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Roles and regulation of the mucus barrier in the gut

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Abbreviations: GI, gastrointestinal; SCFA, short chain fatty acids; GalNAc, N-Acetylgalactosamine; MucBP, Mucin binding proteins; LLO, Listeriolysin O; EhCP5, Entamoeba histolytica cysteine protease 5; LPS, Lipopolysaccharide; IBD, Inflammatory bowel disease; UC, Ulcerative colitis; CD, Crohns disease; UPR, unfolded protein response; ERAD, ER-associated protein degradation; FAS, fatty acid synthase

The gastrointestinal tract is coated by a thick layer of mucus that forms the front line of innate host defense. Mucus consists of high molecular weight glycoproteins called mucins that are synthesized and secreted by goblet cells and functions primarily to lubricate the epithelium and protect it from damage by noxious substances. Recent studies have also suggested the involvement of goblet cells and mucins in complex immune functions such as antigen presentation and tolerance. Under normal physiological conditions, goblet cells continually produce mucins to replenish and maintain the mucus barrier; however, goblet cell function can be disrupted by various factors that can affect the integrity of the mucus barrier. Some of these factors such as microbes, microbial toxins and cytokines can stimulate or inhibit mucin production and secretion, alter the chemical composition of mucins or degrade the mucus layer. This can lead to a compromised mucus barrier and subsequently to various pathological conditions like chronic inflammatory diseases. Insight into how these factors modulate the mucus barrier in the gut is necessary in order to develop strategies to combat these disorders.

Introduction

The gastrointestinal (GI) mucosal barrier is made up of epithelial and immune cells that participate with the resident microbiota which together form a barrier to harmful substances.[1] The epithelial cells are covered by a thick layer of mucus, which is produced by goblet cells, and serves as the first line of innate host defense. Mucus forms a protective physical barrier that prevents microorganism and noxious substances from reaching the surface of the epithelium. The major building blocks of the mucus gel are high molecular weight glycoproteins called mucins.[2] The human mucin (MUC) family consists of members designated MUC1 to MUC21[3] and is subdivided into 2 groups: secreted mucins and transmembrane mucins. The secreted/gel-forming mucins (MUC2, MUC5AC, MUC5B and MUC6) are responsible for the formation of the mucus layer over the epithelium. The functions of the transmembrane mucins (e.g. MUC1, MUC4, MUC13 and MUC16) are poorly understood, however, they appear to be involved in various signaling pathways associated with tumorigenesis.[3]

Mucins are produced and stored in granules in the goblet cell cytoplasm. They are then transported to the cell surface and secreted into the lumen from the apical surface of the goblet cell.[4] Mucins are secreted by 2 distinct processes, constitutive/basal secretion and compound exocytosis/regulated secretion. Basal secretion occurs by continuous fusion and release of single mucin granules. Compound exocytosis occurs when goblet cells are exposed to mucin secretagogues or other agents, leading to rapid release of centrally stored mucin granules.[5] Mucin production and secretion are important in maintaining the mucus barrier. A wide range of factors including microbes, microbial products, toxins and cytokines has been shown to regulate these processes thus affecting the mucus barrier.[6,7] In this review we discuss the roles of mucus and factors that can regulate the mucus barrier in the gut.

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Functions of Mucus in the Gut

Barrier function: The gastrointestinal epithelium is covered by a thick mucus gel synthesized by goblet cells in the epithelium. Mucus performs various functions in the gut; particularly, serving as a protective barrier against microbes. Bacteria utilize adhesins that interact and bind to the oligosaccharide side chains on mucins thereby acting as a decoy and immobilizing bacteria, preventing them from reaching and damaging the epithelium. It comes therefore as no surprise that in the absence of a mucus layer, as in *Muc2*$^{-/-}$ mice, colonization of enteric pathogens occurs to a greater extent and more readily than in wildtype (Wt) animals. Particularly, in *Citrobacter rodentium* infection of *Muc2*$^{-/-}$ mice, lack of a mucus barrier resulted in

greater microcolony formation at the mucosal surface and 10–100 fold more *C. rodentium* present in the stool.[8] These increased bacterial burdens correlated to greater macroscopic damage to the epithelium, thickening of the colon, shrinkage of the cecum and occasional focal ulceration. Muc2 deficiency in these animals however, does not significantly affect the total number of bacteria to readily infect the tissue, but predisposes a greater proportion of loosely adherent bacteria to the mucosal surface. Interestingly, microcolony foci in *Muc2*$^{-/-}$ colons generally contained both *C. rodentium* and commensal organisms, whereas Wt mice only contained *C. rodentium*. Additionally, mucin secretions following infection generally result in a flushing of both the pathogenic and commensal species leading to decreases in total luminal bacteria in Wt mice, however this did not occur in *Muc2*$^{-/-}$ animals. In addition to its protective barrier function against bacteria microbes, mucus also prevents other toxic and noxious substances from reaching the epithelial surface. Other functions include serving as a semi permeable gel layer, which allows the exchange of gases, water and nutrients with the underlying epithelium and also maintaining a hydrated layer over the epithelium.[9]

Immune functions: Recent studies have described MUC2 mucin and goblet cells as having roles in immune functions such as antigen sampling and tolerance. In one study,[10] a model was presented in which goblet cells provided the passage of low molecular weight soluble luminal antigens by transcytosis to underlying CD103+CD11b+CX3CR1− dendritic cells (DC). The luminal antigens included components from the diet, commensal and pathogenic organisms, and when delivered to CD103+DC favored IgA production, imprinting of gut lymphocytes and expansion of regulatory T cells (Tregs). This facilitated gut homeostasis and tolerance. Another study[11] later ascribed a role for MUC2 in directly interacting with DCs in the small intestine. MUC2 bound luminal antigens, specifically bacteria, and associated with galectin-3, dectin-1 FcgRIIB complex to suppress inflammatory, but not tolerogenic DC responses. MUC2 did so by activating AKT and GSK3β leading to inhibition of β-catenin, leading to greater transcription of tolerogenic cytokines such as IL-10, TGF-β and REGIIIγ. These effects were noted on both macrophage-like CD103−CD11b+CX3CR1+ DCs and myeloid CD103+CD11b+CX3CR1− DC. This effect was shown *in vitro* through transepithelial dendrites favoring the macrophage-like DC mechanism of antigen uptake.[12] Interestingly, MUC2 administered orally via gavage in *Muc2*$^{-/-}$ animals restored gut homeostasis and the tolerogenic potential of intestinal DCs.[11]

Mucus and Microbiota

The GI tract is home to an immensely complex diversity of microorganisms. The gut is colonized by approximately 1×10^{14} colony forming units (CFU) of bacteria.[13] Some of the microbes bind and colonise the mucus gel via lectin interactions. Attached to mucus, microbiota acts as commensals to the host and also prevents colonisation by pathogenic organisms by occupying empty niches.[14] The dynamic relationship between commensal bacteria and mucins largely impact the course of pathogenesis of enteric bacteria that cause diarrheal disease and enterocolitis. Evidence of this is best exemplified by perturbation of the commensal flora with antibiotics prior to infection with an enteric pathogen, such as *Salmonella* Typhimurium.[15] Despite epithelial cells being highly susceptible to infection *in vitro*, mice that are orally gavaged are generally resistant unless streptomycin treatment precedes infection. Here antibiotic treatment perturbs the microbiota, opening niches to allow for heavy colonization of the epithelium and disease to develop. Indeed, this method has proven useful for a variety of pathogens that previously have been asymtomatic in a mouse model, including enteropathogenic and enterohemorragic *E coli* (EPEC and EHEC).[16] It is likely that perturbing the microbiota subpopulations or opening up niches within the mucus barrier are the causative factors allowing for pathogenesis to proceed. Perturbation of the microbiota with antibiotics can also modulate the mucosal carbohydrate availability often facilitating pathogen expansion in the case of *Salmonella* and *Clostridium.*[17] This is mainly driven free fucose and sialic acid that spikes during antibiotic treatment and favors specific pathogen competitiveness for available niches within the mucosa leading to colonization.

Studies that have compared germ-free mice and conventionally raised animals demonstrated that microbiota have significant effects on mucus composition and thickness. Compared to conventionally raised mice, germ-free mice have fewer goblet cells, which are smaller in size.[18,19] Accordingly, the mucus layer in germ-free mice is relatively thinner than in conventional mice.[20,21] Furthermore, mucins in the small and large intestines of germ-free mice had a higher percentage of neutral and sulphated mucins than those that were conventionally raised.[21,22] When germ-free mice were stimulated with bacterial products [lipopolysaccharide (LPS) and peptidoglycan], the properties of the mucus layer became similar to those of conventional mice.[23] In addition, probiotics such as *Lactobacillus planetarium* induced the expression of MUC2 and MUC3 mucins thus inhibiting adherence of *EPEC Escherichia* coli to the epithelium.[24] Probiotic mixture of *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici* and *L. salivarius* was shown to alter the oligosaccharide composition of mucins in the ileum and duodenum of broilers. Mannose and N-acetylglucosamine decreased linearly while fucose increased linearly with increasing probiotic treatment.[25]

The microbial communities also contribute significantly in host nutrient metabolism providing folate, vitamin K and SCFAs.[26,27] A major function of gut microbiota is to break down non-digestible carbohydrates into SCFAs, particularly acetate, propionate and butyrate that serves as an important energy source for colonocytes.[26,27] Furthermore, butyrate is a potent inhibitor of inflammation, tumor growth and also stimulates mucosal restitution.[28,29] SCFAs have been reported to increase mucus production and secretion. With low concentrations of SCFAs, there was a significant increase in MUC2 expression while higher concentrations decreased production in mice.[30,31] Similarly, *ex vivo* stimulation of colon tissue with 0.05–1mM butyrate induced MUC2 synthesis, whereas higher concentrations returned

MUC2 synthesis to basal levels.[32] Even though the molecular mechanisms behind this increase in MUC2 expression by SCFAs are not well understood, butyrate was shown to induce MUC2 transcription through AP-1 binding and acetylation of histones at the MUC2 promoter.[31]

Pathogen Adhesion to Mucin

Co-evolution of enteric pathogens with hosts have allowed for pathogens to exploit and subvert various mechanisms to access the epithelium. The vast proportion of pathogens utilize flagella to bury through the mucus layer of the intestine to gain access to the epithelium.[33] A well-studied example of this is *Vibrio cholera,* which follows a chemotactic gradient to penetrate the mucus layer, with non-chemotactic or non-flagellated mutants remaining within the luminal side of the mucus barrier.[34] Non-motile stains of *V. cholerae* colonize 10–25 times less efficiently than the Wt counterparts and migrate significantly slower through a mucus gel. Penetration into the mucus gel by polar flagellar movement resulted in the majority of *V. cholerae* losing their flagellar structures, resulting in non-motile cells.[35] Interestingly, this allows *V. cholerae* to assess its environment prior to colonization through the transcriptional regulator HapR, allowing for temporal expression of virulence genes to maximize infection.[35] Bacterial flagella have also been proposed to be a mucin adhesin, particularly in EPEC. Purified and denatured flagellin from EPEC and EHEC can bind to mucins and this phenomenon does not occur in FliC mutants.[36] *Salmonella* Typhimurium uses its fimbriae to bind to mucins, specifically terminal fucose residues. This interaction could be blocked by incubation with *Ulex europaeus* agglutinin lectin or abrogating N-linked glycans.[37] Deletion of the regulatory fimbrial operon, STD, resulted in decreases in the long-term persistence of the *S.* Typhimurium in the cecum of mice, without effecting infection in the small intestine.[38] The large intestine contains more fucosylated mucins due to the expression of Fut2, resulting in *S.* Typhimurium persisting in the cecum of infected mice which inherently increases fecal shedding and transmission via the fecal-oral route.[39]

Entamoeba histolytica, the protozoan parasite responsible for amebiasis, uses an adherence lectin to bind to galactose and N-acetylgalactosamine (GalNAc) residues present in mucin side chains.[40] The affinity of this lectin for GalNAc is greatest for polyvalent species, with $GalNAc_{39}BSA$ being 140,000 greater affinity than the monovalent counterpart. This difference in affinity exceeds what would be predicted based on more GalNAc residues, and instead presents the Gal-lectin binding as being specific for priate multivalent spacing.[41] Accordingly, it has been shown that mucin purified from human colon and LS 174T can inhibit amebic adherence to host cells *in vitro*. Oxidation and glycosidase treatment of purified mucins, specifically using sodium metaperiodate, galactose oxidase, β-galactosidase, or β-N-acetylhexosaminidase, abolished the protective functions of mucins allowing *E. histolytica* to contact host cells.[40]

There is conflicting data on whether bacterial cell surface hydrophobicity predicts the potential of these organisms to adhere to the mucosa and mucus layer. Instead, specific adhesion molecules have been implicated in a variety of bacteria and studied intensely in *Lactobacilli*. Lactic acid bacteria isolated from intestinal niches, particularly *Lactobacilli*, contain mucin-binding proteins (MucBP) that show specificities to the glycans of intestinal mucins. The best studied example is MUB, produced in *L. reuteri*, which is a 353kDa protein with tandem repeats of mucus binding domains approximately 200aa in length. The binding of MUB to mucus was inhibited by addition of the glycoprotein fetuin, suggesting MUB interacts with the carbohydrate side chains of mucins however, the individual moiety has yet to be elucidated.[42] Indeed, 13 *Lactobacilli sp.* have proteins with homology to the mucus-binding domains of MUB and are termed MucBP.[43] Interestingly, the crystal structure of MUB has revealed a striking similarity to Protein L, which allows MUB to bind to a variety of immunoglobulin molecules including secretory IgA.[44] Due to the probiotic nature of lactic acid bacteria and importance of IgA against bacterial surface epitopes in maintaining homeostasis, further investigation is warranted on how MucBP may modulate innate host defense.[45] *L. reuteri* also contains a collagen-binding protein (CnBP) that interacts with intestinal mucin, specifically with α-D-galactose in a lectin-like manner.[46]

Exploitation of Goblet Cells or Mucin Secretion for Pathogenesis

Many pathogens can facilitate tissue invasion by regulating goblet cell function and mucin expression as outlined in **Table 1**. Examples of these include; *Listeria monocytogenes, E. histolytica*, *Nippostrongylus brasiliensis* and *Trichinella spiralis*. Each secretes bioactive factors that can directly or indirectly affect mucin production and secretion.

L. monocytogenes, the gram-positive bacteria responsible for listeriosis, exploits the unique shape of goblet cells during its pathogenesis. *L. monocytogenes* uses the internalin-A surface adhesin to bind E-cadherin, which is located in the adherens junction between adjacent cells.[47] Normally, this moiety is completely inaccessible in a confluent monolayer or intestinal epithelium. However due to the unique shape of goblet cells, E-cadherin is accessible and is subsequently targeted by *L. monocytogenes* allowing paracellular transport of itself into the lamina propria.[48] Previous studies have elucidated loosening of tight junctions during mucin secretion and indeed with *L. monocytogenes*, the majority of goblet cells that had accessible adherens junctions were undergoing exocytosis.[49] *L. monocytogenes* also induces mucin expression via the thiol-activated exotoxin listeriolysin O (LLO).[50] LLO has been shown to induce mucus exocytosis in HT29-MTX cells by an unknown mucin secretory pathway, as it does not activate any of the signaling pathways involved in mucin exocytosis. The transcription factors NF-κB and AP-1 were reportedly not involved in LLO-induced up-regulation of MUC genes in HT29-MTX cells.[51] The toxin was however, shown to bind to a brush border-associated receptor following toxin oligomerization that could account for its exocytosis activity.[50] In addition, LLO up regulated MUC3 gene and protein expression and increased the transcription of MUC4 and MUC12. Secretion of the gel-

Table 1. Mucin expression and secretion in response to pathogens and cytokines

Species	Effector	Pathway	Gene expression	Secretion	Reference
Listeria monocytogenes	Listerio-lysin O	?	MUC3, MUC4, MUC12	+	50
Entamoeba histolytica	?	PKC	?	+	53
Vibrio cholerae	Cholera toxin	cAMP/CREB	?	+	56,57
Clostridium difficile	C difficile toxin A	?	?	Decrease	70
Helicobacter pylori	LPS	PI3K/ ERK. MAPK	Decrease; MUC5AC, MUC1	Decrease	72,73
Lactobacillus spp.	p40	EGFR/ AKT	MUC2, MUC3	+	24
Bacteria spp	LPS/LTA	Src-dependent RAS/ RAF/MEK/ERK	MUC2, MUC5AC, MUC5B	+	106,107
Bacteria spp.	SCFA	AP1	[Low] Increase MUC2, [High] decrease MUC2; MUC1, MUC3, MUC4	+	30,31
Nippostrongylus brasiliensis	IL-4, IL-13	STAT6	MUC2	+	62,63
Trichinella spiralis	Th2 cytokines	STAT6	MUC2, MUC5AC	+	64,65
Trichuris muris	Th2 cytokines	STAT6	MUC2, MUC4	+	66,67
Cytokines	IL-4	STAT6/ MAPK	MUC2,MU5AC	?	115,116,117
	IL-13	STAT6/ MAPK	MUC2,MU5AC	?	119
	IL-6	?	MUC2,MUC5AC,MUC5B,MUC6	+	123
	IL-9	?	MUC2,MUC5AC	?	124
	IL-10	?	MUC2	?	126
	IL-22	STAT3	MUC3,MUC10,MUC13	?	125
	IFN-γ	STAT1/6	MUC1	?	129
	TNF-α	PI3K/AKT/NFkB/ MAPK	MUC2, MUC5AC, MUC5B, MUC6	+	123
	IL-1β	PKC/MEK/ ERK/PI3K	MUC2, MUC5AC	+	57

forming mucin MUC5AC was also increased in response to the toxin.[51]

E. histolyica causes mucus hypersecretion to deplete mucin stores such that intimate contact with the mucosa may occur.[52,53] *E. histolytica* is an enteric protozoan parasite that can colonize the mucus layer and exist as a harmless commensal.[40] However, it can breach the mucosal barrier and invade the underlying epithelium causing amoebic colitis and dysentery.[40] Using a gerbil model of amebic colitis, we have shown that *E. histolytica* stimulated potent secretion of mucin leading to mucin depletion in goblet cells before invading the epithelium.[54] *E. histolytica* also triggers mucin secretion from LS 174T cells (goblet cell-like colon cancer cell line) by a contact dependent mechanism involving protein kinase C (PKC).[53] These studies have been extended *in vivo* in mouse colonic loop model where a strong secretagogue response was observed containing both mucin and non-mucin components[55] similar to cholera toxin. *V. cholerae* evokes massive mucin secretion from intestinal goblet cells via increases in intracellular cAMP.[56] This leads to the activation of CREB (*cAMP response element-binding* protein) and stimulation of mucin secretion.[57] Mucin release can be partially hindered with either Protein Kinase A inhibitors or microtubule perturbation.

Another intestinal parasite that has been shown to induce mucin changes during infection is the gastrointestinal nematode *N. brasiliensis.* It is a natural parasite of rats that matures during migration from the lung to the small intestine. *N. brasiliensis* was shown to cause goblet cell hyperplasia and increase mucus production leading to its entrapment and expulsion. *N. brasiliensis* infection also induces qualitative changes of the oligosaccharide chains of MUC2 mucin, and induces the production of acidic mucins at the expense of neutral mucins. These events coincide with expulsion of the worm.[58,59,60] The structural change in mucin caused by this parasite may be driven by $CD4^+$ T cells. Pre-treatment of mice with anti-CD4 antibody the day before infection with *N. brasiliensis* significantly reduced the production of intestinal mucus, changes in glycosylation and expulsion of the worm.[61] *N. brasiliensis* expulsion is also dependent on IL-13 whereby the parasite induces cytokine production by immune cells that significantly increases mucus production leading to its expulsion.[62,63] Goblet cell hyperplasia and increased mucus production have also been described in infections with *T. spiralis*[64,65], *Trichuris muris*[66,67] and *Ascaris galli*.[68] *T. spiralis* infection was accompanied by increased MUC2 and MUC5AC production in the colon. In Muc2 deficient mice, *T. spiralis* induced potent Muc5ac secretion in the colon.[66] Muc5ac appears to be crucial in worm expulsion as its loss prevented clearance of the infection.

Inhibition of Mucus Secretion During Pathogenesis

Pathogens have also adapted mechanisms to inhibit mucin secretion to enhance pathogenesis, particularly *Clostridium difficile* and *Helicobacter pylori*. Pre-treatment with*C. difficile* toxin A, which is responsible for barrier dysfunction and causing severe inflammatory enteritis, effectively blocks the mucin secretagouge activity of a variety of agonists, notably forskolin and calcium ionophore.[69] Interestingly, only induced mucin secretion was effected with no hindrance to constitutive exocytosis. The mechanisms that *C. difficile* toxin A acts is likely upstream of calcium, perhaps the cytoskeleton or exocytosis machinery.[70]

H. pylori is an ulcer-causing bacterium that resides in the mucus gel layer covering the gastric mucosa. The bacterium alters the structure of the mucin molecule and causes abnormal gene expression of MUC1, MUC5AC and MUC6 which are the predominant mucins expressed in the stomach.[71] In addition, it

decreases gastric mucin synthesis[72] and exocytosis,[73] leading to a decrease in the thickness of the mucus gel. Studies in vivo showed a decrease of approximately 20% in the thickness of gastric mucus in *H. pylori*-infected patients.[74] Exposure of the human colonic epithelial goblet cell line, HT29-CL^{-}16E, to viable *H. pylori* also markedly decreased mucin biosynthesis and secretion in a dose-dependent manner.[73] The decrease in mucin secretion was achieved by pre-treating cell monolayers with *H. pylori* and inducing compound exocytosis via forskolin and ionophore.[73] In gastric cell lines, *H. pylori* also suppressed the expressions of MUC1 and MUC5AC.[75] Moreover, when gastric mucosal segments from rats were treated with LPS from *H. pylori*, mucin glycosylation and sulfation were significantly inhibited. This effect of *H. pylori* can disrupt the structure of the mucus gel.[76,77] *H. pylori* can also modulate the mucus barrier by changing its viscoelastic properties. The organism survives in the mucus layer by producing urease, which hydrolyses urea to yield ammonia thus increasing the pH of its environment.[78] Studies have shown that the rheological property of mucus is pH dependent: at neutral pH it is a viscous solution but a gel in acidic conditions[79,80] *H. pylori* has been shown to increase the pH of the immediate environment resulting in reduced viscoelasticity of the mucus gel, thereby compromising the integrity of the mucus barrier and alleviating movement[81] A summary of how microbes and other factors modulate the mucus barrier is depicted in **Figure 1**.

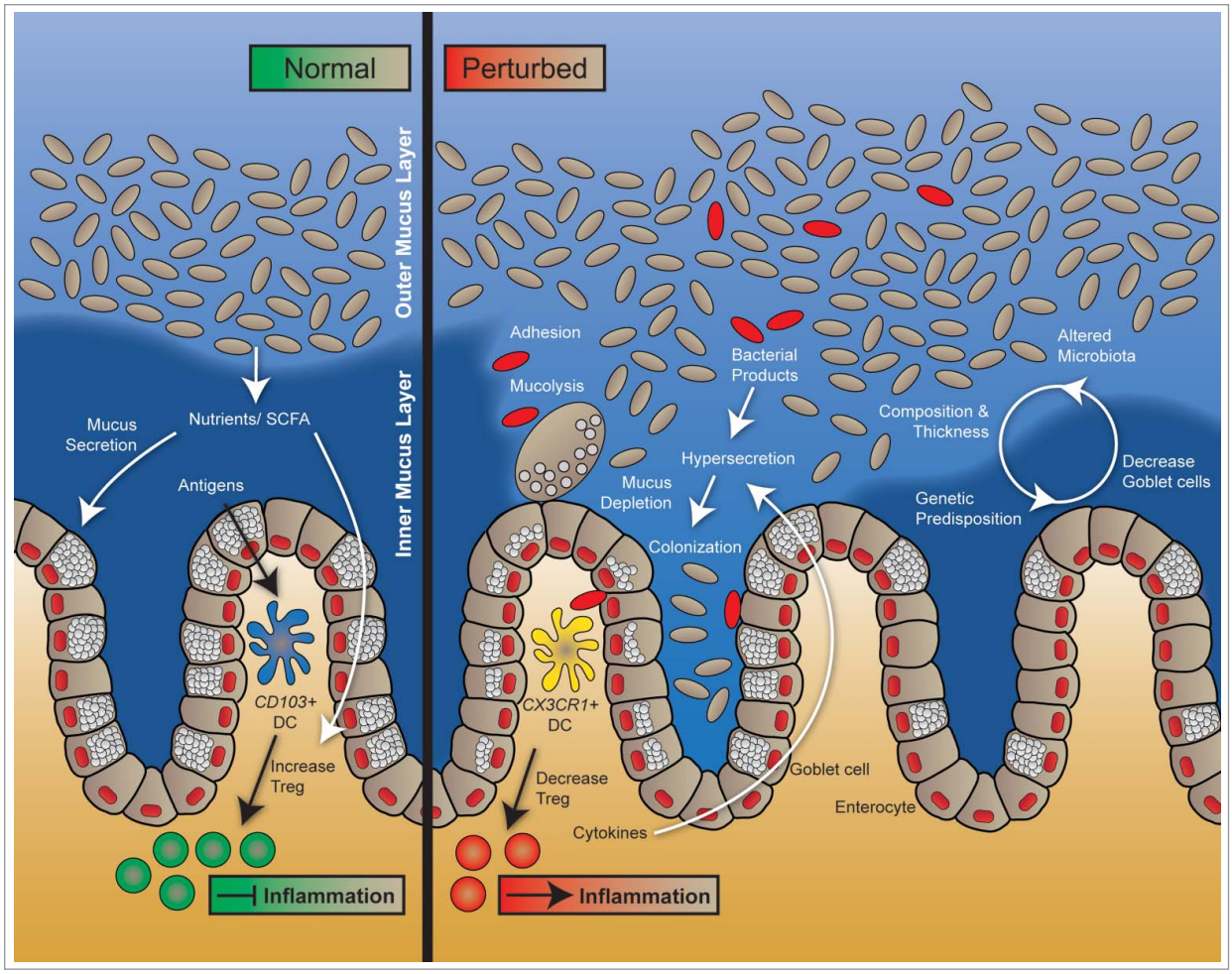

Figure 1. The mucus layer under normal and perturbed conditions. The gut mucosa is covered by a thick layer of mucus that acts as a protective barrier against harmful substances. The barrier consists of 2 layers, an inner mucus layer and an outer mucus layer. Commensal and environmental microbes colonize and remain in the outer mucus layer by binding to mucus via lectin-like molecules, while the inner mucus layer is relatively sterile. Mucus is composed of high molecular weight glycoproteins called mucins. Mucins are produced and packaged into secretory granules within the goblet cell cytoplasm and secreted at the apical membrane. Under normal conditions, goblet cells continuously produce mucus to maintain a functional mucus layer; however, genetic and environmental factors can modulate goblet cell function and mucus production. Short chain fatty acids (SCFAs) produced by commensal microorganisms not only provide nutrients for colonocytes but also increase mucus production and secretion. Furthermore, SCFAs also modulate immune homeostasis and tolerance in the intestines. Under perturbed conditions during infection, pathogens can induce mucin hypersecretion, inhibit mucus production, degrade mucus or induce changes in mucin glycosylation that can lead to disruption of the mucus barrier. Altered barrier function can subsequently cause commensal and pathogenic microbes as well as microbial products to translocate to the epithelial surface. This in turn can trigger immune cell response and cytokine production leading to inflammation.

MUC2 Degradation by Microbes

Numerous pathogens have evolved specific mechanisms to subvert and penetrate the mucus barrier in disease pathogenesis. A wide array of enteropathogens contains a class of serine proteases know as SPATE (serine protease autotransporters of Enterobacteriaceae) that cleave glycoproteins, including MUC2. During ETEC *E coli* infection, EatA (enterotoxinogenic *E. coli* autotransporter A) is sufficient to degrade intestinal mucin and accelerate toxin delivery to the cell surface. Interestingly, EatA is immunogenic and successfully protects vaccinated animals from ETEC virulence and decreased small intestinal colonization.[82] Enteroaggregative *E. coli* and *Shigella flexeri* have been shown to contain a highly homologous protein termed Pic, which similarly can degrade various glycoproteins including mucins, induce mucus release and enhance colonization of the mucosa.[83–85] *E. histolytica* utilizes cysteine proteases, particularly cysteine protease 5 (EhCP5), as part of its pathogenesis during colonization and penetration of the mucus barrier. This virulence protein is absent in non-pathogenic *E. dispar*.[86] Amoebic virulence and invasiveness is directly correlated to the activity of EhCP5 such that parasites lacking this protease are less efficient at forming amoebic liver abscess.[87,88] Prior to invasion, *E. histolytica* must first traverse the mucus barrier, a function that is accomplished by EhCP5 whereby the protease alters the viscoelastic and protective properties of MUC2 mucin. It does so by targeting the C-terminal domain of MUC2, a region that is poorly glycosylated and susceptible to proteolysis.[89] *E. histolytica* cysteine proteases cleave MUC2 at 2 sites in the C-terminal domain, resulting in liberation of the dimer at the C-terminal domain and mono associated N-terminal trimers.[90] This event significantly altered the protective functions of MUC2 *in vitro* leading to greater encounters with the host cells.[91] Similar mechanisms are likely involved for other parasites at mucosal surfaces such as *Trichomonas* and *Giardia*.[92–94]

The spectrum of glycans that can be digested by different bacteria varies and the metabolome likely reflects the coordinated efforts of the mucosal community rather than specific species. Some species are better suited for a broad range of glycans such as *Bacteroides thetaiotaomicron*, thus maximizing survival within the host. Glycosylation of MUC2 in the intestine is complex with over 100 different O-linked glycans present on the apoprotein.[95] These carbohydrates are composed of monosaccharides ranging from 2–12 monomers and are generally based on core 1–5 structures with the colon predominantly core-3.[96] There appears to be variation in the glycosylation pattern along the GI tract, with the small intestine containing core-4 highly fucosylated glycans, core-2 sulpho-lewis in the colon and blood group H/A in the cecum and ileum.[97] Sulphation of mucins generally confers resistance to degradation by both host proteases and bacterial glycosidases, and has even been implicated in conferring protection in newborns.[98,99] Sialic acid terminal residues confer a negative charge to mucins and appear to inhibit proteolysis to some degree however, bacterial species contain sialidases/neuraminidases to get around this.[100] Perturbation of glycosylation has devastating effects on barrier integrity and defense as evidenced by mice lacking core 3β1, 3-N-acetylglucosaminyltransferase. These mice are unable to synthesize core-3 glycans and as a result, Muc2 expression is significantly reduced and the barrier rendered highly susceptible to experimental colitis and adenocarinoma.[101] This mouse model has also been used in the context of enterocolitis induced by *Salmonella*, where transgenic animals harbour similar pathogen burdens but much greater barrier disruption. Impairment of glycosylation can also occur through interruption of the sulfate autotransporter, NaS1. In a mouse model, these knockout mice have increased intestinal permeability, are highly susceptibility to experimental colitis and develop systemic infections when challenged with *C. jejuni* as a result of reduced sulfomucin content.[102] Sulfation of mucins, in addition to being age-dependant, may be dynamic during infections such as rotavirus, where greater sulfomucins are present in infected animals and this confers protection by inhibiting infection.[103]

Regulation of Mucins by Bacterial Products

A number of bacterial products including LPS, flagellin and lipoteichoic acids have been implicated in mucin gene regulation. LPS is found on the outer membrane of gram-negative bacteria anchored by a lipid moiety called Lipid A.[104] Recognition of LPS by LPS-binding protein (LBP), CD14 and TLR4 (Toll-Like Receptor)[104] leads to a strong pro-inflammatory response in mammalian cells. Several studies have linked LPS to the induction of mucin expression. Supernatants from cultures and purified LPS from *P. aeruginosa and E. coli* have been shown to up-regulate endogenous MUC2, MUC5AC and MUC5B in NCI-H292 cells[105], and in human HT29-MTX colon carcinoma cells.[106] LPS has been shown to induce mucin gene expression by binding to TLR4 and LBP. LBP then binds to CD14 leading to activation of Src-dependent Ras/Raf/MEK/ERK/pp90rsk pathway.[107] This pathway leads to the activation of NF_KB and subsequent mucin transcription.[108] Similar to LPS, flagellin from gram-negative bacteria induces mucin upregulation through the Ras pathway. Flagellin binds to the surface receptor Asialo-GM1, which leads to the release of ATP that binds to G protein-coupled receptor (GPCR). This activates phospholipase C leading to induction of the Ras pathway and subsequent mucin transcription.[107,109] Lipoteichoic acid, a component of the cell wall of gram-positive bacteria also induces mucin expression in a pathway similar to the 2 mentioned above. By binding to the G-protein-coupled platelet-activating factor receptor, lipoteichoic acid activates ADAM 10 (a metalloprotease).[110] ADAM 10 in turn cleaves the transmembrane heparin-binding EGF, which then activates the epidermal growth factor receptor (EGFR). EGFR activates the Ras/Raf/MEK/ERK/pp90 rsk/NF-κB pathway leading to mucin transcription.[110]

Regulation of Mucins by Cytokines

Cytokines are bioactive factors that are secreted by a wide range of cells including immune, epithelial, endothelial cells and

fibroblasts when activated and in contact with pathogen associated molecular patterns (PAMPs). They play important roles in regulating cellular processes in the cell under normal and pathological conditions. In particular, cytokines regulate various inflammatory responses. Cytokines are broadly classified into type 1/Th1 cytokines and type 2/Th2 cytokines. Generally, Th1 cytokines favor the development of cellular immune response and includes IL-2, 1L-12 and IFN-γ while Th2 cytokines favor a strong humoral immune response and includes IL-4, 1L-6, 1L-10 and IL-13.[111,112] Cytokines bind to specific receptors and generally activate the JAK (Janus kinase)/STAT pathway.[113,114] A large number of cytokines including interleukin (IL)-1β, IL-4, IL-6, 1L-13 and TNF-α are known to regulate mucin synthesis/exocytosis and shown in **Table 1**.

Type 2 cytokines such as IL-4 and IL-13 are important inducers of goblet cell metaplasia in experimental animals[115–117] and have been shown to induce mucin gene expression both *in vitro* and *in vivo*. They up-regulate MUC2 and MUC5AC gene expression by binding to the IL-4 receptor and subsequently activating STAT6.[7] Furthermore, they also up-regulate MUC2 transcription in human colonic cancer cells through NF-κB activation mediated by MAPK (mitogen-activated protein kinase).[118] IL-4 and IL-13 also up-regulate intestinal trefoil factor (ITF), a goblet cell product important in mucus stabilization, mucosal protection and mucosal repair in HT-29 CL.16E and HT29 cells through the STAT6 pathway.[119] Although these cytokines have been shown to up-regulate mucin genes in colonic cell lines, they seem to have different effects in other cell types. For example, in a human pulmonary carcinoma cell line (NCI-H292), IL-4 up-regulated MUC2 expression and also upregulated MUC5AC expression in mouse airway epithelial cells in vivo.[116] In contrast, MUC5AC and MUC5B expression was markedly decreased by IL-4 in human tracheobronchial cells.[120] Like IL-4, IL-13 appears to have different effects in different cell types as well. In guinea pig tracheal epithelial cells, IL-13 induced goblet cell hyperplasia and increased MUC5AC secretion.[121] It however decreased MUC5AC expression in human nasal epithelial cells.[122] Other Th2 cytokines like IL-6, IL-9, IL-10 and IL-22 have also been shown to regulate mucin expression. IL-6 increased the expression of the gel-forming mucins, MUC2, MUC5AC, MUC5B and MUC6 and stimulated secretion in LS180 cells.[123] IL-9 induced increased expression of MUC2 and MUC5AC expression in airway epithelial cells.[124] IL-22 stimulated production of MUC1, MUC3, MUC10 and MUC13 mucins in a STAT3-dependent pathway, leading to rapid alleviation of local intestinal inflammation.[125] In addition, IL-22 induced restitution of goblet cells in a STAT3-dependent manner under inflammatory conditions.[125] Finally, the anti-inflammatory cytokine, IL-10 was recently shown to enhance MUC2 folding in goblet cells thereby maintaining the integrity of the mucus gel.[126]

Several Th1 cytokines regulate mucin biosynthesis and secretion notably, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and IL-1β. IFN-γ in particular was shown to promote the expression of MUC1 in a number of cell types including ovarian[127] and prostate[128] carcinoma cells. Like most of the Th2 cytokines, IFN-γ activates the JAK/STAT pathway. Upon binding to its receptor, IFN-γ activates JAK, which in turn activates STAT1 and STAT6. This leads to NF-κB activation or direct binding of STAT to the MUC1 promoter, leading to increased MUC1 expression.[129] IFN-γ also inhibited cholera-toxin induced mucin exocytosis in HT29-CL.16E cell without affecting constitutive mucin secretion or MUC2 gene expression.[130]

TNF-α and IL-1β are known to be major regulators of gel-forming mucins. These 2 cytokines are commonly involved in inflammatory diseases with TNF-α also implicated in intestinal epithelial cell apoptosis. TNF-α stimulated mucin secretion in LS180 cells and increase the expression of MUC2, MUC5AC, MUC5B, and MUC6.[123] Similarly, IL-1β stimulated rapid mucin exocytosis in HT29-Cl.16E cells in a dose-dependent manner and increased mucin gene expression. Similar effects were observed in colonic LS180 cell line and in perfused rat colons.[123] TNF-α upregulates MUC2 expression through PI3K/Akt/NF-κB pathway.[131] Using pharmacological inhibitors and genetic inhibition of various pathways, it was showed that IL-1β and TNF-α induced MUC5AC over-expression by activating MSK1 (mitogen- and stress activated protein kinase 1), CREB (cAMP response element binding protein) and CRE signaling pathways in human nasal epithelial cells.[57]

ER stress and Mucin Regulation in IBD

IBD, which includes Crohn's disease (CD) and ulcerative colitis (UC), are chronic and relapsing diseases of the intestine. CD is characterized by the formation of fistulas, transmural inflammation and is generally localized to the ileum, however it can affect any area of the GI tract. UC presents as mucosal localized inflammation and predominantly in the distal colon. Both disorders predispose individuals to an increased risk for developing colorectal cancer.[132] The etiological trigger of inflammation is unknown; however it is likely the combinatorial input of predisposed genetic factors, the microbiome, and an ill-mounted immune response. Early studies on risk alleles associated with IBD identified the 7q22 locus, which harbours numerous mucin genes such as MUC3A, MUC3B, MUC12, MUC16 and MUC17 as susceptibility loci in Crohn's disease.[133,134] Missense polymorphisms in MUC1 and MUC2 have also been identified as predisposing to CD and indeed, in an animal model single point mutations have been shown to alter the properties of MUC2, as in the Winnie transgenic mouse.[135,136] Winnie mice were generated using N-ethyl-N-nitrosourea (ENU) and contain a single point mutation in the D3 domain of MUC2 N-terminal to the PTS tandem repeat domain. This mutation results in alteration of N-terminal oligomerisation and thus improper folding of the native MUC2 protein. Histopathogically, Winnie mice present with smaller goblet cells filled with non-glycosylated MUC2 precursor[136] and therefore have less mucus secretions into the lumen.

Disproportionate shifts in the commensal microbiota have been well reported in both UC and CD, especially with the advent of next-generation sequencing.[137] Additional studies have

recently identified a greater abundance of mucosa-associated bacteria in both CD and UC. This correlated to increases in specific mucolytic bacteria, particularly the Ruminococcus family.[138] Fecal samples collected from patients with UC have greater mucinase activity than controls or CD patients.[139] Additionally, patients with active UC (but not those in remission) have altered O-linked glycosylation of MUC2 resulting in smaller, less complex glycans on the apoprotein.[140]

The extent of this phenomenon was correlated to the severity of inflammation and also disease outcome. This altered glycosylation pattern in those patients in relapse could affect the ability for specific species of bacteria to adhere to and colonize the mucus layer.[141] In that study, fecal samples from UC patients in remission or active disease were compared to healthy controls using the M-SHIME model which facilitates long term *in vitro* quantification of mucin covered microcosms. They found that *Lactobacilli* spp from samples of active UC had an inability to colonize mucin-covered microcosms, along with variations in the metabolomes of UC patients in relapse versus remission.

Alterations to the mucus barrier or biosynthesis of mucins likely play a role in the onset and persistence of IBD. One of the hallmarks of UC and CD is goblet cell pathology. In CD, the mucus layer is thicker, possibly from goblet cell hyperplasia and MUC2 expression is increased irrespective of inflammation.[142] The MUC2 produced, however, has an altered structure as the oligosaccharide chain length is reduced by about 50%.[143] The overall effect of this is that, even though there is increased MUC2 output, the altered structure leads to loss of its viscoelastic properties and a reduced barrier function. In UC however, the mucus layer is thin due to decreased MUC2 production and secretion. This decrease is due to a reduction in goblet cell number and small goblet cell thecae.[144] The cells contain fewer mucin granules which are filled with non-glycosylated MUC2 precursor.[136] Recent studies with animal models have shown that a change in the amount and composition of the mucus barrier, leads to IBD-like syndrome.[135,136] A study using electron microscopy showed that partially synthesized or misfolded mucins accumulate within the ER of the goblet cells giving the ultrastructural appearance of vacuolization.[145] The accumulation of these immature glycoproteins cause ER stress in the cells and lead to a reduced production of mature mucin for secretion.[136] Decreased MUC2 output due to ER stress can diminish the mucus barrier and ultimately trigger inflammation. Since MUC2 is essential in maintaining the integrity of the mucus barrier, the activation of ER stress response can also ultimately trigger inflammation.[146] ER stress is therefore quickly becoming a growing area of interest in intestinal inflammation and IBD as ER stress has been reported in IBD patients.[136,147]

ER stress and The Unfolded Protein Response

As mentioned above, recent studies have shown that ER stress is important in goblet cell function and regulation of the mucus barrier. The ER is responsible for synthesis of polypeptides into functional proteins.[148] Because of the complex processes involved in proper protein folding, the ER is equipped with a number of enzymes such as the protein-disulfide isomerase (PDI), anterior gradient 2 (AGR2) and chaperones including glucose-regulating peptide 78 (GRP78), GRP94, calnexin and calreticulin to aid in protein synthesis.[149,150] In spite of these aids available, a small proportion of proteins still misfold in the ER. The ER however, has mechanisms to remove misfolded proteins.[151] When the ER is unable to rapidly remove these proteins and they accumulate, a condition known as ER stress is reached.

ER stress has become an interesting topic lately as it has been linked to a number of inflammatory diseases including cancer, diabetes and IBD.[152] When ER stress is reached, a cascade of reactions, known as the unfolded protein response (UPR) is activated in order to restore correct folding and ER homeostasis. The ER chaperone GRP78 is the key protein that activates the UPR process.[153] Under normal conditions, GRP78 is bound to the N-termini of the 3 UPR initiating molecules [PKR (double-stranded RNA-dependent protein kinase)-like ER kinase] (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6), to prevent their aggregation, keeping them in an inactive state.[154] When unfolded proteins accumulate in the ER however, GRP78 dissociates from these sensors and bind to the unfolded proteins. This causes the UPR initiating molecules to become activated, initiating the UPR. The UPR initiating molecules eventually activate various transcription factors that translocate to the nucleus and induce transcription of genes required to restore ER homeostasis.[155,156,157] Ultimately, the UPR restores ER homeostasis by; attenuation of protein translation to reduce the amount of proteins trafficking to the ER, up-regulation of ER chaperones to restore protein folding and up-regulation of ER-associated protein degradation (ERAD) genes to increase degradation of misfolded proteins.[158] Alternatively, when ER stress is prolonged and ER homeostasis is not achieved, the UPR leads to up-regulation of pro-apototic factors such as CCAAT/-enhancer-binding protein homologous protein (CHOP) and c-Jun-N-terminal kinase (JNK) which drives the cell to apoptosis. [159]

Various studies link ER stress in goblet cells to mucus barrier dysfunction and development of inflammation. Due to the large size and complexity of mucins, they are extremely susceptible to misfolding in the ER which can eventually lead to ER stress. Thus, goblet cells are one of the major cells that tend to undergo ER stress in the intestinal epithelium. Using various animal models, ER stress in goblet cells has been shown to diminish the integrity of the mucus barrier by reducing biosynthesis and secretion of mucins.[136,160,161] **Figure 2** shows the effect of ER stress on the mucus barrier. Recently, 2 mutant mouse strains, Winnie and Eeyore, derived from ENU mutagenesis have provided some insights into the link between ER stress, mucin production and intestinal inflammation. These mice possess missense mutation in the MUC2 gene and have a defect in MUC2 folding, leading to accumulation of misfolded MUC2 precursor in the ER, ER stress and activation of the UPR.[136] ER stress in these mice causes reduction in MUC2 biosynthesis by goblet cells due to the translational block induced by the UPR. This subsequently leads to

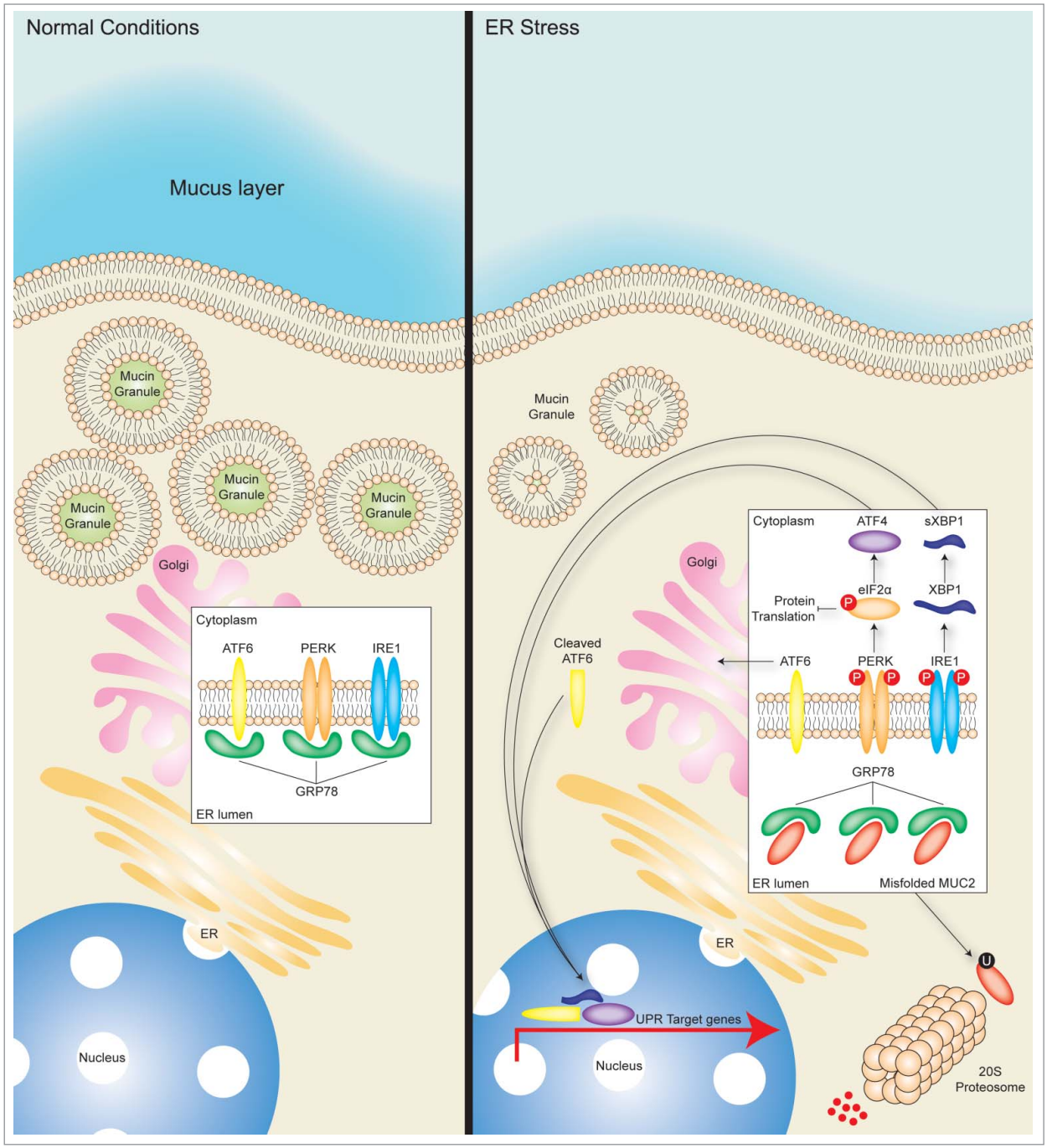

Figure 2. Endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in goblet cells. Various factors can induce ER stress in goblet cells, which can lead to a reduction in mucin synthesis and a diminished mucus layer. During ER stress, the UPR is activated to restore ER homeostasis. Glucose-regulating peptide 78 (GRP78) dissociates from the UPR initiating molecules and binds misfolded proteins. [PKR (double-stranded RNA-dependent protein kinase)-like ER kinase] (PERK), upon dissociation from GRP78 becomes active and phosphorylates eukaryotic initiation factor-2α (eIF2α) leading to inhibition of mRNA translation. This reduces the protein load trafficking to the ER. The mRNA encoding the transcription factor activating transcription factor 4 (ATF4) is however translated. ATF4 then moves to the nucleus to up-regulate UPR genes. On becoming active, inositol-requiring enzyme 1 activates Xbox binding protein (XBP1). Activated XBP1 then translocate to the nucleus and up-regulates UPR target genes. Activating transcription factor 6 (ATF6) moves to the Golgi apparatus after dissociation from GRP78, where it is cleaved by proteases to form an active transcription factor, which moves to the nucleus to modulate UPR gene expression. ER stress causes misfolding of mucins in the ER. Furthermore, inhibition of mRNA synthesis by the PERK arm can diminish mucin translation. The overall effect is that, decreased synthesis of mucins can lead to fewer mucin granules and a thinner mucus layer.

decreased amount of secreted MUC2 and reduction in efficiency of the mucus barrier.[136]

Similar to the MUC2 mutant mice, mice deficient in the ER-resident enzyme, fatty acid synthase (FAS), in their colonic epithelium have a defective mucus barrier and enhanced inflammation.[160] FAS is important in lipidation of MUC2 and its deficiency results in loss of MUC2 palmitoylation and increased MUC2 misfolding. As a result, there is impaired MUC2 production and secretion in these mice.[160] These effects were associated with ER stress as mRNAs for XBP1 and CHOP were significantly increased in the colons of the mice.[160] *In vitro* knockdown of FAS in LS 174T cells also significantly increased the expression levels of GRP78, CHOP, and XBP1 further suggesting that ER stress might play a role in the reduced mucus secretion and inflammation seen in FAS-deficient mice.[160] Similarly, mice deficient in AGR2 which is crucial in disulphide bond formation during mucin folding, also showed ER stress, causing decreased goblet cell mucin biosynthesis and secretion and enhanced inflammation.[161] Despite these studies, the effect of high mucin production and secretion on ER stress has not been well described.

As ER stress regulates goblet cell function, bioactive factors that affect goblet cell ER stress can potentially modulate mucin production and secretion. Recent studies have shown the effect of some cytokines on ER stress and protein folding. In particular, IL-10 has been shown to regulate the mucus barrier by inhibiting ER stress and protein misfolding.[126] IL-10 is a Th2 cytokine with anti-inflammatory functions and also inhibits the synthesis of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α.[162,163] The effect of IL-10 on ER stress was investigated *in vivo* and *in vitro* where IL-10 enhanced protein folding and decreased ER stress thereby reducing intestinal inflammation.[126] These authors also noted that IL-10 enhanced MUC2 folding and decreased ER stress in Winnie mice. In addition, IL-10 up-regulated genes important in MUC2 folding, thus maintaining correct MUC2 folding and secretion. Furthermore IL-10 upregulated genes in the ERAD system, enhancing removal of misfolded MUC2. Finally they also reported that IL-10 acts by activating STAT1 and STAT3 after binding to the IL-10 receptor (IL-10R1) which leads to the suppression of protein misfolding in the ER, reducing UPR signaling.[126] This study provides evidence that IL-10 regulates the mucus barrier by enhancing MUC2 production and reducing ER stress, thus helping the intestines to maintain the mucus barrier. IL-10 also modulated the UPR by inhibiting nuclear translocation of ATF6 in a p38-mediated fashion.[147]

Other cytokines have also been reported to affect ER stress indirectly mostly by inducing oxidative stress, a condition known to increase protein misfolding and ER stress. IL-1β, TNF-α and IFN-γ induce reactive oxygen species (ROS) and nitric oxide (NO) production that in turn increases ER stress in fibrosarcoma cells and pancreatic β cells. Thus it is not surprising that IFN-γ and TNF-α were shown to increase ER stress in human intestinal epithelial cells *in vitro*.[164] It was also shown that similar to IL-10, dexamethasone enhanced MUC2 folding, reduced ER stress and up-regulated ERAD genes thereby enhancing degradation of misfolded proteins.[165] In summary, cytokines that induce ER stress in goblet cells inhibit proper mucin folding and reduce output of mucin while those that inhibit ER stress promote folding of mucins and hence enhance mucin biosynthesis and secretion to help maintain an intact and functional mucus barrier in the intestines.

Autophagy and Mucus Regulation

ER stress and the UPR has also been linked to various pathways, for example, autophagy. Autophagy is a catabolic mechanism that involves degradation of dysfunctional organelles and cytosolic macromolecules through the action of lysosomes.[166] Autophagy is activated in situations of cellular stress such as nutrient and growth factor deprivation and therefore important in cellular homeostasis. Importantly, autophagy serves as a host defense mechanism against bacteria as it is involved in the degradation and clearance of intracellular pathogens.[167,168] Recent studies have shown that autophagy is required for mucus secretion by intestinal goblet cells, making this process important in maintaining the mucus barrier.[169] In particular, Atg5, Atg7 and LC3β proteins were shown to be involved in the formation of autophagosomes that were required for efficient mucus secretion. In addition, the generation of ROS derived from NADPH (nicotinamide adenine dinucleotide phosphate) oxidases, was vital to the secretion process, concluding that autophagy regulates mucin secretion through ROS, which is in part generated by LC3-positive vacuole-associated NADPH oxidases.[169] These data showed the importance of autophagic proteins in regulating goblet cell secretion, however, the upstream signaling pathway that activated the autophagy machinery is yet to be determined.

Autophagy is involved in bacteria clearance, and thus the process is influenced by several pattern recognition receptors (PRRs) like the TLRs and NLRs (Nod–like receptors). Studies have shown that NOD1 and NOD2 interact with the autophagy protein ATG16L1, which is an important component of a large protein complex (Atg5-Atg12/Atg16L1) essential for the autophagic process.[170] These NLRs recruit ATG16L1 to the plasma membrane where they can target intracellular pathogens at the point of entry.[170] NLRs may thus be involved in signaling that could regulate autophagy-dependent mucus exocytosis. A member of the NLR family and the inflammasome pathway, NLRP6, has been shown to promote autophagy-dependent mucus secretion from goblet cells. NLRP6 participates in activation of the inflammasome and thus, provides defense against infection and tumorigenesis among others. The inflammasome is a complex of proteins that recruits the adaptor protein ASC (Apoptosis-associated speck-like protein containing a CARD) and activates caspase-1, which subsequently cleaves and activates IL-1β and IL-18.[171] A recent study[172] showed that NLRP6 affects the mucus barrier by regulating mucin secretion. Mice that were deficient in NLRP6, ASC, or caspase-1 lacked a continuous inner mucus layer compared to their wild type counterparts thus increasing their susceptibility to *C. rodentium* infection. The authors attributed the thinner mucus layer to impaired mucus

granule secretion since the number of goblet cells in NLRP6 deficient mice was increased and mucin gene expression was not abnormally altered. NLRP6 inflammasome modulated mucus granule exocytosis and were found to be essential for autophagy in intestinal cells. NLRP6 was critical for maximum conversion of the cytosolic form of microtubule-associated protein 1 light chain (LC3I) to its phosphatidylethanolamine conjugate (LC3II), a process required for the formation of autophagosomes.[172] Taken together, these studies showed that NLRP6 inflammasome is critical in maintaining autophagy in the intestinal epithelium that is important in mucus secretion and maintaining the mucus barrier.

Concluding Remarks

The classical dogma of the mucus layer as being a static physical barrier to noxious substances and bacteria has gone away in favor of a model that supports this barrier as being a highly dynamic and multifunctional regulator of gut homeostasis. In accordance with this model, complex interactions between the diverse microbiota is beginning to be elucidated, and interesting new paradigms on how this affects pathogen colonization and IBD is forthcoming. How pathogens exploit the mucus layer and goblet cells have provided novel insights into disease pathogenesis. Furthermore, recent insights into the immune functions of goblet cells and MUC2 can potentially be explored to develop relevant treatments for chronic gut inflammatory disorders. The cost at which mucin is produced, particularly in the context of ER stress, outlines the functional importance of this molecule despite the detrimental effects on goblet cells. Looking forward in the field of mucin biology, it is essential to determine the broad reaching effects of the mucus barrier, as it relates to innate host defense, homeostasis of intestinal environments and pathogen defense.

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No potential conflicts of interest were disclosed.

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