The exploitation of human glycans by Group A Streptococcus

Anuk D. Indraratna1,2, Arun Everest-Dass3, Danielle Skropeta1,2, Martina Sanderson-Smith1,2,*

1 Illawarra Health and Medical Research Institute, Northfields Ave, Keiraville New South Wales 2522, Australia
2 School of Chemistry and Molecular Bioscience, Molecular Horizons, University of Wollongong, Northfields Avenue, Keiraville, New South Wales, 2522, Australia
3 Institute for Glycomics, Griffith University, Gold Coast Campus, Parklands Drive, Southport, Queensland, 4215, Australia

*Corresponding author: Illawarra Health and Medical Research Institute, Bld 32, University of Wollongong, Northfields Avenue, Keiraville, New South Wales, 2522, Australia. Tel: +61 2 45981935, E-mail: martina@uow.edu.au

Abstract

Host carbohydrates, or glycans, have been implicated in the pathogenesis of many bacterial infections. Group A Streptococcus (GAS) is a Gram-positive bacterium that readily colonises the skin and oropharynx, and is a significant cause of mortality in humans. While the glycointeractions orchestrated by many other pathogens are increasingly well-described, the understanding of the role of human glycans in GAS disease remains incomplete. Although basic investigation into the mechanisms of GAS disease is ongoing, several glycointeractions have been identified and are examined herein. The majority of research in this context has focussed on bacterial adherence, however, glycointeractions have also been implicated in carbohydrate metabolism; evasion of host immunity; biofilm adaptations; and toxin-mediated haemolysis. The involvement of human glycans in these diverse avenues of pathogenesis highlights the clinical value of understanding glycointeractions in combatting GAS disease.

Keywords: Group A Streptococcus, glycans, glycointeractions, N-glycans, glycosidases, bacteria

Introduction

Carbohydrates, or glycans, are the most abundant macromolecules in nature (Nelson and Cox 2013). In the cellular environment, glycans are abundant at epithelial surfaces and mediate many vital biological processes (Varki 1993). While carbohydrates can exist in a number of forms, the term ‘glycan’ commonly refers to oligosaccharides built enzymatically from a limited array of monosaccharide substrates. Glycans can occur freely, but more commonly occur as conjugates upon other biomolecules such as lipids and proteins. It is estimated that over 50% of human proteins are post-translationally modified by glycosylation, whereby glycans are typically conjugated to proteins in the endoplasmic reticulum and processed in the Golgi (Apweiler, Her-Terris and Simons 1995; Shivshankar et al. 2019). Glycosylation influences the proteome in many ways, including cellular localisation; enzymatic function and substrate specificity; membrane receptor specificity; protein folding; and quality control (Scheiffele, Peranen and Simons 1995; Skropeta 2009; Hebert and Molinari 2012; Xu and Ng 2015; de Haas et al. 2020). In addition to modulating protein structure and function, glycans mediate many crucial interactions between cells (reviewed in Varki 2001). Glycans added to asparagine residues are termed N-glycans, whereas O-glycans are a structurally distinct class typically added to serine or threonine, and less frequently to tyrosine. Historically speaking, the study of carbohydrates in biology has lagged behind that of nucleic acids and proteins. Unlike genomics or proteomics, respectively, glycomics is limited by the inability to amplify material from the source, and by the low abundance of individual molecules within a sample. These difficulties are compounded by the remarkable diversity and intrinsic complexity generated from the non-linear, non-template-driven biosynthesis of glycans. Glycobiology is an evolving discipline and modern technologies have enabled researchers to partially overcome these challenges, allowing more sensitive and insightful investigations into the structures and roles of glycans. The growing appreciation of glycans is reflected in a range of biological contexts, in some cases leading to the development of therapeutic strategies (reviewed in Shriver, Raguram and Sasisekharan 2004; Shibuya et al. 2019; Tortorici et al. 2019). In the narrower context of microbiology, the importance of host glycans in pathogenesis is now well-established for a number of clinically important pathogens such as coronaviruses, Influenza A virus, Escherichia coli, Helicobacter pylori, Pseudomonas aeruginosa, Neisseria meningitidis, Neisseria gonorrhoeae and Streptococcus pneumoniae (Schembri et al. 2001; Shivshankar et al. 2009; King 2010; Magalhaes and Reis 2010; Wilks et al. 2012; Kattoo et al. 2016; Seib, Jennings and Day 2018; Semchenko et al. 2019; Tortorici et al. 2019). While further study is needed to elucidate the role of host glycans in Group A Streptococcal (GAS) disease, there are several GAS-mediated glycointeractions reported in the literature, which have not been reviewed.

Streptococcus pyogenes (Group A Streptococcus; GAS) is a Gram-positive species which is a major cause of infectious disease in humans. Superficial disease occurs primarily as pharyngitis, which
has an estimated annual incidence of 616 million cases worldwide (Carapetis et al. 2005), but may also manifest as scarlet fever or impetigo. Invasive infection involves penetration of GAS into deeper tissues, causing severe disease states with poor survival rates, such as necrotising fasciitis and streptococcal toxic shock syndrome. Auto-immune complications, such as acute glomerulonephritis, acute rheumatic fever, and rheumatic heart disease, also contribute heavily to the global burden of disease. In a 2005 report, the most recent global epidemiological survey to date, GAS caused over half a million deaths and ranked in the top ten infectious causes of mortality worldwide (Carapetis et al. 2005). Translational research such as vaccine development is ongoing in the effort to curtail GAS disease (Rivera-Hernandez et al. 2016; Dale and Walker 2020), and basic research into the host–pathogen relationship remains crucial in informing potential therapeutic and prophylactic strategies. The interactions governing this relationship are complex and involve a vast array of host elements, including glycans. The exploitation of epithelial glycans is now recognised as an important facet of pathogenesis in many microorganisms (reviewed in (Poole et al. 2018)). Relative to other infections, this aspect of GAS disease has not been investigated to the same extent. However, there is a growing body of work implicating human glycans in GAS adherence to host tissue, particularly via the streptococcal M protein (Section 2). Other types of glycointeractions discussed herein include the identified and putative scavenging of simple and complex carbohydrates from the pharynx (Section 3); evading host immunity through deglycosylation of IgG and interrupting GAG:chemokine interactions (Section 4); the involvement of host glycans in biofilm formation (Section 5); and the haemolytic activity of the GAS toxin Streptolysin O (Section 6).

Adherence to host tissue

Adherence of bacteria to epithelial surfaces is the first step in colonisation, and is a complex, multimodal process involving the specific binding of host molecules. Strong adherence is particularly necessary in the oropharynx, in order for bacteria to withstand the mechanical stresses of mucosal and salivary flow. A number of GAS adhesins have been characterised, including lipoteichoic acid (Ofek et al. 1975; Tylewska, Fischetti and Gibbons 1988; Courtney et al. 1992), collagen-binding protein (Visai et al. 1995) and laminin-binding protein (Terao et al. 2002). Several other virulence factors not primarily considered as adhesives have been ascribed adhesion properties, such as M protein (Sanford, Davison and Ramsay 1982; Tylewska, Fischetti and Gibbons 1988; Courtney et al. 1992; Courtney et al. 1994; Wang and Stinson 1994a; Okada et al. 1995; Eyal et al. 2003; Smeesters, McMillan and Srirakasph 2010) and the cysteine protease SpeB (Hytonen et al. 2001). In addition to the primarily proteinaceous ligands of these adhesins such as fibrinogen, several glycan targets have also been identified (Table 1).

Host glycosaminoglycans are bound by the M protein

The streptococcal M protein is a highly variable surface protein that forms the basis of categorising GAS isolates in three systems of increasing specificity: emm-pattern, emm-cluster and emm-type (Hollingshead et al. 1994; Sanderson-Smith et al. 2014). As the M protein is highly expressed by most GAS, it initially drew interest as a potential target for GAS vaccine development. Classically, the M protein has been regarded primarily as an immune evasion molecule (reviewed in (Fischetti 1989; Smeesters, McMillan and Srirakasph 2010)), though it is likely to have additional roles in GAS pathogenesis as it binds a wide range of ligands at the host cell surface and in extracellular matrix (ECM) in vitro. Its role in adherence to pharyngeal tissue, however, remains disputed in the literature. While there are several reports of M protein-negative mutants (M(-)) exhibiting decreased adherence to human cells in vitro (Ellen and Gibbons 1972; Tylewska, Fischetti and Gibbons 1988; Wang and Stinson 1994b; Schragger et al. 1998; De Oliveira et al. 2017), other authors report no difference in adherence between M(+) and wild-type (M(+)) (Caparon et al. 1991; Courtney et al. 1994). Conversely, a 2013 study demonstrated that a M(-) mutant demonstrated greater attachment to pharyngeal cells relative to its M(+) counterpart (Anderson et al. 2014), further obfuscating the role of M protein as an adhesin. The highly variable amino acid sequence between M proteins within the species (Smeesters, McMillan and Srirakasph 2010; McMillan et al. 2013) may explain these contrasting reports and underline the difficulties in achieving a consensus regarding the contribution of the M protein to adherence. This is further complicated by the inconsistency of in vitro models of infection employed in GAS research. Nevertheless, it is clear that the M protein is involved in adherence in some cases, and efforts to characterise and validate this role are ongoing.

Although the majority of research focusses on host proteins as ligands of the M protein, there are several descriptions of the M protein as a glycan-binding protein, or lectin. Glycosaminoglycans (GAGs) are among the non-protein ligands recognised by M protein and have been identified as targets of bacterial adhesins in several species (reviewed in (Wadstrom and Ljungh 1999; Menozzi et al. 2002)). They are large carbohydrate molecules composed primarily of polymerised amino and uronic sugars, expressed on eukaryotic cell surfaces and in ECM. Early research identified human GAGs, such as heparin, as targets of GAS binding, following observations that exogenous heparin and heparan sulphate inhibited attachment of GAS surface proteins to proteoglycans on basement membranes in vitro (Bergey and Stinson 1988). It has since been shown that the streptococcal M1 protein is responsible for the binding of various GAGs, including heparin, heparan sulphate and dermatan sulphate (Frick, Schmidtchen and Sjobring 2003) (Fig. 1). Dermatan sulphate, an abundant glycan in the skin, was bound by the majority of the 49 different M proteins tested. Furthermore, enzymatic removal of GAGs from human epithelia significantly reduced bacterial adherence, suggesting that the lectin properties of the M protein promote attachment to host tissue. However, the effect of removing these GAGs upon adherence was not investigated in M-negative mutants.

Histo-blood group antigens are involved in GAS adherence

Glycans other than GAGs are also targeted by streptococcal lectins. The M protein of throat-tropic M1 GAS specifically binds the ABO(H) histo-blood group antigens (HBGAs; Fig. 1) which are abundant in the oropharynx and saliva of most individuals (Slomani and Slomani 1978; Ravn and Dabelsteen 2000). HBGAs binding M1 is thought to be mediated by the B repeat domains (De Oliveira et al. 2017). In vitro experiments have shown that M1 protein exhibits the highest affinity for H antigen-bearing structures (De Oliveira et al. 2017). In the same study, the corresponding M1 GAS isolate demonstrated the greatest association with H-expressing primary human buccal epithelial (HBE) cells. Such data implicate the specific binding of these glycans by M protein as an important process in adherence. Furthermore, M3 and M12 proteins of emm-types frequently isolated from throat infections have also been shown to bind HBGAs with additional experiments showing binding of members of the Lewis antigen family,
which is another system of human blood group antigens (Fig. 1) (De Oliveira et al. 2019). The M protein-expressing GAS strains were shown to preferentially associate with HBE cells expressing H-antigen. While it is tempting to hypothesise that blood group may influence host susceptibility, it is unclear how influential HBGA binding is for adherence to the oropharynx, as epidemiological studies have not found significant correlations between GAS pharyngitis and HBGA status (Haverkorn and Goslings 1969; Cooling 2015). Interestingly however, there are reports of higher GAS carriage and tonsillectomy in non-secretors: individuals who lack oropharyngeal HBGA expression and comprise ∼20% of the population (Mourant and MAD 1954; Watkins 1980; Jaff 2010). Secretors, who represent the majority, possess Lewis A or X (Le(A), Le(B), Le(X)) sialic acid, whereas non-secretors instead express only Lewis A or X (Le(A), Le(B), Le(X)) sialic acid. Adherence was significantly reduced when GAS was pre-treated with sialic acid, but not other sugars, attenuated this binding. In a similar approach, adherence of GAS to human pharyngeal cells was shown to be mediated by M6 protein, as it was observed in wild-type (M(+-)) but not isogenic M-knockout mutant M(-) (Ryan, Pancholi and Fischetti 2001). Notably, pre-treatment of bacteria with sialic acid, but not other carbohydrates, attenuated this binding. In a similar approach, adherence of GAS to human pharyngeal cells was shown to be mediated by M6 protein, as it was observed in wild-type (M(+-)) but not isogenic M-knockout mutant M(-) (Ryan, Pancholi and Fischetti 2001).

### Sialic acid residues are specifically recognised by GAS lectins

The association of GAS with HBE cells has been shown to decrease significantly following the pre-treatment of cells with Sialidase A, an exoglycosidase which removes terminal sialic acids (De Oliveira et al. 2019). This suggests that host sialic acid residues are targeted by GAS lectins as a mechanism of adherence (Fig. 1). Sialic acid refers to a large family of nine-carbon sugar residues that exhibit marked structural and functional diversity (reviewed in Cohen and Varki 2011). The most commonly described sialic acid is N-acetylmuramic acid, which occurs abundantly upon glycolipids and termini of N- and O-glycans. As described in detail later, the utilisation of sialic acid is a strategy employed by many pathogenic species (reviewed in Severi, Hood and Thomas 2007). In addition to its acquisition in the context of immune evasion and nutrient acquisition, sialic acid may also be a target of bacterial adhesins, as seen in S. pneumoniae (Barthelson et al. 1995) and pathologically related species which occupies the nasopharynx.

The importance of sialic acid has been exhibited by the finding that an M6 GAS strain binds to human mucins through sialic acid residues (Ryan, Pancholi and Fischetti 2001). Mucins are heavily O-glycosylated glycoproteins abundant in saliva and pharyngeal epithelia (Berggård and Werner 1958; Leach 1963) and are also the predominant carrier of sialic acid and HBGAs in these tissues (Frakobphol et al. 1993). Mucin-binding by GAS was attributed to M6 protein, as it was observed in wild-type (M(+-)) but not the isogenic M knock-out mutant M(-) (Ryan, Pancholi and Fischetti 2001). Notably, pre-treatment of bacteria with sialic acid, but not other sugars, attenuated this binding. In a similar approach, adherence of M6 GAS to human pharyngeal cells was shown to be mediated by M6 protein and sialic acid interactions, particularly α2-6-linked sialic acid. Adherence was significantly reduced when GAS was pre-treated with sialic acid at a minimum inhibitory concentration...
Figure 1. Streptococcal M proteins contain lectin domains with varying affinity for diverse host glycan receptors. Proteoglycan components of the ECM such as heparan and dermatan sulphate have been identified as targets of various M proteins. Smaller motifs such as the Lewis and blood group antigens are bound by many M proteins, as are monosaccharides such as galactose, sialic acid and fucose.

In addition to sialic acid, other monosaccharides have been implicated in GAS adherence to host tissue (Fig. 1). Wild-type M1 and M12 GAS have been shown to bind to D-galactose-sepharose beads, whereas M(-) mutants exhibited no binding (Wadström and Tylewska 1982). The same authors reported impeded adherence of cells with monensin, an inhibitor of O-glycosylation (Tartakoff 1983; Yanagishita and Hascall 1985) during growth and pre-treatment of GAS with exogenous MUC1, respectively (Hafez, Abdel-Wahab and El-Fouhil 2010). MUC1 is known to be heavily glycosylated with diverse glycoprofiles depending on tissue type (Shimizu and Yamauchi 1982; Patton, Gendler and Spicer 1995; Parry et al. 2006), but the source of MUC1 in this study is ambiguous. It is thus unclear which carbohydrate moieties are responsible for the MUC1-mediated inhibition, though these data suggest that GAS lectins, including the M6 protein, bind to sialic acid. Similarly, it is apparent that the M protein binds mucin O-glycans which most likely display a range of monosaccharide ligands.

Other monosaccharides may be involved in GAS adherence

In treatment of cells with monensin, an inhibitor of O-glycosylation (Tartakoff 1983; Yanagishita and Hascall 1985) during growth and pre-treatment of GAS with exogenous MUC1, respectively (Hafez, Abdel-Wahab and El-Fouhil 2010). MUC1 is known to be heavily glycosylated with diverse glycoprofiles depending on tissue type (Shimizu and Yamauchi 1982; Patton, Gendler and Spicer 1995; Parry et al. 2006), but the source of MUC1 in this study is ambiguous. It is thus unclear which carbohydrate moieties are responsible for the MUC1-mediated inhibition, though these data suggest that GAS lectins, including the M6 protein, bind to sialic acid. Similarly, it is apparent that the M protein binds mucin O-glycans which most likely display a range of monosaccharide ligands.

Other monosaccharides may be involved in GAS adherence

In addition to sialic acid, other monosaccharides have been implicated in GAS adherence to host tissue (Fig. 1). Wild-type M1 and M12 GAS have been shown to bind to D-galactose-sepharose beads, whereas M(-) mutants exhibited no binding (Wadström and Tylewska 1982). The same authors reported impeded adherence of cells with monensin, an inhibitor of O-glycosylation (Tartakoff 1983; Yanagishita and Hascall 1985) during growth and pre-treatment of GAS with exogenous MUC1, respectively (Hafez, Abdel-Wahab and El-Fouhil 2010). MUC1 is known to be heavily glycosylated with diverse glycoprofiles depending on tissue type (Shimizu and Yamauchi 1982; Patton, Gendler and Spicer 1995; Parry et al. 2006), but the source of MUC1 in this study is ambiguous. It is thus unclear which carbohydrate moieties are responsible for the MUC1-mediated inhibition, though these data suggest that GAS lectins, including the M6 protein, bind to sialic acid. Similarly, it is apparent that the M protein binds mucin O-glycans which most likely display a range of monosaccharide ligands.
of M1 and M12 GAS to primary pharyngeal cells after pre-treating the bacteria with D-galactose, with the effect abrogated in corresponding M1 and M12 mutants. This effect in primary pharyngeal cells was not observed in HBE cells, suggesting the presence of distinct receptors for GAS adhesions between the cell types. Earlier work had demonstrated the binding of M6 protein to fucosylated glycoproteins on human laryngeal cells (Wang and Stinson 1994a). Pre-treatment of laryngeal cells with α-L-fucosidase reduced binding of M protein to the cell surface by 80%, which was the largest effect relative to other glycosidase pre-treatments. In whole bacterial experiments, various glycosidase treatments of HBE cells have been shown to attenuate association, in a manner dependent on both GAS serotype and blood group antigen expression (De Oliveira et al. 2019). Considered together, these data support the notion that host glycan expression patterns mediate GAS colonisation in an M protein-dependent manner. However, given the remarkable diversity of M proteins in GAS, research into a broad range of M proteins is warranted in order to holistically elucidate the importance of blood group antigen recognition in GAS adherence. The importance of the lectin-glycan interactions in mediating GAS adherence is exemplified by recent evidence, whereby N-glycans released from saliva were shown to inhibit association of M1, M3 and M12 GAS to HBE cells (De Oliveira et al. 2019). Glycan-based inhibitors may thus represent a promising area for the development of novel therapeutics.

Other streptococcal lectins

In addition to the M protein, two other GAS proteins have been suggested as lectin-like adhesins based on their capacity to bind host glycopolypeptides. Many host glycopolypeptides, such as fbronecin and laminin, which are expressed at the epithelial surface and/or in ECM, are implicated in GAS adherence (Simpson and Beachey 1983; Switalski et al. 1984; Stanislawski et al. 1985; Terao et al. 2002). However, these proteins are bound specifically by GAS adhesins in a peptide- rather than glycan-dependent manner. Hytönen et al. however, found that an initially unidentified streptococcal surface protein could bind a range of bovine glycopolypeptides (thryoglobulin, fetuin, asialofetuin, submaxillary mucin), a phenomenon they described as ‘strepadhesin’ activity (Hytönen et al. 2000). This protein exhibited no binding to non-glycosylated proteins (horse myoglobin and bovine erythrocyte carbonic anhydrase), suggesting that glycoprotein-binding was mediated by glycan, rather than peptide, recognition. These authors later convincingly demonstrated that the protein responsible was SpeB (Hytönen et al. 2001), a well-documented streptococcal cytotoxic protease with activity on both streptococcal and host proteins, previously thought to exist exclusively in a secreted form (Łukomska et al. 1997; Łukomska et al. 1998; Nelson, Garbe and Collin 2011). Importantly, it was demonstrated that binding to glycopolypeptides could be inhibited by other glycoproteins, suggesting SpeB recognises a common structure across the four glycopolypeptides tested. Given the sequence diversity of the glycopolypeptides tested, it is likely that this common structure is a glycan epitope. However, this hypothesis is yet to be conclusively proven as preliminary experimentation by Hytönen et al. with glycans derived from the parent glycoproteins did not appreciably inhibit glycoprotein binding by SpeB.

Strepadhesin activity was later observed in SpeB-negative GAS mutants. Another protein, termed PulA, was identified, with glycoprotein-binding behaviour highly similar to SpeB (Hytönen, Haataja and Finne 2003). It was identified as a pullulanase, a pullulan-degrading enzyme, based on functional assays and sequence similarity to other pullulanases. Pullulan is a polysaccharide in which maltotriose units (three glucose molecules with α1–4 glycosidic linkage) are polymerised by an α1–6 glycosidic linkage, produced by some fungal species but absent in humans (Cheng, Demirci and Catchmark 2011). As the strepadhesin phenomenon was initially observed in experiments using bovine glycopolypeptides, Hytönen et al. later corroborated its relevance in the human context by examining the association of isogenic GAS mutants deficient in SpeB and PulA, respectively, with four human oropharyngeal cell lines (Hytönen, Haataja and Finne 2006). Of the two GAS strains employed in this work (M2 and M49 Emm-), both PulA-deficient mutants associated significantly less with human cells from all four lines compared to the respective wild-type strains. The corresponding analysis of SpeB-knockout mutants did not reach statistical significance, suggesting that when both SpeB and PulA are expresses, PulA is the major mediator of strepadhesin activity. Interestingly, PulA contains the Gram-positive cell-wall anchoring LPXTG motif, strongly suggesting that it is expressed at the bacterial surface and directed towards host substrates. PulA was shown to be active against pullulan and starch in vitro, but not glycogen (Hytönen, Haataja and Finne 2003) which is the closest polysaccharide analogue in humans (Calder 1991).

PulA has since been further investigated, with more recent research by Shelburne III et al. demonstrating increased adherence to pharyngeal epithelial cells of wild-type M1 GAS relative to pulA knockouts (Shelburne III et al. 2011). The pulA gene is well-conserved within GAS (Davies et al. 2019) and interestingly, pulA transcripts are elevated in saliva, and detectable in humans with pharyngitis (Shelburne III et al. 2011). Moreover, pullulanase-specific antibodies are present in the sera of GAS-infected patients (Reid et al. 2001; Hytönen, Haataja and Finne 2003). PulA is thus expressed during infection, despite the absence of pullulan substrate in the human pharynx, suggesting that it contributes significantly to adherence. This hypothesis is supported by an in vivo study in which colonisation of the murine oropharynx was significantly decreased for an isogenic pulA knockout compared to wild-type (Shelburne III et al. 2011). PulA is thus likely to be an important example of a lectin involved in the early stages of GAS disease. Given its α-glucan degrading properties and its upregulation in saliva, it may well have other roles in pathogenesis, such as in metabolism.

Carbohydrate metabolism

GAS is an unusually versatile pathogen with regard to its capacity to persist and cause disease in a variety of microenvironments within the human host. Given this adaptability to diverse nutritional landscapes, the metabolic activities and requirements of GAS represents an area of interest in fundamental research (reviewed in (Pancholi and Caparon 2016)). GAS is primarily reliant on the glycolytic pathway (Pancholi and Caparon 2016), however, free glucose is not readily available in the two primary sites of GAS colonisation: the oropharynx (Gough et al. 1996; Wood et al. 2004); and skin (Calderon-Santiago et al. 2015). Within the GAS genome, a number of pathways for the utilisation of diverse carbohydrates have been identified, with approximately 15% of the GAS genome dedicated to carbohydrate transport and metabolism (Shelburne III et al. 2006; Davies et al. 2019). These includes several phosphoenolpyruvate-dependent phosphotransferase systems, responsible for the cellular import of non-glucose mono- and disaccharides (Fig. 2). Many of the encoded products are predicted to be glycoside hydrolases: enzymes responsible for cleavage of glycosidic bonds. Glycoside hydrolases are classified into numbered families (GH) based on sequence and structural
Figure 2. GAS sugar transport and major catabolite repression pathway in the oropharynx. Some mechanisms of degrading complex host substrates have been described and suggested (A). Glucose and several non-glucose substrates can be imported by GAS via dedicated transporters (B), many of which are phosphoenolpyruvate (PEP)-dependent phosphotransferase systems (PTS); multi-component assemblies illustrated as one unit for simplicity. In the oropharynx, low concentrations of glucose (1) result in decreased phosphorylation at the serine-46 (S46P) residue of heat-stable protein (HPr) (2). HPr-S46P is a co-repressor of carbon catabolite protein A (CcpA) which enhances binding to catabolite repression (cre) sites (3). Decreased phosphorylation of HPr results in complex disassembly (4), enabling increased transcription of sugar transport factors (5A) and altering transcription of non-metabolic virulence factors (5B). All sugars are depicted using the universal symbol nomenclature for glycans, except ascorbate which is not included in the nomenclature. Spy_xxxx assignments are based on GAS M1 strain MGAS5005, in which the N-acetylglucosamine transporter is yet to be identified. All genes are core GAS genes as per Davies et al. 2019 unless otherwise indicated. Gene not conserved in all strains,† Gene not functional in all strains,∗ Gene is conserved in all strains but is yet to be functionally characterised.

similarity, which enable prediction of activity and substrate specificity (Henrissat and Davies 1997; Lombard et al. 2014). The identification of a putative mannosidase, for example, within a bacterial genome, suggests the utilisation of mannose as a non-glucose carbohydrate. As this system relies on in silico prediction and not experimental validation, further research is necessary to conclusively establish the suite of glycan substrates available for GAS metabolism and holistically elucidate the relevant biochemical pathways. The foundations for such investigation are discussed herein.
Metabolism of simple carbohydrates

Maltose, mannose, lactose and galactose have been identified as simple carbohydrate alternatives to glucose, with GAS possessing the relevant enzymes required for their import and eventual entry into the glycolytic pathway (Shelburne III et al. 2006; Loughman and Caparon 2007; Shelburne III et al. 2007a, 2008a,b). However, the mechanisms by which GAS acquires mannose and galactose from the oropharyngeal environment remain unclear. Streptococcus pneumoniae expresses a number of exoglycosidases that provide a fermentable source of carbon in the absence of glucose (Burnaugh, Frantz and King 2008). Though these pathways should not be inferred to occur in GAS, S. pneumoniae is, in this regard, a well-studied Gram-positive inhabitant of the oropharynx. Metabolism of simple carbohydrates

While the distribution of genes associated with sialic acid acquisition and utilisation are limited almost entirely to mammalian colonisers (Almagro-Moreno and Boyd 2009), suggesting that sialic acid exploitation conveys a significant competitive advantage in colonisation. Although predominantly exhibited by commensals and pathogens that occupy the intestinal tract, the exploitation of host-derived sialic acid also occurs in non-intestinal pathogens such as N. gonorrhoeae and S. pneumoniae (Mubaiwa et al. 2017). The latter in particular has been investigated in-depth for its utilisation of host sugars (reviewed in (King 2010)). In addition to metabolism, the sialic acid-degrading enzyme NanA of S. pneumoniae has been implicated in adherence, biofilm formation and resistance to neutrophil-mediated killing (King, Hippe and Weiser 2006; Burnaugh, Frantz and King 2008; Parker et al. 2009; Brittan et al. 2012; Wren et al. 2017). Given the common niche of the two organisms, it is reasonable to hypothesise that the acquisition of sialic acid, along with other monosaccharides, occurs in GAS. A sialic acid-binding protein has been identified in GAS and its expression is upregulated in saliva (Shelburne III et al. 2005; Vir- taneva et al. 2005). The gene responsible is well-conserved (Davies et al. 2019), but it and its related genes have not been characterised. In addition to this, there are early reports of extracellular neuraminidase activity detected in GAS supernatants (Davis, Baig and Ayoub 1979; Potter et al. 1982), but these have not been further validated and no candidate genes have been proposed, to date. While there is thus some evidence of sialic acid utilisation by GAS, further investigation is needed. Given the importance of this capacity in many human colonisers, and the abundance of sia- lylated glycans in the oropharynx, an examination of sialic acid utilisation may be a promising area for novel research into GAS pathogenesis.

Metabolism of complex carbohydrates

In addition to monosaccharides, complex carbohydrates may also be the target of bacterial scavenging pathways. For example, S. pneumoniae can utilise human hyaluronic acid (HA) as a carbon source through the action of hyaluronate lyase (Marion et al. 2012). The core genome of GAS also encodes a hyaluronate lyase (hylA) (Davies et al. 2019), but the enzyme is inactive in the majority ofemm-types due primarily to a well-conserved amino acid substitution (Hynes, Johnson and Stokes 2009; Henningham et al. 2014). Importantly, GAS is encapsulated with HA, which is widely regarded as an essential virulence factor in invasive disease-causing isolates (Cunningham 2000; Cole et al. 2011). However, Henningham and colleagues identified that M4 GAS, anemm-type frequently associated with invasive disease, is unencapsulated and possess functional hylA (Henningham et al. 2014). The authors draw further attention to the ostensible mutual exclusivity between intact HA capsule and hylA; expression of functional hylA in invasive, capsulated M1 5448 GAS resulted in loss of capsule. The evolutionary reasons for why M4 GAS appears to have lost the capacity to synthesise HA capsule while retaining the functional hylA remains unclear, though its potential role in metabolism can be speculated. Active hyaluronidase expressed by GAS can degrade murine HA in vitro and in vivo, releasing the monosaccharide product N-acetylglucosamine (GlcNac) (Fig. 2A). (Starr and Engleberg 2006). It has been shown that minimal media supplemented solely with GlcNac supports the growth of wild-type GAS and the corresponding hyaluronidase-deficient mutant (Starr and Engleberg 2006). However, only the wild-type strain could grow in media where intact HA was the exclusive carbon source. Host or bacterial HA may therefore be an example of the diverse range of carbohydrate sources that GAS may exploit in the oropharynx (Fig. 2). Further investigation into HA as a substrate involved a
core gene (Davies et al. 2019) whose product had initially been annotated as a putative hyaluronidase based on the presence of a domain associated with hyaluronan degradation. Interestingly, a study of the GH84 enzyme encoded by hyl revealed that it had no activity upon hyaluronan, but was active upon O-GlcNAc moieties of mammalian glycoproteins (Sheldon et al. 2006). Notably, the authors present no examination of its activity upon N-linked glycans despite GlcNAc being a common terminus of pharyngeal N-glycans. The Gram-positive intestinal pathogen Clostridium perfringens possesses four genes encoding GH84 enzymes, all of which are active on N-glycans (Pluvinage et al. 2020). It is thus conceivable that the GH84 enzyme in GAS is active on the N- and O-glycans present in the pharyngeal epithelia. The example of C. perfringens also illustrates that the GH domain classification should be taken as predictive, rather than definitive, with regard to the substrate specificity of an enzyme. There are a number of putative glycosidases encoded within the GAS genome identified by the GH domain approach that are yet to be functionally validated (Table 2).

In a substantial body of work, Shelburne III et al. have extensively studied GAS carbohydrate metabolism. They demonstrated that growth in saliva significantly alters the transcriptome as compared to laboratory media, including increased transcription of genes responsible for complex carbohydrate acquisition and metabolism (Shelburne III et al. 2005). In vivo transcriptome analyses of GAS-infected nonhuman primates revealed similar trends (Virtaneva et al. 2005). The maE gene was selected for further investigation and was shown to be a key component of a complex carbohydrate transport system for maltodextrins (Shelburne III et al. 2006, 2007a), which are constituents of α-glucan polymers such as starch, pullulan and glycogen. While pullulan is not present in humans, glycogen and starch are abundant in the oropharynx. Maltotriose availability be due in part to amyA and PulA, two α-glucanases secreted by GAS (Shelburne III et al. 2009). Of the two, amyA is primarily responsible for starch hydrolysis (Shelburne III et al. 2009). However, the amyA gene is not well-conserved across the species and it appears dispensable for oropharyngeal colonisation in mice (Shelburne III et al. 2009). Instead, it is likely that host salivary amylases, active on polysaccharides from the diet, are primarily responsible for the accumulation of maltotriose in the oropharynx (Roberts and Whelan 1960; Humphrey and Williamson 2001). Streptococcal MaE was identified as the major transporter of maltotriose and longer maltodextrins and is expressed at substantially greater levels in saliva as compared to glucose- or nutrient-rich medium. Notably, maE knockout resulted in decreased growth in human saliva and decreased oropharyngeal colonisation in mice (Shelburne III et al. 2006). The scavenging of maltodextrins as a carbon source in a niche devoid of glucose is thus an important aspect of GAS pathogenesis. The transcription of genes responsible for complex carbohydrate utilisation is regulated through the involvement of factors such as catabolite control protein A, MalR and SplR/S, whose activities are linked to the production of seemingly unrelated virulence factors (Shelburne III et al. 2007b, 2008a, c, 2011). Thus, the differential availability of host glycans not only drives the regulation of carbohydrate-utilising pathways, but also directly influences virulence, a phenomenon observed in other streptococcal species (reviewed in Shelburne III et al., 2008b).

**Table 2.** Characterised and putative exoglycosidases within the core GAS genome. *Spy number refers to the annotated sequence numbers in the genome of M1 GAS strain MGAS5005 (accession no. CP000017.2). Gene annotations are from Tables S3 and S4 (Supporting Information) in (Davies et al. 2019). GH family refers to the classification of conserved or characterised glycoside hydrolases based on the presence of conserved amino acid domains (Henrissat and Davies 1997; Lombard et al. 2014).

| Spy* | Gene* | Annotated/characterised as | GH Family† | Substrate |
|------|-------|---------------------------|------------|-----------|
| 0476 | bgIA  | β-glucosidase              | GH1        | Not characterised |
| 1304 | lacZ  | β-galactosidase           | GH2        | Not characterised |
| 1312 | Unnamed| 6-phospho-β-glucosidase   | GH1        | 6-phospho-β-glucose (Stepper et al. 2013) |
| 1314 | hyl   | N-acetylglucosaminidase   | GH4        | O-GlcNAc (Sheldon et al. 2006) |
| 1316 | Unnamed| α-mannosidase             | GH12S      | Not characterised |
| 1317 | Unnamed| α-1-3-mannosyl linkages   | GH38       | α1-3-mannosyl linkages (Suits et al. 2010) |
| 1632 | lacG  | 6-phospho-β-galactosidase | GH1        | Not characterised |

**Immune evasion**

**EndoS**

In addition to adherence, host glycans may be exploited in the context of immune evasion. As an example, *S. pneumoniae* actively degrades host glycans to evade the immune system, via exoglycosidases whose activities confer resistance to neutrophils (Dalia, Standish and Weiser 2010). In contrast to exoglycosidases, which remove terminal monosaccharides from larger oligosaccharides, endoglycosidases cleave oligosaccharides, or remove the entire oligosaccharide from its parent glycoprotein. Human immunoglobulin G (IgG) is one such glycoprotein, bearing an N-glycan on an arginine residue (Asn-297) within the Fc region (Tao and Morrison 1989). Although all IgG molecules are modified with N-glycans in all healthy individuals, there is evidence that the identities of N-glycans at Asn-297 are subclass-specific (Bakovic et al. 2013; de Haan et al. 2017). In 15%–25% of individuals, a single N-glycan is also present in the Fab region (van de Boovenkamp et al. 2016). Many studies have collectively established the importance of Fc region N-glycosylation. In mice and in humans, IgG lacking Fc glycosylation has impaired or ablated capacities to activate complement, interact with macrophage Fc receptors; and trigger antibody mediated cytotoxicity (Koide. Nose and Muramatsu 1985; Nose and Wizell 1983; Leatherbarrow et al. 1985; Tao and Morrison 1989; Walker et al. 1989; Radaev and Sun 2001). Therefore, the ability to deglycosylate human IgG may confer a survival advantage for bacterial pathogens.

Endo-β-N-acetylglucosaminidase (EndoS) is a 108 kDa secreted protein expressed in GAS and encoded by *ndoS* with endoglycosidase activity specifically against the N-glycan on the Fc region of IgG (Fig. 3). It was first described by Collin and Olsén, who demonstrated its activity on both soluble IgG and IgG bound to the bacterial surface (Collin and Olsen 2001). These authors also showed that EndoS-deficient mutants could not hydrolyse IgG glycans, suggesting that EndoS is the only IgG-specific endoglycosidase ex-
pressed by GAS. It has been shown that functional EndoS significantly enhances survival of GAS in human blood ex vivo, an effect attributed in part to the observation that EndoS-treated human IgG exhibits impaired binding to Fc receptors and is unable to activate the classical complement pathway in vitro (Collin et al. 2002). EndoS has not been comprehensively studied in vivo, with only two animal studies reported, to date. In interpreting these studies, it should be noted that the mouse IgG N-glycome is more variable than in humans and glycosylation profiles are highly strain-specific (Krištić et al. 2018). The repertoire of glycans however, does not differ markedly between humans and mice, and when comparing certain subclasses there are notable similarities in glyco-patterns (Zaytseva et al. 2018).

The more recent and more comprehensive of the two mouse studies performed to date suggest EndoS provides an advantage in the context of adaptive immunity. Naegeli and colleagues immunised mice with M1 protein prior to subcutaneous infection with an M1 strain (AP1), reporting significantly greater survival, decreased weight loss and decreased bacterial load in the ndoS mutant, relative to wild-type (Naegeli et al. 2019). Notably, this effect was not apparent in naive mice, where the only difference observed between the two strains was a modest increase in splenic bacterial load following wild-type infection; weight loss, skin bacterial load and survival were similar between both groups (Naegeli et al. 2019). The ostensible irrelevance of ndoS in M1 infection of naïve mice is in accord with an earlier study (Sjogren et al. 2011), which employed wild-type M1 5448, a globally prevalent serotype frequently isolated from severe, invasive GAS disease (Aziz and Kotb 2008). The authors observed no significant difference in 10-day lethality following intraperitoneal infection between wild-type and ndoS mutant strains (Sjogren et al. 2011). In the same study, the mouse experiment was repeated with a less virulent, naturally EndoS-deficient strain (NZ131; M49 (McShan et al. 2008)) transformed with ndoS. The ndoS-expressing strain exhibited significantly greater 10-day lethality compared to the untransformed control. The authors speculate that this strain-biased effect of EndoS may be due to the overexpression of ndoS in the transformed strain, relative to S44 wild-type. Interestingly, it has since been shown that this M49 strain expresses a similar endoglycosidase, EndoS2 that has not been described for any other emm-type (Sjogren et al. 2013). EndoS2 acts on human α-1-acid glycoprotein in addition to IgG and has distinct activity and glycoform selectivity (Sjogren et al. 2015). Ostensibly only expressed in a minority of GAS strains, EndoS2 nevertheless represents a further example of an immunomodulatory interaction of host glycans in GAS infection. ndoS is highly conserved among GAS (Davies et al. 2019), suggesting an important role in bacterial fitness or pathogenicity. Notably, it has been shown that EndoS is not involved in nutrient acquisition (Collin and Olsen 2001), confirming its primary role as an immunomodulatory factor.

The importance of EndoS and EndoS2 in infection is unclear and warrants further scrutiny, particular in the context of human infection. In a study involving throat swab samples from GAS pharyngitis patients and blood samples from sepsis patients, the authors observed significantly higher levels of glycan-hydrolysed IgG relative to healthy controls in both kinds of samples (Naegeli et al. 2019).
Figure 4. Proposed model for the recognition of glycans in erythrocyte membranes by the pore-forming toxin SLO (A) and its co-toxin SPN (B). In the absence of streptococcal NAD+ glycohydrolase (SPN), secreted streptolysin O (SLO) polymers assemble at local or distal erythrocyte membranes by first binding to a galactose residue (A). Locally secreted SPN binds to a hypothetical glycan receptor and serves as the site for SLO polymer assembly in the absence of galactose (B). In both cases, the assembled SLO polymers interact with membrane cholesterol prior to pore insertion.

et al. 2019). The authors also reported decreased virulence of EndoS knockout mutant GAS (AP1, M1 serotype) in a mouse model. The inherent limitations of mouse models for GAS disease necessitates the examination of human samples, as conducted here. However, analyses were limited by patient availability and confounded by variation in the GAS strains responsible for infection. Further investigation is thus necessary to provide clarity about the expression and role of EndoS during infection. Human infection models, such as the Controlled Human Infection for Vaccination Against Streptococcus (CHIVAS) trial (Osowicki et al. 2019) exemplify a platform conducive for the rigorous exploration of these kinds of host-pathogen interactions without the limitations inherent in hospital samples. However, CHIVAS primarily facilitates the study of the host-pathogen relationship in the oropharynx, where EndoS is perhaps less relevant as compared to more severe infection. Thus, alternative approaches such as non-human primate studies may be necessary to elucidate the importance of EndoS in GAS pathogenesis.
SpyCEP

Streptococcus pyogenes cell envelope protease (SpyCEP) is a GAS virulence factor implicated in the pathogenesis of severe GAS disease. It is upregulated in invasive isolates and has been identified as a major cause of the hallmark diminished neutrophil recruitment at sites of necrotising infection (Edwards et al. 2005; Turner et al. 2009). SpyCEP cleaves the human chemokine Interleukin-8 (CXCL8) at the C-terminus, impeding neutrophil chemotaxis and recruitment. Although SpyCEP does not directly interact with host glycans, its capacity to confer protection from innate immunity relies heavily on the involvement of host GAGs in neutrophil functions. It has been established that the C-terminal portion of CXCL8 is responsible for binding host GAGs and that the N-terminal region interacts with its cognate receptors, CXCR1 and CXCR2 (Gayle 3rd et al. 1993; Ahuja, Lee and Murphy 1996). The mechanisms for how SpyCEP-mediated C-terminal cleavage impede chemokine:receptor interactions, however, have only recently been explored. It has been proposed by Goldblatt et al. that surface-bound GAGs sequester intact CXCL8 in close proximity to its receptors, enabling more efficient chemotaxis (Goldblatt et al. 2019). The authors evidence the necessity of the CXCL8:GAG interaction by demonstrating aberrant, impaired neutrophil migration following the enzymatic removal of host GAGs, or SpyCEP-mediated CXCL8 cleavage, respectively. SpyCEP thus exploits the dependence of neutrophils upon host glycans in the process of chemokine binding, effectively attenuating this crucial component of the innate immune response. SpyCEP and EndoS/EndoS2 are examples of mechanisms that interrupt host glycosylation to defend GAS against the human immune system. These mechanisms are protective in nature, as opposed to pathways that directly elicit host cell damage such as those mediated by bacterial toxins.

The Streptolysin O toxin

Many bacterial toxins interact with host glycan structures, including the cholera, Shiga, tetanus, and pneumolysin toxins (reviewed in (Schiavo and van der Goot 2001; Kulkarni, Weiss and Iyer 2010; Poole et al. 2018)). Streptolysin O (SLO) is a cholesterol-dependent, pore-forming cytolysin secreted by GAS with lytic activity against a range of mammalian cells (Sierig et al. 2003; Ahmad, Lutfullah and Ali 2011). Toxicity is exerted by SLO-mediated translocation of streptococcal NAD+ glycohydrolase (SPN) into the target cell (Madden, Ruiz and Caparon 2001; Bricker et al. 2002). While its recognition of membrane cholesterol has long since been established, SLO has more recently been identified as a lectin, with reported binding of a number of physiologically relevant human glycans. It was demonstrated that exogenous lacto-N-neotetraose (LNNT) inhibited SLO binding to erythrocyte membranes (Shewell et al. 2014). LNNT terminates with a galactose residue and is present as a ceramide on the surface of human neutrophils (Schwarting and Marcus 1977). Interestingly, it has been demonstrated that SLO-induced haemolysis can be inhibited by a galactose-binding lectin isolated from the marine invertebrate Aplysia kurodai (AKL) (Hasan et al. 2014). This inhibition was dose-dependent and was suppressed with exogenous galactose, suggesting that AKL and SLO compete for terminal galactose in the erythrocyte membrane. Further research showed that mutation of SLO residues at the predicted glycan-binding sites impaired or abolished haemolytic activity on erythrocytes, despite retaining affinity to target cell membranes (Mozola and Caparon 2015). Furthermore, SLO was unable to form pores in cells lacking galactose-containing glycoproteins or glycolipids. Taken together, these results strongly implicate the involvement of host galactose in SLO pore formation and haemolysis. Co-expression of SPN rescued pore-forming ability in the absence of galactose, indicating that galactose is not necessary for SLO binding in the presence of SPN (Fig. 4B). However, SPN is also predicted to contain a glycan-binding domain (Kelley and Sternberg 2009; Smith et al. 2011) and notably, point mutation within this putative domain abolished pore-forming ability. Considered together, these studies suggest that the haemolytic activity exerted by SLO and SPN relies on recognition of host membrane glycans.

Later research employing glycan microarray analysis, a technique increasingly utilised in GAS-glycome research (Indraratna et al. 2020), revealed that SLO binds a range of host glycans, with especially high affinity for B antigen type IV pentasaccharide (Shewell et al. 2020). Although B type IV pentasaccharide does not occur on erythrocytes, it is a galactose-bearing oligosaccharide similar to type I or II antigen abundant on erythrocyte membranes in individuals of B blood type. Notably, pre-incubation of SLO with this pentaose completely abolished haemolytic activity (Shewell et al. 2020). Further research may inform the therapeutic plausibility of harnessing this glycan-mediated negation of SLO, given its contribution to invasive GAS disease.

Biofilms

The formation of biofilms in both commensal and pathogenic contexts is an important aspect of the host-bacteria relationship. Biofilms exhibit markedly different characteristics to their planktonic counterparts, including phenotypic heterogeneity, resistance to host immunity, presence of persister cells and antibiotic tolerance, all of which contribute to increased virulence (reviewed in Dufour, Leung and Lévesque 2010). Although there is notable paucity of research in the roles of and processes concerning GAS biofilms, there is considerable evidence of biofilm formation and consequent antibiotic tolerance in clinical GAS infections (reviewed in Vyas et al. 2019). The involvement of host and bacterial glycans with respect to biofilm formation and phenotype is a niche that is well-explored in only a few pathogenic species, such as P. aeruginosa (Tielker et al. 2005; Wheeler et al. 2019) and S. pneumoniae (Trappetti et al. 2009; King 2010). With regard to the former, several glyco-based molecules have shown biofilm-modulating properties in vitro, including a C-fucosyl derivative, rhamnose-binding protein and mannose-conjugated polymers (Consoli et al. 2011; Fu et al. 2019; Limquoco et al. 2020). There has been far less investigation into the role of host glycans in GAS biofilm formation, despite the abundance of glycans in the oropharynx. One 2020 study however, demonstrated a change in GAS biofilm phenotype following modification of the host surface glycome in vitro (Vyas et al. 2020). Indiscriminate removal of N-glycans from the surface of human pharyngeal monolayers resulted in increases in biofilm biomass. Further experiments demonstrated that enzymatic removal of terminal mannose and sialic acid residues, respectively, also resulted in increases in biofilm biomass across a diverse selection of emm-types. Moreover, M12 biofilms grown on these exoglycosidase pre-treated surfaces resulted in a two-fold increase in penicillin tolerance relative to control biofilms. The mechanisms by which host glycans influence antibiotic susceptibility are yet to be uncovered but represent a promising avenue for future innovative research.

Concluding remarks

The involvement of the host glycome in GAS infection remains only partially uncovered. However, it is clear that many streptococcal factors exploit host glycans in achieving a range of
pathogenic outcomes, including adherence to host tissue; carbohydrate acquisition within the oropharyngeal niche; degradation of host IgG; biofilm formation and SLO-mediated haemolysis. The examination of the many glycointeractions involved in these processes also reveals the substantial paucities in the understanding of the role of human carbohydrates in GAS disease. Many of these gaps represent promising opportunities for novel research into GAS pathogenesis and more generally, may advance our broader understanding of the host–pathogen relationship.

**Funding**

AI is the recipient of an Australian Postgraduate Award.

**Conflicts of interest.** The authors have no conflicts of interest to declare.

**REFERENCES**

Ahmad A, Lutfullah G, Ali R. Structural and functional features of Streptolysin O. *Int J Bioinf Res Appl* 2011; 7: 427–44.

Ahuja SK, Lee JC, Murphy PM. CXC chemokines bind to unique sets of selectivity determinants that can function independently and are broadly distributed on multiple domains of human interleukin-8 receptor B: determinants of high affinity binding and receptor activation are distinct. *J Biol Chem* 1996; 271: 225–32.

Almagro-Moreno S, Boyd EF. Insights into the evolution of sialic acid catabolism among bacteria. *BMC Evol Biol* 2009; 9: 118.

Anderson EL, Cole JN, Olson J et al. The fibrinogen-binding M1 protein reduces pharyngeal cell adherence and colonization phenotypes of M1T1 Group A Streptococcus. *J Biol Chem* 2014; 289: 3539–46.

Apweiler R, Hermjakob H, Sharon N. On the frequency of pro-alpha2,3-sialyltransferase activity within the oropharyngeal niche; degradation and receptor activation are distinct. *J Biol Chem* 1996; 271: 225–32.

Ahuja SK, Lee JC, Murphy PM. CXC chemokines bind to unique sets of selectivity determinants that can function independently and are broadly distributed on multiple domains of human interleukin-8 receptor B: determinants of high affinity binding and receptor activation are distinct. *J Biol Chem* 1996; 271: 225–32.

Almagro-Moreno S, Boyd EF. Insights into the evolution of sialic acid catabolism among bacteria. *BMC Evol Biol* 2009; 9: 118.

Anderson EL, Cole JN, Olson J et al. The fibrinogen-binding M1 protein reduces pharyngeal cell adherence and colonization phenotypes of M1T1 Group A Streptococcus. *J Biol Chem* 2014; 289: 3539–46.

Apweiler R, Hermjakob H, Sharon N. On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1999; 1473: 4–8.

Aziz RK, Kotb M. Rise and persistence of global M1T1 clone of Streptococcus pyogenes. *Emerg Infect Dis* 2008; 14: 1511–7.

Bakovic MP, Selman MH, Hoffmann M et al. High-throughput IgG fc N-glycosylation profiling by mass spectrometry of glycopeptides. 2013; 12: 821–31.

Barthelson R, Mobasser A, Zopf D et al. Adherence of Streptococcus pneumoniae to respiratory epithelial cells is inhibited by sialylated oligosaccharides. *Infect Immun* 1998; 66: 1439–44.

Bergery EJ, Stinson MW. Heparin-inhibitable basement membrane-binding protein of Streptococcus pyogenes. *Infect Immun* 1988; 56: 1715–21.

Berggaard I, Werner IAOS. The carbohydrate constituents of human saliva. 1958; 16: 43–50.

Bricker AL, Cywes C, Ashbaugh CD et al. NAD+-glycohydrolase acts as an intracellular toxin to enhance the extracellular survival of group a Streptococcus. 2002; 44: 257–69.

Brittan JL, Buckeridge TJ, Finn A et al. Pneumococcal neutramidase A: an essential upper airway colonization factor for Streptococcus pneumoniae. *Mol Oral Microbiol* 2012; 27: 270–83.

Burnaugh AM, Frantz LJ, King SJ. Growth of Streptococcus pneumoniae on human glycoconjugates is dependent upon the sequential activity of bacterial exoglycosidases. *J Bacteriol* 2008; 190: 221–30.

Calder PC. Glycogen structure and biogenesis. *Int J Biochem* 1991; 23: 1335–52.

Calderon-Santiago M, Priego-Capote F, Turck N et al. Human sweat metabolomics for lung cancer screening. *Anal Bioanal Chem* 2015; 407: 5381–92.

Caparon MG, Stephens DS, Olsen A et al. Role of m protein in adherence of Group A Streptococcus. *Infect Immun* 1991; 59: 1811–7.

Carapetis JR, Steer AC, Mulholland EK et al. The global burden of group A Streptococcal diseases. *Lancet Infect Dis* 2005; 5: 685–94.

Cheng KC, Demirci A, Catchmark JM. Pullulan: biosynthesis, production, and applications. *Appl Microbiol Biotechnol* 2011; 92: 29–44.

Cohen M, Varki AJ. O-ajoib. The sialome—for more than the sum of its parts. 2010; 14: 455–64.

Cole JN, Barnett TC, Nizet V et al. Molecular insight into invasive group A Streptococcal disease. *Nat Rev Microbiol* 2011; 9: 724–36.

Collin M, Olsen A. EndoS, a novel secreted protein from Streptococcus pyogenes with endoglycosidase activity on human IgG. *EMBO J* 2001; 20: 3046–55.

Collin M, Svensson MD, Sjoholm AG et al. EndoS and SpeB from Streptococcus pyogenes inhibit immunoglobulin-mediated opsonophagocytosis. *Infect Immun* 2002; 70: 6646–51.

Consoli GML, Granata G, Cafiso V et al. Multivalent calixarene-based C-fucosyl derivative: a new *Pseudomonas aeruginosa* biofilm inhibitor. *Tetrahedron Lett* 2011; 52: 5831–4.

Cooling L. Blood groups in infection and host susceptibility. *Clin Microbiol Rev* 2015; 28: 801–70.

Courtney HS, Bronze MS, Dale JB et al. Analysis of the role of M24 protein in Group-a Streptococcal adhesion and colonization by use of omega-interpon mutagenesis. *Infect Immun* 1994; 62: 4868–73.

Courtney HS, von Hunolstein C, Dale JB et al. Lipoteichoic acid and m protein: dual adhesins of Group A Streptococci. *Microbiol Pathog* 1992; 12: 199–208.

Cunningham MW. Pathogenesis of group A Streptococcal infections. *Clin Microbiol Rev* 2000; 13: 470–511.

Dale JB, Walker MJ. Update on group a Streptococcal vaccine development. *Curr Opin Infect Dis* 2020; 33: 244.

Dalia AB, Standish AJ, Weiser JN. Three surface exoglycosidases from Streptococcus pneumoniae, NanA, BgaA, and StrH, promote resistance to opsonophagocytic killing by human neutrophils. *Infect Immun* 2010; 78: 2108–16.

Davies MR, McIntyre L, Mutreja A et al. Atlas of Group A Streptococcal vaccine candidates compiled using large-scale comparative genomics. *Nat Genet* 2019; 51: 1035–43.

Davis L, Baig MM, Ayoub EM. Properties of extracellular neuraminidase produced by Group A Streptococcus. *Infect Immun* 1979; 24: 780–6.

de Haan N, Reiding KR, Krištić J et al. The N-glycosylation of mouse immunoglobulin g (IgG)-fragment crystallizable differs between IgG subclasses and strains. 2017; 8: 608.

de Haas P, Hendriks W, Lefeber DJ et al. Biological and technical challenges in unraveling the role of N-Glycans in immune receptor regulation. *Front Chem* 2020; 8: 55.

De Oliveira DM, Hartley-Tassell L, Everest-Dass A et al. Blood group antigen recognition via the Group A Streptococcal m protein mediates host colonization. *mBio* 2017; 8.

De Oliveira DMP, Everest-Dass A, Hartley-Tassell L et al. Human glycan expression patterns influence Group A Streptococcal colonization of epithelial cells. *FASEB J* 2019; 33: 10808–18.

Dube DH, Bertozzi CR. NDRd. Glycans in cancer and inflammation—potential for therapeutics and diagnostics. 2005; 4: 477–88.

Dufour D, Leung Y, Lévesque CMJ et al. Bacterial biofilm: structure, function, and antimicrobial resistance. 2010; 22: 2–16.

Edwards RJ, Taylor GW, Ferguson M et al. Specific C-terminal cleavage and inactivation of interleukin-8 by invasive disease isolates of Streptococcus pyogenes. *J Infect Dis* 2005; 192: 783–90.

Ellen RP, Gibbons RJ. M protein-associated adherence of Streptococcus pyogenes to epithelial surfaces: prerequisite for virulence. *Infect Immun* 1972; 5: 826–30.
Eyal O, Jadoun J, Bitler A et al. Role of M3 protein in the adherence and internalization of an invasive Streptococcus pyogenes strain by epithelial cells. FEMS Immun Med Microbiol 2003;38:205–13.

Fischetti VA (1989) Streptococcal m protein: molecular design and biological behavior. Clin Microbiol Rev 2:285–314.

Frick IM, Schmidtchen A, Sjobring U. Interactions between m proteins of Streptococcus pyogenes and glycosaminoglycans promote bacterial adhesion to host cells. Eur J Biochem 2003;270:2303–11.

Fu T-K, Ng S-K, Chen Y-E et al. Rhamnose binding protein as an antibacterial agent—Targeting biofilm of Pseudomonas aeruginosa. Mar Drugs 2019;17:355.

Gayle R, Sleath PR, Srinivason S et al. Importance of the amino terminus of the interleukin-8 receptor in ligand interactions. J Biol Chem 1993;268:7283–9.

Goldblatt J, Lawrenson RA, Muir L et al. A requirement for neutrophil glycosaminoglycans in chemokine: receptor interactions is revealed by the streptococcal protease SpeCYP. 2019;202:3246–55.

Gough H, Luke GA, Beeley JA et al. Human salivary glucose analysis by high-performance ion-exchange chromatography and pulsed amperometric detection. Arch Oral Biol 1996;41:141–5.

Hafez MM, Abdel-Wahab KS, El-Fouhil DF. Augmented adherence and internalization of group a streptococcal pyogenes to influenza a virus infected MDCK cells. J Basic Microbiol 2010;50 Suppl 1: S46–57.

Hasan I, Watanabe M, Ishizaki N et al. A galactose-binding lectin isolated from aplysia kurodai (sea hare) eggs inhibits streptolysin-induced hemolysis. Molecules 2014;19:13990–4003.

Haverkorn MJ, Goslings WR. Streptococci, ABO blood groups, and secretor status. Am J Hum Genet 1969;26:360–75.

Hebert DN, Molinari M. Flagging and docking: dual roles for N-terminus of the interleukin-8 receptor in ligand interactions.

Hafner JC, Ruiz N, Caparon M. Cytolysin-mediated translocation of a multigene family in group a Streptococcus pyogenes. Mol Microbiol 2006;59:961–74.

Holleran MJ, Tekell J, Zaat AC. Augmented adherence and internalization of group a streptococcal pyogenes to influenza A virus infected MDCK cells. J Basic Microbiol 2010;50 Suppl 1: S46–57.

Henningham A, Yamaguchi M, Aziz RK et al. Mutual exclusivity of hyaluronan and hyaluronidase in invasive Group A Streptococcus. J Biol Chem 2014;289:32303–15.

Hollingshead SK, Arnold J, Readdy TL. Glycosyltransferases in the human airway: Current and future studies. Trends Biochem Sci 2012;37:404–10.

Hytonen J, Haataja S, Finne J. Streptococcus pyogenes glycoprotein-binding strepdesain activity is mediated by a surface-associated carbohydrate-degrading enzyme, pullulanase. Infect Immun 2003;71:784–93.

Hytonen J, Haataja S, Finne J. Use of flow cytometry for the adhesion analysis of Streptococcus pyogenes mutant strains to epithelial cells: investigation of the possible role of surface pullulanase and cysteine protease, and the transcriptional regulator rgg. BMC Microbiol 2006;6:18.

Hytonen J, Haataja S, Gerlach D et al. The SpeB virulence factor of streptococcus pyogenes, a multifunctional secreted and cell surface molecule with strepdesain, laminin-binding and cysteine protease activity. 2001;39:512–9.

Hytonen J, Haataja S, Isomaki P et al. Identification of a novel glycoprotein-binding activity in Streptococcus pyogenes regulated by the mga gene. Microbiology 2000;146:31–9.

Indraratna A, De Oliveira DM, Hartley-Tassell LE et al. Investigation of group a streptococcal interactions with host glycan structures using high-throughput techniques: glycan microarray analysis using recombinant protein and whole cells. In: Group A Streptococcus. Springer, 2020, 145–51.

Jaf MSJf. Higher frequency of secretor phenotype in o blood group–its benefits in prevention and/or treatment of some diseases. 2010;5:901.

Kuturo L, Nguyen-Khuong T, Everest-Dass A et al. Glycan involvement in the adhesion of Pseudomonas aeruginosa to tears. Exp Eye Res 2016;145:278–88.

Kelley LA, Sternberg ME. Protein structure prediction on the web: a case study using the phyre server. Nat Protoc 2009;4:363–71.

King SJ, Hippe KR, Weiser JN. Deglycosylation of human glycoconjugates by the sequential activities of exoglycosidases expressed by Streptococcus pneumoniae. Mol Microbiol 2006;59:961–74.

Kurlkarni AA, Weiss AA, Iyer SS. Glycan-based high-affinity ligands for toxins and pathogen receptors. Med Res Rev 2010;30:327–93.

Leach SA. Release and breakdown of sialic acid from human salivary mucin and its role in the formation of dental plaque. Nature 1963;199:486–7.

Leatherbarrow RJ, Rademaker TW, Dwek RA et al. Effector functions of a monoclonal aglycosylated mouse IgG2a: binding and activation of complement component C1r and interaction with human monocyte fc receptor. Mol Immunol 1985;22:407–15.

Limqueco E, Passos Da Silva D, Reichhardt C et al. Mannose-conjugated polymer targeting P. aeruginosa biofilms. ACS Infect Dis 2020;6:2866–71.

Lombard V, Golaconda Ramulu H, Drula E et al. The carbohydrate-active enzymes database (CAZY) in 2013. Nucleic Acids Res 2014;42:D490–495.

Loughman JA, Caparon MG. Comparative functional analysis of the lac operons in Streptococcus pyogenes. Mol Microbiol 2007;64:269–80.

Lukomski S, Burns EH, Wyde PR et al. Genetic inactivation of an extracellular cysteine protease (SpeB) expressed by Streptococcus pyogenes decreases resistance to phagocytosis and dissemination to organs. Infect Immun 1998;66:771–6.

Lukomski S, Sreevatsan S, Amberg C et al. Inactivation of Streptococcus pyogenes extracellular cysteine protease significantly decreases mouse lethality of serotype M3 and M49 strains. J Clin Invest 1997;99:2574–80.

Madden JC, Ruiz N, Caparon M. Cystolysin-mediated translocation (CMT): a functional equivalent of type III secretion in Gram-positive bacteria. Cell 2001;104:143–52.

Magalhaes A, Reis CA. Helicobacter pylori adhesion to gastric epithelial cells is mediated by glycan receptors. Braz J Med Biol Res 2010;43:611–8.

Marion C, Stewart JM, Tazi MF et al. Streptococcus pneumoniae can utilize multiple sources of hyaluronic acid for growth. Infect Immun 2012;80:1390–8.

McMillan DJ, Dreze PA, Vu T et al. Updated model of group a streptococcus m proteins based on a comprehensive worldwide study. Clin Microbiol Infect 2013;19:E222–229.
Pluvinage B, Massel PM, Burak K et al. Genotype sequence of a nephritogenic and highly transformable M49 strain of streptococcus pyogenes. J Bacteriol 2008;190:7773–85.

Menozzi FD, Pete K, Bifani P et al. Enhanced bacterial virulence through exploitation of host glycosaminoglycans. Mol Microbiol 2002;43:1379–86.

Mourant AE, MAD PJ TB. Distribution of histo-blood group antigens. J Cell Biol 1960;19:777–85.

Mozola CC, Caparon MG J. Dual modes of membrane binding direct pore formation by Streptolysin O. 2015; 97:1036–50.

Mubaiwa TD, Semchenko EA, Hartley-Tassell LE et al. The sweet side of the pathogenic Neisseria: the role of glycans interactions in colonisation and disease. Pathog Dis 2017;75:ftx063.

Naegeli A, Bratians E, Karlsson C et al. Streptococcus pyogenes evades adaptive immunity through specific IgG glycan hydrolysis. J Exp Med 2019;216:1615–29.

Nelson DC, Garbe J, Collin MJ et al. Cysteine proteinase SpeB from streptococcus pyogenes—a potent modulator of immunologically important host and bacterial proteins. 2011;392:1077–88.

Nelson DL, Cox MM. Lehninger Principles of Biochemistry. W.H. Freeman and Company, New York. 2013.

Nose M, Wigzell H. Biological significance of carbohydrate chains on mononuclear antibodies. Proc Natl Acad Sci 1983;80:6632–6.

Ofeik I, Beachey EH, Jefferson W et al. Cell membrane-binding properties of group a streptococcal lipoteichoic acid. J Exp Med 1975;141:990–1003.

Okada N, Liszewski MK, Atkinson JP et al. Membrane cofactor protein (CD46) is a keratinocyte receptor for the M-Protein of the Group A Streptococcus s. Proc Natl Acad Sci 1995;92:2489–93.

Osowicki J, Azzopardi KJ, Baker C et al. Controlled human infection for vaccination against Streptococcus pyogenes (CHIVAS): establishment of a group a Streptococcus pharyngitis human infection study. Vaccine 2019;37:3485–94.

Pancholi V, Caparon M. Streptococcus pyogenes metabolism. In: Ferretti J, Stevens DL, Fischetti VA (eds). Pathogenic and Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center, 2016.

Parker D, Soong G, Planet P et al. The NanA neuraminidase of Streptococcus pneumoniae is involved in biofilm formation. Infect Immun 2009;77:3722–30.

Parry S, Hanisch FG, Leir SH et al. N-Glycosylation of the MUC1 mucin in epithelial cells and secretions. Glycobiology 2006;16:623–34.

Patton S, Gendler SJ, Spicer AP. The epithelial mucin, MUC1, of milk, mammary gland and other tissues. Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes 1995;1241:407–23.

Pluvinage B, Massel PM, Burak K et al. Structural and functional analysis of four family 84 glycoside hydrolases from the opportunistic pathogen Clostridium perfringens. Glycobiology 2020;30:58–68.

Poole J, Day CJ, von Itzstein M et al. Glycointeractions in bacterial pathogenesis. Nat Rev Microbiol 2018;16:440–52.

Potter EV, Shaughnessy MA, Poon-King T et al. Streptococcal neuraminidase and acute glomerulonephritis. Infect Immun 1982;38:1196–202.

Prakopbil A, Leffer H, Fisher SJ et al. A novel molecular weight human mucin is the primary salivary carrier of ABH, lea, and leb blood group antigens. 1993;4:325–33.

Radaev S, Sun PD. Recognition of IgG by FC gamma receptor. The role of fc glycosylation and the binding of peptide inhibitors. J Biol Chem 2001;276:16478–83.

Ravn V, Dabelestein E. Tissue distribution of histo-blood group antigens. APMIS 2000;108:1–28.

Reid SD, Green NM, Buss JK et al. Multilocus analysis of extracellular putative virulence proteins made by Group A Streptococcus: population genetics, human serologic response, and gene transcription. Proc Natl Acad Sci 2001;98:7552–7.

Rivera-Hernandez T, Pandey M, Henningham A et al. Differing efficiencies of lead group a streptococcal vaccine candidates and full-length m protein in cutaneous and invasive disease models. MBio 2016;7.

Roberts PJ, Whelan WJ. The mechanism of carbohydrate action. S. Action of human salivary alpha-amylase on amylopectin and glycogen. Biochem J 1960;76:246–53.

Ryan PA, Pancholi V, Fischetti VA. Group A Streptococci bind to mucin and human pharyngeal cells through sialic acid-containing receptors. Infect Immun 2001;69:7402–12.

Sanderson-Smith M, De Oliveira DM, Guglielmini J et al. Systematic and functional classification of Streptococcus pyogenes that serves as a new tool for molecular typing and vaccine development. J Infect Dis 2014;210:1325–38.

Sanford BA, Davison VE, Ramsay MA. Fibrinogen-mediated adherence of Group A Streptococcus to influenza a virus-infected cell cultures. Infect Immun 1982;38:513–20.

Scheiffele P, Peranen J, Simons K. N-Glycans as apical sorting signals in epithelial-cells. Nature 1995;378:96–98.

Schembri MA, Kjaergaard K, Sokurenko EV et al. Molecular characterization of the Escherichia coli FimH adhesin. J Infect Dis 2001;183 Suppl 1:S28–31.

Schiavo G, van der Goot FG. The bacterial toxin toolkit. Nat Rev Mol Cell Biol 2001;2:530–7.

Schrager HM, Alberti S, Cywes C et al. Hyaluronic acid capsule carbohydrate utilization contributes to the persistence of Group A Streptococcus to CD44 on human keratinocytes. J Clin Invest 1998;101:1708–16.

Schwartz GC, Marcus DM. The reactions of antibodies to paragloboside (lacto-N-neotetraosylceramide) with human erythrocytes and lymphocytes. J Immunol 1977;118:1415–9.

Seib KL, Jennings MP, Day CJ. Characterizing the meningococcal glyco-interactome: what is new?. Future Medicine 2018;13:279–82.

Semchenko EA, Everest-Dass AV, Jen FE et al. Glycointeractome of Neisseria gonorrhoeae: identification of host glycans targeted by the gonococcus to facilitate adherence to cervical and urethral epithelial cells. mBio 2019;10:e01339–01319.

Severi E, Hood DW, Thomas GH. Sialic acid utilization by bacterial pathogens. Microbiology 2007;153:2817–22.

Shebloune SA, Davenport MT, Keith DB et al. The role of complex carbohydrate catabolism in the pathogenesis of invasive Streptococcus. Trends Microbiol 2008b;16:518–25.

Shebloune SA, Fang H, Okorafor N et al. MalE of Group A Streptococcus participates in the rapid transport of maltotriose and longer maltodextrins. J Bacteriol 2007a;189:2610–7.

Shebloune SA, Keith D, Horstmann N et al. A direct link between carbohydrate utilization and virulence in the major human pathogen group a streptococcus s. Proc Natl Acad Sci 2008c;105:1698–703.

Shebloune SA, Keith DB, Davenport MT et al. Contribution of AmyA, an extracellular α-glucan degrading enzyme, to group a streptococcal host-pathogen interaction. 2009;74:159–74.

Shebloune SA, Keith DB, Davenport MT et al. Molecular characterization of group a streptococcus maltodextrin catabolism and its role in pharyngitis. Mol Microbiol 2008a;69:436–52.

Shebloune SA, Okorafor N, Sitikiewicz I et al. Regulation of polysaccharide utilization contributes to the persistence of Group A Streptococcus in the oropharynx. Infect Immun 2007b;75:2981–90.

Shebloune SA, Sahasrobbajane P, Suber B et al. Niche-specific contribution to Streptococcal virulence of a malr-regulated carbohydrate binding protein. 2011;81:500–14.
Shelburne SA, Sumby P, Sitkiewicz I et al. Central role of a bacterial two-component gene regulatory system of previously unknown function in pathogen persistence in human saliva. Proc Natl Acad Sci 2005;102:16037–42.

Shelburne SA, Sumby P, Sitkiewicz I et al. Maltodextrin utilization plays a key role in the ability of Group A Streptococcus to colonize the oropharynx. Infect Immun 2006;74:4605–14.

Sheldon WL, Macauley MS, Taylor EJ et al. Functional analysis of a Group A Streptococcal glycoside hydrolase spy1600 from family 84 reveals it is a β-N-acetylgalactosaminidase and not a hyaluronidase. Biochem J 2006;399:241–7.

Shewell LK, Day CJ, Jen FE-C et al. All major cholesterol-dependent cytolysins use glycans as cellular receptors. 2020;6.eaaaz4926.

Shewell LK, Harvey RM, Higgins MA et al. The cholesterol-dependent cytolysins pneumolysin and Streptolysin O require binding to red blood cell glycans for hemolytic activity. Proc Natl Acad Sci 2014;111:E5312–5320.

Shimizu M, Yamauchi K. Isolation and characterization of mucin-like glycoprotein in human milk fat globule membrane. J Biochem 1982;91:515–24.

Shivshankar P, Sanchez C, Rose LF et al. Novel laminin-binding protein of Streptococcus pyogenes, lab, is involved in adhesion to epithelial cells. Infect Immun 2002;70:993–7.

Shuter AM. Perturbation of vesicular traffic with the carboxylic ionophore monensin. Clin Cell 1983;32:1026–8.

Terao Y, Kawabata S, Kunitomo E et al. Novel laminin-binding protein of Streptococcus pyogenes, lab, is involved in adhesion to epithelial cells. Infect Immun 1989;143:2595–601.

Tartakoff AM. Biological roles of glycans. Adv Drug Delivery Rev 2015;73:192–202.

Visai L, Bozzini S, Raucci G et al. Emerging role of the interleukin-8 cleaving enzyme SpyCEP in clinical Streptococcus pyogenes infection. J Infect Dis 2009;200:555–63.

Tylewsk SK, Fischetti VA, Gibbons RJ. The role of the M-protein to epithelial cells differs from that of lipoteichoic acid. 1988;16:209–16.

van de Bovenkamp FS, Hafkenscheid L, Kispens T et al. Emerging importance of IgG fab glycosylation in immunity. J Immunol 2016;196:1435–41.

Varki A. Biological roles of glycans. Glycobiology 2017;27:3–49.

Varki A. Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 1993;3:97–130.

Virtaneva K, Porcella SF, Graham MR et al. Longitudinal analysis of the Group A Streptococcus transcriptome in experimental pharyngitis in cynomolgus macaques. Proc Natl Acad Sci 2009;102:9014–9.

Vyas HK, Indraratna AD, Everest-Dass A et al. The role of glycans in pathogenic bacteria–potential for targeted covalent therapeutics and imaging agents. 2014;50:4659–73.

Wadstrom T, Ljung B. Glycosaminoglycan-binding microbial proteins in tissue adhesion and invasion: key events in microbial pathogenicity. J Med Microbiol 1999;48:223–33.

Wadstrom T, Tylewska SJCM. Glycoconjugates as possible receptors for Streptococcus pyogenes. 1982;7:343–6.

Walker MR, Lundy J, Thompson KM et al. Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition of
by human cells expressing FcγRI and/or FcγRII receptors. Biochem J 1989;259:347–53.

Wang JR, Stinson MW. M protein mediates Streptococcal adhesion to HEp-2 cells. Infect Immun 1994b;62:442–8.

Wang JR, Stinson MW. Streptococcal M6 protein binds to fucose-containing glycoproteins on cultured human epithelial cells. Infect Immun 1994a;62:1268–74.

Watkins WM. Biochemistry and genetics of the ABO, Lewis, and P blood group systems. Adv Hum Genet 1980;10:1–136.

Wheeler KM, Carcamo-Oyarce G, Turner BS et al. Mucin glycans attenuate the virulence of Pseudomonas aeruginosa in infection. Nature Microbiology 2019;4:2146–54.

Wilks S, de Graaf M, Smith DJ et al. A review of influenza haemagglutinin receptor binding as it relates to pandemic properties. Vaccine 2012;30:4369–76.

Wood DM, Brennan AL, Philips BJ et al. Effect of hyperglycaemia on glucose concentration of human nasal secretions. Clin Sci (Colch) 2004;106:527–33.

Wren JT, Blevins IK, Pang B et al. Pneumococcal neuraminidase a (NanA) promotes biofilm formation and synergizes with influenza a virus in nasal colonization and middle ear infection. Infect Immun 2017;85:e01044–01016.

Xu C, Ng DT. Glycosylation-directed quality control of protein folding. Nat Rev Mol Cell Biol 2015;16:742–52.

Yanagishita M, Hascall VC. Effects of monensin on the synthesis, transport, and intracellular degradation of proteoglycans in rat ovarian granulosa cells in culture. J Biol Chem 1985;260:5445–55.

Yesilkaya H, Manco S, Kadioglu A et al. The ability to utilize mucin affects the regulation of virulence gene expression in Streptococcus pneumoniae. FEMS Microbiol Lett 2008;278:231–5.

Zähner D, Hakenbeck RJ Job. The Streptococcus pneumoniae beta-galactosidase is a surface protein. 2000;182:5919–21.

Zaytseva OO, Jansen BC, Hanić M et al. M1gG Gly (mouse IgG glycosylation analysis)-a high-throughput method for studying Fc-linked IgG N-glycosylation in mice with nanoUPLC-ESI-MS. 2018;8:1–9.