



Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract

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ABSTRACT

Glycosylation is a key modification of proteins and lipids and is involved in most intermolecular and intercellular interactions. The gastrointestinal mucus gel is continuous and can be divided into two layers: a secreted loosely associated layer and a layer firmly attached to the mucosa. In addition, the membrane-bound glycosylated proteins and lipids create a glycocalyx, which remains adherent on each cell and is dynamic and responsive to the physiological state and environment of the cell. The secreted glycans form a mucus gel layer that serves as a physicochemical sensor and barrier network and is primarily composed of mucins and associated peptides. These glycans protect gut epithelial cells from chemical, biological and physical insults and are continuously renewed. Pathogens colonise and invade the host epithelial cells using protein–protein and glycan–lectin interactions. During the process of colonisation and infection, the glycosylation state of both host and pathogen change in response to the presence of the other. This complex modulation of glycan expression critically determines pathogenesis and the host response in terms of structural changes and immune response. In addition, by influencing host immunity and gut glycosylation, the microbiota can further effect protection against pathogens. In this review, the roles of host glycosylation in interactions with two prevalent bacterial pathogens, *Campylobacter jejuni* and *Helicobacter pylori*, are discussed to illustrate important concepts in pathogenesis.

INTRODUCTION

Beyond genes and proteins

For the past 60 years, the central dogma of molecular biology has emphasised the importance of the transcription of DNA to RNA and translation of RNA into amino acids to form proteins. By their physical and chemical nature, DNA, RNA and proteins are linear molecules and are synthesised using a pre-existing template. However, with the completion of sequencing of several organisms' genomes, it has emerged that the complement of genes alone does not define the complexity of an organism and its interactions with other organisms. Once amino acid chains are synthesised, they can undergo over 200 covalent modifications, so-called post-translational modifications, which determine the biological, physical and chemical properties of the proteins.¹ The realisation that proteins are heavily modified, and that these modifications are

critical to their structure and function, has led to a shift in emphasis from primarily studying genes and proteins to the analysis of post-translational modifications, such as phosphorylation, sulfation and glycosylation.

What is glycosylation?

Glycosylation is one of the most prevalent modifications mediated by complex enzymatic machinery, whereby glycans (sugars) are covalently attached to specific amino acid sites of proteins. Mammalian glycosylation takes place on nascent protein chains when they are passing through the endoplasmic reticulum (ER) and the Golgi complex. Subsequently, these proteins and lipids are transported to different organelles in the cell, to the cell membrane, or secreted from the cell.

All cells, from bacteria to human, are covered in glycans. Glycosylation leads to the formation of monomeric and multimeric glycan linkages which are essential for cell viability, biochemical communication and normal function. The resulting glycome encompasses a diverse and abundant repertoire of glycans, which form one of the four fundamental macromolecular components of all cells (together with nucleic acids, proteins and lipids). Glycans have important biological functions in protein maturation and turnover, cell adhesion and trafficking, and receptor binding and activation. Protein glycosylation has crucial roles in most physiological processes and diseases, including cell signalling, cellular differentiation and adhesion. Dysfunction leads to pathogenesis of infectious diseases and development of chronic diseases, for example, cancer, neurological disorders and severe inflammation.²

The diversity and complex characteristic of glycans on glycoconjugates is derived from the many ways in which monosaccharides can be linked together during their synthesis to form higher-order structures (figure 1B). Unlike nucleic acids and proteins, which are synthesised on the basis of a template and are linear molecules, glycan synthesis is not template-based and glycans can be both linear and branched. They can produce a large number of isomers because of the possibility of linkage and anomeric isomers. These isomers have very different three-dimensional structures and biological activities. A monosaccharide can be involved in more than two glycosidic linkages, thus serving as a branch point.

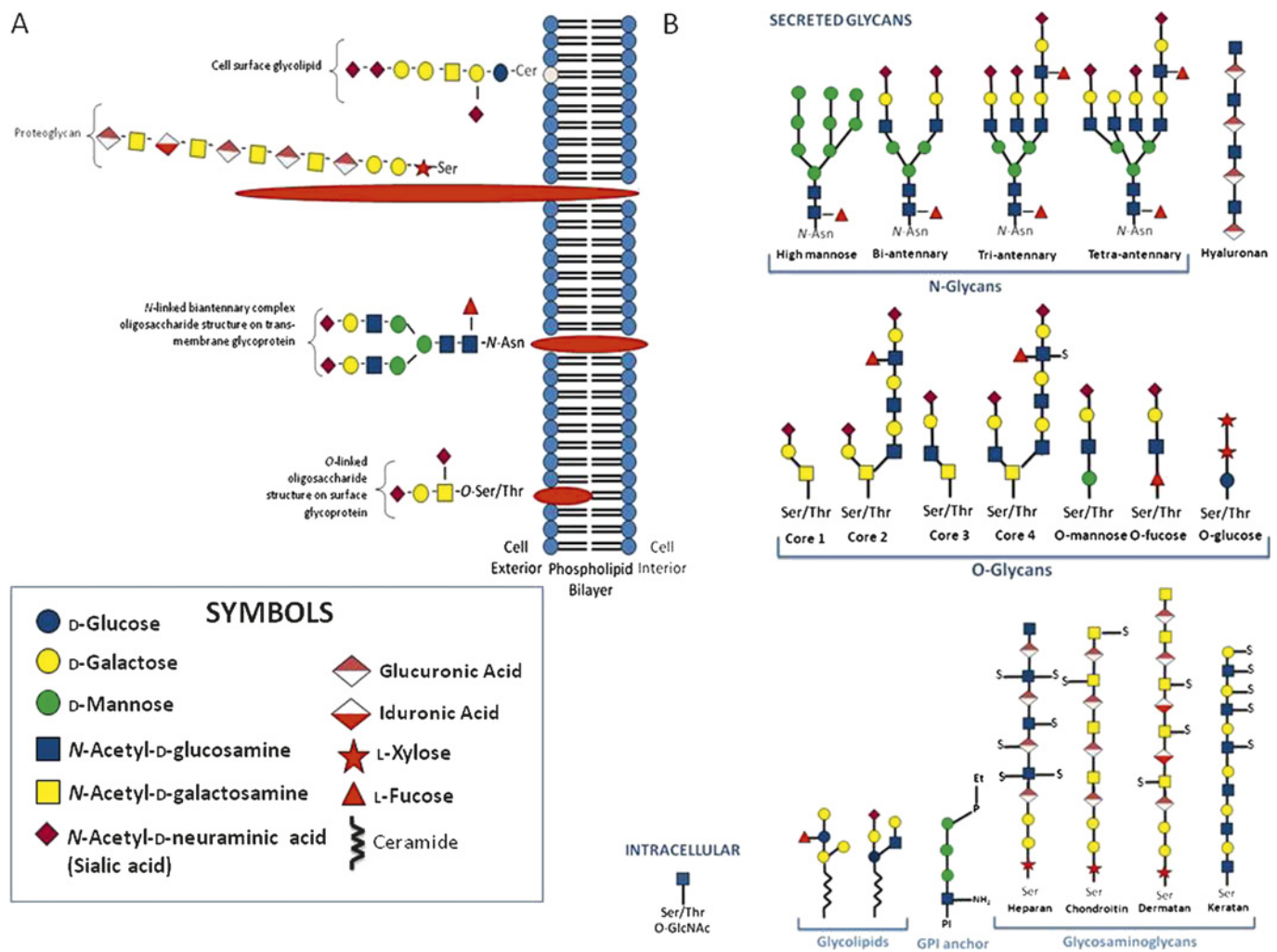


Figure 1 Glycosylation on the mammalian cell surface. (A) Diagrammatic representation of glycosylation on glycoconjugates of the mammalian cell surface including glycolipid, proteoglycan, N-linked and O-linked glycoprotein molecules. (B) The types of mammalian glycans, their diversity and complexity. Glycan structures of the six classes of secretory glycan (N-glycans, hyaluronan, O-glycans, glycolipids, glycosylphosphatidylinositol (GPI) anchor and glycosaminoglycans) and the single intracellular glycan, O-linked β -N-acetylglucosamine (O-GlcNAc), are shown. Representative examples of each type are indicated using the symbol nomenclature for monosaccharides (see symbols). PI is phosphatidylinositol, Et-P denotes a phosphoethanolamine linkage, and -s denotes the sulfation positions of the glycosaminoglycan chains.

The common occurrence of branched sequences is unique to glycans and contributes to their structural diversity. The glycosidic linkage is the most flexible part of a disaccharide structure. Thus, a disaccharide of well-defined primary structure can adopt multiple conformations in solution that differ in the relative orientation of the two monosaccharides. The combination of structural rigidity and flexibility is typical of complex carbohydrates and, more than likely, essential to their biological function.^{2,3}

Glycans are linked to other biomolecules, such as lipids or amino acids within polypeptides, through glycosidic linkages to form glycoconjugates. The glycan may be a single monosaccharide or an oligosaccharide. The attachment of many glycans to a polypeptide scaffold creates tremendous diversity among glycoproteins (figure 1). Glycoconjugates therefore make up a highly dynamic system capable of participating in a wide range of biological interactions.³

Nature of sugars and glycosylation—glycoproteins, proteoglycans and glycolipids

Mammalian glycoconjugates are classified on the basis of whether the glycans are attached to proteins or lipids (figure 1A). In the case of protein glycosylation, there are two major types of glycoprotein, N-linked and O-linked (figure 1B). In N-linked glycoproteins, the glycosylation takes place on an asparagine residue when asparagine is found in the following consensus motif: Asn-X-Ser/Thr (where X can be any amino acid but proline). N-Linked glycoproteins go through a complex series of attachment events in the ER and the Golgi complex, with the resultant glycoprotein carrying a mixture of glycan structures varying in length from five to 15 sugars. These structures are primarily composed of N-acetyl-D-glucosamine (GlcNAc), D-mannose (Man), D-galactose (Gal), L-fucose (Fuc) and sialic acid also known as neuraminic acid (N-acetylneuraminic acid; Neu5Ac) (figure 1B). Several cell-surface-bound as well as

secreted proteins are N-glycosylated—for example, erythropoietin and immunoglobulins.³ Unlike N-linked glycosylation, O-linked glycosylation takes place entirely in the Golgi complex where a single *N*-acetyl-D-galactosamine (GalNAc) residue is enzymatically attached to the Ser/Thr residue on folded proteins (figure 1B). In general, O-linked glycan chains are shorter than N-linked glycan chains but are composed of the same monosaccharides as used in N-linked glycosylation. Cell-surface-bound and secreted mucins are examples of O-linked glycoproteins (figure 2).⁴ These heavily O-glycosylated proteins carry clusters of GalNAc-based glycans in repetitive Ser- and Thr-rich VNTR (variable number of tandem repeats) domains. These complex oligosaccharide structures have been arbitrarily assigned three main domains: the core region, which comprises the innermost two or three sugars of the glycan chain proximal to the peptide; the backbone region, which mainly contributes to the length of the chains formed by uniform elongation; and the peripheral or terminal region, which exhibits a high degree of structural complexity (figure 2).⁴ Mucins are the most prominent host molecules involved in host–pathogen

interactions and are discussed in more detail in the following sections.

Proteoglycans are a subclass of glycoproteins, but the distinction between the two is not very clear, as both carry a protein backbone with glycans attached at specific amino acids. In the case of proteoglycans, several acidic disaccharide-repeating units are attached to the protein backbone. These disaccharide units (called glycosaminoglycans) are composed of a hexosamine and a hexose or a hexuronic acid (glucuronic acid or iduronic acid). Proteoglycans are integral components of the extracellular matrix; examples include chondroitin sulfate, dermatan sulfate and heparan sulfate.⁵

A glycolipid (also referred to as glycosphingolipid) is a glycoconjugate in which glycan chains are attached to the lipid ceramide with glucose (Glc) or Gal as the first sugar, thereby forming glucosylceramide and galactosylceramide. Glycolipids are ubiquitous components of membranes, and their distribution and structures vary depending on the location in the organism.⁶

During the process of colonisation and infection, the glycosylation state of both host and pathogen change in response to the presence of the other.

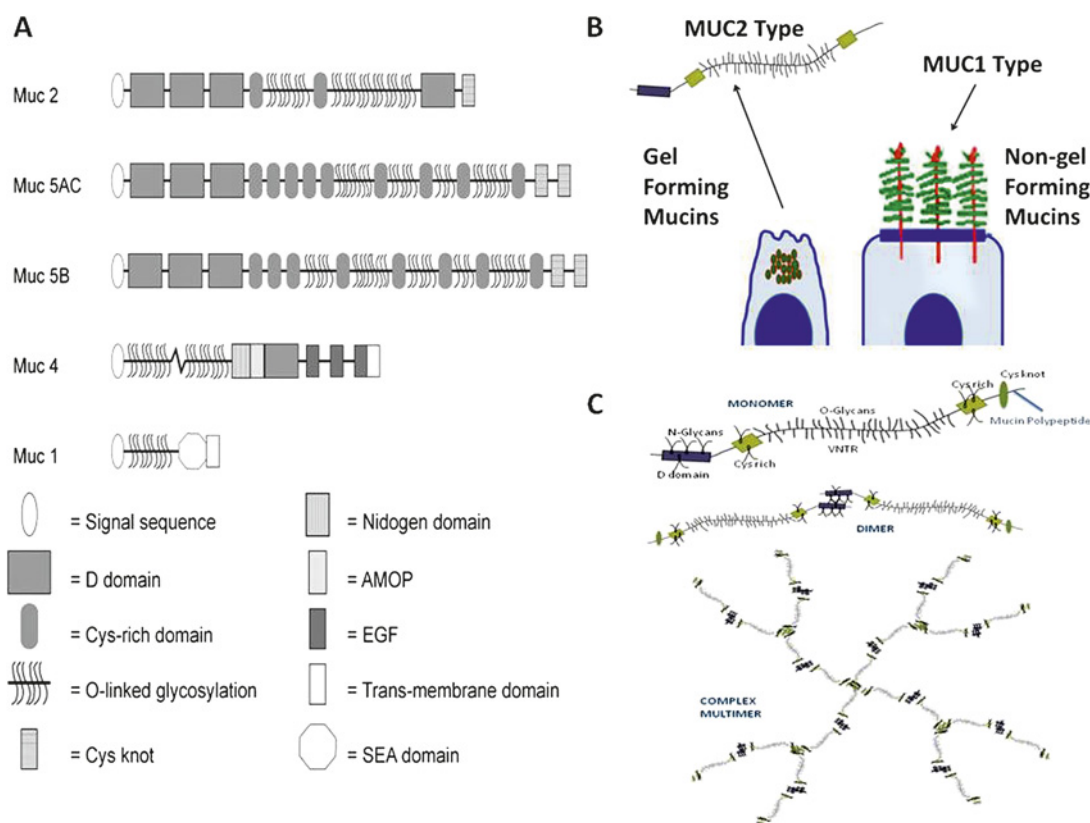


Figure 2 Mucin structure and glycosylation. (A) Schematic representation of structures of secreted and cell-membrane-associated mucins showing different domains. (B) Cartoon showing distribution and structure of gel-forming secreted, non-gel-forming secreted, and membrane-bound mucins. (C) Schematic representation of the complex glycosylated structure and multimeric assembly of a gel-forming secreted mucin. Immediately after its synthesis and translocation into the endoplasmic reticulum, the mucin polypeptide chain is N-glycosylated and forms disulfide-bonded dimers through its C-terminal Cys-rich domains. The dimers are then transported to the Golgi complex, and the tandem repeat domains are O-glycosylated. Once the glycosylated dimers reach the trans-Golgi compartments, they are assembled into disulfide-bonded multimers through their N-terminal D-domains. The Y-shaped structures on the N-terminal D-domains and the C-terminal Cys-rich domains represent N-linked oligosaccharides. The squiggly lines perpendicular to the polypeptide chain represent O-linked oligosaccharides in the tandem repeat domains.

This complex modulation of glycan expression critically determines pathogenesis and the host response in terms of structural changes and immune response. The aim of this paper is to review the roles of glycosylation of the gastrointestinal (GI) tract in colonisation and invasion of this mucosa by bacteria. In particular, the gastroduodenal pathogen, *Helicobacter pylori*, and the enteric pathogen, *Campylobacter jejuni*, will be used as examples of acute and chronic bacterial infections, respectively, to illustrate important concepts concerning the role of GI glycosylation in pathogenesis.

MUCUS LAYER AND EPITHELIAL GLYCOCALYX AS BARRIERS TO BACTERIAL INFECTION

Mucus layer

The mucosal surface of the GI tract, which is a complex ecosystem of resident microbiota, immune cells and an epithelium covered by a mucus layer, is the largest body surface (200–300 m²) in contact with the external environment.⁷ The adherent gastrointestinal mucus gel in vivo is continuous and can be divided into two layers: a loosely adherent layer and a layer firmly attached to the mucosa (figure 3A). It ranges in thickness from 300 µm in the stomach and 700 µm in the intestine,⁸ and represents the front-line defence barrier between the external environment and tissues of the host.⁹ The relative thickness of the two component layers of the mucus gel varies for different regions of the gut. Thus, in the stomach, the thickness of the firmly adherent mucus component is 80 and 154 µm for the corpus and antrum, respectively, with an overlying layer of loosely adherent mucus of similar thickness (figure 3A). The gastric pathogen *H pylori* has been shown to reduce the rate of renewal of the mucus layer, as well as cause an acid-induced increase in its renewal rate. The mucus gel is the thickest in the colon, and the rate of mucus accumulation is highest. In the small intestine, the mucus layer is thin and discontinuous.^{8–9} This viscous, supramucosal gel presents a highly hydrated, mechanical and lubricative barrier, as well as a matrix for immune-related and antimicrobial proteins. It is not static, since the outermost loosely adherent mucus layer is continually removed by movement of the luminal contents of the GI tract and renewed. The rate of renewal of the mucus layer varies in different parts of the GI tract (figure 3A).^{8–9} Thus, the mucus layer protects the underlying epithelial cells against chemical, enzymatic, microbial and mechanical insult, and serves as a physical gel to inhibit and entrap invading microbes and aid clearance. In addition, in the stomach, mucus acts as an unstirred layer in which bicarbonate ions secreted by the surface epithelium counteract protons diffusing from the lumen into the gel, causing the pH in the mucus layer to range from acidic in the lumen to neutral at the cell surface which, in turn, can influence bacterial colonisation (figure 3B).¹⁰ For example, *H pylori* usually resides within the one-fourth zone

(0–25 µm) of mucin gel above the epithelium in close proximity to the cells where the pH ranges from 5 to 7 (figure 3B).¹⁰

The protective physicochemical properties of mucus are substantially attributable to the high carbohydrate content of mucins, which also presents considerable potential for interaction between microbial lectins and O-linked glycans. In addition, glycosidases and other enzyme activities secreted or induced by microbes have the capacity to directly modify these glycans after the mucins have been secreted or exported to the cell membrane.^{11–12} This strategy is significant as a mechanism for generating binding ligands and in the provision of sugars for bacterial metabolism.¹³ Bacterial interaction with the supramucosal gel can lead to chronic colonisation of the mucus. On one hand, the mucosal microbiota blocks access to the underlying cells, and on the other, in the case of pathogens, necessitates the engagement of gel-penetration strategies before cellular adhesion.⁹ It is therefore surprising that the relevance of this layer to colonisation and invasion of the mucosal epithelium has not historically been of higher priority.

Glycocalyx

Underneath the mucus layer, the second line of defence is constituted by the epithelial glycocalyx, which is partially integrated with the overlying gel (figure 3A).¹⁴ The glycocalyx represents an array of highly diverse glycoproteins and glycolipids expressed on the membrane of epithelial cells. The glycocalyx is highly variable from tissue to tissue as well as in thickness (intestinal microvilli tips, 100–500 nm; lateral microvilli surface, 30–60 nm) (figure 3A).⁸ The glycosylation machinery of mucosal epithelia not only varies with the cell type but is also influenced by the sub- and supramucosal environment—for example, hormonal status, inflammation and microbial colonisation have all been shown to influence the glycosylation of epithelial glycoproteins.^{15–18} Like the mucus gel, the glycocalyx is constantly renewed; the average turnover time of the human jejunal glycocalyx is 6–12 h. In the gut lumen, the glycocalyx interacts with the overlying mucus layer, the gastric-biliary juices and the resident probiotic microflora to limit colonisation by pathogens. The complex glycosylated structures expressed within the glycocalyx serve as receptors and form the primary site for bacterial adhesion. For example, the carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) expressed on the apical surface of ileal epithelial cells serves as a receptor for adherent-invasive *Escherichia coli* in Crohn disease.¹⁹ Expression of such glycosylated ligands can in turn be modulated by bacterial infection and inflammation, further promoting colonisation.

Bacterial colonisation and adherence

To colonise mucosal surfaces and invade the epithelium, microbes commonly interact with glycan structures of the host glycocalyx.^{11–16} Bacterial fimbriae (or pili), various outer membrane

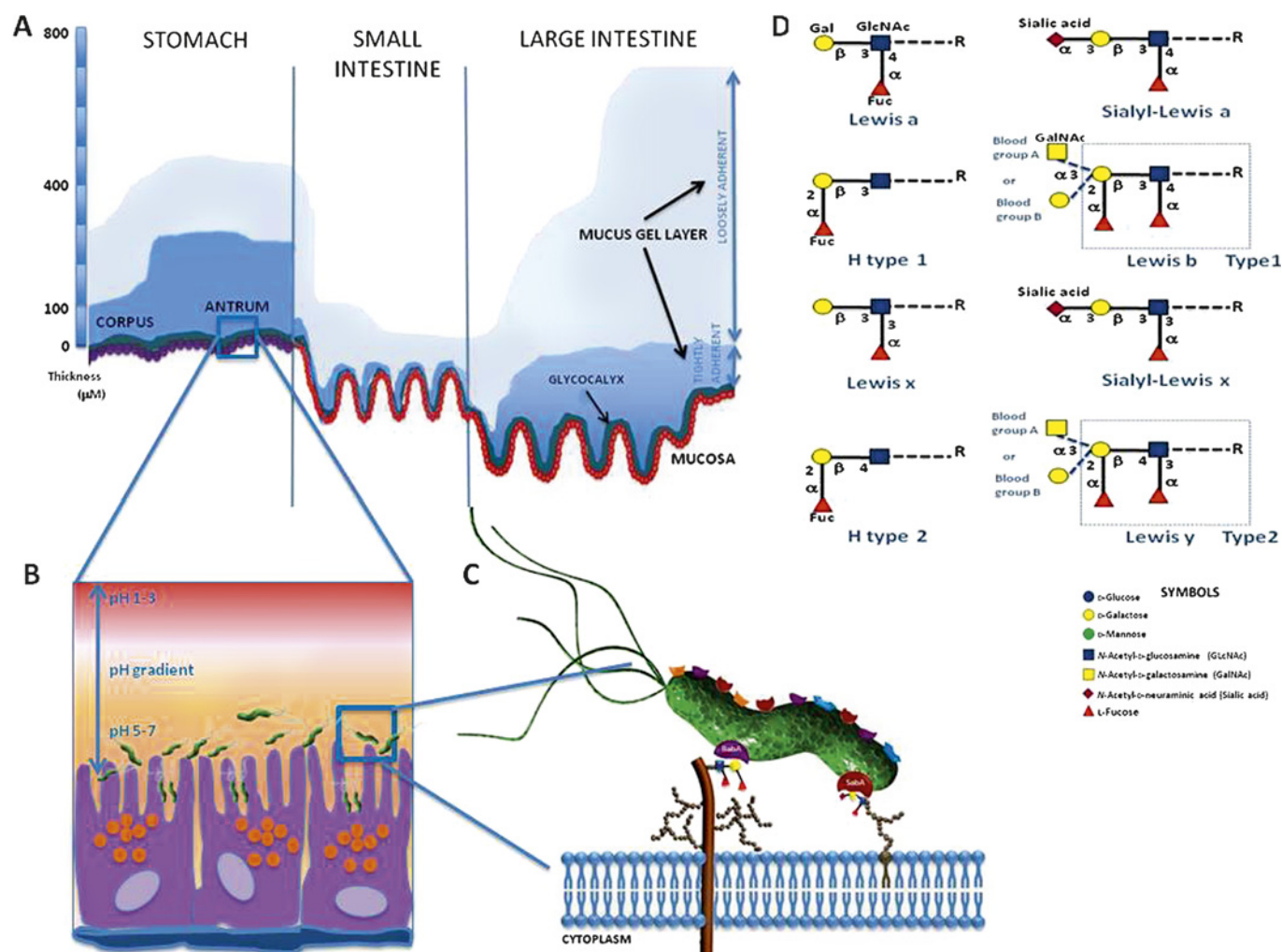


Figure 3 The gastrointestinal mucus layer and its role in bacterial adhesion. (A) Diagram showing the thickness of the two mucus gel layers in vivo in the stomach and small and large intestine. The mucus gel layer is continuous throughout the gastrointestinal tract and does not follow the contours of the villi in the intestine. The membrane-associated glycocalyx is also shown. (B,C) Glycan-mediated adhesion of *Helicobacter pylori* to gastric epithelial cells. The figure illustrates some of the characterised bacterial–glycan interactions that contribute to successful colonisation of the gastric mucosa. (D) Schematic representation of the terminal ABH structures and Lewis epitopes present in O-glycans, which act as receptors for *H. pylori* adhesins, BabA and SabA.

proteins and cell wall components—for example, lipopolysaccharides (LPSs)—may all function as adhesins. Adhesion to host cells is considered to be mediated by hydrophobic interactions, cation-bridging whereby divalent cations counteract the repulsion of the negatively charged surfaces of bacteria and host, and receptor-ligand binding.^{7–11} One of the most extensively studied mechanisms of adhesion is the binding of bacterial lectins to the corresponding glycosylated receptors associated with host cells.¹⁰ Bacteria may have multiple adhesins with different carbohydrate specificities, and modulation of surface receptor density, kinetic parameters, or topographical distributions of these receptors on cell membranes regulate adhesion. After *H. pylori* enters the stomach, infection occurs in three crucial steps: establishing colonisation; evading host immunity; and invading gastric mucosa. Urease activity and motility mediated by the flagella of *H. pylori* are important in penetrating the mucus gel and harbouring colonies beneath the gastric mucus in niches adjacent to the epithelium.

Several putative adhesins attach the organism to the gastric epithelium and prompt the succeeding processes for evading host immunity and invading the mucosa.^{20–21} *H. pylori* expresses at least six lectin-like adhesins for recognition of the gastric mucosa, of which the blood group-binding adhesin (BabA), which binds to Lewis b (Le^b) and H-1 antigens,²⁰ and sialic acid-binding adhesin (SabA), which binds sialyl-Le^x in inflamed tissue,²¹ are most important for colonisation (figure 3C). Human Lewis antigens represent terminal modifications on mucin-type O-glycans. Their structure is composed of two different backbones (figure 3D). The type 1 chain contains Gal and GlcNAc composed as Gal-β(1,3)-GlcNAc, while the type 2 chain has Gal-β(1,4)-GlcNAc, which is also termed N-acetyl-lactosamine (LacNAc). Adding α-Fuc as a side chain to the backbone structure on the 1–4 location, on both 1–2 and 1–4 locations, and on both 2–2 and 2–4 locations gives rise to Lewis a (Le^a), Le^b and sialyl-Le^a antigens, respectively (figure 3D). Based on the backbone structure of the type 2 chain, the

addition of α -Fuc as a side chain at the 1–3 location on both 1–2 and 1–3 locations, or on both the 2–2 and 2–3 locations, gives rise to the Lewis x (Le^x), Lewis y (Le^y) and sialyl- Le^x antigens, respectively (figure 3D).²²

The distribution of type 1 antigens such as Le^a and Le^b is primarily on the surface of the gastric epithelium. This is the primary site of ligation for the *H pylori* adhesion, BabA. BabA-positive status and its binding to Le^b have been associated with severe gastric injury, high *H pylori* density, and severe clinical outcomes.²³ The fucosylated H-type 1 and Le^b antigens are naturally expressed on the gastric mucosa of secretor or Lewis-positive individuals. The secretor status depends on an active FUT2 enzyme, an $\alpha(1,2)$ -fucosyltransferase that catalyses the addition of terminal $\alpha(1,2)$ -fucose residues. Inactivating mutations in the secretor gene affect 20% of the human population (known as non-secretors) and have been associated with reduced susceptibility to infections by *H pylori*.^{23 24} Nearly 95% of the BabA-encoding *H pylori* adapt to any of the Le^b sub-types, and such strains are termed ‘generalists’. A small subset of *H pylori* strains express BabA, but cannot adhere to the A- Le^b antigen. Such strains are called ‘specialist’.²³ BabA can also act as a mediator for *H pylori* binding to MUC5AC, even in non-secretors or those without Le^b . In contrast, type 2 antigens may be located deeper in the glands, such as mucous, chief, or parietal cells. Le^b is weakly expressed on the surface epithelium during chronic inflammation, as well as in gastric atrophy (atrophic gastritis) and intestinal metaplasia. Under these clinical conditions, there is an upward migration of Le^x associated with atrophy of the epithelium. During chronic inflammation or in atrophic gastritis, *H pylori* binds to Le^x and sialyl- Le^x via SabA adhesin. SabA production and SabA-mediated adhesion are associated with severe disease outcomes, including gastric atrophy, intestinal metaplasia and the development of gastric cancer. The minimal structure required for SabA adhesin binding was shown to be NeuAc α 2-3Gal (figure 3C,D).²⁵

H pylori CagL binds to the integrin, $\alpha 5 \beta 1$, to form an injection pile by which CagA translocates into the cytosol of gastric epithelium, using a type IV secretory system. Other known *H pylori* adhesins include neutrophil-activating protein, which binds to sulfated carbohydrate structures,^{26 27} a 25 kDa adhesin that binds the glycoprotein laminin in the extracellular matrix,^{28 29} as well as adherence-associated lipoprotein A and B (AlpA/B)^{10 30} and HorB,³¹ whose gastric receptors have not been identified. Moreover, *H pylori* lectins bind molecules in the oral cavity, such as salivary agglutinin, that may influence re-infection of the gastric mucosa from this secondary infection reservoir.³² At least four protein adhesins, which differ substantially in activity and importance depending on the anatomical site within the orogastric route, the mucin-type, the environmental pH and the gastric disease status, are involved in *H pylori* binding to mucus-related oligosaccharides.³³ The adhesive interactions are not

limited to protein adhesins, as *H pylori* LPS interacts and binds with the gastric epithelium³⁴ through recognition by the host lectin, galectin-3.³⁵ As occurs with protein adhesins,³⁶ environmental pH, particularly low pH, can influence expression of the LPS ligand involved in the latter interaction.³⁷ In addition, this bacterium recognises a range of glycosylated molecules, not only glycoproteins and oligosaccharide moieties, but also proteoglycans and glycolipids.¹⁰ In contrast, the nature and specificities of the lectins expressed by intestinal-colonising *C jejuni* appear less diverse,^{38 39} possibly reflecting the lesser mucus and tissue diversity of the jejunal environment (figure 3A). Nevertheless, although individual adhesion–receptor binding is usually of low affinity, clustering of adhesins and receptors can cause multivalency effects resulting in strong attachment.

The O antigen of *H pylori* LPS shares structural homology with the Lewis blood group antigens expressed in human gastric mucosa, including both type 1 chains, namely Le^a and H-type 1, and type 2 chains, such as Le^x and Le^y . The expression of these antigens undergoes phase variation, with different bacterial fucosyltransferases being switched on and off, contributing to a dynamic glycosylation even in the same host. It has been proposed that Le^x structures expressed by the *H pylori* O antigen side chain may further promote adhesion to gastric epithelial cells.^{37 40}

Collectively, ligand–receptor binding can have different consequences for bacterial pathogens during colonisation of the GI tract and whether the resultant infection is acute or chronic. Adhesion can affect bacteria by stimulation/inhibition of growth, as well as induction of other adhesive structures and proteins required for invasion, whereas the effects of adhesion on host cells can be altered morphology, fluid loss, induction of cytokine release, upregulation of cell adhesion molecules, and apoptosis.⁷ In addition, infection and associated inflammation can induce changes in mucosal glycosylation,^{41 42} including the release of carbohydrates for bacterial utilisation. An example of this occurs in *H pylori* infection whereby human α -L-fucosidase is secreted which liberates L-Fuc from host cells, which becomes available for *H pylori* to synthesise mammalian Lewis antigens,⁴³ which contribute to pathogenesis of the bacterium.^{40 44}

Of the range of GI barrier-related molecules with which bacterial pathogens interact, mucins as a common family of molecules present in secreted mucus and in the glycocalyx are receiving increasing attention and will form the predominant focus for further discussion in this review.

PROPERTIES AND CHARACTERISTICS OF MUCINS

General attributes of mucins

The polymeric scaffolding of mucus is formed by secreted mucin glycoproteins. However, it is important to note that certain mucins (eg, MUC1) can be membrane-bound and hence form a major constituent of the epithelial glycocalyx of a variety of mucosal tissues (figure 2B). Mucin-type molecules consist of a core protein moiety (apomucin) within

which a number of carbohydrate chains are attached to Ser, Thr and Pro residues by glycoside bonds forming O- and N-linked oligosaccharides^{15 45} (figure 2C). Mucins have a high molecular mass (5×10^5 to 30×10^6), and more than 50% (often 70–80%) of the mucin molecular mass is composed of carbohydrate.⁹ Each mucin carries of the order of 100 different oligosaccharide structures. These carbohydrate chains are often clustered into highly glycosylated domains, giving the mucin a 'bottle-brush' appearance (figure 2C). In secreted mucins, these O-linked oligosaccharides are concentrated in large peptide domains of repeating amino acid sequences rich in Ser and Pro.¹⁵ The size and number of repeats vary between mucin types, and in many of the mucin genes there is polymorphism in the number of repeats (VNTR) (table 1)^{8 9} (figure 2C). Thus, the size of such mucins can differ substantially between individuals and could represent a basis for differences in infection susceptibility between hosts.

Mucin categories

Mucins can be divided into three distinct subfamilies: (i) secreted gel-forming mucins; (ii) cell surface mucins; (iii) secreted non-gel-forming mucins (table 1). The gel-forming mucins (eg, MUC2, MUC5AC, MUC6 and MUC19) are a major constituent of mucus and confer its viscoelastic properties. Gel-forming mucins contain N- and C-terminal Cys-rich domains, which are involved in homo-oligomerisation mediated by intermolecular disulfide bonds, which is likely to result in web-like supramolecular structures critical for the rheological properties of the mucus gel^{4 8 46 47} (figure 2C). The extensive O-glycosylation of secreted mucins gives protection from proteolytic enzymes and, because of the dense glycosylation, induces a relatively extended conformation, which causes these secreted

oligomeric molecules to occupy large volumes equivalent in size to small bacteria.⁴⁸ In addition to the secreted mucins, cell surface mucin glycoproteins (eg, MUC1, MUC3A/B, MUC4, MUC12 and MUC13) are expressed on the apical membrane of all mucosal epithelial cells, representing major constituents of the glycocalyx^{8 15} (figure 2A). They are monomeric and may be shed into the overlying mucus and contribute to viscosity but not elasticity of the gel.⁹ In general, their large VNTR domains (table 1) predict that they form rigid elongated structures, which together with their high expression, indicate they are a predominant constituent of the glycocalyx. Cell surface mucins associate with the cell membrane by an integral transmembrane domain and possess short cytoplasmic tails that associate with cell cytoskeletal proteins and are important for transducing external signalling into the mammalian cell (eg, with MUC1 and MUC4).^{7 17} The secreted non-oligomerising mucins include the MUC7 salivary mucin (table 1).

Mucin glycosylation

The oligosaccharide content and carbohydrate structures of mucins vary according to cell lineage, tissue location and developmental stage. O-Linked glycans contain 1–20 residues, which occur both as linear and branched structures (table 2). The carbohydrate chain is initiated with a GalNAc residue linked to Ser or Thr, to which is added the core structures followed by the backbone region produced by elongation units of either type 1 or type 2 LacNAc.^{9 15} The chains are terminated by Fuc, Gal, GalNAc or Neu5Ac residues in the peripheral region, forming histo-blood-group antigens such as A, B, H, Le^a, Le^b, Le^x, Le^y, as well as sialyl-Le^a and sialyl-Le^x structures (figure 3D).^{22 44} The carbohydrate structures present depend on which glycosyltransferases are expressed in the cells—that is, by the genotype of the individual.²² The H-1 structure is made by the *secretor* (*Se*) gene product; the majority of humans worldwide carry this structure and are thus referred to as secretors. Individuals may also express the *Lewis* gene (90% of the Caucasian population), and, provided that they are also secretors, will modify the H-1 antigen to Le^b, whereas if they are non-secretors, Le^a will be expressed on type 1 chains. Also, mucins contain a smaller number of N-linked oligosaccharides that have been implicated in folding, oligomerisation or surface localisation⁸ (figure 2C).

The terminal structures of mucin oligosaccharides are highly heterogeneous and vary between/within species and even with tissue location within a single individual. For example, in the gastric mucosa, Le^a and Le^b blood-group antigens mainly appear on the surface epithelium on MUC5AC, whereas the Le^x and Le^y antigens are expressed in mucous, chief and parietal cells of the glands, co-localised with MUC6.^{49 50} Also, mucin glycosylation changes with infection progress; mucins from a healthy human stomach express low levels of sialic acid and sulfate and are therefore predominantly neutral, but prolonged infection

Table 1 Distribution of mucins in the gastrointestinal tract and in associated tissues

Mucin	Distribution	Chromosomal location	Tandem repeat size (No of amino acids)
Secreted gel-forming			
MUC2	Small intestine, colon	11p15.5	23
MUC5AC	Stomach	11p15.5	8
MUC5B	Salivary glands, gallbladder	11p15.5	29
MUC6	Stomach, duodenum, gallbladder, pancreas	11p15.5	169
MUC19	Sublingual gland, submandibular gland	12q12	19
Secreted non-gel-forming (monomeric)			
MUC7	Salivary glands	4q13-q21	23
Cell surface			
MUC1	Stomach, gallbladder, pancreas, duodenum, colon	1q21	20
MUC3A/B	Small intestine, colon, gall bladder, duodenum	7q22	17
MUC4	Colon, stomach	3q29	16
MUC12	Colon, small intestine, stomach, pancreas	7q22	28
MUC13	Colon, small intestine, stomach	3q13.3	27
MUC15	Small intestine, colon, foetal liver	11p14.3	None
MUC16	Peritoneal mesothelium	19p13.3	156
MUC17	Small intestine, colon, duodenum, stomach	7q22	59
MUC20	Colon	3q29	18

Table 2 Common O-linked oligosaccharide structures on mucins

Nomenclature	Structure	Schematic representation
Core type		
Core 1	-Gal β 1-3GalNAc α 1-Ser/Thr	
Core 2	-Gal β 1-3(-GlcNAc β 1-6)GalNAc α 1-Ser/Thr	
Core 3	-GlcNAc β 1-3GalNAc α 1-Ser/Thr	
Core 4	-GlcNAc β 1-3(-GlcNAc β 1-6)GalNAc α 1-Ser/Thr	
LacNAc elongation units		
Type 1 chain	-Gal β 1-3GlcNAc β 1-	
Type 2 chain	-Gal β 1-4GlcNAc β 1-	
Branching		
i-antigen	-Gal β 1-4GlcNAc β 1-3Gal β 1- (unbranched)	
I-antigen	-Gal β 1-4GlcNAc β 1-3(-Gal β 1-4GlcNAc β 1-6)Gal β 1- (branched)	
Terminal structures		
Blood group H	Fuca1-2Gal β 1-	
Blood group A	Fuca1-2(-GalNAc α 1-3)Gal β 1-	
Blood group B	Fuca1-2(-Gal α 1-3)Gal β 1-	
Type 1 chain-based		
Lewis ^a	Gal β 1-3(-Fuca1-4)GlcNAc β 1-	
Lewis ^b	Fuca1-2Gal β 1-3(-Fuca1-4)GlcNAc β 1-	
Sialyl-Lewis ^a	Neu5Ac α 2-3Gal β 1-3(-Fuca1-4)GlcNAc β 1-	
Type 2 chain-based		
Lewis ^x	Gal β 1-4(-Fuca1-3)GlcNAc β 1-	
Lewis ^y	Fuca1-2Gal β 1-4(-Fuca1-3)GlcNAc β 1-	
Sialyl-Lewis ^x	Neu5Ac α 2-3Gal β 1-4(-Fuca1-3)GlcNAc β 1-	
Sulfation		
3-Sulfation	HSO ₃ -3Gal β 1-	
6-Sulfation	HSO ₃ -6GlcNAc β 1-	
Examples of combined epitopes		
H-1 antigen	Fuca1-2Gal β 1-3GlcNAc β 1-	
Sialylated type 2 chain	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-	

Fuc, L-fucose; Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; Neu5Ac, N-acetylneuraminic acid.

with *H pylori* decreases fucosylation and increases sialylation of mucins.^{21 51} However, the sialylation pattern returns to normal after successful *H pylori* eradication therapy. The structural diversity of glycosylation has been suggested to allow the host to cope with diverse and changing pathogen challenges.¹¹ Notably, host susceptibility to a specific bacterial pathogen and subsequent disease severity can vary between individuals expressing different histo-blood groups. This has been demonstrated for *H pylori* infection in rhesus monkeys, which shares strong similarities to the natural history of *H pylori* infection in humans.²⁴ This bacterium has an ability to vary and adapt its surface characteristics, including expression of glycosylation and outer membrane proteins to aid infection in humans and in animal models.^{35 52–55} A strong correlation has been found between the adhesive properties of *H pylori* endemic in specific human populations and the mucin-related blood group structures expressed by those populations,⁵¹ as well as the ability of the bacterial adhesins to vary to aid colonisation.^{10 23 56}

Changes in the expression levels and glycosylation patterns of mucins have been associated with several diseases, including carcinomas. In gastric cancer, alterations in mucin polypeptide expression have been reported: loss of expression of MUC5AC, increased mucin heterogeneity, and glycosylation changes, including exposure of simple mucin-type carbohydrates.⁵⁷ The majority of gastric carcinomas are preceded by a precancerous stage, characterised by sequential development of atrophic gastritis leading to intestinal metaplasia, and dysplasia. Intestinal metaplasia consists of replacement of the gastric mucosa by an epithelium that histologically resembles the intestinal mucosa and is characterised by the presence of absorptive cells, Paneth cells, and goblet cells secreting sialo-mucins and/or sulfo-mucins and corresponds to the small intestine phenotype.⁵⁸ Altered mucin expression patterns reported in intestinal metaplasia include under-expression of MUC1, MUC5AC and MUC6 and de novo expression of MUC2.⁵⁸

BACTERIAL INTERACTIONS WITH MUCINS

Effects on mucins and their synthesis

Mucus is secreted by specialised goblet cells (also known as mucin-secreting cells).^{59–61} Through the expression of different mucins, variations in mucin glycosylation and the co-secretion of mucin-associated molecules, the nature of the mucus varies at different anatomical locations and constitutes a responsive system that can be adapted to local physiological requirements, including response to bacterial colonisation and infection.^{7–9 15 60} The mucins are produced by cells in the epithelial surface and/or by glands located in the submucosal connective tissues, and secretion occurs via both constitutive and regulated pathways.^{8 15} The constitutive pathway continuously secretes sufficient mucin to maintain the mucus layer, whereas the regulated pathway affords a massive discharge of mucus in response to environmental and/or patho-

physiological stimuli such as bacterial infection and inflammation. Although release is regulated by activation of signalling pathways by a number of secretagogues (including acetylcholine, vasoactive intestinal peptide and neurotensin), activation can occur by bacterial-induced inflammatory mediators (eg, interleukin (IL)-1, IL-4, IL-6, IL-9, IL-13, interferons, tumour necrosis factor α and nitric oxide).^{7 8 59 61} The responsiveness to cytokines and other immune regulators provides a link between mucin-based defence, innate mucosal immunity and mucosal inflammatory responses.⁸ Stimulated mucin release occurs rapidly and is accompanied by a hundredfold or so expansion of secretory granules.

Changes in goblet cell function and in the chemical composition of intestinal mucins have been detected in response to a broad range of luminal insults, including changes in the normal microbiota and intrusion of enteric pathogens.^{41 42} Probiotic bacteria have been shown to be capable of enhancing mucin secretion in colonic epithelial cells,⁶² thus potentially boosting this defensive mechanism against pathogens. There is evidence that a variety of microbial products can stimulate increased production of mucins by mucosal epithelial cells.^{61–63} Also, germ-free mice exhibit changes in mucin gene expression, mucus composition and mucus secretion in response to intestinal bacteria or host-derived inflammatory mediators.^{64 65} Therefore, it can be surmised that alterations in mucus secretion and composition, including mucin glycosylation, represent important mechanisms for unfavourably changing the niche occupied by a mucosal pathogen. Successful pathogens, however, are capable of countering this mechanism. For example, *H pylori* has been shown to decrease mucin exocytosis,⁶⁶ and LPS of this bacterium has been shown to affect mucin synthesis⁶⁷ and the macromolecular nature of the mucin secreted,⁶⁸ thereby representing a potential strategy by which the bacterium can favourably modulate mucin composition of the mucus barrier and the glycocalyx.

Specifically, *H pylori* causes an aberrant expression of human MUC1, MUC5AC and MUC6 in infected gastric mucosa,^{69 70} in particular with downregulation of MUC5AC and upregulation of MUC6,⁷¹ both of which are secreted mucins. A similar downregulation of MUC5AC has been found in rhesus monkeys, which have strong similarities to mucin glycosylation in humans.⁷² The reduced MUC5AC synthesis induced by *H pylori* can impair the gastric mucus gel,⁹ but the upregulation of MUC6 may represent an antimicrobial host response, since the O-linked glycans of MUC6 display antimicrobial activity against *H pylori*.⁷³ The basis for this is the inhibition of biosynthesis of the essential bacterial membrane lipid, cholesterol- α -D-glucopyranoside, by the terminal $\alpha(1\rightarrow4)$ -linked GlcNAc of MUC6 interacting with the respective enzyme.⁷⁴ A close association with secreted MUC5AC and membrane-bound MUC1 is considered important for *H pylori* colonisation of the gastric mucosa.^{75 76}

H. pylori is bound by secreted MUC5AC through the bacterial outer membrane protein, BabA, by recognising fucosylated Le^b expressed in this mucin.⁴⁹ Although, on the one hand, aiding *H. pylori* colonisation of the mucus layer, this binding may, on the other hand, limit colonisation to the mucus gel, and hence reduce the number of *H. pylori* bacteria interacting with the epithelial surface by blocking BabA recognition of structures in the epithelial glycocalyx. Nevertheless, *H. pylori* is capable of binding cell surface MUC1,^{75–77} and thus bacterial contact with the epithelial surface can be mediated in a mucin-dependent manner.

Other bacterial effects on mucins

In addition to direct interactions with mucins and their synthesis, bacteria can use other strategies to overcome the gel-based barrier function of the mucus layer. Flagellation is a shared characteristic of the vast majority of successful mucosal pathogens that are highly motile, such as *C. jejuni* and *H. pylori*.⁷⁸ Motility, along with the corkscrew morphology of these bacteria, allows them to burrow through the mucus layer to reach the epithelium.⁷⁹ Indeed, *H. pylori* that have dysfunctional flagella have a greatly reduced ability to infect.⁸⁰ Combined with motility, degradative enzymes, such as fucosidases, mucinases (mucin-degrading enzymes), glycosylsulfatases, sialidases, sialate *O*-acetylsterases and *N*-acetylneuraminidases, are produced by a broad spectrum of bacterial pathogens that destabilise the mucus as well as removing mucin decoy carbohydrates for bacterial adhesins.^{9–11–12–81} The size of the mucus polymer is important for the formation of entangled gels and the viscous properties of mucus,^{8–15} and thus degradation of mucins will result in localised disintegration of mucus, thereby aiding bacterial penetration of the epithelium.

Supporting the view of the mucus gel as an effective infection barrier, avoidance of this layer has been suggested as a further strategy used by certain mucosal pathogens of the GI tract.⁸ Although M cells are only a very small percentage of mucosal epithelial cells, they are the major point of attachment and/or entry used by a large proportion of enteric bacteria, including *C. jejuni*.⁸² Intestinal M cells, which capture and present microbial antigens to the underlying lymphoid tissue, do not produce gel-forming mucins and have a thin glycocalyx.⁸³ M cells selectively express the sialyl-Le^a antigen, whereas Le^a is expressed on all epithelial cells of the GI tract.^{84–85} Thus, M cells with a different profile of glycocalyx glycoproteins^{84–85} represent a focal point of entry for mucosal pathogens. Once M cells are invaded or mucus is penetrated, a further strategy of GI tract pathogens is to disrupt the tight junctions between adjacent mucosal cells, thereby exposing the vulnerable lateral membranes not protected by the glycocalyx. Such a mechanism is used by *H. pylori*, which affects the permeability of cellular tight junctions,⁸⁶ penetrates intercellular junctions, and, mediated by a bacterial lectin and LPS, binds to the

glycoprotein laminin in the extracellular matrix and basement membrane.^{28–87} The latter interaction has further significance since *H. pylori* LPS inhibits recognition of laminin by an epithelial cell receptor (integrin), which has been hypothesised to contribute to lack of mucosal integrity.⁸⁸

Cell surface mucins in protection

Although the cell surface mucins can act as recognition molecules for bacterial adhesins, these mucins may also show other protective effects against pathogenic bacteria. For example, MUC1 has been shown to protect p53-expressing epithelial cells in vitro from the effects of *C. jejuni* cytolethal distending toxin, a genotoxin, and, as demonstrated in *Muc1*^{−/−} mice, lowers gastric colonisation by *C. jejuni*.⁸⁹ It should be noted that glycocalyx mucins are capable of initiating intracellular signalling in response to bacteria, reflecting their reporter function, as well as their barrier role as constituents of the glycocalyx on the apical surface of mucosal epithelial cells.^{7–17} Importantly, the cytoplasmic domain of MUC1 can be cleaved, translocates to mitochondria, and, together with the p53 transcription factor, modulates the cell cycle and protects against the apoptotic response to genotoxic stress.^{90–91} This protective effect, first identified in cancer research, has been suggested to have evolved as part of the natural epithelial defence against microbial genotoxins.⁸

Another protective mechanism of cell surface mucins that has been hypothesised, again exemplified by MUC1, involves their role as releasable decoy ligands for mucosal pathogens attempting to anchor to the glycocalyx. Supportive evidence for such a role of MUC1 has been accumulated for both *C. jejuni*⁸⁹ and *H. pylori*.⁷⁷ Although *H. pylori* can bind MUC1, primary murine gastric epithelial cells expressing MUC1 bind fewer *H. pylori* than murine *Muc1*^{−/−} cells.⁷⁷ These, at first apparently conflicting, results can be explained if MUC1 acts as a releasable decoy; the bacteria bind MUC1 on normal gastric epithelial cells, but this is then shed by the host⁹²; however, owing to the absence of MUC1 on *Muc1*^{−/−} murine cells, the bacteria attach to other epithelial receptors and hence are not lost. The findings are reflected in pathology, since *Muc1*^{−/−} mice develop fivefold greater *H. pylori* colonisation density, which is maintained for at least 2 months, whereas heterozygous mice have a lower level of gastric MUC1 expression and intermediate colonisation densities.⁷⁷ Moreover, comparing *H. pylori*-positive patients, those with MUC1 polymorphisms exhibiting short extracellular mucin domains are more likely to develop gastritis than those without,⁷⁵ because the shorter MUC1 molecules will allow greater access of the bacterium to the cell surface. Likewise, infection experiments in *Muc1*^{−/−} mice have shown greater susceptibility to infection, GI barrier transit, and pathology by *C. jejuni* than in normal mice.⁸⁹ Collectively, the results are consistent with the importance of MUC1 in shielding the epithelial surface from pathogen contact.

Mucin-associated molecules in bacterial infection

In addition to mucins, other mucosa-associated molecules that involve carbohydrate–ligand interactions can play an important role in determining the success of bacterial colonisation of epithelial surfaces.¹⁵ An example of such molecules is trefoil factors.⁹ These factors, comprising TFF1–3, are a group of small proteins involved in wound repair and protection of the integrity of the epithelial barrier; they are secreted by mucus-secreting cells and expressed in a site-specific manner in the GI tract.⁹³ TFF1 segregates with MUC5AC in the gastric foveolar epithelium, TFF2 with MUC6 in the glandular epithelium in the distal stomach, whereas TFF3 and MUC2 are co-expressed by the goblet cells of the large and small intestine. The role of these factors in infection is exemplified in *H pylori* colonisation of the gastric mucosa. *H pylori* has been shown to bind avidly to dimeric TFF1, and this interaction enables binding to gastric mucin, suggesting that TFF1 acts as a linker molecule binding to both *H pylori* and MUC5AC.^{94–95} Although the *H pylori*–TFF1 interaction appears well-founded, recognition by TFF1 has been ascribed to Glc- and Man-related structures within *H pylori* LPS by *in vitro* testing.⁸⁹ However, this requires further examination because the sugar Man is absent from this LPS,^{40–44} but is present in bacterial cell surface mannans which co-purify with LPS.⁹⁶

Another group of molecules, the surfactant proteins, have relevance to antibody-independent pathogen recognition and clearance from the GI tract. Surfactant protein D (SP-D), a member of the collectin family, is a C-type lectin that is found in mucus-secreting cells within gastric pits and with maximal expression at the luminal surface.^{97–98} Levels of expression of SP-D are significantly increased in *H pylori*-associated antral gastritis compared with normal human gastric mucosa, and co-localises with infecting *H pylori* bacteria.⁹⁶ *SP-D*^{−/−} mice are more susceptible to *Helicobacter* colonisation, and exhibit decreased inflammation and immune response to infection, emphasising the

important influence on the establishment of infection.⁹⁹ Functioning as a lectin, SP-D binds *H pylori* by recognition of the carbohydrate-bearing O-chain of LPS, resulting in bacterial aggregation and immobilisation,^{97–100} thus aiding eventual phagocytosis and clearance. Nevertheless, to evade this important mechanism of innate immune recognition, escape variants within the infecting *H pylori* population can arise^{98–100} with modifications in their O-chain glycosylation, thus decreasing bacterial interaction with SP-D and aiding colonisation.¹⁰⁰ This represents an important example of changes in bacterial glycosylation to overcome lectin-based microbial recognition in the GI mucosa.

CONCLUSIONS

GI glycosylation in health and disease

The GI tract and its mucosa is a complex ecosystem of resident microbiota, resident and recruited immune cells, and a responsive mucus layer and epithelium. In addition to the barrier function of the mucus gel and glycocalyx against pathogens, protection is augmented by the production of antimicrobial peptides, including defensins, by the host.^{7–101} For example, expression of the human cathelicidin LL-37¹⁰² and human β -defensin 2¹⁰³ is upregulated in the gastric mucosa of *H pylori*-infected patients.

Not to be overlooked, the microbiota may influence host antimicrobial peptide production. Angiogenin-4, a molecule with bactericidal activity produced by Paneth cells, is induced by *Bifidobacterium thetaiotaomicron*, a dominant member of the gut microbiota.¹⁰⁴ Also, *Bifidobacterium* cell wall proteins have been shown to induce β -defensin 2 production from human intestinal epithelial cells.⁷ Moreover, Gram-negative bacterial components, such as LPS, peptidoglycan¹⁰⁵ and protein derived from flagella (FliC), can induce expression of human β -defensin 2,¹⁰⁶ and hence is likely to be induced by resident intestinal colonisers. Although members of the resident microbiota have been described as inducing inhibition of other bacterial species, including pathogens, by the production of the antimicrobial compounds microcins and colicins,^{7–107} invading pathogens can also produce such compounds to shape the GI tract flora. *H pylori* produces a ceropin-like peptide, Hp(2-20),¹⁰⁸ to which it is resistant, compared with other Gram-negative bacteria, because of modification of a membrane lipoglycan moiety.¹⁰⁹

In general, the gut microbiota is no longer considered inert and stagnant but evolving, and influences the homeostasis of the host,^{7–110–111} for example, by influencing the host immunity through the T regulatory cell response.^{110–111} The capsular polysaccharide A of the intestinal symbiont *Bacteroides fragilis* induces protection from experimental colitis, produced by *Helicobacter hepaticus*, through induction of tolerogenic T cells.¹¹² Also, members of the microbiota can limit anti-inflammatory responses and colonisation,¹¹³ and establish a balanced relationship with the host.¹¹⁰ Overall, it has been deduced that commensal

Key points

- ▶ All cells, including those of the gastrointestinal tract, are covered in a glycocalyx composed of glycolipids, glycoproteins and proteoglycans.
- ▶ Mucins are a major class of glycoproteins that are both cell-membrane-bound and secreted to form a gel.
- ▶ Mucins are key molecules in acute and chronic infections of the gut, and their glycosylation changes in response to microbial pathogens.
- ▶ The supramucosal gel is a physicochemical sensor responsive to the environment that acts as a highly hydrated, mechanical and lubricative barrier and a matrix for immune-related and antimicrobial proteins.
- ▶ Bacterial surface proteins, adhesins, bind to carbohydrate moieties on host cells and affect the adhesion and colonisation process.
- ▶ Glycan–lectin interactions are emerging as key elements in gut pathogenesis.
- ▶ The glycocalyx and carbohydrate components of the mucus gel are responsive to pathogens, which can promote or hinder adhesion and infection.

bacteria have evolved specialised molecules, such as polysaccharide A of *B fragilis*, to induce tolerogenic responses that coordinate tolerance of the microbiota in the gut, in contrast with pathogen colonisation.¹¹¹ This represents another level of communication with the host that can be utilised by the microbiota.

By modifying GI glycosylation, the gut microbiota can further influence this barrier against pathogens. Seminal work showed that, in germ-free rodents, the mucus layer is reduced by half and is less stable,¹¹⁴ and has an altered glycosylation profile compared with conventional hosts.^{18–41} The use of cDNA microarray analysis has shown the participation of a well-established intestinal microbiota in regulation of the gut mucus layer and composition.⁶⁵ It has been suggested that the resident microbiota can affect goblet cell dynamics and mucin composition directly, by the release of bioactive factors, and indirectly by activating host cells.^{7–41} For example, Gram-negative LPS increases the expression of mRNA of MUC5AC and MUC5B and stimulates their secretion.⁶¹ A bacterial quorum-sensing signal molecule of Gram-negative bacteria may stimulate production of MUC5AC. A strain of *Lactobacillus plantarum*, a Gram-positive subdominant species of the microbiota, increases expression of MUC2 and MUC3 mRNA,¹¹⁵ which has been shown to inhibit the cell attachment of enteropathogenic *E coli* (EPEC) strains.¹¹⁶ Moreover, a cocktail of probiotic bacteria (*Lactobacillus* and *Bifidobacterium* spp.) has been shown to attenuate *C jejuni* association with, and translocation through, mucus-secreting intestinal cells in vitro.¹¹⁷

Therefore, in considering glycosylation as contributing to the structures and barrier functions of the GI tract against pathogens, the influence of the resident microbiota promises to be a subject of developing interest and potential.

Glycosylation and glycosylated receptors as novel therapeutic targets

Biomedical applications of glycobiology have focused greatly on the roles of carbohydrates as receptors for adhesion of pathogens and toxins to host cells, appearance as tumour-associated markers, the regulation of inflammation, and the circulatory half-life of plasma glycoproteins and glycosylated therapeutic proteins. Many approaches have been adopted to modulate biological systems involving carbohydrates and lectin interactions. The most common involve the utilisation of glycans or glycomimetics to regulate the activity of lectins. The other approaches include modulation of genes and enzymes involved in glycosylation pathways and the use of glycotopes as vaccination targets.¹¹⁸ The success of sialic acid analogues (zanamivir and oseltamivir—Relenza and Tamiflu, respectively) as inhibitors of influenza virus sialidase shows promise for the use of glycan analogues against pathogens.¹¹⁹

The increasing antibiotic resistance associated with *H pylori* eradication by antimicrobial therapy is raising the need to search for alternative strategies. As *H pylori* uses the host glycan receptors for

adhesion and infection, a strategy of carbohydrate-dependent inhibition of *H pylori* colonisation using carbohydrate-binding mimics (bio-mimics) will have a therapeutic and/or prophylactic effect on *H pylori* infection. Although *H pylori*-induced changes in host glycosylation have been implicated in the progression of gastric disease, the molecular mechanisms by which *H pylori* modulates host cell glycosylation is not known. In addition, the potential of using synthetic carbohydrates to inhibit *H pylori* adhesion to glycan receptors on gastric epithelial cells needs to be explored. Characterisation of the glycosylation genes and glycan receptors essential for *H pylori* adhesion will allow identification of individuals at highest risk of developing *H pylori* infection-associated diseases, as well as provide the proof of concept towards the design of new infection eradication strategies based on inhibition of bacterial adhesion to gastric epithelial cells.

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REFERENCES

1. Mann M, Jensen ON. Proteomic analysis of post-translational modifications. *Nat Biotechnol* 2003;**21**:255–61.
2. Varki A, Lowe JB. Biological Roles of Glycans. In: Varki A, Cummings RD, Esko JD, et al, eds. *Essentials of Glycobiology*. 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 2009:75–88.
3. Walsh G, Jefferis R. Post-translational modifications in the context of therapeutic proteins. *Nat Biotechnol* 2006;**24**:1241–52.
4. Perez-Vilar J, Hill RL. The structure and assembly of secreted mucins. *J Biol Chem* 1999;**274**:31751–4.
5. Bartlett AH, Park PV. Proteoglycans in host-pathogen interactions: molecular mechanisms and therapeutic implications. *Expert Rev Mol Med* 2010;**12**:e5.
6. Hakomori S. Structure, organization, and function of glycosphingolipids in membrane. *Curr Opin Hematol* 2003;**10**:16–24.
7. Liévin-Le Moal V, Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin Microbiol Rev* 2006;**19**:315–37.
8. Linden SK, Sutton P, Karlsson NG, et al. Mucins in the mucosal barrier to infection. *Mucosal Immunol* 2008;**1**:183–97.
9. Carrington SD, Clyne M, Reid CJ, et al. Microbial interactions with mucus and mucins. In: Moran AP, Holst O, Brennan PJ, von Itzstein M, eds. *Microbial Glycobiology: Structures, Relevance and Applications*. London, UK: Academic Press, 2009:655–71.
10. Linden SK, Arnqvist A, Teneberg S, et al. *H. pylori* adhesion to the gastric surface. In: Sutton P, Mitchell H, eds. *Helicobacter Pylori in the 21st Century*. Wallingford, UK: CABI Publishers, 2010:248–68.
11. Hooper LV, Gordon JL. Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity. *Glycobiology* 2001;**11**:1R–10R.
12. Sonnenburg JL, Xu J, Leip DD, et al. Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* 2005;**307**:1955–9.
13. Sonnenburg JL, Angenent LT, Gordon JL. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nat Immunol* 2004;**5**:569–73.
14. Hattrop CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 2008;**70**:431–57.

15. **Corfield AP**, Carroll D, Myerscough N, *et al.* Mucins in the gastrointestinal tract in health and disease. *Front Biosci* 2001;**6**: D1321–57.
16. **Corfield AP**, Wiggins R, Edwards C, *et al.* A sweet coating — how bacteria deal with sugars. *Adv Exp Med Biol* 2003;**535**:3–15.
17. **Singh PK**, Hollingsworth MA. Cell surface-associated mucins in signal transduction. *Trends Cell Biol* 2006;**16**:467–76.
18. **Sharma R**, Schumacher U. Carbohydrate expression in the intestinal mucosa. *Adv Anat Embryol Cell Biol* 2001;**160**:1–91.
19. **Barnich N**, Carvalho FA, Glasser AL, *et al.* CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. *J Clin Invest* 2007;**117**:1566–74.
20. **Ilver D**, Arngqvist A, Ogren J, *et al.* *Helicobacter pylori* adhesion binding fucosylated histo- blood group antigens revealed by retagging. *Science* 1998;**279**:373–7.
21. **Mahdavi J**, Söndén B, Hurtig M, *et al.* *Helicobacter pylori* adhesin in persistent infection and chronic inflammation. *Science* 2002;**297**:573–8.
22. **Green C**. The ABO, Lewis and related blood group antigens; a review of structure and biosynthesis. *FEMS Microbiol Immunol* 1989;**1**:321–30.
23. **Aspholm-Hurtig M**, Dailide G, Lahmann M, *et al.* Functional adaptation of BabA, the *H. pylori* ABO blood group antigen binding adhesin. *Science* 2004;**305**:519–22.
24. **Lindén S**, Mahdavi J, Semino-Mora C, *et al.* Role of ABO secretor status in mucosal innate immunity and *H. pylori* infection. *PLoS Pathogens* 2008;**4**:e2.
25. **Yamaoka Y**. Roles of *Helicobacter pylori* BabA in gastroduodenal pathogenesis. *World J Gastroenterol* 2008;**14**:4265–72.
26. **Teneberg S**, Miller-Podraza H, Lampert HC, *et al.* Carbohydrate binding specificity of the neutrophil-activating protein of *Helicobacter pylori*. *J Biol Chem* 1997;**272**:19067–71.
27. **Namavar F**, Sparrius M, Veerman EC, *et al.* Neutrophil-activating protein mediates adhesion of *Helicobacter pylori* to sulfated carbohydrates on high-molecular-weight salivary mucin. *Infect Immun* 1998;**66**:444–7.
28. **Valkonen KH**, Wadström T, Moran AP. Identification of the *N*-acetylneuraminylactose- specific laminin-binding protein of *Helicobacter pylori*. *Infect Immun* 1997;**65**:916–23.
29. **Moran AP**, Broaders SA, Rapa A, *et al.* In vivo expression of the 25-kDa laminin- binding protein of *Helicobacter pylori*. *FEMS Immunol Med Microbiol* 2005;**43**:331–7.
30. **Odenbreit S**, Till M, Hofreuter D, *et al.* Genetic and functional characterization of the alpAB gene locus essential for the adhesion of *Helicobacter pylori* to human gastric tissue. *Mol Microbiol* 1999;**31**:1537–48.
31. **Snelling WJ**, Moran AP, Ryan KA, *et al.* HorB (HP0127) is a gastric epithelial cell adhesin. *Helicobacter* 2007;**12**:200–9.
32. **Issa S**, Moran AP, Ustinov SN, *et al.* O-linked oligosaccharides from salivary agglutinin: *Helicobacter pylori* binding sialyl-Lewis x and Lewis b are terminating moieties on hyperfucosylated oligo-*N*-acetylglucosamine. *Glycobiology* 2010;**20**:1046–57.
33. **Lindén SK**, Wickström C, Lindell G, *et al.* Four modes of adhesion are used during *Helicobacter pylori* binding to human mucins in the oral and gastric niches. *Helicobacter* 2008;**13**:81–93.
34. **Edwards NJ**, Monteiro MA, Faller G, *et al.* Lewis X structures in the O antigen side- chain promote adhesion of *Helicobacter pylori* to the gastric epithelium. *Mol Microbiol* 2000;**35**:1530–9.
35. **Fowler M**, Thomas RJ, Atherton J, *et al.* Galectin-3 binds to *Helicobacter pylori* O- antigen: it is upregulated and rapidly secreted by gastric epithelial cells in response to *H. pylori* adhesion. *Cell Microbiol* 2006;**8**:44–54.
36. **Yamaoka Y**, Ojo O, Fujimoto S, *et al.* *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. *Gut* 2006;**55**:775–81.
37. **Moran AP**, Knirel YA, Senchenkova SN, *et al.* Phenotypic variation in molecular mimicry between *Helicobacter pylori* lipopolysaccharides and human gastric epithelial cell-surface glycoforms. Acid-induced phase variation in Lewis^x and Lewis^y expression by *H. pylori* lipopolysaccharides. *J Biol Chem* 2002;**277**:5785–95.
38. **Ruiz-Palacios GM**, Cervantes LE, Ramos P, *et al.* *Campylobacter jejuni* binds intestinal H(O) antigen (Fuc α 1,2Gal β 1,4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. *J Biol Chem* 2003;**278**:14112–20.
39. **Hu L**, Kopecko DJ. Cell biology of human host cell entry by *Campylobacter jejuni*. In: Nachamkin I, Szymanski CM, Blaser MJ, eds. *Campylobacter*. 3rd edn. Washington, DC: ASM Press, 2008:297–313.
40. **Moran AP**. Lipopolysaccharides of *Helicobacter pylori*: importance in gastric adaptation and pathogenesis. In: Sutton P, Mitchell H, eds. *Helicobacter Pylori in the 21st Century*. Wallingford, UK: CABI Publishers, 2010:190–211.
41. **Deplancke B**, Gaskins HR. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am J Clin Nutr* 2001;**73**:1131S–41S.
42. **Lindén SK**, Florin THJ, McGuckin MA. Mucin dynamics in intestinal bacterial infection. *PLoS One* 2008;**3**:e3952.
43. **Liu TW**, Ho CW, Huang HH, *et al.* Role of α -L-fucosidase in the control of *Helicobacter pylori*-infected gastric cancer cells. *Proc Natl Acad Sci USA* 2009;**106**:14581–6.
44. **Moran AP**. Relevance of fucosylation and Lewis antigen expression in the bacterial gastroduodenal pathogen *Helicobacter pylori*. *Carbohydr Res* 2008;**343**:1952–65.
45. **Seregini E**, Botti C, Massaron S, *et al.* Structure, function and gene expression of epithelial mucins. *Tumori* 1997;**83**:625–32.
46. **Perez-Vilar J**, Eckhardt AE, DeLuca A, *et al.* Porcine submaxillary mucin forms disulphide-linked multimers through its amino-terminal D domains. *J Biol Chem* 1999;**273**:1442–9.
47. **Lidell ME**, Johansson ME, Mörgelin M, *et al.* The recombinant C-terminus of the human MUC2 mucin forms dimers in Chinese-hamster ovary cells and heterodimers with full-length MUC2 in LS 1747 cells. *Biochem J* 2003;**372**:335–45.
48. **Jentoft N**. Why are proteins O-glycosylated? *Trends Biochem Sci* 1990;**15**:291–4.
49. **Lindén S**, Nordman H, Hedenbro J, *et al.* Strain- and blood group-dependent binding of *Helicobacter pylori* to human gastric MUC5AC glycoforms. *Gastroenterology* 2002;**123**:1923–30.
50. **Nordman H**, Davies JR, Lindell G, *et al.* Gastric MUC5AC and MUC6 are large oligomeric mucins that differ in size, glycosylation and tissue distribution. *Biochem J* 2002;**364**:191–200.
51. **Ota H**, Nakayama J, Momose M, *et al.* *Helicobacter pylori* infection produces reversible glycosylation changes to gastric mucins. *Virchows Arch* 1998;**433**:419–26.
52. **Moran AP**, Ferris JA, Perepelov AV, *et al.* Adaptation of Lewis antigen expression in LPS of *Helicobacter pylori* from experimentally infected rhesus monkeys. *J Endotoxin Res* 2004;**10**:315–16.
53. **Wirth HP**, Yang M, Sanabria-Valentín E, *et al.* Host Lewis phenotype-dependent *Helicobacter pylori* Lewis antigen expression in rhesus monkeys. *FASEB J* 2006;**20**:1534–6.
54. **Nilsson C**, Skoglund A, Moran AP, *et al.* An enzymatic ruler modulates Lewis antigen glycosylation of *Helicobacter pylori* LPS during persistent infection. *Proc Natl Acad Sci USA* 2006;**103**:2863–8.
55. **Nilsson C**, Skoglund A, Moran AP, *et al.* Lipopolysaccharide diversity evolving in *Helicobacter pylori* communities through genetic modifications in fucosyltransferases. *PLoS One* 2008;**3**:e3811.
56. **Solnick JV**, Hansen LM, Salama NR, *et al.* Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. *Proc Natl Acad Sci USA* 2004;**101**:2106–11.
57. **Yasui W**, Sentani K, Motoshita J, *et al.* Molecular pathobiology of gastric cancer. *Scand J Surg* 2006;**95**:225–31.
58. **Reis CA**, David L, Correa P, *et al.* Intestinal Metaplasia of Human Stomach Displays Distinct Patterns of Mucin (MUC1, MUC2, MUC5AC, and MUC6) Expression1. *Cancer Res* 1999;**59**:1003–7.
59. **Forstner G**. Signal transduction, packaging and secretion of mucins. *Annu Rev Physiol* 1995;**57**:585–605.
60. **Corfield AP**, Myerscough N, Longman R, *et al.* Mucins and mucosal protection in the gastrointestinal tract: new prospects for mucins in the pathology of gastrointestinal disease. *Gut* 2000;**47**:589–94.
61. **Smirnova MG**, Guo L, Birchall JP, *et al.* LPS up-regulates mucin and cytokine mRNA expression and stimulates mucin and cytokine secretion in goblet cells. *Cell Immunol* 2003;**221**:42–9.
62. **Cabellero-Franco C**, Keller K, De Simone C, *et al.* The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2007;**292**:G315–22.
63. **Lemjabbar H**, Basbaum C. Platelet-activating factor receptor and ADAM10 mediate responses to *Staphylococcus aureus* in epithelial cells. *Nat Med* 2002;**8**:41–6.
64. **Fukushima K**, Sasaki L, Ggawa H, *et al.* Colonization of microflora in mice: mucosal defence against luminal bacteria. *J Gastroenterol* 1999;**34**:54–60.
65. **Comelli EM**, Simmering R, Faure M, *et al.* Multifaceted transcriptional regulation of the murine intestinal mucus layer by endogenous microbiota. *Genomics* 2008;**91**:70–7.
66. **Micots I**, Augeron C, Laboisse CL, *et al.* Mucin exocytosis: a major target for *Helicobacter pylori*. *J Clin Pathol* 1993;**46**:241–5.
67. **Slomiany BL**, Slomiany A. Cytosolic phospholipase A2 activation in *Helicobacter pylori* lipopolysaccharide-induced interference with gastric mucin synthesis. *IUBMB Life* 2006;**58**:217–23.

68. **Slomiany BL**, Liao YH, Lopez RA, *et al.* Effect of *Helicobacter pylori* lipopolysaccharide on the synthesis of sulphated gastric mucin. *Biochem Int* 1992;**27**:687–97.
69. **Byrd JC**, Yunker CK, Xu QS, *et al.* Inhibition of gastric mucin synthesis by *Helicobacter pylori*. *Gastroenterology* 2000;**118**:1072–9.
70. **Morgenstern S**, Koren R, Moss SF, *et al.* Does *Helicobacter pylori* affect gastric mucin expression? Relationship between gastric antral mucin expression and *H. pylori* colonization. *Eur J Gastroenterol Hepatol* 2001;**13**:19–23.
71. **Byrd JC**, Yan P, Sternberg L, *et al.* Aberrant expression of gland-type gastric mucin in the surface epithelium of *Helicobacter pylori*-infected patients. *Gastroenterology* 1997;**113**:455–64.
72. **Cooke CL**, An HJ, Kim J, *et al.* Modification of gastric mucin oligosaccharide expression in rhesus macaques after infection with *Helicobacter pylori*. *Gastroenterology* 2009;**137**:1061–71.
73. **Kawakubo M**, Ito Y, Okimura Y, *et al.* Natural antibiotic function of a human gastric mucin against *Helicobacter pylori* infection. *Science* 2004;**305**:1003–6.
74. **Lee H**, Wang P, Hoshino H, *et al.* Alpha1,4GlcNAc-capped mucin-type O-glycan inhibits cholesterol α -glucosyltransferase from *Helicobacter pylori* and suppresses *H. pylori* growth. *Glycobiology* 2008;**18**:549–58.
75. **Vinall LE**, King M, Novelli M, *et al.* Altered expression and allelic association of the hypervariable membrane mucin MUC1 in *Helicobacter pylori* gastritis. *Gastroenterology* 2002;**123**:41–9.
76. **Van den Bovenkamp JH**, Mahdavi J, Korteland-Van Male AM, *et al.* The MUC5AC glycoprotein is the primary receptor for *Helicobacter pylori* in the human stomach. *Helicobacter* 2003;**8**:521–32.
77. **McGuckin MA**, Every AL, Skene CD, *et al.* Muc1 mucin limits both *Helicobacter pylori* colonization of the murine gastric mucosa and associated gastritis. *Gastroenterology* 2007;**133**:1210–18.
78. **Josenshans C**, Suerbaum S. The role of motility as a virulence factor in bacteria. *Int J Med Microbiol* 2002;**291**:605–14.
79. **Moran AP**. Pathogenic properties of *Helicobacter pylori*. *Scand J Gastroenterol* 1996;**31**(Suppl 215):22–31.
80. **Ottemann KM**, Lowenthal AC. *Helicobacter pylori* uses motility for initial colonization and to attain robust infection. *Infect Immun* 2002;**70**:1984–90.
81. **Corfield AP**, Wagner SA, Clamp JR, *et al.* Mucin degradation in the human colon: production of sialidase, sialate O-acetyltransferase, N-acetylneuraminidase, aryltransferase, and glycosulfatase activities by strains of fecal bacteria. *Infect Immun* 1992;**60**:3971–8.
82. **Jones B**, Pascopella L, Falkow S. Entry of microbes into the host: using M cells to break the mucosal barrier. *Curr Opin Immunol* 1995;**7**:474–8.
83. **Neutra MR**, Mantis NJ, Frey A, *et al.* The composition and function of M cell apical membranes: implications for microbial pathogenesis. *Semin Immunol* 1999;**11**:171–81.
84. **Lelouard H**, Reggio H, Mangeat P, *et al.* Mucin-related epitopes distinguish M cells and enterocytes in rabbit appendix and Peyer's patches. *Infect Immun* 1999;**67**:357–67.
85. **Lelouard H**, Reggio H, Roy C, *et al.* Glycocalyx on rabbit intestinal M cells displays carbohydrate epitopes from Muc2. *Infect Immun* 2001;**69**:1061–71.
86. **Terrés AM**, Pajares JM, Hopkins AM, *et al.* *Helicobacter pylori* disrupts epithelial barrier function in a process inhibited by protein kinase C activators. *Infect Immun* 1998;**66**:2943–50.
87. **Valkonen KH**, Wadström T, Moran AP. Interaction of lipopolysaccharides of *Helicobacter pylori* with basement membrane protein laminin. *Infect Immun* 1994;**62**:3640–8.
88. **Slomiany BL**, Piotrowski J, Rajah G, *et al.* Inhibition of gastric mucosal laminin receptor by *Helicobacter pylori* lipopolysaccharide: effect of nitecapone. *Gen Pharmacol* 1991;**22**:1063–9.
89. **McAuley JL**, Linden SK, Png CW, *et al.* MUC1 cell-surface mucin is a critical element of the mucosal barrier to infection. *J Clin Invest* 2007;**117**:2313–24.
90. **Ren J**, Agata N, Chen D, *et al.* Human MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. *Cancer Cell* 2004;**5**:163–75.
91. **Wei X**, Xu H, Kufe D. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. *Cancer Cell* 2005;**7**:167–78.
92. **Lindén SK**, Sheng YH, Every AL, *et al.* MUC1 limits *Helicobacter pylori* infection both by steric hindrance and by acting as a releasable decoy. *PLoS Pathog* 2009;**5**:e1000617.
93. **Wright NA**, Hoffman W, Otto WR, *et al.* Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. *FEBS Lett* 1997;**408**:121–3.
94. **Clyne M**, Dillon P, Daly S, *et al.* *Helicobacter pylori* interacts with the human single-domain trefoil protein TFF1. *Proc Natl Acad Sci USA* 2004;**101**:7409–14.
95. **Reeves EP**, Ali T, Leonard P, *et al.* *Helicobacter pylori* lipopolysaccharide interacts with TFF1 in a pH-dependent manner. *Gastroenterology* 2008;**135**:2043–54.
96. **Ferreira JA**, Azevedo NF, Viesira MJ, *et al.* Identification of cell-surface mannans in a virulent *Helicobacter pylori* strain. *Carbohydr Res* 2010;**345**:830–8.
97. **Murray E**, Khamri W, Walker MM, *et al.* Expression of surfactant protein D in the human gastric mucosa and during *Helicobacter pylori* infection. *Infect Immun* 2002;**70**:1481–7.
98. **Moran AP**, Khamri W, Walker MM, *et al.* Role of surfactant protein D (SP-D) in innate immunity in the gastric mucosa: evidence of interaction with *Helicobacter pylori* lipopolysaccharide. *J Endotoxin Res* 2005;**11**:357–62.
99. **Khamri W**, Worku ML, Anderson AE, *et al.* *Helicobacter* infection in the surfactant protein D-deficient mouse. *Helicobacter* 2007;**12**:112–23.
100. **Khamri W**, Moran AP, Worku ML, *et al.* Variations in *Helicobacter pylori* lipopolysaccharide to evade the innate immune component surfactant protein D. *Infect Immun* 2005;**73**:7677–86.
101. **Salzman NH**, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. *Semin Immunol* 2007;**19**:70–83.
102. **Hase K**, Murakami M, Iimura M, *et al.* Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against *Helicobacter pylori*. *Gastroenterology* 2003;**125**:1613–25.
103. **Hamanaka Y**, Nakashima M, Wada A, *et al.* Expression of human beta-defensin 2 (hBD-2) in *Helicobacter pylori* induced gastritis: antibacterial effect of hBD-2 against *Helicobacter pylori*. *Gut* 2001;**49**:481–7.
104. **Hooper LV**, Stappenback TS, Hong CV, *et al.* Angiogenins: a new class of microbicidal proteins involved in innate immunity. *Nat Immunol* 2003;**4**:269–73.
105. **Vora P**, Youdim A, Thomas LS, *et al.* Beta-defensin 2 expression is regulated by TLR signaling in intestinal epithelial cells. *J Immunol* 2004;**173**:5398–405.
106. **Ogushi K**, Wada A, Niidome T, *et al.* Gangliosides act as co-receptors for Salmonella enteritidis FliC and promote FliC induction of human beta-defensin 2 expression in Caco-2 cells. *J Biol Chem* 2004;**279**:12213–19.
107. **Duquesne S**, Petit V, Peduzzi J, *et al.* Structural and functional diversity of microcins, gene-encoded antibacterial peptides from enterobacteria. *J Mol Microbiol Biotechnol* 2007;**13**:200–9.
108. **Putsep K**, Branden CI, Boman HG, *et al.* Antibacterial peptide from *H. pylori*. *Nature* 1999;**398**:671–2.
109. **Moran AP**, Trent MS. *Helicobacter pylori* lipopolysaccharides and Lewis antigens. In: Yamaoka Y, ed. *Helicobacter Pylori: Molecular Genetics and Cellular Biology*. Norfolk, UK: Caister Academic Press, 2008:7–36.
110. **Round JL**, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009;**9**:313–23.
111. **Round JL**, O'Connell RM, Mazmanian SK. Coordination of tolerogenic immune responses by the commensal microbiota. *J Autoimmun* 2010;**34**:J220–5.
112. **Mazmanian SK**, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008;**453**:620–5.
113. **Chow J**, Mazmanian SK. A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe* 2010;**7**:265–76.
114. **Szentkuti L**, Riedesel ML, Enss K, *et al.* Pre-epithelial mucus layer in the colon of conventional and germ-free rats. *Histochem J* 1990;**22**:491–7.
115. **Mack DR**, Ahne S, Hyde L, *et al.* Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells *in vitro*. *Gut* 2003;**52**:827–33.
116. **Mack DR**, Michail S, Wei S, *et al.* Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. *Am J Physiol* 1999;**276**:G941–50.
117. **Alemka A**, Clyne M, Shanahan F, *et al.* Probiotic colonization of mucus-adherent HT29MTX12 cells attenuates Campylobacter jejuni virulence properties. *Infect Immun* 2010;**78**:2812–22.
118. **Dwek RA**, Butters TD, Platt FM, *et al.* Targeting glycosylation as a therapeutic approach. *Nat Rev Drug Discov* 2002;**1**:65–75.
119. **Merry T**, Astrautsova S. Alternative approaches to antiviral treatments: focusing on glycosylation as a target for antiviral therapy. *Biotechnol Appl Biochem* 2010;**56**:103–9.