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The gastrointestinal mucus system in health and disease

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Abstract

Mucins—large, highly glycosylated proteins—are important for the luminal protection of the gastrointestinal tract. Enterocytes have their apical surface covered by transmembrane mucins and goblet cells produce the secreted gel-forming mucins that form mucus. The small intestine has a single unattached mucus layer. In cystic fibrosis, this layer becomes attached, accounting for the intestinal manifestations of this disease. The stomach and colon have two layers of mucus; the inner layer is attached and the outer layer is less dense and unattached. In the colon, the outer mucus layer is the habitat for commensal bacteria. The inner mucus layer is impervious to bacteria and is renewed every hour by surface goblet cells. The crypt goblet cells have the ability to reconstitute the mucus layer by secretion, for example after an ischaemic challenge. Proteases of certain parasites and some bacteria can cleave mucins and dissolve the mucus as part of their pathogenicity. The inner mucus layer can, however, also become penetrable to bacteria by several other mechanisms, including aberrations in the immune system. When bacteria reach the epithelial surface, the immune system is activated and inflammation is triggered. This mechanism might occur in some types of ulcerative colitis.

Introduction

The gastrointestinal tract is an amazing organ: it can digest food but does not digest itself; it harbours more bacteria than there are cells in the human body, yet does not allow the bacteria to take over despite their rapid multiplication; and it can handle relatively strong hydrochloric acid without denaturing the stomach. The mechanisms behind these amazing skills vary, but a major reason is the uttermost defence line of the gastrointestinal tract—the mucus.¹ The proximal part of the digestive tract, the mouth and oesophagus, is, like the skin, protected by multiple layers of tight and largely inert squamous epithelium, which is flushed by mucus from salivary and other glands. By contrast, the rest of the gastrointestinal tract has a single layer of very active cells. The major protection of this vulnerable cellular compartment is by mucus covering these cells and by the glycocalyx,^{2,3} which is both built by and around mucins.

The gastrointestinal tract mucus was studied relatively intensely during the 1960s–1980s,^{4,5} a period that is not covered here. However, more recently it has been less well appreciated or understood that the gut is covered with mucus. Here, we provide an overview of the

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Review criteria We searched our in-house database of 9,600 references for suitable articles. In addition we searched PubMed using the following terms “mucin”, “mucus”, “gastrointestinal tract”, “intestine”, “colon” and “colitis”. We searched for articles dating back to the time of the cloning of the mucin genes in the 1990s.

mucus system along the gastrointestinal tract and discuss the role of mucus in health and disease.

Mucins

Mucin domain

The major building blocks in mucus are mucins, which are large, highly glycosylated proteins (Figure 1).^{6–10} Typically, these mucins are >80% carbohydrate, and are concentrated into mucin domains.¹¹ These domains are built on a protein core that is rich in the amino acids proline, serine and threonine (called PTS sequences). These sequences are often called VNTRs (variable number tandem repeats), as the amino acid sequences are often repeated in tandem, although this is not always the case. As the VNTR designation suggests, the length can vary, but as these are encoded within one exon the length is genetically determined.^{11,12} PTS sequences can be very long; for example the largest one in the MUC2 mucin is about 2,300 amino acids.¹³ The hydroxyl group of the amino acids threonine and serine become the attachment sites for GalNAc (*N*-acetylgalactosamine), which is added in the Golgi apparatus. The repertoire of peptidyl-GalNAc transferases is unique for each organ and cell type.¹⁴ These GalNAc residues are then extended and branched by a series of other glycosyltransferases, generating a very complex mixture of glycan epitopes.^{15,16} Once this glycosylation process is finished at the end of the Golgi apparatus, the mucin domains have extended into long rods. We have estimated that the 2,300 amino acids of the large MUC2 mucin domain make it about 0.45 μm long and the total length of the two mucin domains is 0.55 μm (Table 1).¹⁷ These long stiff rods are so densely covered with glycans that their protein core is totally protected from degradation by proteases. The mucin domain glycans bind a lot of water and thereby generate most of the typical gel-like properties of mucins.

Transmembrane mucins

In addition to one or several mucin domains, all mucins have domains that give them specific properties. Transmembrane mucins have a transmembrane domain that enables them to be anchored in the cell membrane (Figure 1, Table 1). These mucins have one large N-terminal mucin domain and a short cytoplasmic C-terminus.^{6,7} On the outer side of the membrane, these mucins either have a SEA-domain (sea urchin-enterokinase-agrin domain; the SEA-mucins are MUC1, MUC3, MUC12, MUC13 and MUC17) or NIDO-AMOP-vWD domains (only MUC4). Interestingly, both of these domains are cleaved during biosynthesis, but are held together by strong noncovalent bonds. The SEA domain is cleaved during folding by an autocatalytic mechanism that does not require additional enzymes.^{18–21} The two parts are held together by four β -pleated sheets—two from the outer part of the mucin domain and two from the membrane anchored part—these parts can be pulled apart by mechanical stress avoiding rupturing of the cell membrane as the force that can disrupt the SEA domain is smaller than the forces necessary for disrupting the membrane.^{21,22} The cleavage of MUC4 takes place in the vWD (von Willebrand D) domain. The mechanisms involved are less clear, but both enzymatic and autocatalytic processes have been suggested.^{23,24}

The transmembrane mucins are found at the apical side of epithelial cells. MUC3, MUC12 and MUC17 probably build the glycocalyx, sometimes also called fuzz, on the microvilli of enterocytes.^{3,25,26} This hypothesis has not been shown formally, but the length of their extended mucin domains is in accordance with the thickness of the glycocalyx. MUC13 is also abundant in enterocytes, but has only a short mucin domain.²⁷

The normal functions of the transmembrane mucins are only partly understood. The enterocyte glycocalyx probably forms a protective brush for the periciliary space on the

ciliated cells of the lung,²⁸ and probably acts as a diffusion barrier in the gastrointestinal tract, ensuring a stable apical cell membrane milieu and inhibiting the undesired passage of large molecules.^{3,7} MUC1, which is present in the stomach epithelium, has protective effects against *Helicobacter pylori* infection.²⁹ MUC1 is also a well-known cancer cell antigen that can modulate growth and apoptosis; it relocates from the apical membrane and contributes to tumour cell behaviour with its large cytoplasmic tail interacting with β -catenin and other molecules involved in cancer development.^{6,30–33} MUC3, MUC12 and MUC17 all have cytoplasmic tails that interact with different PDZ-proteins, which are regulators of apical organization and inward and outward shuttling of proteins, especially ion channels.^{34–36} In addition to a role in protection, the transmembrane mucins are probably involved in apical cell surface sensing and signaling.^{7,37}

Gel-forming mucins

The gel-forming mucins all have central mucin domains that are flanked by an N-terminal part (involved in oligomerization) and a C-terminal, forming dimeric structures (Figure 1, Table 1). This group of mucins uses their N-termini and C-termini to form large polymers that together with the mucin domains form the gels that are typical of mucus and are of paramount importance for protection of the gastrointestinal tract.^{1,8,13} In fact, the evolutionary appearance of this group of mucins probably coincides with the formation of a simple digestive system.¹¹ This coincidental development could make sense considering that the intestine can digest all types of bonds between amino acids in food, whilst at the same time the intestine itself is left unaffected. The major intestinal mucin, MUC2, is resistant to endogenous proteases; the central mucin domains are protected by glycans, and the N-termini and C-termini are stabilized with numerous cross-links between cysteine amino acids. Notably, digestive enzymes are unable to digest glycans other than starch and some disaccharides, leaving the mucins intact. The main functions of the gel-forming mucins are to build the mucus that together with additional components protect and lubricate the gastrointestinal tract. Caldara *et al.*³⁸ suggest that native fresh mucus is non-sticky and non-hydrophobic and that the sticky and hydrophobic properties of mucus appear after the mucus had been stored or purified.

An overview of the mucus systems

The organization of the protective mucus system varies markedly along the digestive tract (Figure 2, Table 2).³⁹ In the mouth, the salivary glands produce MUC5B and MUC7, which lubricate ingested food for passage through the oesophagus.^{40–44} The stomach has a two-layered mucus system, composed of an inner, attached mucus and an outer, unattached, loose mucus layer, both built by the MUC5AC mucin produced by the superficial epithelium.^{39,45,46} Glands in the stomach and duodenum secrete the gel-forming mucin MUC6.^{47,48} The small intestine has, in contrast to the stomach and colon, only one type of surface mucus, again composed of MUC2. This mucus is unattached and easy to remove.¹³ Finally, the colon again has a two-layered mucus system, with an inner dense and attached mucus layer and an outer, loose and unattached mucus, both built by MUC2. The same mucin, MUC2, thus behaves differently in the small and large intestine.^{25,49,50}

Colon: mucus layer organization

Of the intestinal mucus layers, most is known about the colon and MUC2. This mucin is built by monomers that have a mass of about 2.5 MDa, of which 20% is the protein core and the rest is glycans. The central mucin domains make the monomers into ~0.6 μ m long stiff rods.¹⁷ These monomers are covalently interlinked as dimers in their C-terminus and as trimers in their N-terminus, thus forming large net-like structures.^{51,52} Before release into the gut lumen, MUC2 is stored in the goblet cell granulae assembled on a ring-like platform

formed by calcium dependent interactions between MUC2 N-termini.¹⁷ Upon release, the calcium is chelated, probably by bicarbonate, and the mucin unfolds like an umbrella and expands >1,000-fold in volume. The formed nets then spontaneously organize into flat sheets that have a tendency to stack on top of each other, like roof tiles, to form the lamellar inner mucus layer (Figure 1).⁵³ This layer remains anchored to the epithelial cells: it cannot be aspirated off (thus originally called firmly attached); it is highly organized; and it does not allow bacteria to penetrate. The inner mucus layer is mainly formed and renewed by surface goblet cells in the colon.⁵⁴ The thickness of this layer is several hundred micrometers in humans, but less in rodents. This inner layer is thus renewed from the epithelial side and has a turnover time of about an hour.⁵⁴

At the luminal border of the inner mucus layer, it is converted into the outer mucus layer at a relatively sharp demarcation line (Figures 2 and 3). As the outer layer is formed from the inner layer, it contains the same components, but interestingly it has markedly different properties: it is loose, unattached, and allows bacteria to penetrate. The transition from firm to loose form is probably due to proteolytic cleavages within the MUC2 mucin as this transition can be partly inhibited by protease inhibitors.⁵⁰ These cleavages allow the mucin to expand 3–4 times in volume without disrupting the polymeric network, which is still held together by cysteine disulfide bonds.⁵⁰ The exact mechanisms of conversion from inner to outer mucus layer are still only poorly understood, but are known to be generated by the host itself and not by bacteria since the outer mucus layer also exists in germ-free mice.⁵⁰

Stomach: mucus and hydrochloric acid

Like the colon, the stomach has a two-layered mucus system.³⁹ The inner mucus layer, built around MUC5AC, acts as a diffusion barrier for hydrochloric acid.⁵⁵ The surface epithelial cells secrete bicarbonate, creating a pH gradient from the acidic lumen to neutral pH at the cell surface.^{56,57} Hydrochloric acid is produced in the glands and is secreted together with MUC6 and pepsin. Interestingly, these gland secretions are able to cross the surface inner mucus layer through what looks like temporary canals.^{58,59} These canals are subsequently immediately closed, but leave an imprint in the surface mucus. How the glands can withstand the very high proton concentration (pH 1–2) is not understood, but it is likely that MUC6 is important. Whether MUC6 also contributes to the formation of the inner gastric mucus layer is controversial.^{60,61}

Small intestine: mucus and ion secretion

The small intestine has a loose, unattached mucus layer. Like the colon, this layer is formed by MUC2, but the small intestinal mucus layer has similar properties to the outer, not the inner colon mucus layer (Ermund *et al.* unpublished work). How the same mucin can behave so differently in these two intestinal segments is still not understood, although it is possible that MUC2 is processed differently.

To understand the ingenuity of the different organization of the mucus layers in the small intestine and colon, one has to remember their different functions and environment. The small intestine, except for the distal ileum, is kept sterile, whilst also enabling absorption of nutrients. By contrast, the colon is stably colonized with a complex flora that has an important role in metabolism. The small intestine normally limits bacterial exposure by flushing (that is, mucus and liquid secretions—mainly chloride through the CFTR channel) coordinated with intense motor activity. This motor activity is accomplished by the so-called migrating motor complex (MMC), generated by the enteric nervous system. The MMC generates long segments of maximal motor activity and secretion that move slowly down the small intestine.^{62,63} This activity probably physically moves the loose mucus and its bound bacteria distally into the colon.

To further minimize the risk of contact between virulent bacteria and the epithelium, the small intestinal mucus contains high concentrations of antibacterial peptides and proteins secreted by both Paneth cells and enterocytes.⁶⁴ These antibacterial substances kill or entrap bacteria and thus keep the epithelial cells relatively free from bacterial contact.^{65,66} The mucus layer is an important component of this protection as it limits diffusion and generates a gradient of antibacterials from the epithelial side.⁶⁷ The crypts of the small intestine, where stem cells are located, are especially vulnerable as there is no physical barrier present as in the colon. The Paneth cells at the bottom of these crypts not only secrete antibacterial peptides and lysozyme, but also scaffolding proteins such as MUC2 and DMBT1.⁶⁸ Combined secretion of mucins and fluid from the crypts help to ensure that this compartment normally remains sterile.

Role of mucus in health and disease

Cystic fibrosis

The CFTR channel, which preferentially secretes chloride, has a key role in protecting the small intestine from bacterial invasion. The severe inherited disease cystic fibrosis is caused by a nonfunctional CFTR channel.⁶⁹ This disease affects all organs that produce mucus. Problems with recurrent lung infections dominate the clinical course, although the gastrointestinal tract is also affected in many patients. Around 10% of newborns with cystic fibrosis are diagnosed with meconium ileus at birth and some adults with cystic fibrosis also have problems with distal intestinal obstruction syndrome (DIOS).⁷⁰ These entities are characterized by an acute and complete or incomplete obstruction by inspissated mucus localized to the ileocaecum.⁷¹ Experimental animal models of cystic fibrosis (mice or pigs) tend to have a relatively severe intestinal phenotype.^{72–74} In mice with cystic fibrosis, the mucus was found to be attached to the epithelium.⁷⁵ This mucus phenotype was normalized by exposing it to buffers containing about 100 mM bicarbonate. Conversely, in wild-type mice, the mucus could be turned into a cystic-fibrosis-like mucus phenotype by removing bicarbonate. Thus, the altered mucus in the cystic fibrosis mice was attributed to a lack of bicarbonate owing to the dysfunctional CFTR channel (in health, this channel also secretes bicarbonate). Bicarbonate probably acts by precipitating calcium ions, which seems to be necessary for the correct unfolding of stored mucins and formation of a proper gel.¹⁷

That the small intestinal mucus was found to remain attached to the epithelium in the absence of a functional CFTR channel could explain why patients with cystic fibrosis sometimes develop DIOS. If the mucus remains attached and cannot be moved forward by peristaltic movement, obstruction is easy to understand. Mouse models of cystic fibrosis can actually be kept alive with PEG (polyethylene glycol) in their drinking water, which is an osmotic laxative that is also used to treat DIOS.^{73,76} Given that the function of the small intestinal mucus is to trap and transport bacteria distally, one would predict an increased load of bacteria in the distal small intestine of patients with cystic fibrosis. In fact, an increased bacterial load has been observed both in mouse models of cystic fibrosis and in patients with cystic fibrosis (estimated from hydrogen breath tests).⁷⁷ Furthermore, cystic fibrosis mice that become sick can be rescued with antibiotics,^{78,79} and anecdotal observations indicate that patients with cystic fibrosis often experience relief from intestinal pain during antibiotic treatment for lung disease. These observations from mice and humans elegantly illustrate the importance of the small intestinal mucus system for protecting the intestine from bacteria.

Commensal bacteria in the colon

If the small intestine is designed to limit the growth of bacteria and transport these organisms distally, the large intestine is made to harbour the 10^{13} – 10^{14} commensal bacteria.

MMC movements do not occur in the colon; instead the enteric nervous system generates vigorous peristaltic waves (mass movements) that relentlessly move towards the rectum, eventually triggering the defecation reflex. These waves are physically powerful and carry mucus with them. Thus, the inner mucus layer that physically separates bacteria from the epithelial cells (by 50 μm in mouse and several hundred micrometers in humans) is of utmost importance for homeostasis in the colon.⁵⁰

It should also be remembered that the outer mucus layer serves as the habitat and a partial food source for these bacteria. Complex polysaccharides in food, as well as MUC2, provide complex glycans that can be released by the numerous bacterial exoglycosidases that remove one monosaccharide at a time.^{80,81} Fortunately this is a slow process, but once the mucin protein core is reached the mucus gel is dissolved by bacterial proteases. This process is of mutual interest for bacteria and host because the bacteria make short-chain fatty acids from the degraded material and, depending on the bacterial species, deliver acetate, propionate and butyrate as energy sources for the host epithelium.⁸²

The commensal bacteria of humans is different from other animals and consists of about 50% Firmicutes and 30% Bacteroidetes.⁸⁰ This composition is probably not random as each species seems to have mechanisms for selecting its microbiota, as illustrated by cross colonizing germfree zebra fish and mice.⁸³ How such a selection can take place is not understood, but the observation that humans have a constant repertoire of MUC2 glycans in the colon suggests that glycan epitopes might be one such mechanism.¹⁶

Pathogenic organisms in the colon

An inner mucus layer in the colon that is impervious to bacteria is a major obstacle for pathogenic microorganisms. Infection requires highly specialized virulence systems, which explains the relatively limited number of such organisms. The rodent pathogen *Citrobacter rodendum* is an example of a bacteria that is able to pass and reside under the mucus layer,⁸⁴ in a similar way as *Helicobacter pylori* has specialized mechanisms allowing it to reside under the inner gastric mucus layer.⁸⁵

Helminth infections of the small intestine are a common problem worldwide, and at least one-quarter of the world's population is infected with an intestinal worm of some sort. Clearance of the infection is associated with increased numbers of goblet cells and mucus secretion, as demonstrated in animal models infected with *Trichinella spiralis*, *Nippostrongylus brasiliensis* and *Hymenolepis diminuta*.^{86–90} The importance of mucus for clearance has also been shown in a mouse infected with *Trichuris muris*; this species is closely related to *Trichuris trichuria*, which infects the human colon.^{91,92} Protection is mediated by $\text{T}_\text{H}2$ cytokines with microRNA as a regulator.^{93,94}

The parasite *Entamoeba histolytica* is of particular as this large organism not only reaches but is also able to penetrate the colonic epithelium.⁹⁵ Studies of its virulence factors have revealed that the parasite first uses a lectin-like adhesin to attach to the mucus, probably onto the outer side of the inner mucus layer. This adhesion triggers the secretion of a protease—specific to *E. histolytica*—that is capable of cleaving MUC2 at one of its most vulnerable positions (that lacks cysteine disulfide bond crosslinks).^{96,97} This mechanism means that this particular enzyme can dissolve the inner mucus layer, which is a necessary second step for invading the host. However, this vulnerable spot in MUC2 is not totally unprotected as there are glycosylation sites in the vicinity of the cleavage site. Glycosylation can block cleavage, but it seems that these sites are not always fully utilized (van der Post, personal communication). These glycosylation sites could be one explanation for the observation that only a minority of all patients infected with *E. histolytica* actually get invasive disease. It is possible that other microorganisms might also have developed clever

methods to circumvent the protective function of the inner colon mucus layer formed by MUC2.

Colonic ischaemia and compound exocytosis

Ischemia of the colon can be caused by disease or during surgery. Colonic ischemia has a more favourable outcome than ischemia of the small intestine.^{98,99} The reason for this difference has been addressed;¹⁰⁰ 1 h of colonic ischemia led to detachment of the inner mucus, enabling bacteria to reach the epithelium and penetrate into the normally sterile crypts. However, when the tissue was reperfused, the crypt goblet cells released their stored mucin granulae and the mucus cleaned the crypts of bacteria and restored a functional mucus layer (Figure 3). Goblet cells thus seem to tolerate ischemia relatively well and when circulation is restored, they release their mucins by so-called compound exocytosis.¹⁰¹ This mode of mass release can also be induced by other mechanisms, for example by acetylcholine.^{2,102} The mass release of mucins is in line with the fact that colon crypt goblet cells accumulate large amounts of MUC2. These mucin granulae have a slower turnover, about 6–8 h compared to about 3 h for surface goblet cells, and normally do not release their contents until the cells have reached the crypt opening.⁵⁴ These observations suggest that crypt goblet cells are charged with large amounts of mucus available for release in the crypts when exposed to hazards. After ischaemia–reperfusion, the crypt goblet cells are totally emptied and restoration of the mucin granulae is slow.¹⁰⁰ Repetitive challenges with short intervals will thus be difficult to handle for the colonic epithelium, with implications for colonic surgery.^{100,103}

Ulcerative colitis

Ulcerative colitis, a form of IBD, is restricted to the colon and is associated with commensal microbiota. The current concept is that contact of bacteria and bacterial antigens with the lamina propria immune cells triggers and drives an inflammatory immune reaction.¹⁰⁴ The vast majority of research into the pathophysiology of ulcerative colitis has concentrated on the immunological response and not many papers have addressed the alternative hypothesis of a defective inner mucus layer leading to increased bacterial contact with the epithelium.¹⁰⁵ This idea is actually supported by the observation that mice lacking MUC2 develop severe colitis and eventually colon cancer.^{50,106,107} In mice without MUC2, there is no mucus layer at all and bacteria are in direct contact with epithelial cells. Bacteria are also found deep in the crypts and inside the epithelial cells of these animals, something that is never seen in healthy animals.⁵⁰ Other mouse models with defective mucus all develop colitis.^{108–110} It is, therefore, conceivable that the main problem in at least some patients with ulcerative colitis is a defective mucus layer rather than a hyperactive immune system (Figure 4).

The most common animal model for ulcerative colitis in mice or rats is to administer dextran sodium sulphate (DSS) in their drinking water. Normally, the animals develop inflammation after 3–5 days; it has been shown that the inner mucus layer, in both mouse and human colon, becomes penetrable to bacteria more or less directly after exposure to DSS.¹¹¹ This enables bacteria to reach the epithelial cells. The observation that DSS increases the penetrability of the inner mucus layer to bacteria and beads the size of bacteria without changing its thickness shows that mucus properties can be altered. We have observed that several mouse models with defective ion transport (NHE3; sodium–hydrogen exchanger 3) or cytokine (for example, IL-10) production have seemingly intact mucus that turns out to be penetrable to bacteria.¹¹² This phenomenon has also been observed in patients with active ulcerative colitis.¹¹² Moreover, in the proximal colon at least, major changes in the epithelial reactivity to secretagogues are seen in patients with ulcerative colitis in stable remission.¹¹³ Together, these findings imply that the inner mucus layer is a dynamic system that can be

affected by changes in local ion concentrations, thus participating in complex and poorly understood crosstalk with mucosal ion transport systems.

A number of other animal models that develop spontaneous colitis and ulcerative-colitis-like symptoms exist. A single unifying mechanism for these models does not exist, and it is likely that a number of defects lead to colonic inflammation. Of these models, several can be linked to dysfunction of the mucus system. The observation that mice that lack the master regulator XBP-1 of endoplasmic reticulum stress develop colitis has triggered a lot of interest.¹¹⁴ Given that the endoplasmic reticulum stress system is linked to inflammation, this connection is possible. Two mouse models that develop spontaneous colitis were shown to have mutations in MUC2 that caused misfolding and accumulation of this protein in the endoplasmic reticulum, something that triggers endoplasmic reticulum stress.^{108,115} AGR2 is assumed to be an endoplasmic reticulum resident disulfide isomerase that is essential for proper MUC2 production and its deletion also causes colitis.^{116,117} Together, the available information suggests that several components of the secretory pathway are important for colonic homeostasis, although the mechanisms are not clarified. One should remember that because of its high number of disulfide bonds and high degree of glycosylation, MUC2 is one of the most complex proteins synthesized in the body. Increased demand, as a result of aggressive bacteria for example, might ‘push’ this complex synthesis, leading to release of suboptimally assembled MUC2 and generation of a low-quality inner mucus layer (Figure 4). However, the colonic mucus system and layers also contain a number of other components that contribute to the mucus properties in as-yet undetermined ways.

Conclusions

The gastrointestinal mucus system is the first line of defence against bacteria, but its organization varies along the tract. The small intestinal mucus layer, with its antibacterial peptides and proteins, is freely transported distally together with trapped bacteria. These disarmed bacteria are then cleared by motor activity and fluid secretion. The colon handles its large bacterial load with a two-layered mucus system, in which the inner layer normally remains impenetrable to bacteria. Defective function of the inner colonic mucus layer might be a previously unexplored pathophysiological mechanism in ulcerative colitis and infectious diseases, knowledge that could lead to novel therapeutic principles.

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Key points

Gastrointestinal mucus is the first line of defence against bacteria

The mucus layer in the small intestine is freely movable and carries bacteria distally

In cystic fibrosis, the small intestinal mucus is not freely movable, which might explain the intestinal symptoms of this disease

The colon handles its large bacterial load with a two-layered mucus system, in which the inner layer normally remains impenetrable to bacteria.

Defective functioning of the inner mucus layer of the colon might be a pathophysiological mechanism for colitis and infectious diseases

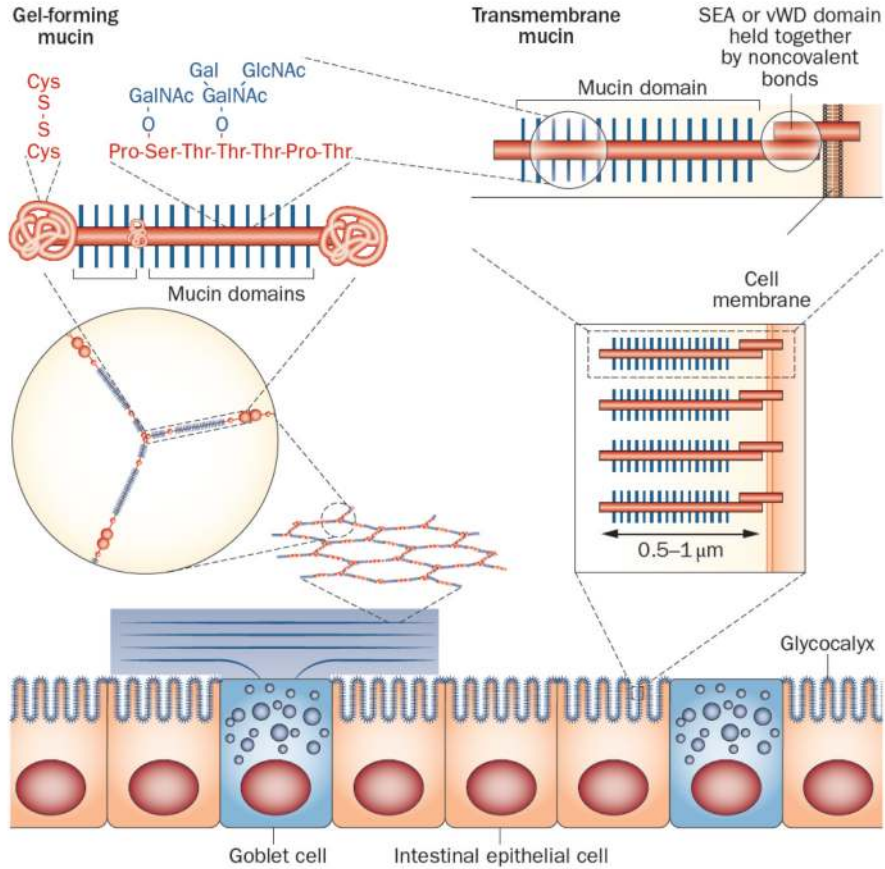


Figure 1. Simplified model of a gel-forming mucin produced by goblet cells and transmembrane mucins attached to the apical membrane of enterocytes. Left: the MUC2 mucin polymer is packed in the granulae (blue) of the goblet cells and during secretion expands to form flat ring-like structures that stack under each other. The enlarged ring shows the oligomeric nature of the MUC2 polymer, and above the MUC2 monomer is shown with its central mucin domains and exemplified O-glycans. The MUC2 N-termini and C-termini are held together with numerous disulphide bonds. Right: the apical side of the intestinal enterocytes are covered by a glycocalyx made up of mucins that are anchored in the cell membrane. The long and extended mucin domains make up the glycocalyx. The cleaved and associated SEA and vWD domains are indicated as well as the mucin domain. Abbreviations: SEA: sea urchin-enterokinase-agrin domain; vWD, von Willebrand domain.

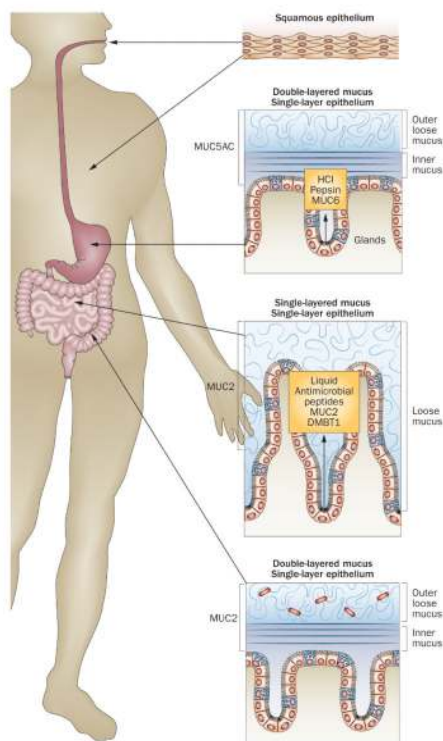


Figure 2. The organization of epithelium and mucus in the gastrointestinal tract. The squamous epithelium of the mouth and oesophagus is washed by mucus from the salivary glands. The stomach and colon have a two-layered mucus system with an inner, attached mucus and an outer, unattached, loose mucus layer. The small intestine on the other hand has only one type of mucus that is unattached and loose.

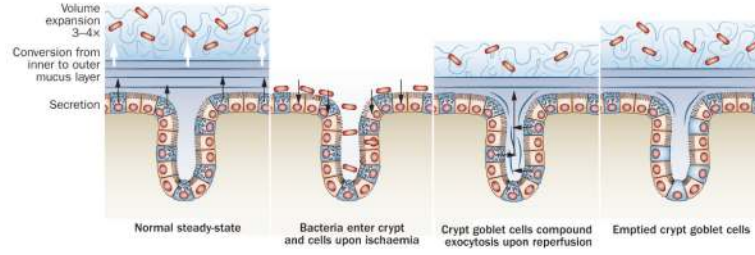


Figure 3. Mucus secretion from the colon surface epithelium and crypts during ischaemia and reperfusion. a | Normal steady state renewal of the inner mucus layer by the surface goblet cells. This inner mucus layer is converted and expanded to the outer mucus layer. b | Upon ischaemia the mucus layers are lost and bacteria can enter the crypt and epithelial cells. c | Upon reperfusion, the crypt goblet cells release their stored mucins and a new inner and outer mucus layer are formed at the same time as the bacteria in the crypt are washed out. d | After reperfusion, the crypt goblet cells remain emptied for several hours as replenishing of the mucin granulae is slow. During this period, the tissue will be more vulnerable to another episode of ischemia.

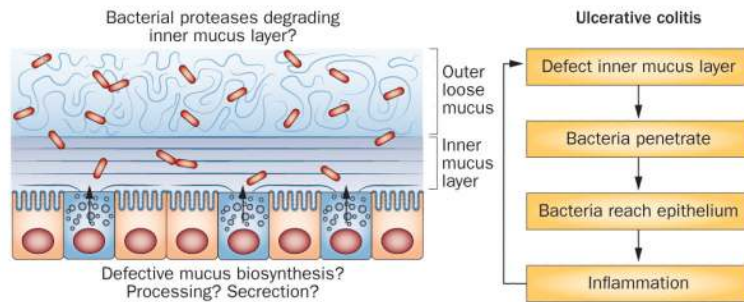


Figure 4. Model of mechanisms that could affect the inner mucus layer properties and potentially cause ulcerative colitis. The inner mucus layer normally separates the bacteria from the epithelial cells. If this inner mucus layer is absent or penetrable to bacteria, the load of bacteria on the epithelium increases, which will trigger an immune reaction by the subepithelial immune system.

Table 1

Mucins in the gastrointestinal tract.

Mucin* (and type)	Protein domains [‡]	Number of amino acids	Number of mucin domains (and estimated length of mucin domains [§])	Cell type expression	Function	Reference
MUC1 (transmembrane)	1 SEA	~1,250	1 (~200 nm)	Epithelial cells	Signalling, protection	30, 118–124
MUC2 (gel-forming)	4 VWD, 2 CysD, 1 CK	~5,200	2 (~550 nm)	Goblet cells Paneth cells	Protection, lubrication, entrapment	13, 50, 125, 126
MUC3 ^{II} (transmembrane)	1 SEA	>2,550	1 (>350 nm)	Enterocytes	Apical surface protection	127 – 129
MUC4 (transmembrane)	1 NIDO, 1 AMOP, 1 VWD	~5,300	1 (~800 nm)	Epithelial cells Goblet cells	Signalling, protection	130-133
MUC5AC (gel-forming)	4 VWD, 11 CysD, 1 CK	>5,050	11 (>350 nm)	Mucous cells	Protection, lubrication, entrapment	57, 134–138
MUC5B (gel-forming)	4 VWD, 7 CysD, 1 CK	~5,700	7 (~550 nm)	Mucous cells Goblet cells	Protection, lubrication, entrapment	139 – 141
MUC6 (gel-forming)	1 VWD, 1 CK	~2,400	1 (~250 nm)	Mucous cells	Protection, lubrication, entrapment	142 – 144
MUC7 (gel-forming)	None	377	1 (~230 nm)	Mucous cells	Protection	41, 145
MUC12 (transmembrane)	1 SEA	~5,500	1 (~1000 nm)	Enterocytes	Apical surface protection	26
MUC13 (transmembrane)	1 SEA	512	1 (~30 nm)	Enterocytes	Apical surface protection?	27, 146
MUC16 (transmembrane)	33 SEA	~22,000	1 (~2400 nm)	Epithelial cells	Apical surface protection	147, 148
MUC17 (transmembrane)	1 SEA	~4,500	1 (~800 nm)	Enterocytes	Apical surface protection	149

* Human mucin nomenclature is used. A curated database of mucin gene and protein sequences can be found at www.medkem.gu.se/mucinbiology/ databases.

[‡]In addition to mucin domains (glycosylated PTS domains).

[§]Total length of all mucin domains. Length = 0.2nm/amino acid.

^{II}Two MUC3 genes A and B have been proposed to exist.¹⁵⁰ Abbreviations: AMOP, adhesion associated domain in MUC4 and other proteins; CysD, Cystein D domain interrupting mucin domains; CK, cysteine knot; GF, Gelforming; SEA, sea urchin-enterokinase-agrin domain, and agrin domain; NIDO, nidogen-like domain; S, Secreted (not polymeric); VWD, von Willebrand.

Table 2

Mucus along the gastrointestinal tract

Mucus	Section of gastrointestinal tract				References
	Mouth	Stomach	Small intestine	Large intestine	
Mucus layers	None	Outer, unattached layer Inner, attached layer	Single, unattached layer	Outer, unattached layer Inner, attached layer	39,50,57,151
Gel-forming mucins: <ul style="list-style-type: none"> • Surface/crypt • Gland • Paneth cells 	NF MUC5B NA	MUC5AC MUC6 NA	MUC2 MUC6 MUC2	MUC2 (MUC5B) NF NA	25,42-50,152-154
Transmembrane mucins: <ul style="list-style-type: none"> • Epithelial cells 	MUC1, MUC16	MUC1	MUC1, MUC3, MUC4, MUC12, MUC13, MUC17	MUC1, MUC3, MUC4, MUC12, MUC13, MUC17	25– 27,30,34,49,124,133,149,152,153,155–158
Secreted mucins	MUC7	NF	NF	NF	40 – 42

Abbreviation: NA, not applicable. NF, not found.