Characterising the meningococcal glycointeractome: what's new?

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Body of article

Neisseria meningitidis is a Gram-negative bacterium that asymptotically colonises the upper respiratory tract of approximately 10-20% of the population. However, it can also cause life threatening invasive meningococcal disease that typically presents as meningitis or septicaemia [1]. Vaccines are available to prevent disease caused by five of the six main meningococcal serogroups (serogroups A, B, C, W, Y) and the bacteria is susceptible to antibiotics [2]. Regardless, there is still a high burden of disease, with an estimated 1.2 million cases and 135,000 deaths each year worldwide. This is likely due to several factors, including the incomplete uptake of available vaccines, the absence of a serogroup X vaccine, and the fact that invasive infection is often difficult to diagnose at early stages and can rapidly progress to a life-threatening disease before antibiotics can be administered. As such, there is an ongoing need for a better understanding of meningococcal pathogenesis to aid development of improved therapeutics and vaccines.

Carbohydrates (also called glycans, oligo/polysaccharides or sugars) have long been known to play an important role in host-pathogen interactions [3]. With respect to *N. meningitidis*, there are several glycoconjugates expressed on its surface; it has a polysaccharide capsule, a lipooligosaccharide (LOS), and glycosylated outer membrane proteins, including type IV fimbriae (pili) and a surface expressed nitrite reductase, called AniA [reviewed in 4, 5]. On the host side, human microenvironments encountered by *N. meningitidis* have a complex and extensive glycocalyx [6, 7] and association with host glycans has been reported in several studies of meningococcal virulence factors [reviewed in 4, 5]. These interactions include the serogroup B vaccine antigen NHBA that binds to heparin [8] and heparan sulfate proteoglycans (HSPGs) [9]. These interactions contribute to serum survival and adherence to epithelial cells, respectively. Another key example of glycan targeting by Neisserial adhesins are the opacity proteins, Opc and Opa, which have been reported to bind heparin, HSPGs, galactose, sialic acids, and the extracellular matrix glycoproteins fibronectin and vitronectin [reviewed in 4, 5].

Glycan array technology was recently used as a systematic approach to identify interactions between *N. meningitidis* and host glycans and thereby define the meningococcal glycointeractome. The serogroup B wild-type strain MC58 is reported to bind to 223 of the 367 glycan structures on the glycan array, including blood group antigens, mucins, gangliosides and glycosaminoglycans (GAGs) [10]. Of the interactions revealed in this study, only approximately 30% of the binding events can be explained by previously known meningococcal lectin activity. Newly identified interactions include binding to mannosyl structures, complex galactose (Gal) structures and hyaluronan GAGs. Using strains lacking expression of key outer membrane structures (i.e., pili, Opc and capsule mutants, and LOS variants), further glycan array studies were performed to determine the meningococcal surface factors responsible for the observed binding to host glycans. In addition, surface plasmon resonance (SPR) and isothermal calorimetry (ITC) analysis was performed with isolated versions of the same set of bacterial structures to confirm binding, and to determine the kinetics of the interactions.

Recent evidence suggests that high affinity glycan-glycan interactions may be a common mechanism of bacterial adherence to host cells [11]. To determine whether glycan-glycan interactions occur during *N. meningitidis*-host cell contact, the major surface glycoconjugates, polysaccharide capsule and LOS, were investigated. The structures of the polysaccharide capsules expressed by *N. meningitidis* strains is the basis for serogroup classification, and is the immunising antigen in the serogroup A, C, W and Y vaccines. The serogroup B capsular polysaccharide ((α 2-8)-linked polysialic acid) is similar to host glycans (e.g. neural cell adhesion molecule (NCAM)), and is therefore poorly immunogenic and has not been used as a vaccine antigen. Capsule provides the meningococcus with protection from innate and adaptive immune responses and is crucial for invasive disease [1]. Glycan array analysis showed that binding to 40 glycans was lost in a capsule knockout strain Δ 3 [12], relative to the serogroup B MC58 wild-type strain, suggesting that the

capsule may directly mediate binding to several glycans [10]. Furthermore, the $\mathcal{C}3$ acapsulate strain bound 43 additional structures relative to the capsulate wild-type strain, suggesting that some interactions are blocked by the presence of the dense high molecular weight polyanionic polysaccharide coating the bacterial surface. While both the wild-type and acapsulate strain bound glycans from all classes on the array, they differed in their ability to recognise blood group antigens. The acapsulate strain bound to blood group A antigen structures, while the capsulate wild-type strain bound to the B and H blood group antigens, indicating variability in blood type preference of acapsulate versus capsulate strains. Blood group antigens are widely distributed in the human body, and are among the most common terminal glycans found on red blood cells, airway epithelial cells and mucins. As such, there have been numerous studies investigating whether blood type or blood antigen secretion status can affect the clinical outcome or susceptibility to pathogenic bacteria and viruses [13]. These new findings may indicate a novel role of host blood group status in the carriage of either capsulate or acapsulate *N. meningitidis*.

The LOS of *N. meningitidis* is made up of lipid A, a core oligosaccharide (containing 3-deoxy-D-manno-oct-2-ulosonic acid and heptose residues), and variable short oligosaccharides chains. The differences in the composition of these oligosaccharides form the basis of meningococcal immunotyping (L1-L12) and results from the phase variable expression of several glycosyltransferases [14]. Glycan binding by two natural LOS phase variants was investigated, the L3 LOS and the truncated L8 LOS, which lacks the terminal lacto-*N*-neotetraose structure of L3 LOS due to the phase variation of the LgtA glycosyltransferase [14]. *N. meningitidis* strains expressing either the L8 or L3 immunotype LOS bound a broad range of glycans on the array with a total of 208 glycans common between the strains. Purified LOS structures were used to better characterise the direct binding of the LOS to glycan structures on the array. Glycans bound by L3 and L8 LOS were almost mutually exclusive. The GAG hyaluronan, which is widely distributed in the human body, was bound by both L3 and L8 LOS. This indicates that the LOS of *N. meningitidis* regardless of immunotype is one of the surface structures responsible for the hyaluronan binding noted in the initial MC58 wild-type array screen. L3 LOS bound short-chain host glycans displaying a terminal galactose, e.g. lacto-*N*-biose I, the Thomsen–Friedenreich (TF) antigen, and large chain fucosylated glycans containing an *N*-acetyllactosamine (Gal β 1-4GlcNAc) epitope, including lacto-*N*-fucopentaose III, a Lewis X pentasaccharide. L3 LOS also bound a α 2-8 linked sialic acid trisaccharide and the GAG heparin. SPR analysis revealed that the interaction between L3 LOS and the TF antigen is the highest affinity glycan-glycan interaction reported to date (K_D = 13nM). TF antigen is the most common mucin O-glycan core (core 1) structure and is found on most cell types [3]. Unlike L3 LOS, purified L8 LOS did not bind any glycans with terminal GlcNAc or glucose, and recognised mostly GAGs and sialylated glycans including colominic acid ((α 2-8)-linked polysialic acid), an analogue of the *N. meningitidis* serogroup B capsular polysaccharide. These direct glycan-glycan interactions suggest distinct roles for meningococcal immunotypes with respect to host-pathogen interactions and intra-meningococcal interactions.

To further investigate protein-glycan interactions during *N. meningitidis*-host cell contact, a comparison of the glycan binding profile of strains with and without Opc expression (strains $\mathcal{C}2$ and $\mathcal{C}9$, respectively [12]) was performed. This revealed a loss of binding to 43 glycans in the absence of Opc, which included terminal Gal structures, sialylated glycans and GAGs. ITC analysis revealed that several of these interactions were high affinity (with a dissociation constant (K_D) below 5 μ M). Opc bound 2,6 sialylactose (K_D of $\sim$ 710 nM) and sialyl LewisX (K_D of $\sim$ 590 nM) with affinities similar to the previously observed for these interactions [15]. In addition, novel glycan binding by Opc was observed to chondroitin-6-sulfate polymer (K_D $\sim$ 630 nM) and lacto-*N*-neotetraose (K_D $\sim$ 1.7 μ M) [10]. Chondroitin-6-sulfate is found in all human extra cellular matrix, and is the major GAG expressed within the central nervous system (CNS) [16], suggesting the potential for Opc-mediated meningococcal adherence to cells within the CNS during meningitis. Lacto-*N*-neotetraose is an integral component of glycosphingolipids such as paragloboside, and is

also the precursor of the ABO and P1 blood group antigens. As mentioned above, ABO Blood group antigens are found on epithelial cells and mucins and interactions with these glycans may be important for nasopharyngeal colonization.

Pili are adhesins, and in *Neisseria* pili glycosylation enhances adherence to epithelial cells and endothelial cells [17], and is required for platelet activating factor receptor-mediated adherence to human airway epithelial cells [18]. To investigate potential pili-based glycointeractions, two pili mutants were examined in a MC58 wild-type and capsule mutant background. Over 50 interactions were lost when comparing the pili mutant strain with the isogenic wild-type strain, regardless of the presence or absence of capsule. The ability of capsule to suppress meningococcal interactions with the host cells described above, and is thought to be due to the polyanionic nature of the polysaccharide. Capsule expression is crucial for meningococcal survival during invasive disease, while the non-encapsulated state is favoured during colonisation [1]. The observed variation in glycan binding by the different pili mutants suggests flexibility in the roles that pili play in these interactions, depending on disease stage. Interestingly, the non-encapsulated pili mutant lost binding to asialo GM1 and GalNAc β 1-4Gal, implying that meningococcal pili mediate these interactions. Asialo GM1 is highly expressed in regenerating respiratory epithelia and is a potential target for meningococcal adherence to these cells. Several other pathogens that colonise the respiratory tract are known to target asialo GM1 (or -GM2), and these interactions are also mediated by pili of *Pseudomonas aeruginosa* [19].

As the field of glycobiology has expanded, it has become apparent that the human glycome is extensive and is an important target for interactions with various bacterial pathogens. Recent analysis has revealed that *N. meningitidis* binds host glycans from many different structural and functional classes [10], highlighting the diverse glycointeractions that may occur during meningococcal colonisation and disease. The meningococcal proteins and/or glycans that are responsible for all the newly identified glycan interactions are currently under further investigation, and could potentially be targeted for the development of novel antibiotics or vaccines to prevent meningococcal disease.

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