immunity to meningococcus. II. Development of natural immunity. J. Exp. Med. 129, 1327-1346 (1969b).

Griffiss, J.M., O'Brien, J.P., Yamasaki, R., Williams, G.D., Rice, P.A., Schneider, H.: Physical heterogeneity of neisserial lipooligosaccharides reflects oligosaccharides that differ in apparent molecular weight, chemical composition, and antigenic expression. Infect. Immun. 55, 1792-1800 (1987a).

Griffiss, J.M., Schneider, H., Mandrell, R.E., Jarvis, G.A., Kim, J.J., Gibson, B., and Apicella, M.A.: The immunochemistry of neisserial LOS. Antonie van Leeuwenhoek 53, 501-7 (1987b).

Gu, X.X., Tsai, C.M., and Karpas, A.B.: Production and characterization of monoclonal antibodies to type 8 lipooligosaccharide of *Neisseria meningitidis*. J. Clin. Microbiol. 30, 2047-2053 (1992).

Hakomori, S. and Kannagi, R.: Carbohydrate antigens in higher animals. In: Handbook of experimental immunology (Ed. Weir, D.M.). Blackwell Scientific, London (1986).

Hammerschmidt, S., Birkholz, C., Zahringer, U., Robertson, B.D., van Putten, J., Ebeling, O., and Frosch, M.: Contribution of genes from the capsule gene complex (cps) to lipooligosaccharide biosynthesis and serum resistance in *Neisseria meningitidis*. Mol. Microbiol. 11, 885-896 (1994).

Harrison, L.M., Morris, J.A., Telford, D.R., Brown, S.M., and Jones, K.: The nasopharyngeal bacterial flora in infancy: Effects of age, gender, season, viral upper respiratory tract infection and sleeping position. FEMS Immunol. Med. Microbiol. 25, 19-28 (1999).

Holme, T., Rahman, M., Jansson, P.E., and Widmalm, G.: The lipopolysaccharide of *Moraxella catarrhalis* structural relationships and antigenic properties. Eur. J. Biochem. 265, 524-529 (1999).

Holten, E., Bratlid, D., and Bovre, K.: Carriage of *Neisseria meningitidis* in a semi-isolated arctic community. Scand. J. Infect. Dis. 10, 36-40 (1978).

Jennings, H.J., Johnson, K.G., and Kenne, L.: The structure of an R-type oligosaccharide core obtained from some lipopolysaccharides of *Neisseria meningitidis*. Carbohydr. Res. 121, 233-241 (1983).

Jennings, M.P., Srikhanta, Y.N., Moxon, E.R., Kramer, M., Poolman, J.T., Kuipers, B., and van der Ley, P.: The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis*. Microbiology 145, 3013-3021 (1999).

Jones, D.M., Borrow, R., Fox, A.J., Gray, S., Cartwright, K.A., and Poolman, J.T.: The lipooligosaccharide immunotype as a virulence determinant in *Neisseria meningitidis*. Microb. Pathog. 13, 219-224 (1992).

Jonsson, I., Holme, T., Krook, A., Rahman, M., and Thoren, M.: Variability of surface-exposed antigens of different strains of *Moraxella catarrhalis*. Eur. J. Clin. Microbiol. Infect. Dis. 11, 9199-9122 (1992).

Jonsson, I., Holme, T., Krook, A., and Thoren, M.: Serological cross-reactions between *Moraxella* (*Branhamella*) *catarrhalis* and other oropharyngeal bacteria. Eur. J. Clin. Microbiol. Infect. Dis. 12, 289-293 (1993).

Jonsson, I., Holme, T., and Krook, A.: Significance of isolation of *Moraxella catarrhalis* in routine cultures from the respiratory tract in adults: Antibody response studied in a whole cell EIA. Scand. J. Infect. Dis. 26, 553-558 (1994).

Katial, R.K., Brandt, B.L., Moran, E.E., Marks, S., Agnello, V., and Zollinger, W.D.: Immunogenicity and safety testing of a group B intranasal meningococcal native outer membrane vesicle vaccine. Infect. Immun. 70, 702-707 (2002).

Kim, J.J., Mandrell, R.E., and Griffiss, J.M.: *Neisseria lactamica* and *Neisseria meningitidis* share lipooligosaccharide epitopes but lack common capsular and class 1, 2, and 3 protein epitopes. Infect. Immun. 57, 602-608 (1989).

Kim, J.J., Phillips, N.J., Gibson, B.W., Griffiss, J.M., and Yamasaki, R.: Meningococcal group A lipooligosaccharides (LOS): Preliminary structural studies and characterization of serotype-associated and conserved LOS epitopes. Infect. Immun. 62, 1566-1575 (1994).

Kogan, G., Uhrin, D., Brisson, J.R., and Jennings, H.J.: Structural basis of the *Neisseria meningitidis* immunotypes including the L4 and L7 immunotypes. Carbohydr. Res. 298, 191-199 (1997).

Kremastinou, J., Tzanakaki, G., Velonakis, E., Voyiatzi, A., Nickolaou, A., Elton, R.A., Weir, D.M., and Blackwell, C.C.: Carriage of *Neisseria meningitidis* and *Neisseria lactamica* among ethnic Greek school children from Russian immigrant families in Athens. FEMS Immunol. Med. Microbiol. 23, 13-20 (1999a).

Kremastinou, J., Tzanakaki, G., Pagalis, A., Theodondou, M., Weir, D.M., and Blackwell, C.C.: Detection of IgG and IgM to meningococcal outer membrane proteins in relation to carriage of *Neisseria meningitidis* or *Neisseria lactamica*. FEMS Immunol. Med. Microbiol. 24, 73-78 (1999b).

Mandrell, R.E., Griffiss, J.M., and Macher, B.A.: Lipooligosaccharides (LOS) of *Neisseria gonorrhoeae* and *Neisseria meningitidis* have components that are immunochemically similar to precursors of human blood group antigens. Carbohydrate sequence specificity of the mouse monoclonal antibodies that recognize crossreacting antigens on LOS and human erythrocytes. J. Exp. Med. 168, 107-126 (1988).

Mandrell, R.E., Kim, J.J., John, C.M., Gibson, B.W., Sugai, J.V., Apicella, M.A., Griffiss, J.M., and Yamasaki, R.: Endogenous sialylation of the lipooligosaccharides of *Neisseria meningitidis*. J. Bacteriol. 173, 2823-2832 (1991).

Mandrell, R.E., Griffiss, J.M., Smith, H., and Cole, J.A.: Distribution of a lipooligosaccharide-specific sialyltransferase in pathogenic and non-pathogenic *Neisseria*. Microb. Pathog. 14, 315-327 (1993).

Mandrell, R.E. and Apicella, M.A.: Lipo-oligosaccharides (LOS) of mucosal pathogens: Molecular mimicry and host-modification of LOS. Immunobiology 187, 382-402 (1993).

Marcus, D.M., Naiki, M., and Kundu, S.K.: Abnormalities in the glycosphingolipid content of human Pk and p erythrocytes. Proc. Natl. Acad. Sci. USA 73, 3263-3267 (1976).

Marsh, W.L. and Jenkins, W.J.: Anti-i: A new cold antibody. Nature 188, 753-755 (1960).

Marsh, W.L.: Anti-i, a cold antibody defining the Ii relationship in human red cells. Br. J. Haematol. 7, 200-209 (1961).

Masoud, H., Perry, M.B., and Richards, J.C.: Characterization of the lipopolysaccharide of *Moraxella catarrhalis*. Structural analysis of the lipid A from *M. catarrhalis* serotype

A lipopolysaccharide. Eur. J. Biochem. 220, 209-216 (1994).

McLeod Griffiss, J., Brandt, B.L., Saunders, N.B., and Zollinger, W.: Structural relationships and sialylation among meningococcal L1, L8, and L3,7 lipooligosaccharide serotypes. J. Biol. Chem. 275, 9716-9724 (2000).

Michon, F., Beurret, M., Gamian, A., Brisson, J.R., and Jennings, H.J.: Structure of the L5 lipopolysaccharide core oligosaccharides of *Neisseria meningitidis*. J. Biol. Chem. 265, 7243-7247 (1990).

Murphy, T.F. and Bartos, L.C.: Surface-exposed and antigenically conserved determinants of outer membrane proteins of *Branhamella catarrhalis*. Infect. Immun. 57, 2938-2941 (1989).

Olsen, S.F., Djurhuus, B., Rasmussen, K., Joensen, H.D., Larsen, S.O., Zoffman, H., and Lind, I.: Pharyngeal carriage of *Neisseria meningitidis* and *Neisseria lactamica* in households with infants within areas with high and low incidences of meningococcal disease. Epidemiol. Infect. 106, 445-457 (1991).

Pavliak, V., Brisson, J.R., Michon, F., Uhrin, D., and Jennings, H.J.: Structure of the sialylated L3 lipopolysaccharide of *Neisseria meningitidis*. J. Biol. Chem. 268, 14146-14152 (1993).

Plested, J.S., Makepeace, K., Jennings, M.P., Gidney, M.A., Lacelle, S., Brisson, J., Cox, A.D., Martin, A., Bird, A.G., Tang, C.M., Mackinnon, F.M., Richards, J.C., and Moxon, E.R.: Conservation and accessibility of an inner core lipopolysaccharide epitope of *Neisseria meningitidis*. Infect. Immun. 67, 5417-5426 (1999).

Prokop, O. and Uhlenbruck, G.: Human blood and serum groups. McClaren and Sons, London (1965).

Race, R.R. and Sanger, R.: Blood groups in man (6th edition). Backwell Scientific Publications, Oxford (1975).

Rahman, M. and Holme, T.: Antibody response in rabbits to serotype-specific determinants in lipopolysaccharides from *Moraxella catarrhalis*. J. Med. Microbiol. 44, 348-354 (1996).

Rahman, M., Holme, T., Jonsson, I., and Krook, A.: Human immunoglobulin isotype and IgG subclass response to different antigens of *Moraxella catarrhalis*. APMIS 105 213-220 (1997).

Rahman, M.M., Stephens, D.S., Kahler, C.M., Glushka, J., and Carlson, R.W.: The lipooligosaccharide (LOS) of *Neisseria meningitidis* serogroup B strain NMB contains L2, L3, and novel oligosaccharides, and lacks the lipid-A 4'-phosphate substituent. Carbohydr. Res. 307, 311-324 (1998a).

Rahman, M., Jonsson, A.B., and Holme, T.: Monoclonal antibodies to the epitope alpha-Gal-(1-4)-beta-Gal-(1-3) of *Moraxella catarrhalis* LPS react with a similar epitope in type IV pili of *Neisseria meningitidis*. Microb. Pathog. 24, 299-308 (1998b).

Saez-Nieto, J.A., Dominguez, J.R., Monton, J.L., Cristobal, P., Fenoll, A., Vazquez, J., Casal, .J, and Taracena, B.: Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in a school population during an epidemic period in Spain. Hygiene (London) 94, 279-288 (1985).

Saunders, N.B., Shoemaker, D.R., Brandt, B.L., Moran, E.E., Larsen, T., and Zollinger, W.D.: Related Immunogenicity of intranasally administered meningococcal native outer membrane vesicles in mice. Infect. Immun. 67, 113-119 (1999).

Schneider, H., Hale, T.L., Zollinger, W.D., Seid, R.C. Jr., Hammack, C.A., and Griffiss, J.M.: Heterogeneity of molecular size and antigenic expression within lipooligosaccharides of individual strains of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Infect. Immun. 45, 544-549 (1984).

Scholten, R.J., Kuipers, B., Valkenburg, H.A., Dankert, J., Zollinger, W.D., and Poolman, J.T.: Lipo-oligosaccharide immunotyping of *Neisseria meningitidis* by a whole-cell ELISA with monoclonal antibodies. J. Med. Microbiol. 41, 236-243 (1994).

Siddiqui, B. and Hakomori, S.: A ceramide tetrasaccharide of human erythrocyte membrane reacting with anti-type XIV pneumococcal polysaccharide antiserum. Biochim. Biophys. Acta 330, 147-155 (1973).

Simmons, G., Martin, D., Stewart, J., and Bremner, D.: Carriage of *Neisseria lactamica* in a population at high risk of meningococcal disease. Epidemiol. Infect. 125, 99-104 (2000).

Tsai, C.M. and Civin, C.I.: Eight lipooligosaccharides of *Neisseria meningitidis* react

with a monoclonal antibody which binds lacto-N-neotetraose (Gal beta 1-4 GlcNAc beta 1-3 Gal beta 1-4 Glc). Infect. Immun. 59, 3604-3609 (1991).

Vaneechoutte, M., Verschraegen, G., Claeys, G., Weise, B., and Van den Abeele A.M.: Respiratory tract carrier rates of *Moraxella* (*Branhamella*) *catarrhalis* in adults and children and interpretation of the isolation of *M. catarrhalis* from sputum. J. Clin. Microbiol. 28, 2674-2680 (1990a).

Vaneechoutte, M., Verschraegen, G., Claeys, G., and Van Den Abeele, A.M.: Serological typing of *Branhamella catarrhalis* strains on the basis of lipopolysaccharide antigens. J. Clin. Microbiol. 28, 182-187 (1990b).

Varaine, F., Caugant, D.A., Riou, J.Y., Konde, M.K., Soga, G., Nshimirimana, D., Muhirwa, G., Ott, D., Hoiby, E.A., Fermon, F., and Moren, A.: Meningitis outbreaks and vaccination strategy. Trans. R. Soc. Trop. Med. Hyg. 91, 3-7 (1997).

Wakarchuk, W., Gilbert, M., Martin, A., Wu, Y., Brisson, J.-R., Thibault, P., and Richards, J.C.: Structure of an α2,6-sialylated lipooligosaccharide from *Neisseria meningitidis* immunotype L1. Eur. J. Biochem. 254, 626-633 (1998).

Wiels, J., Fellous, M., and Tursz, T.: Monoclonal antibody against a Burkitt lymphoma-associated antigen. Proc. Natl. Acad. Sci. USA 78, 6485-6488 (1981).

Wiener, A.S.: Blood group distributions. Ann. Intern. Med. 79, 137 (1973).