Mucins in the mucosal barrier to infection

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The mucosal tissues of the gastrointestinal, respiratory, reproductive, and urinary tracts, and the surface of the eye present an enormous surface area to the exterior environment. All of these tissues are covered with resident microbial flora, which vary considerably in composition and complexity. Mucosal tissues represent the site of infection or route of access for the majority of viruses, bacteria, yeast, protozoa, and multicellular parasites that cause human disease. Mucin glycoproteins are secreted in large quantities by mucosal epithelia, and cell surface mucins are a prominent feature of the apical glycocalyx of all mucosal epithelia. In this review, we highlight the central role played by mucins in accommodating the resident commensal flora and limiting infectious disease, interplay between underlying innate and adaptive immunity and mucins, and the strategies used by successful mucosal pathogens to subvert or avoid the mucin barrier, with a particular focus on bacteria.

MUCINS—AN INTEGRAL PART OF THE MUCOSAL BARRIER

Mucosal epithelial tissues have evolved multiple mechanisms of defense in response to their vulnerability to microbial attack due to their exposure to the external environment. The mucosal epithelial cells form a contiguous lining that acts as a barrier between the moist exterior environment and the remainder of the host. In addition, these cells, both constitutively and in response to microbes, together with underlying leukocytes, secrete many defensive compounds into the mucosal fluid, including mucins, antibodies, defensins, protegrins, collectins, cathlecidins, lysozyme, histatins, and nitric oxide.[1–3] Together, these different defensive compounds form a physical barrier and have direct antimicrobial activity, and the ability to opsonize microbes to aid clearance. Mucin glycoproteins, however, can fulfill all of these roles individually.

Mucosal pathogens, almost by definition, have evolved mechanisms to subvert these mucosal defensive measures. The first barrier the pathogen encounters is the highly hydrated mucus gel that covers the mucosal surface and protects the epithelial cells against chemical, enzymatic, microbial, and mechanical insult. Mucosal surfaces are coated with a layer of viscous mucus ranging in thickness from 10 μm in the eye[4] and trachea[5] to 300 μm in the stomach and 700 μm in the intestine.[6–8] This mucus layer is not static but moves to clear trapped material. In the gastrointestinal tract, the outer mucus layer is continually removed by movement of the luminal contents, whereas in the respiratory tract cilia drive its movement. Mucin glycoproteins produced by mucus-producing cells in the epithelium or submucosal glands are the major macromolecular constituent of mucus and are responsible for the viscous properties of the mucus gel. In addition to forming a relatively impervious gel, which acts as a lubricant, a physical barrier, and a trap for microbes, mucus provides a matrix for a rich array of antimicrobial molecules.

Underneath the mucus layer, the cells present a dense forest of highly diverse glycoproteins and glycolipids, which form the glycocalyx. Membrane-anchored cell-surface mucin glycoproteins are a major constituent of the glycocalyx in all mucosal tissues. The glycocalyx is highly variable from tissue to tissue; for example, the glycocalyx of human intestinal microvilli tips is thick (100–500 nm) in comparison with the glycocalyx of the lateral microvilli surface (30–60 nm).[9,10] The oligosaccharide moieties of the molecules forming the glycocalyx and the mucus layer are highly diverse, and the average turnover time of the human jejunal glycocalyx is 6–12 h.[11] Consequently, both the secreted and adherent mucosal barriers are constantly renewed and could potentially be rapidly adjusted to changes in the environment, for example, in response to microbial infection.

MUCIN BIOSYNTHESIS AND STRUCTURE

The tremendous energy investment by mucosal tissues in the production of mucins in the basal state, but particularly in response to infection, is testimony to the importance of these

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glycoproteins. Epithelial mucins are a heterogenous family of large complex glycoproteins containing a dense array of *O*-linked carbohydrates typically comprising over 70% of their mass. These glycans are concentrated in large peptide domains of repeating amino-acid sequences rich in serine and threonine. The size and number of repeats vary between the mucins, and in many of the genes there are genetic polymorphisms in the number of repeats (variable number of tandem repeats or VNTR polymorphisms), which means the size of individual mucins can differ substantially between individuals. Each mucin is thought to form a filamentous protein carrying typically 100s of complex oligosaccharide structures,[12] giving the mucin a "bottle-brush" appearance. To date, at least 16 human mucins have been included in the family, and the expression profile of mucins varies between tissues with the gastrointestinal tract showing the highest and most diverse expression (see **Table 1**).

Mucins can be divided into three distinct subfamilies: (a) secreted gel-forming mucins, (b) cell-surface mucins, and (c) secreted non-gel-forming mucins (**Table 1**). Gel-forming mucins, which are the major constituent of mucus and confer its viscoelastic properties, are encoded by a cluster of four highly related genes on chromosome 11[13–15] and a similar gene on chromosome 12.[16] Gel-forming mucins contain N- and C-terminal cysteine-rich domains that are both involved in homo-oligomerization mediated by inter-molecular disulfide bonds.[17,18] The current model for mucin oligomerization is that dimerization occurs rapidly during biosynthesis in the endoplasmic reticulum preceding or concomitant with *N*-glycosylation but before *O*-glycosylation in the Golgi apparatus, which in turn is followed by multimerization of dimers.[19] Oligomerization is likely to produce either extended filamentous structures or, more probably, web-like molecular structures likely to be critical to the rheological properties of the mucus gel.[20–24] The extended conformation caused by dense glycosylation enables the molecules to occupy large volumes, with the secreted oligomeric mucins occupying volumes equivalent to those of small bacteria.[25] The secreted non-oligomerizing mucins include the MUC7 salivary mucin, which can self-aggregate but is not thought to contribute significantly to mucus properties, and the MUC8 respiratory mucin, which has not been fully characterized to date.

There are 11 known genes encoding cell-surface mucins expressed by a wide diversity of mucosal tissues, with substantial redundancy in many tissues (see **Table 1**). Cell-surface mucins are present on the apical membrane of all mucosal epithelial cells and contain large extracellular VNTR domains predicted to form rigid elongated structures. Together with their high expression, this indicates that these molecules are likely to be a prominent, probably dominating, constituent of the glycocalyx and may provide a barrier that limits access of other cells and large molecules to the cell surface. During synthesis, most cell-surface mucins appear to be cleaved into two subunits in a region known as an SEA module, which is often flanked by epidermal growth factor-like domains.[26] Structural studies of the MUC1 SEA module suggest that the cleavage occurs via autoproteolysis and that the two subunits remain non-covalently associated throughout biosynthesis.[27] However, importantly, the extracellular α-subunit can be shed from the cell surface either mediated via a second distinct cleavage event[28,29] or perhaps via physical shear forces separating the two domains at the original cleavage site as suggested by Macao *et al*.[27] Mutation

Table 1 Tissue distribution of mucins

Mucin	Distribution	References
Secreted gel forming		
MUC2	Small intestine, colon, respiratory tract, eye, middle ear epithelium	231–235
MUC5AC	Respiratory tract, stomach, cervix, eye, middle ear epithelium	235–239
MUC5B	Respiratory tract, salivary glands, cervix, gallbladder, seminal fluid, middle ear epithelium	235,236, 240–244
MUC6	Stomach, duodenum, gallbladder, pancreas, seminal fluid, cervix, middle ear epithelium	235,243, 245–247
MUC19	Sublingual gland, submandibular gland, respiratory tract, eye, middle ear epithelium	16,235,248
Secreted non-gel forming (monomeric)		
MUC7	Salivary glands, respiratory tract, middle ear epithelium	235,249,250
Cell surface		
MUC1	Stomach, breast, gallbladder, cervix, pancreas, respiratory tract, duodenum, colon, kidney, eye, B cells, T cells, dendritic cells, middle ear epithelium	235,251–256
MUC3A/B	Small intestine, colon, gall bladder, duodenum, middle ear epithelium	235,243, 257,258
MUC4	Respiratory tract, colon, stomach, cervix, eye, middle ear epithelium	235,255, 259–261
MUC12	Colon, small intestine, stomach, pancreas, lung, kidney, prostate, uterus	32,262
MUC13	Colon, small intestine, trachea, kidney, appendix, stomach, middle ear epithelium	35,235,262
MUC15	spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocyte, bone marrow, lymph node, tonsil, breast, fetal liver, lungs, middle ear epithelium	235,263
MUC16	Peritoneal mesothelium, reproductive tract, respiratory tract, eye, middle ear epithelium	235,264–267
MUC17	Small intestine, colon, duodenum, stomach, middle ear epithelium	235,268
MUC20	Kidney, placenta, colon, lung, prostate, liver, middle ear epithelium	235,269

of the cleavage site inhibits MUC1 shedding in transfected mammary and respiratory epithelial cells without affecting cell surface expression, indicating the importance of the initial cleavage in shedding.[30] Furthermore, most cell-surface mucin genes appear to undergo alternative splicing and also encode directly secreted isoforms lacking transmembrane and cytoplasmic domains.[31–34] These isoforms are stored in subapical granules and in goblet cell thecae and are secreted both constitutively and following stimuli. Consequently, due to shedding and direct secretion, cell-surface mucins can also be seen as components of secreted mucus.[35]

MUC1 is the most extensively studied membrane-associated mucin and is the most ubiquitously expressed across all mucosal tissues. MUC1 has been estimated to be 200–500 nm in length (depending on the number of tandem repeats), suggesting it will tower above other molecules attached to the plasma membrane.[36] MUC1 associated with the cell surface is constantly internalized (0.9% of the surface fraction min^{-1}) and recycled.[37] Internalization occurs by clathrin-mediated endocytosis, and alterations in *O*-glycan structure stimulate endocytosis and intracellular accumulation.[38] During recycling, sialic acid is added to the premature form of MUC1.[37] Complete sialylation requires several rounds of recycling, one cycle taking approximately 2.5 h.[37] Pulse-chase experiments indicate that the half-life of MUC1 in the plasma membrane is 16–24 h,[37,39] suggesting that the average MUC1 molecule recycles up to 10 times before release.[37] Recycling rates vary between cell lines and possibly environmental conditions and have not been measured in non-transformed cells, making it very difficult to extrapolate to the real rate of recycling and cell-surface half-life *in vivo*. The cytoplasmic tail appears to interact with the cytoskeleton and secondary signaling molecules,[40–42] whereas the extracellular domains of MUC1 and other cell-surface mucins interact with extracellular matrix components and other cells.[43–47]

The cytoplasmic domains of the cell surface mucins are complex, often contain known phosphorylation motifs, and are highly conserved across species, suggesting important intracellular functions. The best-studied mucin in this regard is MUC1, which has been explored mainly in terms of its role in cancer cell biology rather than in mucosal defense. We and others have shown phosphorylation of the MUC1 cytoplasmic domain[41,48–52] as well as molecular association with β-catenin,[41,51] linking MUC1 with the Wnt pathway, which is involved in epithelial growth, migration, and wound repair. More recently, it has been shown that the cytoplasmic domain can be cleaved and that the cleaved domain translocates to mitochondria and, together with the p53 transcription factor, to the nucleus, where it modulates the cell cycle and protects against the apoptotic response to genotoxic stress.[53,54] Some pathogenic bacteria produce genotoxins, and thus this protective effect, first identified in cancer cells, may have evolved as part of the natural epithelial defense against microbial genotoxins. We have recently shown that *in vitro* MUC1 protects p53-expressing epithelial cells from the effects of cytolethal distending toxin, a genotoxin produced by *Campylobacter jejuni*.[55,56] *In vivo*, *C. jejuni* more densely colonized the stomachs of *Muc1*$^{-/-}$ mice, but this effect was not seen in isogenic mutants lacking cytolethal distending toxin, indicating that Muc1 lowers gastric colonization at least in part via inhibiting the activity of cytolethal distending toxin.[55] Many of the other cell-surface mucins also contain potential phosphorylation sites and cleavage motifs in the immediate intracellular region of their cytoplasmic domains and may be similarly cleaved. Importantly, there is also evidence that interaction with bacteria can induce phosphorylation of MUC1 *in vitro*.[57] Signaling by the cytoplasmic domains of cell-surface mucins is complex and much remains to be elucidated about their mode of action. However, the evidence to date suggests that these domains are involved in cellular programs regulating growth and apoptosis in mucosal cells perhaps in response to microbes and/or their toxins.

MUCIN GLYCOSYLATION

The carbohydrate structures present on mucosal surfaces vary according to cell lineage, tissue location, and developmental stage. Evidence is emerging that mucin glycosylation can alter in response to mucosal infection and inflammation, and this may be an important mechanism for unfavorably changing the niche occupied by mucosal pathogens. The extensive *O*-glycosylation of the mucins protects them from proteolytic enzymes and induces a relatively extended conformation.[25] The oligosaccharides on secreted mucins are clustered into heavily glycosylated domains (typically 600–1,200 amino acids long) separated by shorter nonglycosylated regions.[25] The *O*-linked glycans contain 1–20 residues, which occur both as linear and branched structures (see **Table 2**). The carbohydrate chain is initiated with an *N*-acetylgalactosamine (GalNAc) linked to serine or threonine and is elongated by the formation of the so-called core structures followed by the backbone region (type-1 and type-2 chains). The chains are typically terminated by fucose (Fuc), galactose (Gal), GalNAc, or sialic acid residues in the peripheral region, forming histo-blood-group antigens such as A, B, H, Lewis-a (Le^a), Lewis-b (Le^b), Lewis-x (Le^x), Lewis-y (Le^y), as well as sialyl-Le^a and sialyl-Le^x structures. Sulfation of Gal and *N*-acetylglucosamine (GlcNAc) residues causes further diversification. In addition to the *O*-linked glycans, mucins contain a smaller number of *N*-linked oligosaccharides, which have been implicated in folding, oligomerization (MUC2), or surface localization (MUC17).[58–60]

The carbohydrate structures present on mucins are determined by the expression of specific glycosyl transferases. Thus, mucin glycosylation is governed by genetics (due to polymorphisms in these enzymes), tissue-specific enzyme expression, and host and environmental factors influencing transferase expression. As an example of the impact of host genotype, the H type-1 structure is made by the *secretor* (Se) gene product, and the majority (80% of Caucasians, all South American Indians and Orientals) carry this structure and are thus referred to as "secretors".[61] Individuals may also express the *Lewis* (Le) gene (90% of the Caucasian population) and, provided that they are also secretors, will modify H type-1 into the Le^b structure.[61,62] If they are nonsecretors, type-1 chains without its blood group antigen H will be turned into Le^a structures.[61,62] The third

Table 2 Common *O*-linked oligosaccharide structures on mucins

Nomenclature	Structure
Core type	
Core 1	-Galβ1-3GalNAcα1-Ser/Thr
Core 2	-Galβ1-3(-GlcNAcβ1-6)GalNAcα1-Ser/Thr
Core 3	-GlcNAcβ1-3GalNAcα1-Ser/Thr
Core 4	-GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα1-Ser/Thr
N-Acetyllactosamine elongation type	
Type 1	-Galβ1-3GlcNAcβ1-
Type 2	-Galβ1-4GlcNAcβ1-
Branching	
i-antigen	-Galβ1-4GlcNAcβ1-3Galβ1- (unbranched)
I-antigen	-Galβ1-4GlcNAcβ1-3(-Galβ1-4GlcNAcβ1-6)Galβ1- (branched)
Terminal structures	
Blood group H	Fucα1-2Galβ1-
Blood group A	Fucα1-2(GalNAcα1-3)Galβ1-
Blood group B	Fucα1-2(Galα1-3)Galβ1-
Terminal structures (Type 1 based)	
Lewis a (Le^a)	Galβ1-3(Fucα1-4)GlcNAcβ1-
Lewis b (Le^b)	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1- (includes H)
Sialyl-Le^a	NeuAc(α2-3)Galβ1-3(Fucα1-4) GlcNAcβ1-
Terminal structures (Type 2 based)	
Lewis x (Le^x)	Galβ1-4(Fucα1-3)GlcNAcβ1-
Lewis y (Le^y)	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1- (includes H)
Sialyl-Le^x	NeuAcα2-3 Galβ1-4(Fucα1-3) GlcNAcβ1-
Sulfation	
3 Sulfation	HSO_3-3Galβ1-
6 Sulfation	HSO_3-6GlcNAcβ1-
Examples of combined epitopes	
H-type 1	Fucα1-2Galβ1-3GlcNAcβ1-
Sialylated type 2	NeuAcα2-3Galβ1-4GlcNAcβ1-

Se phenotype with simultaneous expression of Le^a and Le^b antigens has been described as the "weak-Secretor" (Se^w) phenotype.[63,64] The dual expression of Le^a/Le^b is a consequence of an enzymatically weak Se-transferase in combination with an intact Le-transferase.[63,64] The terminal structures of mucin oligosaccharides are highly heterogeneous and vary between/within species and between and even within tissues. The array of oligosaccharide structures on individual mucin molecules is also somewhat determined by stochastic events as the mucin protein moves through the Golgi apparatus.[65] This structural diversity may allow the mammalian host to cope with diverse and rapidly changing pathogens, as reflected by the observation that susceptibility to specific pathogens differs between people with different histo-blood groups,[66] as exemplified by the observations that individual Se phenotype may determine the ratio of infection as well as the course and severity of urinary tract infections, Norwalk virus induced acute gastroenteritis and *Helicobacter pylori*-induced gastric diseases.[67–69] There is also a strong correlation between distinct adhesive properties of *H. pylori* endemic in specific human populations and the mucin blood group carbohydrate structures expressed by these populations.[70] These differences in the external barrier to infection can be equated with the diversity in underlying innate and adaptive immunity (e.g., polymorphisms in MHC, cytokines), which is thought to have evolved for the same reasons.

REGULATION AND MODULATION OF THE MUCIN BARRIER

The gel-forming 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.[71] Both gel-forming and cell-surface mucins show constitutive and inducible gene expression in mucosal epithelial cells. The promoters of the MUC genes have generally not been fully characterized, although partial promoter characterization is available for human *MUC1*,[72–74] *MUC2*,[75–81] *MUC3*,[82] *MUC4*,[83–85] *MUC5AC*,[79,86] and *MUC5B*.[87] Differential regulation of individual mucin genes is evident between different mucosal tissues and throughout differing regions of the larger epithelial tracts. For example, differing promoter regions are involved in the differential regulation of constitutive *MUC2* expression in the small and large intestines.[78] Expression of cell-surface and gel-forming mucins can be upregulated by inflammatory cytokines such as interleukin (IL)-1β, IL-4, IL-6, IL-9, IL-13, interferons, tumor necrosis factor-α, nitric oxide, and other uncharacterized inflammatory factors.[82,88–110] Responsiveness to these cytokines provides a link between mucins, innate mucosal immunity, and mucosal inflammatory responses. Neutrophils can also stimulate increases in production of both gel-forming and cell-surface mucins by mucosal epithelial cells via neutrophil elastase.[111–116] Microbial products can stimulate increased production of mucins by mucosal epithelial cells.[103,117–120] In fact, there is evidence that adherence of probiotic bacteria upregulates cell-surface mucin expression *in vitro*,[121,122] perhaps representing an important part of the mechanism by which probiotic bacteria limit infection by pathogens. In contrast, the lipopolysaccharide of the pathogen *H. pylori* decreases mucin synthesis in gastric epithelial cells *in vitro* via activation of cPLA-2,[123] representing a mechanism by which a pathogen can favorably modulate the mucus barrier.

The constitutive pathway continuously secretes sufficient mucin to maintain the mucus layer, whereas the regulated pathway affords a massive discharge as a response to environmental and/or (patho)physiological stimuli, including cholinergic stimuli, inflammatory cytokines, prostaglandins, lipopolysaccharide, bile salts, nucleotides, nitric oxide, vasoactive intestinal peptide, and neutrophil elastase.[93,95,103,113,124–131] Stimulated mucin release can occur immediately and is accompanied by hydration, resulting in approximately a hundredfold expansion in volume of the secretory granule contents.[132,133] Shedding of the large extracellular α-subunits of cell-surface mucins from the cell surface and release of secreted splice variants of cell-surface mucins are less well understood. The protease ADAM17 (also known as TACE) has been shown to trigger shedding of MUC1 in endometrial cells in response to tumor necrosis factor-α,[28,134] and the matrix metalloproteinase-1 also appears to be an effective MUC1 sheddase.[29]

In addition to regulation of their synthesis and release, mucins are regulated in terms of their glycosylation. Altering mucin carbohydrates may block mechanisms that pathogens use to subvert the mucin barrier. Tumor necrosis factor-α alters sialylation of mucins produced by a tracheal cell line[135] and expression of both fucosyltransferases and α-2,3-sialyltransferases by normal bronchial mucosal explants.[136] In respiratory epithelial cells, the Th2 cytokines IL-4 and IL-13 increase expression of core 2 β-1,6-*N*-acetylglucosaminyltransferase, which forms β-1,6-branched structures, including core 2, core 4, and blood group I antigen.[137] In addition, glycosylation changes occur during infection/inflammation, for example, in individuals with cystic fibrosis or chronic bronchitis,[138] as well as *H. pylori*-infected individuals.[69,139] The inflammation-associated mucin sialylation shown in patients with *H. pylori* infection returns to the normal pattern following successful bacterial clearance with antibiotics.[140] In rhesus monkeys that share strong similarities in mucin glycosylation and the natural history of *H. pylori* infection with humans,[141] *H. pylori* infection induces time-dependent changes of mucosal glycosylation that alter the *H. pylori* adhesion targets.[69] Such fine-tuned kinetics of host glycosylation dynamically modulate host–bacterial interactions, appearing to balance the impact of infection and thereby may determine the severity of disease.[69] Another example of dynamic changes in mucins occurs following infection of rats with the intestinal parasite *Nippostrongylus brasiliensis*; infection induces increased production and several alterations in the glycosylation of intestinal Muc2 gel-forming mucin, one of which coincides with expulsion of the parasite.[142–147] These alterations in glycosylation appear to be driven partly by $CD4^+$ T cells, as CD4 but not CD8 depletion blocks the increase in mucin production, change in glycosylation and worm expulsion,[148] and also by T-cell-independent mechanisms.[149] Such changes in mucin glycosylation need to be considered as a component of innate and adaptive immune responses to mucosal infection.

MICROBIAL ADHERENCE TO THE EPITHELIUM

To colonize mucosal surfaces and invade the host, microbes typically must first penetrate the secreted mucus barrier and then either attach to the apical surface of epithelial cells decorated with the cell-surface mucins or release toxins that disrupt epithelial integrity. Bacterial adhesion to host cells can be mediated by hydrophobic interactions, cation bridging (i.e., divalent cations counteracting the repulsion of the negatively charged surfaces of bacteria and host) and receptor ligand binding. One of the most extensively studied mechanisms of bacterial adhesion is via lectins and their corresponding glycosylated receptors. Binding is usually of low affinity, but clustering of adhesins and receptors results in multivalent binding. Fimbriae (or pili), outer membrane proteins, and cell wall components (e.g., lipopolysaccharide) may all function as adhesins. Adhesion can affect the bacteria by stimulation/inhibition of growth as well as induction of other adhesive structures and proteins required for invasion such as secretion systems. On the other hand, effects of adhesion on host cells can include altered morphology, fluid loss, induction of cytokine release, upregulation of adhesion molecules, and apoptosis.[150]

Many bacterial adhesins bind oligosaccharides present on mucins. Whether bacterial–mucin binding events favor the bacteria or the host is a key question. In reality, for some organisms, this may be a truly commensal relationship with benefits for both the bacteria (by facilitating retention in a favorable niche and even by providing mucin oligosaccharides for metabolism) and the host (by retaining bacteria in the outer areas of the mucus barrier where they cannot harm the underlying epithelium and also limiting the niche available for pathogenic bacteria). Numerous interactions between microorganisms and mucins and/or mucin-type carbohydrates have been demonstrated (see **Table 3**). 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. As an example, *H. pylori* binds to mucin oligosaccharides via at least four adhesins, which differ substantially with anatomical site along the oro-gastric infection route, mucin type, pH, and gastric disease status.[139,151–153] Thus, for *H. pylori*, binding to mucins can have differing consequences during colonization of the oral-to-gastric niches and during long-term infection.

MUCINS AS DECOYS FOR MICROBIAL ADHESINS

Mucus hypersecretion ensuing from infection is testament to the role of mucus as a component of host defense. Although mucins are the major macromolecular constituent of mucus and are largely responsible for formation of the mucus gel, the precise nature of their role in host defense has not been well demonstrated empirically. Formation of the mucus gel is important in itself, as it provides a biophysical barrier as well as a matrix supporting the retention of a host of antimicrobial molecules. However, the secreted mucins themselves are likely to function as decoys for adhesins that have been evolved by pathogens to engage the cell surface, as the mucins express many of the oligosaccharide structures found on the cell surface and are constitutively produced in large amounts, constantly washing the mucosal surfaces (**Figure 1**). Some mucins are effective viral agglutinating agents and exogenously applied mucins

Table 3 Characterized interactions between mucins and microbes

Tissue derived mucins	Mucin	Carbohydrate	Microbe	References
Respiratory mucins	MUC1	Sialic acids	*P. aeruginosa, Haemophilus influenzae, S. aureus,* influenza viruses	163,181,270–272
Salivary mucins	MUC5B MUC7 (DMBT1-Muclin)	Sulfated Le^a Sialic acids, Sialyl Le^x, Le^b	*P. aeruginosa, H. pylori, Streptococcus sanguis, Streptococcus gordonii, Actinobacillus actinomycetemcomitans, Streptococcus spp., Candida albicans*	273–280
Gastric mucins	MUC5AC MUC1	A, B, H, Le^b	*H. pylori*	139,151,176,281,282
Intestinal mucins	MUC2		Enterotoxigenic *Escherichia coli,* Enteropathogenic *E. coli, Salmonella typhimurium, Shigella boydii, Shigella sonnei, Campylobacter upsaliensis, Yersinia enterolitica, C. albicans,* reoviruses	162,283–290

In most studies, only the tissue origin of the mucin has been determined. Which mucins and carbohydrates are responsible for the binding was only determined for a small proportion of the interactions. The mucin and carbohydrate columns thus do not indicate that all microbes listed interact via these specific structures, but merely that these have been shown to bind to some of the bacteria.

are effective inhibitors of viral infection in *in vitro*-cultured cells.[154,155] *Streptococcus pyogenes*, *Tritrichomonas foetus*, *Tritrichomonas mobilensis*, influenza viruses, reoviruses, adenoviruses, enteroviruses, and coronaviruses bind to sialic acids, which are present both at the epithelial surface and on mucins.[156–165]

Despite the accepted dogma that secreted mucins limit infection, there are few empirical *in vivo* data demonstrating their importance. The only secreted mucin for which genetically deficient animals are available is the intestinal mucin, Muc2. *Muc2*$^{-/-}$ mice develop spontaneous inflammation, presumably due to the absence of the major component of intestinal mucus, leading to increased exposure to the normal intestinal microbial flora.[166,167] As yet, there are no reports of controlled infection experiments in these mice. Further models of secreted mucin deficiency are required to comprehensively determine the importance of secreted mucins in preventing and clearing mucosal infection.

Many pathogens require direct binding to, or penetration of, mucosal epithelial cells to cause pathology. The widest diversity of cell-surface mucin expression is in the mucosal tissues most at risk of infection, such as the gastrointestinal tract, respiratory tract, and eye; notably, nine of the ten cell-surface mucins are expressed in the large intestine, which is the most microbe-rich mucosal environment. Importantly, their ability to be shed from the cell surface has led us to hypothesize that one of the main functions of cell-surface mucins is to act as releasable decoy ligands for microbes attempting to anchor themselves to the glycocalyx. Cell-surface mucins initiate intracellular signaling in response to bacteria, suggesting that they have both a barrier and reporting function on the apical surface of all mucosal epithelial cells.[57] However, until recently, much of the evidence had been circumstantial or restricted to *in vitro* analysis. For example, upregulation of MUC3 expression in colonic cells has been correlated with decreased binding of enteropathogenic *E. coli*.[121,122] *In vitro* studies have shown that expression of MUC1 by transfection inhibits reovirus and adenovirus infection of MDCK cells by up to tenfold.[168,169] Milk can limit bacterial and viral infections of the gastrointestinal tract and this has been attributed in part to the presence of large amounts of cell-surface mucins, chiefly MUC1 and MUC15, in the milk-fat globule membrane.[170–172] *Muc1*$^{-/-}$ mice were reported to display chronic infection and inflammation of the reproductive tract, reducing fertility rates. In this latter study, only normal endogenous bacteria were isolated, suggesting that these species become opportunistic pathogens in the absence of Muc1.[173] In addition, *Muc1*$^{-/-}$ mice were reported to have a high frequency of eye inflammation/infection involving *Corynebacteria*, *Staphylococci* and *Streptococci*,[174] although this could not be duplicated in a different mouse background held in alternative housing conditions.[175]

We recently demonstrated that the intestinal pathogen *C. jejuni* binds to fucosylated mucin oligosaccharides. Controlled infection experiments demonstrated rapid transit of *C. jejuni* across the gastrointestinal barrier and greater intestinal pathology in *Muc1*$^{-/-}$ mice.[55] Bone marrow transplantation studies demonstrated that the increased susceptibility was due to loss of Muc1 on epithelium rather than on leukocytes (which can also express Muc1). Loss of Muc1 had no discernable effects on the abundance or constituency of the intestinal microbial flora. Muc1 appears to prevent *C. jejuni* infection both by protecting cells from the effects of the cytolethal distending toxin (see above) and by acting as a releasable decoy.[55] We have also demonstrated that even though *H. pylori* can bind Muc1, that primary murine gastric epithelial cells expressing Muc1 bind fewer *H. pylori* than *Muc1*$^{-/-}$ cells.[176] This paradoxical result is explained by Muc1 acting as a releasable decoy, i.e., the bacteria bind Muc1 expressed on epithelial cells, which is then shed by the host. Due to the absence of this decoy molecule, *Muc1*$^{-/-}$ mice develop an approximately fivefold greater colonization density of *H. pylori* from the first days following infection that is maintained for at least 2 months. Consequently, *Muc1*$^{-/-}$ mice develop severe gastritis not found in wild-type mice.[176] Heterozygous mice that have a lower level of gastric Muc1

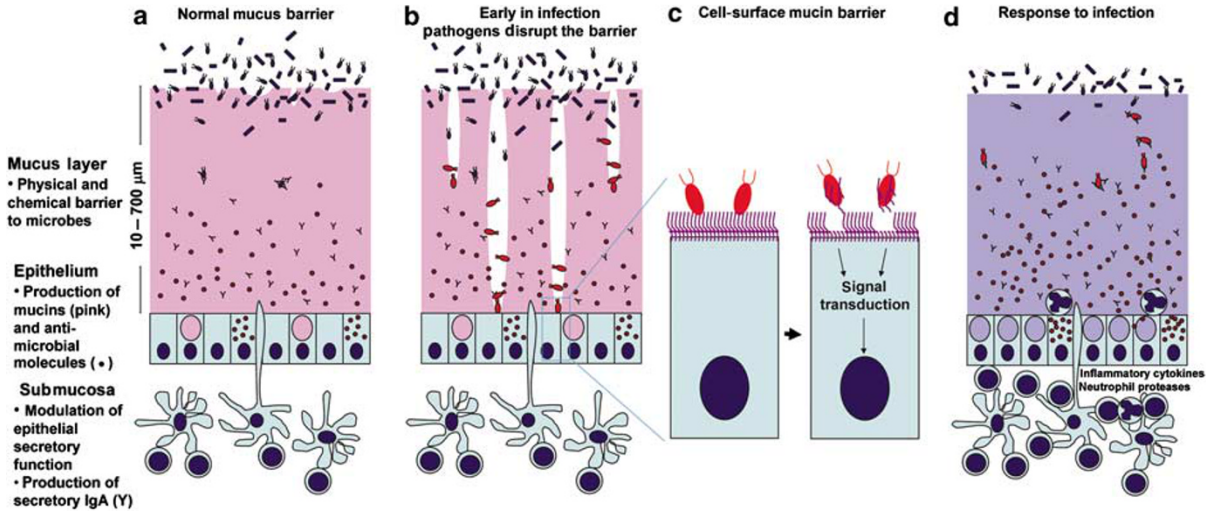

Figure 1 Diagrammatic representation of mucins in the mucosal barrier to infection. (**a**) The normal mucosa is covered with a continuously replenished thick mucus layer retaining host-defensive molecules. Commensal and environmental microbes may live in the outer mucus layer but the layer ensures that contact of microbes with epithelial cells is rare. (**b**) Early in infection, many pathogens actively disrupt the mucus layer and thereby gain access to the epithelial cell surface. In addition, this alters the environment for commensal and environmental microbes and opportunistic pathogenesis may occur. (**c**) Pathogens that break the secreted mucus barrier reach the apical membrane surface, which is decorated with a dense network of large cell-surface mucins. Pathogens bind the cell-surface mucins via lectin interactions and the mucin extracellular domains are shed as releasable decoy molecules. Consequent to contact with microbes and shedding of the extracellular domain, signal transduction by the cytoplasmic domains of the cell-surface mucins modulates cellular response to the presence of microbes. (**d**) In response to infection, there are alterations in mucins that are driven directly by epithelial cells and in response to signals from underlying innate and adaptive immunity. These alterations include goblet cell hyperplasia and increased mucin secretion and altered mucin glycosylation (depicted by the color change) affecting microbial adhesion and the ability of microbes to degrade mucus. These changes in mucins work in concert with other arms of immunity to clear the infection.

protein expression show intermediate colonization densities, which suggests that polymorphisms in *MUC1* or genes that regulate its expression could underlie susceptibility to *H. pylori*-induced pathology in human populations. In fact, in humans, individuals with short *MUC1* alleles (encoding smaller extracellular mucin domains) have a higher propensity to develop gastritis following *H. pylori* infection.[177–179] This may be indicative of lower efficacy of smaller MUC1 extracellular mucin domains allowing increased access of bacteria to the epithelial surface, or these alleles may be surrogate markers of polymorphisms influencing the level of gastric MUC1 expression. Recently, a similar protective role has been demonstrated for the extremely large MUC16 cell-surface mucin in human corneal epithelial cells. Greater binding of *Staphylococcus aureus* occurs to *in vitro*-cultured corneal cells when MUC16 is depleted by RNAi.[180]

Paradoxically, our demonstrations of Muc1-limiting infection in the gastrointestinal tract are opposite to that found in a model of respiratory *Pseudomonas aeruginosa* infection in the lung in which *Muc1*$^{-/-}$ mice have an increased clearance of bacteria and a reduced inflammatory response to infection.[181] MUC1 binds the *P. aeruginosa* flagellin,[182,183] but intriguingly appears to inhibit flagellin-stimulated TLR5-mediated activation of NF-κB by an as yet unclear mechanism requiring the MUC1 cytoplasmic domain.[181,184] Whereas an infection-promoting role of a molecule highly expressed on the apical surface of a broad array of mucosal epithelia appears counterintuitive, an anti-inflammatory role for MUC1 in some tissues is consistent with evolutionary adaptations to clear infection by local defense without potentially damaging inflammation, where possible. Further investigations are required with a broad array of pathogens in multiple tissues to clearly delineate the participation of the family of cell-surface mucins in mucosal defense.

OTHER PROTECTIVE ROLES OF MUCINS

Mucins have direct and indirect roles in defense from infection distinct from their ability to form a physical barrier and act as adhesion decoys. Not only do mucin oligosaccharides bind microbes, but also, in some cases, they either have direct antimicrobial activity or carry other antimicrobial molecules. A mucin oligosaccharide, α-1-4-linked *N*-acetyl-glucosamine, which is expressed by some gastric mucins, has been shown to directly interfere with synthesis of *H. pylori* cell wall components.[185] *H. pylori* must live within gastric mucus to remain protected from luminal gastric pH and prevent expulsion into the intestine. The antimicrobial mucin oligosaccharide probably acts to limit *H. pylori* expansion within gastric mucus. The non-oligomerizing secreted salivary mucin MUC7 has inherent direct candidacidal activity via a histatin domain at its N-terminus.[186,187] In addition, there is evidence for direct binding of antimicrobial molecules such as histatins and statherin by mucins that would help retain the antimicrobial molecules in the correct mucosal microenvironment where they can best protect the host. For example, MUC7 binds statherin and histatin-1,[188] and the other major mucin in saliva, MUC5B, binds histatin-1, -3, and -5 and statherin.[189] Secretory IgA (sIgA) is secreted via mucosal epithelial cells and needs to be

retained in the immediate mucosal environment to maximize exclusion of pathogens. sIgA is retained at high concentrations in mucus where it can efficiently trap the progress of pathogens, although the mechanism(s) for retaining sIgA in mucus are not well understood. Interestingly, secretory component, which is tightly bound to the Fc region of dimeric-IgA to form sIgA, carries oligosaccharide structures similar to those on mucins.[190] In the absence of secretory component carbohydrates, sIgA fails to associate with mucus and fails to prevent infection in a murine respiratory bacterial infection model, substantiating both the physiological importance of sIgA–mucin interactions and the importance of secretory component carbohydrates in maintaining this interaction.[191] It is also tempting to speculate that the poly-anionic mucins bind the poly-cationic antimicrobial defensin peptides that are co-secreted into mucus. Interactions of mucins with other secreted antimicrobial molecules has not been fully explored largely due to difficulties in extracting and purifying mucins in the absence of denaturing agents likely to disrupt such interactions. The cell-surface mucins are an integral component of the glycocalyx where they are likely to interact with proteoglycans and other molecules that could retain host defense molecules in a molecular complex covering the apical cell surface.[192,193] Therefore, other mucins and mucin oligosaccharides may yet prove to have direct and indirect antimicrobial activity. Regardless of whether antimicrobial molecules are retained in mucus by direct binding with mucins or by the biophysical properties of mucus, if mucin synthesis is aberrant or secreted mucins are degraded, the antimicrobial molecules will have impaired efficacy.

SUBVERSION OF THE MUCIN BARRIER BY MUCOSAL PATHOGENS

Perhaps the best evidence for the importance of the mucin barrier to infection is the wide variety of strategies used by microbes to subvert or avoid this barrier. Mucin barrier subversion strategies used by microbes include the production of enzymes capable of degrading mucin core proteins and mucin carbohydrates, and effective motility through mucus gels. Motility is important for bacterial mucosal pathogens to facilitate breaking through the physical mucus barrier. In fact, a vast proportion of mucosal bacterial pathogens are flagellated.[194,195] *H. pylori* that have dysfunctional flagella have a greatly reduced ability to infect.[196] *H. pylori* uses motility for initial colonization and to attain robust infection. In conjunction with motility, degradative enzymes such as glycosulfatases, sialidases, sialate *O*-acetylesterases, *N*-acetyl neuraminate lyases and mucinases are produced by a broad range of bacterial pathogens to destabilize the mucus gel and remove mucin decoy carbohydrates for adhesins.[197–201] The protozoan parasite *Entamoeba histolytica* cleaves the MUC2 mucin, which is the major structural component of the intestinal mucus, and this cleavage is predicted to depolymerize the MUC2 polymers.[202] The size of the polymer is important for the formation of entangled gels and the viscous properties of mucus; consequently, cleavage of the mucin polymer will effectively result in a local disintegration of mucus.[203] There is evidence that these degradative enzymes are critical for microbial pathogenesis. For example, *the Vibrio cholerae* Hap A, which has both mucinolytic and cytotoxic activity, is induced by mucin and required for translocation through mucin-containing gels.[204] The widespread and critically required expression of neuraminidases by a wide variety of sialic acid-binding mucosal viruses underlines the importance of elimination of mucin carbohydrates for their pathogenicity.[160] Lipopolysaccharide from *H. pylori* decreases mucin synthesis,[123] and the mucin carbohydrate-binding adhesins BabA and SabA undergo phase variation and change expression during infection,[153,205] which may allow them to evade this host defense mechanism.

AVOIDANCE OF THE MUCIN BARRIER BY MUCOSAL PATHOGENS

Another strategy commonly used by mucosal pathogens is to avoid the mucin barrier. Intestinal M cells, specifically designed to capture and present microbes to the underlying lymphoid tissue, can be regarded as a hole in the mucin barrier. The dome epithelium in which they lie lacks goblet cells, and therefore does not produce gel-forming mucins, and their apical cell surface has only sparse microvilli and an apparently thin glycocalyx.[206,207] Although no studies have measured the expression of individual cell-surface mucins in M cells, there appear to be differences in the glycocalyx mucins between M cells and adjacent intestinal mucosal epithelial cells. In some species, M cells can be identified by their pattern of lectin binding to specific cell-surface carbohydrates that differ with other mucosal epithelial cells.[208,209] Consequently, even though M cells constitute only a very small percentage of mucosal epithelial cells, they are the major point of attachment and/or entry used by a large number of mucosal pathogens including bacteria (e.g., *S. typhimurium*, *Shigella flexneri*, *Yersinia enterocolitica*, and *V. cholerae*), viruses (e.g., reovirus, HIV-1, and polio virus) and parasites (e.g., Cryptosporidia).[206,210,211] Another strategy used by pathogens to avoid the cell-surface mucin barrier, once mucus is penetrated or M cells are invaded, is to disrupt the tight junctions between adjacent mucosal epithelial cells thereby exposing the vulnerable lateral membranes not protected by the glycocalyx. Such examples include *S. flexneri*,[212] enteropathogenic *E. coli*,[213] *Porphyromonas gingivalis*[214] and *H. pylori*.[215]

MODELS TO INVESTIGATE INTERACTIONS BETWEEN MICROBES AND MUCINS

Numerous models, including cancer cell-lines, organ cultures of gastric biopsies and whole animals have been used to investigate mucin–microbe interactions. Although they express orthologs to most human mucins, the most commonly used laboratory animals such as rats and mice have differing glycosylation of some of their mucins. In fact, it is tempting to speculate that differences in mucin glycosylation between mammalian species may underlie some of the differences in infectivity/pathogenicity for individual microbial pathogens. Murine knockout models are only available for *Muc1*,[216] *Muc2*,[166] and *Muc13* (M.A. McGuckin, unpublished data), and there are also mutants with aberrant Muc2 assembly.[217] Thus, there is a need for more models, as mouse knockouts, although limited by the slightly

different glycosylation, still represent an important way to collect information of the *in vivo* function of mucins in infection. Because human pathogens commonly have adhesins for human carbohydrate structures, it is important to select appropriate models for individual pathogens. For example, the effects of *H. pylori* infection on the mouse are mild, and gastric cancer is not induced even after long-term exposure without other stimuli or genetic defects, although the mouse may develop chronic atrophic gastritis.[218,219] *H. pylori* can colonize the guinea pig and the Mongolian gerbil and cause a severe inflammatory response but does not induce cancer in the absence of exogenous chemical carcinogens.[220] These small animal models are useful to study some aspects of *H. pylori* infection and have the advantage of being relatively cheap. In contrast, rhesus monkeys naturally have persistent *H. pylori* infection leading to loss of mucus, gastritis, gastric ulcers and even cancer.[221–224] In addition, the anatomy and physiology of the GI tract of the rhesus monkey, as well as the expression of mucins and mucin glycosylation are very similar to that in human.[141] However, this model is expensive, the monkeys can have preexisting natural infection, and primate research has a higher level of ethical considerations.

In vitro microbial–mammalian cocultures are used extensively to elucidate the mechanisms by which microbes adhere, invade, and signal to the host, and to examine ensuing mammalian cell responses. These complex interactions are reliant on appropriate gene expression and cellular functioning of both the microbial and mammalian cells. It is therefore critical that appropriate microbial and mammalian cells are used and that the environment created experimentally is as similar to the human mucosal environment as possible. Human cell lines commonly used for *in vitro* infection studies have a highly variable expression of mucins and mucin glycosylation, and generally have very low production and secretion of gel-forming mucins.[225] Investigators using these models need to be aware of these limitations and consider them in interpreting their data. Additional important issues to consider are choice of cell line and, depending on the type of bacteria, oxygen tension.[225,226] With respect to appropriate mucin production, primary human tracheobronchial epithelial cells cultured in an air–liquid interface represent the most physiological cell cultures in which infection studies can currently be undertaken.[227] *Ex vivo*-cultured tissue explants provide another potential avenue for exploring microbial mucin interactions *in vitro*.[228–230]

CONCLUSIONS

The personal repertoire of expression of mucin core proteins and their glycans, mucin allele length, and transient changes in mucin expression and glycosylation in response to infection or stress, as well as variations in environmental conditions may all affect microbial interaction with host mucins and the pathogenic consequences of microbial colonization. Rather than a static barrier, mucins should be considered as a dynamic responsive component of the mucosal barrier that interacts with and responds to other elements of innate and adaptive immunity. Difficulties in working with these complex glycoproteins and the paucity of physiological experimental systems need to be overcome if we are to fully understand the roles of mucins in host defense from infection.

DISCLOSURE

The authors declared no conflict of interest.

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