

## MINIREVIEW

# The sweet side of the pathogenic *Neisseria*: the role of glycan interactions in colonisation and disease

Tsitsi D. Mubaiwa, Evgeny A. Semchenko, Lauren E. Hartley-Tassell, Christopher J. Day, Michael P. Jennings and Kate L. Seib<sup>\*,†</sup>

Institute for Glycomics, Griffith University, Gold Coast, QLD 4222, Australia

\*Corresponding author: Institute for Glycomics, Griffith University, Gold Coast, QLD 4222, Australia. Tel: +61 7 5552 7453; Fax: +61 7 5552 8098,

E-mail: [k.seib@griffith.edu.au](mailto:k.seib@griffith.edu.au)

One sentence summary: Glycosylation of the surface of meningococci and gonococci, and interactions with glycan structures of the host, are essential for pathogenic *Neisseria* colonisation and disease.

Editor: Alison Criss

†Kate L. Seib, <http://orcid.org/0000-0002-7094-3528>

## ABSTRACT

Glycomics is a rapidly growing field that focuses on the structure and function of carbohydrates (glycans) in biological systems. Glycan interactions play a major role in infectious disease, at all stages of colonisation and disease progression. *Neisseria meningitidis*, the cause of meningococcal sepsis and meningitis, and *Neisseria gonorrhoeae*, which causes the sexually transmitted infection gonorrhoea, are responsible for significant morbidity and mortality worldwide. *Neisseria meningitidis* displays a range of surface glycosylations including capsule polysaccharide, lipooligosaccharide and O-linked glycoproteins. While *N. gonorrhoeae* does not have a capsule, it does express both lipooligosaccharide and O-linked glycoproteins. *Neisseria gonorrhoeae* also has the ability to scavenge host sialic acids, while several *N. meningitidis* serogroups can synthesise sialic acid. Surface expressed sialic acid is key in serum resistance and survival in the host. On the host side, the pathogenic *Neisseria* protein adhesins such as Opc and NHBA bind to host glycans for adherence and colonisation of host cells. Essentially, from both the bacterial and host perspective, glycan interactions are fundamental in colonisation and disease of pathogenic *Neisseria*. The key aspects of glycobiology of the pathogenic *Neisseria* are reviewed herein.

**Keywords:** *Neisseria gonorrhoeae*; *Neisseria meningitidis*; glycomics; carbohydrate; lectin; host–pathogen interactions

## INTRODUCTION

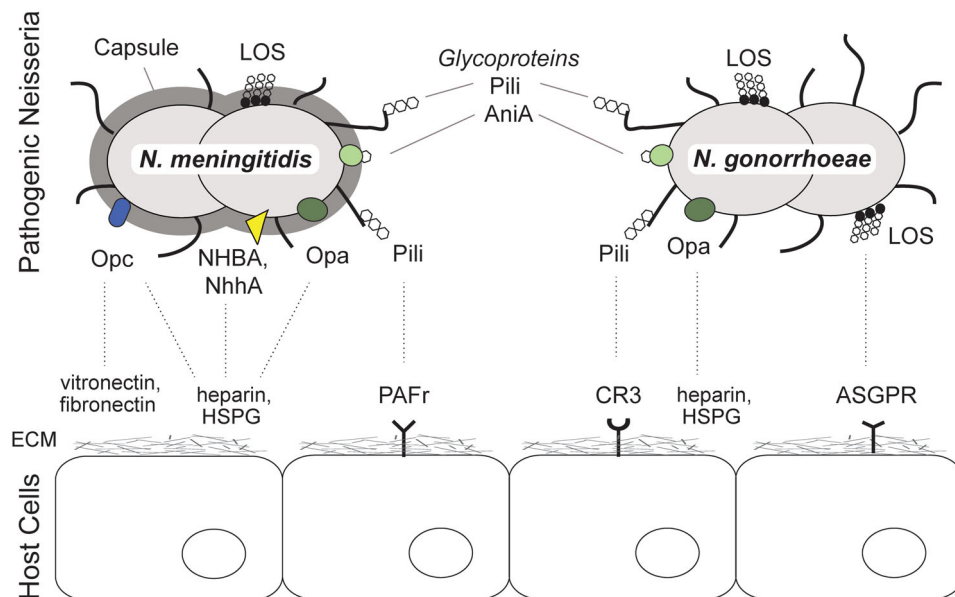
Carbohydrates (glycans) and carbohydrate-recognising proteins (lectins) play significant roles in a diverse range of interactions within organisms, as well as between different organisms (Varki and Sharon 2009). Glycans are extremely diverse; there are 10 basic monosaccharide building blocks, and chains of monosaccharides can vary in length, have a range of different linkages and can be linear or branched. These macromolecules are present in all forms of life, and can be found as free oligo- or polysaccharide chains, or as glycoconjugates when attached to proteins or lipids. Protein glycosylation is one of the most common post-

translational modifications and enhances the functional diversity of proteins produced by an organism. For example, most mammalian cell surface receptors and almost all serum proteins are glycosylated (Apweiler, Hermjakob and Sharon 1999). Consequently, human cells are covered by a glycocalyx, a rich surface coat of glycans (Varki and Sharon 2009), that is important for mediating interactions with other cells and molecules. Furthermore, glycan interactions between microbes and host cells play a major role in bacterial colonisation and disease (Esko and Sharon 2009).

The pathogenic *Neisseria*, *Neisseria meningitidis* and *N. gonorrhoeae*, display extensive glycosylation of bacterial surfaces

Received: 19 March 2017; Accepted: 12 June 2017

© FEMS 2017. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)



**Figure 1.** Schematic of surface glycans of the pathogenic *Neisseria*, and glycan-based interactions with host cells. Glycan structures on the surface of the pathogenic *Neisseria* are shown, and include capsule, lipooligosaccharide (LOS), and the glycosylated Pili and AniA proteins. Glycan-dependent interactions between the pathogenic *Neisseria* and host cells are indicated by a dotted line. ECM, extracellular matrix; HSPG, heparan sulfate proteoglycans; PAFr, platelet activating factor receptor; CR3, complement receptor 3; ASGPR, asialoglycoprotein receptor; NHBA, Neisserial heparin binding antigen.

and structures, and interaction with host glycans is crucial for colonisation and disease. The pathogenic *Neisseria* are closely related obligate human pathogens that share 80%–100% DNA homology, with conservation of the majority of genes (Klee *et al.* 2000). Several of the same bacterial surface structures are involved in initial colonisation of mucosal surfaces by these bacteria (Merz and So 2000); however, they cause distinct diseases. *Neisseria meningitidis* asymptomatically colonises the upper respiratory tract of ~10%–20% of the population, but also poses a significant health problem due to its ability to cause life-threatening meningitis and septicaemia (Rouphael and Stephens 2012). Despite the availability of effective antibiotics, and vaccines for five serogroups, there are an estimated 1.2 million cases and ~135 000 deaths attributed to meningococcal disease, each year, worldwide (Rouphael and Stephens 2012). *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted infection gonorrhoea, is a growing global public health threat. Infection typically results in urethritis in males and cervicitis in females; however, asymptomatic cases are common, and untreated gonorrhoea can lead to serious sequelae, including infertility and increased HIV transmission (reviewed in Edwards *et al.* 2016). There are estimated to be more than 106 million cases of gonorrhoea worldwide each year (World Health Organization and Research 2012). Furthermore, there is no available vaccine and the continuing emergence of antibiotic-resistant strains raises concerns that gonorrhoea may become untreatable in the near future (Unemo and Shafer 2014).

In terms of glycosylated surface structures, *N. meningitidis* is a capsulate organism, and both *N. meningitidis* and *N. gonorrhoeae* express lipooligosaccharide (LOS) and glycosylate some of their proteins (Fig. 1). Several of these structures mediate host-pathogen interactions and are involved in virulence. In addition, human host microenvironments are highly glycosylated (Varki, Esko and Colley 2009) and the pathogenic *Neisseria* targets specific host glycan structures for adherence and colonisation of host cells. In this review, the current knowledge regarding the glycans present on the Neisserial surface and the host

glycans known to interact with *Neisseria* is discussed. There is a focus on *N. meningitidis*, with key points of difference or interest highlighted for *N. gonorrhoeae*. An overview of the glycan-rich host environments inhabited by the pathogenic *Neisseria* highlights the potential for additional glycan-based interactions during various stages of colonisation and disease.

## GLYCAN STRUCTURES OF THE PATHOGENIC NEISSERIA

### Meningococcal polysaccharide capsule

The meningococcal capsule is a high molecular weight polysaccharide, which is linked to the bacterial surface by a glycolipid moiety (Tzeng *et al.* 2005). *Neisseria meningitidis* strains can be classified into 13 serogroups, based on structural differences in their capsular polysaccharides (see Table 1). Six serogroups cause the majority of invasive meningococcal disease worldwide (serogroups A, B, C, W135, X and Y) (Rouphael and Stephens 2012). The polysaccharides differ in their individual monomers and/or linkages between these monomers (Harrison *et al.* 2013) (Table 1). Capsule polysaccharide-based vaccines are available for serogroups A, C, W and Y. However, the  $\alpha(2-8)$ -linked sialic acid homopolymer of the *N. meningitidis* serogroup B capsule is identical to that produced in mammalian cells (Finne, Leinonen and Mäkelä 1983), and due to this molecular mimicry of host glycans, the serogroup B capsule is poorly immunogenic and has not been used in vaccine development.

The polysaccharide capsule is crucial for invasive meningococcal disease (Spinosa *et al.* 2007), and it is universally expressed by strains isolated from the blood and cerebrospinal fluid (Stephens, Greenwood and Brandtzaeg 2007). The meningococcal capsule increases resistance to the complement-mediated bactericidal activity of human serum, and resistance to opsonophagocytosis (reviewed in Lewis and Ram 2014). Carriage strains isolated from the nasopharynx of healthy individuals may or may not be capsulated (Corbett *et al.* 2004). The

**Table 1.** Structures of meningococcal polysaccharide capsules.

Serogroup	Glycan repeat unit	Linkage	Description	Ref
A	ManNAc-1-phosphate	$\alpha 1 \rightarrow 6$ (phosphodiester)	Homopolymer of $\alpha 1 \rightarrow 6$ linked N-acetyl-D-mannosamine-1-phosphate	Liu et al. (1971)
B	Neu5Ac	$\alpha 2 \rightarrow 8$ (glycosidic)	Homopolymer of $\alpha 2 \rightarrow 8$ -linked sialic acid	Bhattacharjee et al. (1975)
C	Neu5Ac	$\alpha 2 \rightarrow 9$ (glycosidic)	Homopolymer of O-acetylated or non-acetylated $\alpha 2 \rightarrow 9$ -linked sialic acid	Bhattacharjee et al. (1975)
D	Not elucidated	–	–	–
E	GalNAc3 -7KDO	$\beta 2 \rightarrow 3$ (glycosidic)	Alternating D-galactosamine (GalN) and 2-keto-3-deoxyoctulosonate (KDO) residues	Bhattacharjee et al. (1978)
H	Gal $\alpha 1$ -2 Gro-3-phosphate	Phosphodiester	Partially O-acetylated Galactosyl glycerol-3-phosphate units	Van Der Kaaden et al. (1984)
I	LGulNAc $\alpha 1$ -3 ManNAcA	$\beta 1 \rightarrow 4$ (glycosidic)	O-Acetylated alternating N-acetyl-guluronic acid and N-acetyl-mannosaminuronic acid residues	Michon et al. (1985)
K	ManNAc $\beta 1$ -4 ManNAcA	$\beta 1 \rightarrow 3$ (glycosidic)	O-Acetylated N-acetylmannosaminuronic acid disaccharide repeat units	Van Der Kaaden et al. (1985)
L	GlcNAc $\beta 1$ -3GlcNAc $\beta 1$ -3GlcNAc-1-phosphate	Glycosidic and Phosphodiester	Heteropolymer of a trimeric repeating unit	Jennings et al. (1983); Litschko et al. (2015)
W-135	Gal $\alpha 1$ -4Neu5Ac	$\alpha 2 \rightarrow 6$ (glycosidic)	Heteropolymer of alternating sequences of D-galactose and sialic acid	Bhattacharjee et al. (1976)
X	GlcNAc-1-phosphate	$\alpha 1 \rightarrow 4$ Phosphodiester	Homopolymer of ( $\alpha 1 \rightarrow 4$ )-linked N-acetylglucosamine-1-phosphate	Bundle et al. (1974)
Y	Glc $\alpha 1$ -4Neu5Ac	$\alpha 2 \rightarrow 6$ (glycosidic)	Heteropolymer of alternating sequences of D-glucose and sialic acid	Bhattacharjee et al. (1976)
Z	GalNAc $\alpha 1$ -1Gro-3-phosphate	Phosphodiester	Monosaccharide glycerol-3-phosphate repeat units	Harrison et al. (2013)

absence of capsule can be either due to the complete absence of the capsule production genes, known as the capsule-null (*cnl*) phenotype (Claus et al. 2002), or due to phase variation (i.e. ON/OFF or graded switching of expression). Two mechanisms of phase variation have been described, slipped strand mispairing of the poly-cytosine tract present within *siaD* and the insertion/excision of the insertion sequence IS1301 in *siaA* (Hammerschmidt et al. 1996a,b). In addition, capsule expression is regulated by RNA thermoregulation, with increased temperature up-regulating *cssA* translation and consequently, capsule expression (Loh et al. 2013). The two-component MisR/MisS system negatively regulates capsule expression, with inactivation of *misR* and *misS* resulting in increased capsule expression (Tzeng et al. 2008). The regulation of capsule is reviewed in detail elsewhere (Bartley and Kahler 2014; Tzeng, Thomas and Stephens 2016).

It has been suggested that capsule may block interactions with host cells due to steric hindrance and masking of surface adhesins involved in intimate cellular interactions, as well due

to the negative charge of its sialic acid residues (Corbett et al. 2004; Virji 2009). Therefore, phase variation and regulation of capsule expression allows the bacteria to adapt to the different stages of infection, i.e. the expression of the capsule is decreased during colonisation but present during invasive disease (Hammerschmidt et al. 1996b; Stephens, Greenwood and Brandtzaeg 2007).

### Meningococcal and gonococcal LOS

LOS makes up ~75% of the outer membrane of Gram-negative bacteria such as the pathogenic *Neisseria* (Gronow and Brade 2001). Neisserial LOS consists of lipid A that anchors the LOS into the outer membrane, an inner core composed of two 3-deoxy-D-manno-2-octulosonic acid (KDO) and two heptose residues (Hep1 and Hep2 residues), and an outer core of oligosaccharide chains ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) attached to the heptose residues (Jennings et al. 1999). The individual sugars in each of the oligosaccharide chains are sequentially added onto the elongating chain by

**Table 2.** Oligosaccharide structures of meningococcal LOS immunotypes.

	$\alpha$ -chain	$\beta$ -chain	$\gamma$ -chain	II PEA	Ref
L1	<sup>a</sup> Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	3P	Di Fabio et al. (1990), Griffiss et al. (2000)
L2	<sup>a</sup> Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	Glc $\beta$ $\alpha$ 1-3	<sup>b</sup> Glc $\beta$ NAC $\alpha$ 1-2	6P	Gamian et al. (1992)
L3	<sup>a</sup> Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	3P	Pavliak et al. (1993)
L4	<sup>a</sup> Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	<sup>b</sup> Glc $\beta$ NAC $\alpha$ 1-2	6P	Kogan et al. (1997)
L5	<sup>a</sup> Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	Glc $\beta$ $\alpha$ 1-3	<sup>b</sup> Glc $\beta$ NAC $\alpha$ 1-2	–	Michon et al. (1990)
L6	<sup>a</sup> 4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	6P	Di Fabio et al. (1990)
L7	Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	6P	Kogan et al. (1997)
L8	Gal $\beta$ 1-Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	3P	Griffiss et al. (2000)
L9	Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	6P	Choudhury et al. (2008)
L10	Gal $\beta$ 1-4GlcNAC $\beta$ 1-3Gal $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	3P/6P	Kim et al. (1994)
L11	Glc $\beta$ 1-4Glc	–	Glc $\beta$ NAC $\alpha$ 1-2	3P&6P	Kim et al. (1994), Mistretta et al. (2010)

<sup>a</sup>Terminal Gal of  $\alpha$ -chain is often partially sialylated.

<sup>b</sup>Partial O-acetylation of the  $\alpha$ -D-GlcNAC (of the  $\gamma$ -chain) observed. Phosphoethanolamine (PEA) substituents at O-3 or O-6 of Hep II = 3P or 6P, respectively.

a series of phase-variable glycosyltransferases, which results in variable lengths of the sugar chain within and between strains (Jennings et al. 1999). LOS variation between Neisserial species or strains also results from the allelic diversity or presence/absence of glycosyltransferases involved in LOS biosynthesis (reviewed in Bartley and Kahler 2014; Bartley et al. 2017). In addition, LOS expression is transcriptionally regulated, with *lgtG* under the control of the MisR/MisS system (Tzeng et al. 2008), while *lst* is expression is repressed at low temperatures via an RNA thermosensor (Loh et al. 2013). Some of the resulting LOS structures contain a terminal lacto-N-neotetraose (LNnT) (Tsai and Civin 1991), which is a human glycan mimic (Moran, Prendergast and Appelmeik 1996). LNnT may be further modified via the addition of a single sialic acid residue (sialylation). Furthermore, Neisserial LOS can vary during infection. For example, male volunteers challenged with a specific gonococcal isolate which expressed non-sialylated LOS were shown to shed a strain expressing a larger, sialylated LOS type (Schneider et al. 1991). LOS variation forms the basis of 12 meningococcal immunotypes (L1–L12, see Table 2); however, there is no LOS typing scheme for *N. gonorrhoeae*.

LOS immunotypes have been linked to meningococcal virulence, and gonococcal LOS plays an important role in pathogenesis and survival. There is a clear role of the sialic acid presence on the LOS in relation to bacterial survival. For example, in *N. meningitidis*, the unsialylated LOS types (L1,8,10) are important for invasion, while the sialylated immunotypes (L3,7,9) are important for survival and dissemination, being less sensitive to complement-mediated killing (Estabrook, Griffiss and Jarvis 1997) and phagocytosis (Unkmeir et al. 2002a) (reviewed in van Putten and Robertson 1995). The presence of a terminal LNnT, resulting from a truncation or deletion of the sialic acid, has been shown to be associated with increased sensitivity to complement (Estabrook, Griffiss and Jarvis 1997).

Similar to *N. meningitidis*, *N. gonorrhoeae*'s LNnT structure is essential for LOS-mediated adherence and invasion. Adherence and invasion into male urethral epithelium cells is mediated by the interaction between the gonococcal LOS with the asialoglycoprotein receptor (C-type lectin) (Harvey et al. 2001). This inter-

action is abated in mutants expressing truncated LOS that lack the LNnT structure, and in strains expressing a terminal sialic acid on their LOS (Harvey et al. 2001). Gonococcal LOS sialylation decreases non-opsonic, opacity-associated uptake by neutrophils (Rest and Frangipane 1992) and allows direct interaction of gonococcal LOS with factor H (Ram et al. 1998), thus promoting serum resistance. Interestingly, LOS sialylation also allows interaction of the pathogenic *Neisseria* with human myeloid cells, via the sialic acid-binding immunoglobulin-like lectins expressed on some phagocytic cells, thereby making the bacteria more susceptible to phagocytosis (Jones, Virji and Crocker 2003).

### Glycosylated proteins

Post-translational modification of proteins with glycan moieties (glycosylation) is ubiquitous in nature and is important for protein function. The PilE subunit of pilin and the nitrite reductase AniA are the best characterised Neisserial glycoproteins. The pathogenic *Neisseria* express type IV pili, which are homopolymeric multimers of a 17–21 kDa polypeptide units (pilin/PilE) that can extend several micrometers from the bacterial cell surface. In *Neisseria*, type IV pilins have been grouped in class I or class II pilin. The class I and II pilin genes are present in different genomic locations (Wormann et al. 2014): class I pilin (but not class II) is recognised by the SM1 antibody (Virji et al. 1989) and class II pilin are restricted to strains from certain clonal complexes (cc1, cc5, cc8, cc11 and cc174) (Wormann et al. 2014). These pilin undergo many post-translational modifications, including glycosylation at serine 63 (Stimson et al. 1995) and glycosylation of up to five sites on class II pilins (Kahler et al. 2001; Gault et al. 2015). Pilin glycosylation involves multiple glycosylation (i.e. *pgl*) genes. Of these, the glycosyltransferases *pglA* and *pglE* are subject to phase variation (Jennings et al. 1998; Power et al. 2003), and consequently the pilin glycan expressed differs across Neisserial strains. For example, pilin can be covalently modified with an O-linked Gal $\beta$ (1-4)Gal $\alpha$ (1-3)-N, N'-diacetylbacillosamine (Gal-Gal-diNAcBac) or Gal $\beta$ (1-4)Gal $\alpha$ (1-3)-glyceramido acetamido trideoxyhexose (Gal-Gal-GATDH) trisaccharide (Chamot-Rooke et al. 2007). While a

Gal( $\alpha$ 1,3)GlcNAc disaccharide is present in this same position on pili of gonococcal and some meningococcal strains (reviewed in Banerjee and Ghosh 2003; Bartley and Kahler 2014). It has also recently been shown that Gal and GlcNAc residues can be O-acetylated, and O-acetylation impacts oligosaccharide chain length (Anonsen et al. 2017).

Neisserial pili are considered to be the primary adhesins involved in early adherence events in colonisation of epithelial cells (Virji 2009). In *N. meningitidis*, pilin glycosylation enhances adherence to epithelial cells (Virji et al. 1993), and is required for platelet activating factor receptor-mediated adherence to human airway epithelial cells (Jen et al. 2013). In *N. gonorrhoeae*, the glycan on the pili subunit interacts directly with the host complement receptor 3 I-domain that is expressed on the surface of epithelial cells. This interaction facilitates bacterial adherence and promotes intracellular survival (Jennings et al. 2011).

The nitrite reductase, AniA, is a glycoprotein that is expressed by all *N. gonorrhoeae* and most *N. meningitidis* strains (Ku et al. 2009; Shewell et al. 2013). AniA is glycosylated at the C-terminus with the trisaccharide digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose (Ku et al. 2009), which is not required for its nitrite reductase function (Ku et al. 2009). However, the O-linked monosaccharide of the AniA glycoprotein is immunodominant and glycosylation of AniA is suggested to be an immune evasion mechanism in which the glycan moiety acts as a 'decoy' to diminish an antibody response against the functional region of the protein (Shewell et al. 2013). A truncated, non-glycosylated, form of AniA induces a non-native immune response against the conserved core region of the protein regardless of the glycosylation state and has been proposed as a vaccine antigen for *N. gonorrhoeae* (Shewell et al. 2013).

Additional glycoproteins have been reported for the pathogenic *Neisseria* via highly sensitive mass spectrometry methods (Vik et al. 2009; Anonsen et al. 2012). However, another study has suggested that for some of the additional proteins identified as glycoproteins, the majority of the protein expressed by the bacteria was essentially unglycosylated (Schulz et al. 2013).

## INTERACTIONS OF THE PATHOGENIC NEISSERIA WITH HOST GLYCANS AND GLYCOPROTEINS

The pathogenic *Neisseria* express a variety of surface structures that interact with host cells. These include the major outer membrane proteins (e.g. pili, the opacity proteins Opc and Opa, and the major porins PorA and PorB) as well as other minor outer membrane structures and adhesins (Merz and So 2000; Virji, 2009). Studies have shown that some of the Neisserial surface structures bind to host glycans and glycoproteins; however, for the latter it is unclear as to whether these interactions are solely protein based, or are also glycan dependent (summarised in Table 3 and Fig. 1). Since these membrane proteins have often undergone post-translational modification by glycosylation, the binding interactions between these bacterial membrane proteins and the host may be either protein:protein, protein:glycan (Esko and Sharon 2009) or glycan:glycan based (Day et al. 2015). The pathogenic *Neisseria* are also known to interact with various other host glycans, such as gangliosides and oligosaccharides, although the specific Neisserial surface structures responsible for this binding are unknown (Table 3).

## Opacity proteins (Opc and Opa)

The opacity proteins, Opc and Opa, are phase variable, integral,  $\beta$ -barrel outer membrane proteins involved in Neisserial adherence and invasion (Carbonnelle et al. 2009; Sa E Cunha, Griffiths and Virji 2010). Opc is not expressed by gonococci (Zhu, Morelli and Achtman 1999) and the *opcA* gene is absent in several meningococcal strains, including the majority of strains in the ST-11/ET-37 and ST-8/Cluster A4 clonal complexes (Seiler et al. 1996). When present in high levels, Opc aids in meningococcal adhesion and invasion into host cells (Virji et al. 1995). *Neisseria meningitidis* strains have 3–4 *opa* genes, while *N. gonorrhoeae* strains may express up to 11 *opa* genes (Bhat et al. 1991). Phase variation of *opa* genes is random and independent, so strains may express any of their repertoire of *opa* genes at any given time (Bhat et al. 1991). The structural variability displayed by these proteins has been linked to functional diversity between Opa variants (reviewed in Billker et al. 2000).

Opc and some Opa proteins can bind the glycosaminoglycans heparin and heparan sulfate proteoglycans (HSPGs) (van Putten and Paul 1995; De Vries et al. 1998). Heparin and heparan sulfate are both highly sulphated glycans, with heparin being found primarily in mast cell granules while heparan sulfate is found in plasma, the extracellular matrix and on cell surfaces of most mammalian cells (Rabenstein 2002). Through its interaction with HSPGs, Opc is able to support, but not sustain, meningococcal adhesion and invasion of host endothelial cells (Virji, Makepeace and Moxon 1994). Due to this and the fact that the interactions are of low affinity, this may not be the only ligand for the opacity proteins. Opc and a few of the Opa variants also interact with extracellular matrix glycoproteins such as vitronectin and fibronectin (Sa E Cunha, Griffiths and Virji 2010). These interactions facilitate association with endothelial cells via the endothelial integrins  $\alpha$ V $\beta$ 3-integrin and  $\alpha$ 5 $\beta$ 1-integrin (the fibronectin and vitronectin receptors, respectively) and result in efficient cellular invasion by meningococci (Virji, Makepeace and Moxon 1994; Unkmeir et al. 2002b) and gonococci (Gómez-Duarte et al. 1997). Furthermore, vitronectin binding also increases meningococcal serum survival by inhibiting the insertion of membrane attack complex into the bacterium (Singh, Su and Riesbeck 2010). The interactions between the gonococcal Opa50 protein and vitronectin can be inhibited by heparin in dose-dependent manner, which suggests that this interaction may be glycan based (Gómez-Duarte et al. 1997).

Both Opa and Opc have been reported to bind a range of other carbohydrate structures including galactose and sialic acids (Moore et al. 2005). Sialic acid residues are typically found at the outermost end of glycan chains of all cell types (Varki 2008). They are a common terminal residue on mucins (Linden et al. 2008) and are an integral component of gangliosides which are abundantly expressed in neuronal tissues (Varki 2008). A majority of Opa proteins also recognise one or more members of the human CD66 carcinoembryonic antigen superfamily; a family of heavily glycosylated cell adhesion proteins which includes the carcinoembryonic antigen-related cell adhesion molecule family (Virji et al. 1996; Sadarangani, Pollard and Gray-Owen 2011). This interaction however is glycan independent (Bos et al. 1998).

## Other adhesins that bind glycans

A range of other Neisserial surface antigens have been shown to bind host glycans or glycoproteins, and these are outlined in Table 3. These include the serogroup B vaccine antigen, the Neisserial heparin binding antigen (NHBA), which binds heparin

**Table 3.** Interactions of meningococcal and gonococcal structures with host glycans and glycoproteins.

Outer membrane structure	Species	Host glycan or glycoprotein	Role of interaction	Ref
Opc	Nm	Fibronectin, vitronectin	Invasion into brain endothelia	Unkmeir <i>et al.</i> (2002b), Sa E Cunha, Griffiths and Virji (2010)
		Heparin/HSPGs	Possible adhesin	De Vries <i>et al.</i> (1998)
		Various oligosaccharides including sialic acid	Adherence to epithelial cells	Moore <i>et al.</i> (2005)
Opa	Nm	Various oligosaccharides	Adherence to epithelial cells	Moore <i>et al.</i> (2005)
Msf	Nm	Vitronectin	Serum survival	Griffiths <i>et al.</i> (2011)
NHBA	Nm	Heparin	Serum survival	Serruto <i>et al.</i> (2010)
		HSPGs	Adherence to epithelial cells	Vacca <i>et al.</i> (2016)
fHBP	Nm	Factor H	Serum survival	Madico <i>et al.</i> (2006); Seib <i>et al.</i> (2009)
NhhA	Nm	Laminin	ND	Scarselli <i>et al.</i> (2006)
		HSPGs	ND	Scarselli <i>et al.</i> (2006)
		Heparan sulphate	ND	Scarselli <i>et al.</i> (2006)
NspA	Nm	Factor H	Serum survival	Lewis <i>et al.</i> (2010)
NadA	Nm	Integrins	ND	Nägele <i>et al.</i> (2011)
PorA	Nm	C4Bp	Serum survival	Jarva <i>et al.</i> (2005)
PorB	Nm	TLR2-TLR1 heterodimer	Immune evasion	Massari <i>et al.</i> (2002)
		Factor H	Serum survival	Lewis <i>et al.</i> (2013)
Pili	Nm	CD46	Unknown	Gill and Atkinson (2004)
		PAFr	Adherence to human airway cells	Jen <i>et al.</i> (2013)
		Ng	Adherence to cervical epithelial cells	Jennings <i>et al.</i> (2011)
Unknown adhesin	Nm	Gangliosides	Adherence to red blood cells	Rumiantsev <i>et al.</i> (1990)
		ECM components	ND	Eberhard <i>et al.</i> (1998)

Nm, *Neisseria meningitidis*, Ng, *Neisseria gonorrhoeae*. HSPG, heparan sulfate proteoglycans; ECM, extracellular matrix; C4Bp, C4 binding protein; TLR, Toll like receptor; PAFr, platelet activating factor receptor; CR3, complement receptor 3. ND, not determined.

(Serruto *et al.* 2010) and heparan sulfate proteoglycans (Vacca *et al.* 2016) via an arginine-rich region. NHBA binding to heparin mediates increased serum resistance (Serruto *et al.* 2010), potentially via interactions between heparin and factor H or C4b binding protein (Sahu and Pangburn 1993; Serruto *et al.* 2010). NHBA is also involved in meningococcal adherence to epithelial cells, via binding to heparan sulfate proteoglycans (Vacca *et al.* 2016). Other minor adhesins such as NadA, Msf, NspA and NhhA also bind host glycans and/or glycoconjugates (see Table 3).

## CONCLUSION

Understanding the glycobiology of the pathogenic *Neisseria* is important for a better understanding of Neisserial pathogenesis, and may ultimately aid improved therapeutic and vaccine development. Surface glycans of the pathogenic *Neisseria* play a key role in resistance to host immune responses by reducing the efficiency of bacterial recognition and killing by the host, and by immune evasion via phase variation and molec-

ular mimicry. These pathogens also harness the host glyco-lyx, which provides opportunities for adherence that are key in colonisation (reviewed in Roupael and Stephens 2012). The full range of glyco-interactions remains to be discovered. A recent study reports that terminal sugars on bacterial LPS/LOS mediate interactions with host cells via direct binding to host glycans (Day *et al.* 2015), and this may also be the case in the pathogenic *Neisseria* and is the subject of current investigation in our laboratory.

## FUNDING

This work was supported by the Australian National Health and Medical Research Council (NHMRC) [Career Development Fellowship 1045235 to KLS, Program Grant 1071659 to MPJ, Project Grant 1099278 to KLS and CJD].

**Conflict of interest.** None declared.

## REFERENCES

- Anonsen JH, Borud B, Vik A et al. Structural and genetic analyses of glycan O-acetylation in a bacterial protein glycosylation system: evidence for differential effects on glycan chain length. *Glycobiology*. 2017, DOI: 10.1093/glycob/cwx032.
- Anonsen JH, Å Vik, Egge-Jacobsen W et al. An extended spectrum of target proteins and modification sites in the general O-linked protein glycosylation system in *Neisseria gonorrhoeae*. *J Proteome Res* 2012;11:5781–93.
- Apweiler R, Hermjakob H, Sharon N. On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database1. *Biochim Biophys Acta* 1999;1473:4–8.
- Banerjee A, Ghosh SK. The role of pilin glycan in neisserial pathogenesis. *Mol Cell Biochem* 2003;253:179–90.
- Bartley SN, Kahler CM. The glycome of *Neisseria* spp.: How does this relate to pathogenesis? In: Davies JK Kahler CM (eds.) *Pathogenic Neisseria: Genomics, Molecular Biology and Disease Intervention*. UK: Caister Academic Press, 2014.
- Bartley SN, Mowlaboccus S, Mullally CA et al. Acquisition of the capsule locus by horizontal gene transfer in *Neisseria meningitidis* is often accompanied by the loss of UDP-GalNAc synthesis. *Sci Rep* 2017;7:44442.
- Bhat KS, Gibbs CP, Barrera O et al. The opacity proteins of *Neisseria gonorrhoeae* strain MS11 are encoded by a family of 11 complete genes. *Mol Microbiol* 1991;5:1889–901.
- Bhattacharjee AK, Jennings HJ, Kenny CP. Structural elucidation of the 3-deoxy-D-manno-octulosonic acid containing meningococcal 29-e capsular polysaccharide antigen using carbon-13 nuclear magnetic resonance. *Biochemistry* 1978;17:645–51.
- Bhattacharjee AK, Jennings HJ, Kenny CP et al. Structural determination of the sialic acid polysaccharide antigens of *Neisseria meningitidis* serogroups B and C with carbon 13 nuclear magnetic resonance. *J Biol Chem* 1975;250:1926–32.
- Bhattacharjee AK, Jennings HJ, Kenny CP et al. Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and BO. *Can J Biochem* 1976;54:1–8.
- Billker O, Popp A, Gray-Owen SD et al. The structural basis of CEACAM-receptor targeting by neisserial Opa proteins. *Trends Microbiol* 2000;8:258–60.
- Bos MP, Kuroki M, Krop-Watorek A et al. CD66 receptor specificity exhibited by neisserial Opa variants is controlled by protein determinants in CD66 N-domains. *P Natl Acad Sci USA* 1998;95:9584–9.
- Bundle DR, Smith IC, Jennings HJ. Determination of the structure and conformation of bacterial polysaccharides by carbon 13 nuclear magnetic resonance. Studies on the group-specific antigens of *Neisseria meningitidis* serogroups A and X. *J Biol Chem* 1974;249:2275–81.
- Carbonnelle E, Hill DJ, Morand P et al. Meningococcal interactions with the host. *Vaccine* 2009;27(Suppl 2):B78–89.
- Chamot-Rooke J, Rousseau B, Lanternier F et al. Alternative *Neisseria* spp. type IV pilin glycosylation with a glyceramido acetamido trideoxyhexose residue. *P Natl Acad Sci USA* 2007;104:14783–8.
- Choudhury B, Kahler CM, Datta A et al. The structure of the L9 immunotype lipooligosaccharide from *Neisseria meningitidis* NMA Z2491. *Carbohydrate Research* 2008;343:2971–9.
- Claus H, Maiden MCJ, Maag R et al. Many carried meningococci lack the genes required for capsule synthesis and transport. *Microbiology* 2002;148:1813–9.
- Corbett A, Exley R, Bourdoulous S et al. Interactions between *Neisseria meningitidis* and human cells that promote colonisation and disease. *Expert Rev Mol Med* 2004;6:1–14.
- Day CJ, Tran EN, Semchenko EA et al. Glycan: glycan interactions: high affinity biomolecular interactions that can mediate binding of pathogenic bacteria to host cells. *P Natl Acad Sci USA* 2015;112:E7266–75.
- De Vries FP, Cole R, Dankert J et al. *Neisseria meningitidis* producing the Opc adhesin binds epithelial cell proteoglycan receptors. *Mol Microbiol* 1998;27:1203–12.
- Di Fabio JL, Michon F, Brisson J-R et al. Structure of the L1 and L6 core oligosaccharide epitopes of *Neisseria meningitidis*. *Can J Chem* 1990;68:1029–34.
- Eberhard T, Virkola R, Korhonen T et al. Binding to Human Extracellular Matrix by *Neisseria meningitidis*. *Infect Immun* 1998;66:1791–4.
- Edwards JL, Jennings MP, Apicella MA et al. Is gonococcal disease preventable? The importance of understanding immunity and pathogenesis in vaccine development. *Crit Rev Microbiol* 2016;42:928–41.
- Esko JD, Sharon N. Microbial lectins: hemagglutinins, adhesins, and toxins. In: Varki A, Cummings RD, Esko JD et al. eds. *Essentials of Glycobiology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2009.
- Estabrook MM, Griffiss JM, Jarvis GA. Sialylation of *Neisseria meningitidis* lipooligosaccharide inhibits serum bactericidal activity by masking lacto-N-neotetraose. *Infect Immun* 1997;65:4436–44.
- Finne J, Leinonen M, Mäkelä PH. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* 1983;2:355–7.
- Gamian A, Beurret M, Michon F et al. Structure of the L2 lipopolysaccharide core oligosaccharides of *Neisseria meningitidis*. *J Biol Chem* 1992;267:922–5.
- Gill DB, Atkinson JP. CD46 in *Neisseria pathogenesis*. *Trends in Molecular Medicine* 2004;10:459–65.
- Gault J, Ferber M, Machata S et al. *Neisseria meningitidis* Type IV pili composed of sequence invariable pilins are masked by multisite glycosylation. *PLoS Pathog* 2015;11:e1005162.
- Gómez-Duarte OG, Dehio M, Guzmán CA et al. Binding of vitronectin to opa-expressing *Neisseria gonorrhoeae* mediates invasion of HeLa cells. *Infect Immun* 1997;65:3857–66.
- Griffiss JM, Brandt BL, Saunders NB et al. Structural Relationships and Sialylation among Meningococcal L1, L8, and L3/7 Lipooligosaccharide Serotypes. *J Biol Chem* 2000;275:9716–24.
- Griffiths NJ, Hill DJ, Borodina E et al. Meningococcal surface fibril (Msf) binds to activated vitronectin and inhibits the terminal complement pathway to increase serum resistance. *Mol Microbiol* 2011;82:1129–49.
- Gronow S, Brade H. Invited review: Lipopolysaccharide biosynthesis: which steps do bacteria need to survive? *J Endotoxin Res* 2001;7:3–23.
- Hammerschmidt S, Hilde R, van Putten JP et al. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. *EMBO J* 1996a;15:192–8.
- Hammerschmidt S, Müller A, Sillmann H et al. Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (*siaD*): correlation with bacterial invasion and the outbreak of meningococcal disease. *Mol Microbiol* 1996b;20:1211–20.
- Harrison OB, Claus H, Jiang Y et al. Description and nomenclature of *Neisseria meningitidis* capsule locus. *Emerg Infect Dis* 2013;19:566–73.

- Harvey HA, Jennings MP, Campbell CA et al. Receptor-mediated endocytosis of *Neisseria gonorrhoeae* into primary human urethral epithelial cells: the role of the asialoglycoprotein receptor. *Mol Microbiol* 2001;**42**:659–72.
- Jarva H, Ram S, Vogel U et al. Binding of the Complement Inhibitor C4bp to Serogroup B *Neisseria meningitidis*. *J Immunol* 2005;**174**:6299–307.
- Jen FEC, Warren MJ, Schulz BL et al. Dual Pili Post-translational modifications synergize to mediate meningococcal adherence to platelet activating factor receptor on human airway cells. *PLoS Pathog* 2013;**9**:e1003377.
- Jennings HJ, Lugowski CW, Ashton FE et al. The structure of the capsular polysaccharide obtained from a new serogroup (I) of *Neisseria meningitidis*. *Carbohydrate Research* 1983;**112**:105–11.
- Jennings MP, Jen FEC, Roddam LF et al. *Neisseria gonorrhoeae* pilin glycan contributes to CR3 activation during challenge of primary cervical epithelial cells. *Cell Microbiol* 2011;**13**:885–96.
- Jennings MP, Srikhanta YN, Moxon ER et al. The genetic basis of the phase variation repertoire of lipopolysaccharide immunotypes in *Neisseria meningitidis*. *Microbiology* 1999;**145**:3013–21.
- Jennings MP, Virji M, Evans D et al. Identification of a novel gene involved in pilin glycosylation in *Neisseria meningitidis*. *Mol Microbiol* 1998;**29**:975–84.
- Jones C, Virji M, Crocker PR. Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. *Mol Microbiol* 2003;**49**:1213–25.
- Kahler CM, Martin LE, Tzeng Y-L et al. Polymorphisms in pilin glycosylation locus of *Neisseria meningitidis* expressing class II pili. *Infect Immun* 2001;**69**:3597–604.
- Kim JJ, Phillips NJ, Gibson BW et al. Meningococcal group A lipooligosaccharides (LOS): preliminary structural studies and characterization of serotype-associated and conserved LOS epitopes. *Infect Immun* 1994;**62**:1566–75.
- Klee SR, Nassif X, Kusecek B et al. Molecular and biological analysis of eight genetic islands that distinguish *Neisseria meningitidis* from the closely related pathogen *Neisseria gonorrhoeae*. *Infect Immun* 2000;**68**:2082–95.
- Kogan G, Uhrin D, Brisson J-R et al. Structural basis of the *Neisseria meningitidis* immunotypes including the L4 and L7 immunotypes. *Carbohydrate Research* 1997;**298**:191–9.
- Ku SC, Schulz BL, Power PM et al. The pilin O-glycosylation pathway of pathogenic *Neisseria* is a general system that glycosylates AniA, an outer membrane nitrite reductase. *Biochem Biophys Res Commun* 2009;**378**:84–9.
- Lewis LA, Ngampasutadol J, Wallace R et al. The meningococcal vaccine candidate Neisserial Surface Protein A (NspA) binds to factor H and enhances meningococcal resistance to complement. *PLoS Pathog* 2010;**6**:e1001027.
- Lewis LA, Vu DM, Vasudhev S et al. Factor H-dependent alternative pathway inhibition mediated by porin B contributes to virulence of *Neisseria meningitidis*. *MBio* 2013;**4**:e00339.
- Lewis LA, Ram S. Meningococcal disease and the complement system. *Virulence* 2014;**5**:98–126.
- Linden SK, Sutton P, Karlsson NG et al. Mucins in the mucosal barrier to infection. *Mucosal Immunol* 2008;**1**:183–97.
- Litschko C, Romano MR, Pinto V et al. The Capsule Polymerase CslB of *Neisseria meningitidis* serogroup L catalyzes the synthesis of a complex trimeric repeating unit comprising glycosidic and phosphodiester linkages. *J Biol Chem* 2015;**290**:24355–66.
- Liu TY, Gotschlich EC, Jonssen EK et al. Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharide. *J Biol Chem* 1971;**246**:2849–58.
- Loh E, Kugelberg E, Tracy A et al. Temperature triggers immune evasion by *Neisseria meningitidis*. *Nature* 2013;**502**:237–40.
- Madico G, Welsch JA, Lewis LA et al. The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance. *J Immunol* 2006;**177**:501–10.
- Massari P, Henneke P, Ho Y et al. Cutting Edge: Immune Stimulation by Neisserial Porins Is Toll-Like Receptor 2 and MyD88 Dependent. *J Immunol* 2002;**168**:1533–7.
- Merz AJ, So M. Interactions of pathogenic *Neisseriae* with epithelial cell membranes. *Annu Rev Cell Dev Biol* 2000;**16**:423–57.
- Michon F, Brisson JR, Roy R et al. Structural determination of the capsular polysaccharide of *Neisseria meningitidis* group I: a two-dimensional NMR analysis. *Biochemistry* 1985;**24**:5592–8.
- Michon F, Beurret M, Gamian A et al. Structure of the L5 lipopolysaccharide core oligosaccharides of *Neisseria meningitidis*. *J Biol Chem* 1990;**265**:7243–7.
- Mistretta N, Seguin D, Thiébaud J et al. Genetic and Structural Characterization of L11 Lipooligosaccharide from *Neisseria meningitidis* Serogroup A Strains. *J Biol Chem* 2010;**285**:19874–83.
- Moore J, Bailey SES, Benmechene Z et al. Recognition of saccharides by the OpaA, OpaD, and OpaB outer membrane proteins from *Neisseria meningitidis*. *J Biol Chem* 2005;**280**:31489–97.
- Moran AP, Prendergast MM, Appelmelk BJ. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. *FEMS Immunol Med Microbiol* 1996;**16**:105–15.
- Nägele V, Heesemann J, Schielke S et al. *Neisseria meningitidis* adhesin NadA targets beta1 integrins: functional similarity to *Yersinia* invasins. *J Biol Chem* 2011;**286**:20536–46.
- Pavliak V, Brisson JR, Michon F et al. Structure of the sialylated L3 lipopolysaccharide of *Neisseria meningitidis*. *J Biol Chem* 1993;**268**:14146–52.
- Power PM, Roddam LF, Rutter K et al. Genetic characterization of pilin glycosylation and phase variation in *Neisseria meningitidis*. *Mol Microbiol* 2003;**49**:833–47.
- Rabenstein DL. Heparin and heparan sulfate: structure and function. *Nat Prod Rep* 2002;**19**:312–31.
- Ram S, Sharma AK, Simpson SD et al. A novel sialic acid binding site on factor H mediates serum resistance of sialylated *Neisseria gonorrhoeae*. *J Exp Med* 1998;**187**:743–52.
- Rest RF, Frangipane JV. Growth of *Neisseria gonorrhoeae* in CMP-N-acetylneuraminic acid inhibits nonopsonic (opacity-associated outer membrane protein-mediated) interactions with human neutrophils. *Infect Immun* 1992;**60**:989–97.
- Rouphael NG, Stephens DS. *Neisseria meningitidis*: biology, microbiology, and epidemiology. *Methods Mol Biol* 2012;**799**:1–20.
- Rumiantsev SN, Avrova NF, Pospelov VF et al. The effect of gangliosides on the adhesive interaction of *Neisseria meningitidis* with human cells. *Zhurnal mikrobiologii, epidemiologii, i immunobiologii* 1990;**29**:29–32.
- Sa E Cunha C, Griffiths NJ, Virji M. *Neisseria meningitidis* Opc invasins binds to the sulphated tyrosines of activated vitronectin to attach to and invade human brain endothelial cells. *PLoS Pathog* 2010;**6**:e1000911.
- Sadarangani M, Pollard AJ, Gray-Owen SD. Opa proteins and CEACAMs: pathways of immune engagement for pathogenic *Neisseria*. *FEMS Microbiol Rev* 2011;**35**:498–514.



- Sahu A, Pangburn MK. Identification of multiple sites of interaction between heparin and the complement system. *Mol Immunol* 1993;**30**:679–84.
- Scarselli M, Serruto D, Montanari P et al. *Neisseria meningitidis* NhhA is a multifunctional trimeric autotransporter adhesin. *Mol Micro* 2006;**61**:631–44.
- Schneider H, Griffiss JM, Boslego JW et al. Expression of paragloboside-like lipooligosaccharides may be a necessary component of gonococcal pathogenesis in men. *J Exp Med* 1991;**174**:1601–5.
- Schulz BL, Jen FEC, Power PM et al. Identification of bacterial protein O-Oligosaccharyltransferases and their glycoprotein substrates. *PLoS One* 2013;**8**:e62768.
- Seib KL, Serruto D, Oriente F et al. Factor H-Binding protein Is important for meningococcal survival in human whole blood and serum and in the presence of the antimicrobial peptide LL-37. *Infect Immun* 2009;**77**:292–9.
- Seiler A, Reinhardt R, Sarkari J et al. Allelic polymorphism and site-specific recombination in the *opc* locus of *Neisseria meningitidis*. *Mol Microbiol* 1996;**19**:841–56.
- Serruto D, Spadafina T, Ciocchi L et al. *Neisseria meningitidis* GNA2132, a heparin-binding protein that induces protective immunity in humans. *P Natl Acad Sci USA* 2010;**107**:3770–5.
- Shewell LK, Ku SC, Schulz BL et al. Recombinant truncated AniA of pathogenic *Neisseria* elicits a non-native immune response and functional blocking antibodies. *Biochem Bioph Res Co* 2013;**431**:215–20.
- Singh B, Su Y-C, Riesbeck K. Vitronectin in bacterial pathogenesis: a host protein used in complement escape and cellular invasion. *Mol Microbiol* 2010;**78**:545–60.
- Spinosa MR, Progidia C, Talà A et al. The *Neisseria meningitidis* capsule is important for intracellular survival in human cells. *Infect Immun* 2007;**75**:3594–603.
- Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet* 2007;**369**:2196–210.
- Stimson E, Virji M, Makepeace K et al. Meningococcal pilin: a glycoprotein substituted with digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose. *Mol Microbiol* 1995;**17**:1201–14.
- Tsai CM, Civin CI. Eight lipooligosaccharides of *Neisseria meningitidis* react with a monoclonal antibody which binds lacto-N-neotetraose (Gal beta 1-4GlcNAc beta 1-3Gal beta 1-4Glc). *Infect Immun* 1991;**59**:3604–9.
- Tzeng Y-L, Datta AK, Strole CA et al. Translocation and surface expression of lipidated serogroup B capsular polysaccharide in *Neisseria meningitidis*. *Infect Immun* 2005;**73**:1491–505.
- Tzeng YL, Kahler CM, Zhang X et al. MisR/MisS two-component regulon in *Neisseria meningitidis*. *Infect Immun* 2008;**76**:704–16.
- Tzeng YL, Thomas J, Stephens DS. Regulation of capsule in *Neisseria meningitidis*. *Crit Rev Microbiol* 2016;**42**:759–72.
- Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014;**27**:587–613.
- Unkmeir A, Kämmerer U, Stade A et al. Lipooligosaccharide and polysaccharide capsule: virulence factors of *Neisseria meningitidis* that determine meningococcal interaction with human dendritic cells. *Infect Immun* 2002a;**70**:2454–62.
- Unkmeir A, Latsch K, Dietrich G et al. Fibronectin mediates Opc-dependent internalization of *Neisseria meningitidis* in human brain microvascular endothelial cells. *Mol Microbiol* 2002b;**46**:933–46.
- Vacca I, Del Tordello E, Gasperini G et al. *Neisserial* heparin binding antigen (NHBA) contributes to the adhesion of *Neisseria meningitidis* to human epithelial cells. *PLoS One* 2016;**11**:e0162878.
- Van Der Kaaden A, Van Doorn-Van Wakeren JIM, Kamerling JP et al. Structure of the capsular antigen of *Neisseria meningitidis* serogroup H. *Eur J Biochem* 1984;**141**:513–19.
- Van Der Kaaden A, Gerwig GJ, Kamerling JP et al. Structure of the capsular antigen of *Neisseria meningitidis* serogrup K. *Eur J Biochem* 1985;**152**:663–8.
- van Putten JP, Paul SM. Binding of syndecan-like cell surface proteoglycan receptors is required for *Neisseria gonorrhoeae* entry into human mucosal cells. *EMBO J* 1995;**14**:2144–54.
- van Putten JpM, Robertson BD. Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. *Mol Microbiol* 1995;**16**:847–53.
- Varki A. Sialic acids in human health and disease. *Trends Mol Med* 2008;**14**:351–60.
- Varki A, Sharon N. Historical background and overview. In: Varki A Cummings RD, Esko JD et al. (eds) *Essentials of Glycobiology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2009.
- Varki A, Esko JD, Colley KJ. Cellular organization of glycosylation. *Essentials of Glycobiology*. In: Varki A, Cummings RD, Esko JD et al. (eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2009.
- Vik Å, Aas FE, Anonsen JH et al. Broad spectrum O-linked protein glycosylation in the human pathogen *Neisseria gonorrhoeae*. *P Natl Acad Sci USA* 2009;**106**:4447–52.
- Virji M. Pathogenic neisseriae: surface modulation, pathogenesis and infection control. *Nat Rev Microbiol* 2009;**7**:274–86.
- Virji M, Heckels JE, Potts WJ et al. Identification of epitopes recognized by monoclonal antibodies SM1 and SM2 which react with all pili of *Neisseria gonorrhoeae* but which differentiate between two structural classes of pili expressed by *Neisseria meningitidis* and the distribution of their encoding sequences in the genomes of *Neisseria* spp. *J Gen Microbiol* 1989;**135**:3239–51.
- Virji M, Makepeace K, Ferguson DJP et al. Carcinoembryonic antigens (CD66) on epithelial cells and neutrophils are receptors for Opa proteins of pathogenic neisseriae. *Mol Microbiol* 1996;**22**:941–50.
- Virji M, Makepeace K, Moxon ER. Distinct mechanisms of interactions of Opc-expressing meningococci at apical and basolateral surfaces of human endothelial cells; the role of integrins in apical interactions. *Mol Microbiol* 1994;**14**:173–84.
- Virji M, Makepeace K, Peak IRA et al. Opc- and pilus-dependent interactions of meningococci with human endothelial cells: molecular mechanisms and modulation by surface polysaccharides. *Mol Microbiol* 1995;**18**:741–54.
- Virji M, Saunders JR, Sims G et al. Pilus-facilitated adherence of *Neisseria meningitidis* to human epithelial and endothelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin. *Mol Microbiol* 1993;**10**:1013–28.
- WHO. *Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in Neisseria gonorrhoeae*. Geneva, Switzerland: World Health Organization, 2012.
- Wormann ME, Horien CL, Bennett JS et al. Sequence, distribution and chromosomal context of class I and class II pilin genes of *Neisseria meningitidis* identified in whole genome sequences. *BMC Genomics* 2014;**15**:253.
- Zhu P, Morelli G, Achtman M. The *opcA* and (*psi*)*opcB* regions in *Neisseria*: genes, pseudogenes, deletions, insertion elements and DNA islands. *Mol Microbiol* 1999;**33**:635–50.