CellPress

Opinion

Sulfide as a Mucus Barrier-Breaker in Inflammatory Bowel Disease?

Noortje Ijssennagger,[1] Roelof van der Meer,[2] and Saskia W.C. van Mil[1,*]

The gut epithelium is covered by mucus consisting of mucin polymers connected via disulfide bonds. The mucus layer limits exposure of epithelial cells to toxins and bacteria. A recent study has shown that sulfide, produced by certain bacteria, reduces disulfide bonds in the mucus network. The resulting breaks in the mucus barrier allow exposure of the epithelium to bacteria and toxins, causing inflammation. In this opinion article we argue that this mechanism may be involved in the etiology and/or severity of inflammatory bowel disease (IBD) because IBD is associated with decreased mucus barrier function, altered microbial species, and increased sulfide concentrations. Increasing the mucus integrity by reducing sulfide concentrations in the intestine may be a novel therapeutic option for IBD.

Trends

The mucus layer lining the colonic epithelium is the first line of defense limiting exposure of epithelial cells to ingested food components, digestive enzymes, and microorganisms.

Mucus consists of a network of mucins produced by intestinal goblet cells. The mucins are interconnected via disulfide bonds into large polymeric sheets forming the mucus layer.

Hydrogen sulfide, produced by sulfate-reducing bacteria (SRB) and some other bacteria, reduces disulfide bonds present in the mucus network, thereby breaking the mucus barrier.

Inflammatory bowel disease (IBD) is characterized by decreased mucus barrier function, which may be due to increased sulfide production by altered microbial species present in IBD patients with active disease.

Lowering hydrogen sulfide concentrations in the gut lumen could represent an exciting potential therapeutic strategy for treating IBD.

The Intestinal Mucus Barrier in Health and Disease

The intestinal wall of mammalian species contains a monolayer of epithelial cells, covered by a mucus layer that serves as a lubricant and a physical barrier between luminal contents and the epithelium. Both the small and large intestine are covered by mucus. However, the mucus covering the small intestine is monolayered and unattached, not covering the entire epithelial surface, whereas the colonic mucus layer consists of a bilayer: an outer less-viscous layer penetrable by bacteria and a viscous and sterile inner layer [1]. Together, the epithelial and mucus layer form the intestinal barrier and the first line of defense against a variety of toxic compounds including bacteria, bacterial products, viruses, and endogenous dietary compounds from the intestinal lumen [1]. Mucus is constituted by highly glycosylated polymeric networks of mucins (MUC proteins) interconnected by disulfide bridges, which are produced by **goblet cells** (see Glossary) in the intestinal epithelium (Box 1). The pore size of this hydrated gel-like structure in the inner mucus layer does not allow penetration of bacteria. In the outer layer, the mucus network is looser due to the activity of proteases, which allows invasion of bacteria (Box 1, Figure I).

Several lines of evidence indicate that a defective mucus layer could lead to **inflammatory bowel disease** (IBD). IBD is characterized by chronic intestinal inflammation and comprises **Crohn's disease** (CD) and **ulcerative colitis** (UC). In Europe and North America, between 550 and 830 per 100 000 persons are affected by IBD, and these numbers are rising [2]. Although the exact etiology is unclear, IBD is thought to result from dysregulated mucosal immune responses triggered by gut bacteria, which are facilitated by defects in intestinal barrier function in genetically predisposed individuals [3]. The first line of evidence that a defective mucus layer may cause IBD is that polymorphisms in several *MUC* genes are associated with IBD [4]. In addition, *Muc2*-deficient mice or mice with missense mutations in *Muc2* spontaneously develop

[1]Department of Molecular Cancer Research, Center for Molecular Medicine, University Medical Center Utrecht, 3508 AB, Utrecht, The Netherlands
[2]Top Institute Food and Nutrition, 6709 PA, Wageningen, The Netherlands

*Correspondence: s.w.c.vanmil@umcutrecht.nl (Saskia W.C. van Mil).

 http://dx.doi.org/10.1016/j.molmed.2016.01.002

Box 1. Mucins as the First Line of Defense Against Pathogens and Bacteria

In the colon, the mucus layer fills the crypt spaces and covers the entire epithelium (Figure I). The colon epithelium is lined by a double-layered mucus layer, consisting of a sterile (non-penetrable) inner layer that is firmly attached to the epithelium and a loose outer layer [1,66]. Mucus consists of a network of two types of interconnected mucins: membrane-bound and secreted mucins. They function to protect epithelial cells from infection, dehydration, and physical injury, as well as to aid passage of materials (e.g., food components). Enterocytes produce mucins that are anchored in the apical membrane via their transmembrane domains [8]. The extracellular domains are heavily *O*-glycosylated at tandem repeats rich in proline (P), threonine (T), and serine (S) (PTS domains), forming the enterocyte apical glycocalyx. In addition, the goblet cells produce and secrete gel-forming mucins in the intestine, predominantly MUC2. The central part of the large MUC2 protein contains two PTS domains to which *O*-glycans are attached, providing MUC2 with a rigid and stiff conformation. The MUC2 proteins polymerize by C-terminal dimerization [67,68] and N-terminal trimerization into large polymeric sheets (see Figure 1 in main text) [69]. These covalently-bound polymers are subsequently tightly packed into regulated secretory vesicles within the goblet cells and secreted into the gut lumen. Normally, a baseline amount of mucins is secreted via exocytosis, but may increase in response to external stimuli, such as microbial factors growth factors, inflammatory cytokines, inflammasomes, etc. [70–74]. Upon secretion into the intestinal lumen, neutralization of the pH and Ca^{2+} sequestering due to bicarbonate secretion leads to unpacking of the covalently joined MUC2 monomers into a polymeric net-like structure [75,76]. The highly glycosylated mucin domains have the crucial biophysical property of binding water (mostly via hydroxyl groups in monosaccharide moieties) which, together with their polymeric nature, generate the gel-like properties. In addition, non-covalent intramolecular interactions have been described; these are proposed to further determine the pore size and gel properties [77]. The pore-size of this inner mucus layer prohibits penetration of bacteria. The continuous production and the renewal of the mucus layer pushes bacteria out toward the lumen. The transformation of the inner mucus layer into the outer mucus layer is due to proteolytic cleavages in the MUC2 network, especially of C-terminal amino acid residues in the outer mucus layer. Although the proteases are not yet identified, more than likely, these are luminal proteases secreted by the host, since germ-free mice still possess the loose outer mucus layer [78].

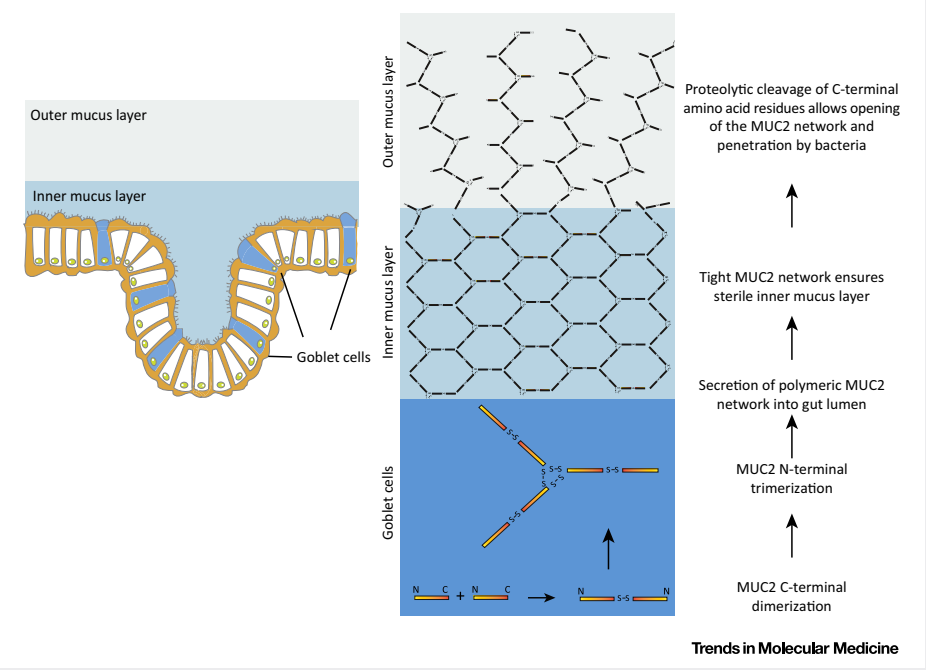

Figure I. The Mucin MUC2 Forms the Inner and Outer Mucus Gel Layers Lining the Colon Epithelium.

Glossary

Crohn's disease (CD): IBD that causes inflammation in any part of the digestive tract.
Enterotypes: three prevalent microbial profiles have been proposed to exist in human colon, and these profiles are called enterotypes.
Glycocholate: the bile acid cholate conjugated with glycine.
Goblet cells: cell type in the intestinal epithelium producing gel-forming mucins.
Heme-supplemented diet: diet supplemented with heme, the pigment of red meat. Dietary heme increases the cytotoxicity of fecal water and injures the cells at the intestinal surface in mice and rats. Subsequently, compensatory hyperproliferation is initiated, which can eventually lead to colorectal cancer.
Inflammatory bowel disease (IBD): chronic inflammation of the intestine. IBD includes ulcerative colitis (UC) and Crohn's disease (CD).
Microbiota: includes (but is not exclusive to) the bacteria that typically inhabit an organ part (e.g., ileal/caecal/colonic microbiota: microorganisms inhabiting the ileum, caecum, and colon, respectively).
Muscularis mucosa: a thin layer of smooth muscle separating the mucosa from the underlying submucosa.
Sulfate-reducing bacteria (SRB): bacteria that reduce sulfate to hydrogen sulfide.
Taurocholate: the bile acid cholate conjugated with taurine.
Ulcerative colitis (UC): IBD that causes chronic inflammation and ulcers in the colon and rectum.
Trisulfides: reduction of disulfide bonds by hydrogen sulfide in mucus leads to the formation of trisulfides.

intestinal inflammation and colon cancer [5–7]. Second, changes in glycosylation and hydration of the mucus network are associated with IBD (reviewed in [8]). Finally, consistent decreases in mucus thickness and % coverage are associated with several nutritional and intestinal disorders, which include enteric infections [9], colon cancer [10,11], intestinal inflammation upon parenteral nutrition (intravenous feeding) [12,13], aging [14], and IBD [10,11,15]. Undoubtedly, increasing mucus barrier integrity can lead to amelioration of epithelial damage and inflammation in these