



Mucins in Intestinal Mucosal Defense and Inflammation: Learning From Clinical and Experimental Studies

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Throughout the gastrointestinal (GI) tract, a distinct mucus layer composed of highly glycosylated proteins called mucins plays an essential role in providing lubrication for the passage of food, participating in cell signaling pathways and protecting the host epithelium from commensal microorganisms and invading pathogens, as well as toxins and other environmental irritants. These mucins can be broadly classified into either secreted gel-forming mucins, those that provide the structural backbone for the mucus barrier, or transmembrane mucins, those that form the glycocalyx layer covering the underlying epithelial cells. Goblet cells dispersed among the intestinal epithelial cells are chiefly responsible for the synthesis and secretion of mucins within the gut and are heavily influenced by interactions with the immune system. Evidence from both clinical and animal studies have indicated that several GI conditions, including inflammatory bowel disease (IBD), colorectal cancer, and numerous enteric infections are accompanied by considerable changes in mucin quality and quantity. These changes include, but are not limited to, impaired goblet cell function, synthesis dysregulation, and altered post-translational modifications. The current review aims to highlight the structural and functional features as well as the production and immunological regulation of mucins and the impact these key elements have within the context of barrier function and host defense in intestinal inflammation.

Keywords: mucins, goblet cell, mucosal defense, intestinal inflammation, enteric infection

INTRODUCTION

The mammalian gastrointestinal (GI) tract harbors a dynamic and complex ecosystem with gut microbes, food particles, foreign substances and the host cells participating in constant interaction. As such, the host requires relentless surveillance and persistent protection in order to maintain strict homeostatic conditions. As the bridge between the internal and external environments, it is no surprise then that roughly 70% of the immune system resides within the GI tract (1). Though these imperative defensive mechanisms span the initial innate responses to the more complex adaptive pathways, the physical aspects of protection should not be overlooked. One such physical aspect is the mucus layer of the GI tract which is responsible for providing lubrication for the passage of food, protecting the underlying epithelium from commensal microbes and establishing a physical barrier against invading pathogens, as well as toxins and other environmental irritants (2, 3). The mucus

layer, particularly through its transmembrane components, also influences several cell signaling pathways that can modulate inflammatory responses, impact cell-cell interactions as well as regulate proliferation, differentiation and apoptosis (4–6).

The intestinal mucus layer is principally comprised of a subset of high molecular weight glycoproteins called mucins, which play a crucial role in physical protection as well as in regulating the concentration and passage of water, ions, and other immune mediators such as antimicrobial peptides (AMPs) and immunoglobulin-A (IgA) within the gut (2, 3, 7). Uniquely, the stomach and colon contain a dual-layer of mucus that is composed of polymeric sheets of these highly glycosylated mucins (**Figure 1**). These two layers can be categorized into the dense inner layer which is firmly attached to the epithelial cells below and impermeable to bacteria, vs. the outer layer which is loosely attached to, and easily removed from, the dense underlying layer (8, 9). This outer layer is also penetrable by bacteria. In contrast, the small intestine contains only one loose layer of mucus, which is penetrable by bacteria [**Figure 1**; (8, 9)].

Goblet cells, as well as the three other principal cells (enterocytes, enteroendocrine cells, and Paneth cells) of the gut mucosa arise from multipotent stem cells at the base of crypts of Lieberkühn (10). Among these unique cell types, goblet cells, are chiefly responsible for the production and preservation of the mucus blanket via mucin production and are heavily influenced by interactions with the immune system (3). Enterocytes also minorly contribute to the production of secreted mucins (11–13). It should also be noted that the distribution and density of goblet cells within the GI tract varies; numbers increase distally and reach a peak in the distal ileum and rectum (14). Within the stomach, mucus production is vital to protect the gastric mucosa from digestive enzymes and the harsh acidic environment of the lumen. Of the five main cell types that contribute to the biochemical milieu of the gastric lumen (including parietal cells, chief cells, and enterochromaffin-like cells), surface mucus cells or foveolar cells, and mucus neck cells are, as the names suggest, the main producers of gastric mucus (15–18). Within the distinct regions of the stomach, the organization of the invaginations that house these cells varies. In the proximal corpus, stem cells

are largely located within the isthmus and are confined to the upper third of the gastric pits. In contrast, stem cells in the distal antrum are typically located in the bottom third of the invagination. In either case, these multipotent progenitor cells can move bidirectionally to either the mucosal surface or the base of the gastric pits differentiating and maturing into the principal epithelial cells of the stomach as they do so (19). Over the course of their migration, those cells destined to become surface mucus and mucus neck cells differentiate and gradually release mucin glycoproteins into the lumen (15).

Given the primary role of the mucus layer in physical defense, the influence that it has on GI inflammatory pathologies is increasingly of interest. Often these pathologies are accompanied by impaired goblet cell function as well as dysregulated mucin biosynthesis with considerable qualitative and quantitative changes (20, 21). One group of diseases that is strongly influenced by the proper function of goblet cells and their secreted mucins is inflammatory bowel disease (IBD) which is broadly classified into Crohn's disease (CD) and ulcerative colitis (UC) (22). These conditions are characterized by chronic inflammation of the GI tract and are increasing in prevalence, particularly as newly industrialized countries become progressively more "westernized" (23). Unfortunately, both cause and cure remain elusive. Colorectal cancer also presents alterations in GI mucin production and function. Lastly, bacterial and parasitic infections, which are more prevalent in developing countries, are also associated with mucin dysfunction and inflammation of the GI tract (3, 24). The present review aims to highlight the structural and functional features as well as the production and immunological regulation of mucins, and the impact these key elements have within the context of barrier function and host defense in intestinal inflammation.

STRUCTURAL FEATURES AND CLASSIFICATION OF MUCINS

Thus far, more than 20 mucin genes have been identified (25). Though the corresponding glycoproteins associated with each of these genes have distinct differences, mucins, in general, share conserved structural features. The protein backbone contains tandem repeat units of varying length consisting of the amino acids proline, serine, and threonine, which create sites for O-glycosylation by O-linked oligosaccharides (26). The majority of these O-linked oligosaccharides are composed of N-acetyl galactosamine (GalNAc), N-acetyl glucosamine (GlcNAc), galactose (Gal), fucose (Fuc), and are often terminated by sialic acids (2, 26, 27).

The O-glycosylation process originates with GalNAc attachment to either a serine or threonine residues within the protein backbone, which can then be further elongated by additional carbohydrate residues. These initial additions can be classified as one of six common "core" regions which encompass the GalNAc-peptide attachment point and any sugars directly linked to this GalNAc. This core region is either terminated by a sialic acid residue or extended to form the backbone and, eventually, the peripheral regions of the glycan, the addition of

Abbreviations: AMP, antimicrobial peptides; IgA, immunoglobulin-A; IL, interleukin; TNF- α , tumor necrosis factor alpha; GalNAc, N-acetyl galactosamine; GlcNAc, N-acetyl glucosamine; Gal, galactose; Fuc, fucose; TLRs, Toll-like receptors; SPDEF, SAM pointed domain-containing ETS transcription factor; NLRs, nucleotide-binding oligomerization domain (NOD)-like receptors; LPS, lipopolysaccharide; LTA, lipoteichoic acid; PAMPs, pathogen associated molecular patterns; LBP, lipopolysaccharide (LPS) binding protein; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; ASGM1, asialoGM1; ILC, innate lymphoid cell; CLP, common lymphoid progenitor; ROR α , retinoic acid receptor-related orphan receptor alpha; GATA3, GATA binding protein 3; Ach, acetylcholine; MR, muscarinic acetylcholine receptors; NR, nicotinic acetylcholine receptor, M3R, type 3 muscarinic receptor; VIP, vasoactive intestinal peptide; VPAC, vasoactive intestinal peptide (VIP) receptor; Chrm3, cholinergic receptor muscarinic 3; NmU, neuromedin U; GWAS, genome-wide association study; FCGBP, IgGFc-binding protein; CLCA1, calcium-activated chloride channel regulator 1; ZG16, zymogen granule protein 16; ESPs, excretory/secretory products; Nmur1, neuromedin U (NmU) receptor 1; LRRK2, leucine-rich repeat kinase 2; KLF4, Kruppel-like factor 4; PC, phosphatidylcholine; C1GalT1, core 1 β 1,3-galactosyltransferase; C3GnT, core 3 β 1,3-N-acetylglucosaminyltransferase; FMT, fecal microbiota transplantation; FAS, fatty acid synthase.

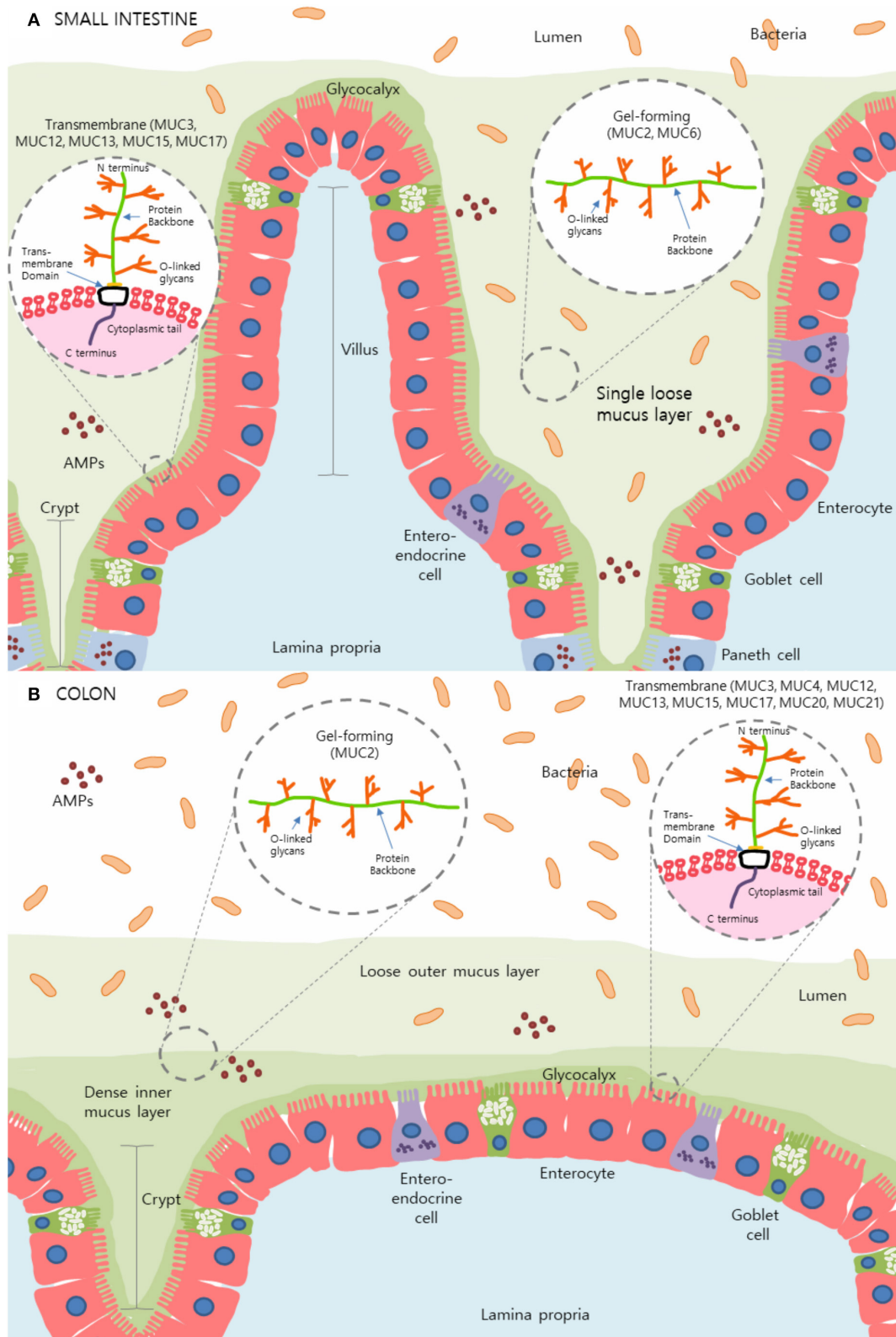


FIGURE 1 | The mucus layer of the small intestine and colon. **(A)** In the small intestine, only one layer of loosely attached mucus is present and is penetrable by resident microbes. **(B)** Primarily produced by goblet cells, colonic mucus is comprised of two layers: an outer layer permeable to bacteria and a tightly adhered inner layer impermeable to bacteria. Here, secreted gel-forming mucins, largely MUC2, are the main components of this mucus layer and provide its viscoelastic properties. *(Continued)*

FIGURE 1 | Transmembrane mucins including MUC3A/B, MUC12, MUC13, MUC15, and MUC17, form a carbohydrate-rich layer called glycocalyx lying between the secreted mucins and the underlying epithelial cells in both the small intestine and colon. Simplified structures of transmembrane mucins and gel-forming mucins can be seen in the magnified sections. Transmembrane mucins are generally comprised of two subunits; the heavily glycosylated and larger extracellular subunit and the shorter subunit consists of a small extracellular domain, a transmembrane domain and a cytosolic compartment. The extracellular protein backbone contains tandem repeat units of varying lengths consisting of the amino acids proline, serine, and threonine which create binding sites for O-linked oligosaccharides. This protein backbone and O-linked glycan structure are also present in secretory/gel-forming mucins.

which marks the termination of O-glycosylation (28). Variation in both the number and type of residues added, as well as, substrate availability and competition among transferases generates immense structural variability in mucin glycoproteins and results in a range from short linear structures to more complex branched forms (28). Within the mucin structure, O-linked oligosaccharides can be added as frequently as one in three amino acids and can substantially increase the molecular weight of mucins and help attract water to the mucus layer. The O-glycosylation of mucins also provides these proteins resistance to the activity of proteases and helps to prevent degradation (28, 29).

Mucins can be broadly classified into gel-forming or transmembrane based on their structural and functional features. Secreted gel-forming mucins, such as MUC2, MUC5AC, MUC5B, and MUC6, are the main components of the mucus layer and provide its viscoelastic properties (**Figure 1**). These mucins undergo homo-oligomerization through the formation of disulfide (S–S) bonds at their cysteine-rich N- and C-terminals aiding in the creation of a flexible network (11, 30). In the intestine, MUC2 is the predominant gel-forming mucin that contributes to the formation of the mucus barrier. MUC5AC, which is normally present in the stomach, can also be upregulated within the intestines during enteric infection (31) suggesting these mucins may have crucial roles at multiple mucosal sites. MUC5B can also be expressed in low levels in the colon while MUC6 is preferentially expressed in the stomach and duodenum (32, 33). MUC7 is a secreted mucin found in saliva and within the oral cavity and is often categorized separately from the rest of the secreted mucins (26). The differential classification of MUC7 is due to its low molecular weight and the fact that it does not significantly contribute to the viscoelastic properties of mucus (34). Unlike other gel-forming mucins, MUC7 lacks cysteine-rich terminals, does not polymerize and exists primarily as a monomeric structure (35).

Transmembrane mucins, such as MUC1, MUC3, MUC4, MUC13, and MUC17, are expressed on the apical surfaces of epithelial cells and form a carbohydrate-rich layer called glycocalyx which acts as a protective barrier between the secreted mucins and the underlying epithelial cells [**Figure 1**; (36, 37)]. The rigid structure of these mucins spans the length of the cell membrane and participates in intracellular signaling with their C-termini located inside the cell (11, 38). Transmembrane mucins are generally comprised of two subunits held together by non-covalent sodium dodecyl sulfate-labile bonds. The larger subunit, which is primarily extracellular, contains serine and threonine repeat units and is heavily glycosylated. The shorter subunit is comprised of a small extracellular domain, a transmembrane domain, and a cytosolic

compartment (39). Although the exact mechanisms remain unknown, transmembrane mucins have been heavily implicated in cell signaling (6). The function MUC1 displays in the downregulation of the Toll-like receptor (TLR)-initiated innate immune response is a well-established instance of cell signaling by transmembrane mucins (40).

GOBLET CELL DIFFERENTIATION, MUCIN PRODUCTION, AND SECRETION

The major specialized intestinal epithelial subtypes including goblet cells, Paneth cells, and enteroendocrine cells, originate from stem cells located at the base of intestinal crypts and are derived from a common secretory precursor cell. Enterocytes, the most abundant cell type within the epithelial layer of the gut, develop from non-secretory lineages of stem cells (41).

Lineage differentiation of these specialized intestinal epithelial cells is, in part, regulated by Notch signaling. Notch is a cell-surface receptor which is responsible for the regulation of various DNA-binding proteins (42). Although the exact mechanism is not known, inhibition of the Notch pathway leads to preferential differentiation of intestinal stem cells into goblet cells, the primary producers of secreted mucins within the GI tract (14, 43, 44).

During their maturation, goblet cells undergo substantial morphological changes as they migrate from the base of intestinal crypts to the villi. At the base of the crypt, stem cells are fated into early goblet cell lineages via Wnt-signaling, which give rise to immature goblet cells. These immature cells are large in size, pyramidal, and contain mucin granules interspersed among the organelles. During maturation, goblet cells begin to lose cytoplasmic volume (14, 45). In this process, SAM pointed domain-containing ETS Transcription Factor (SPDEF) drives terminal differentiation into mature goblet cells (46). The apical region of mature goblet cells, called the theca, is cup-like in appearance and is packed with mucin granules, while the organelles and nucleus congregate to the basal stem of the cell (14, 47). In addition to morphological changes, goblet cell differentiation is also accompanied by alterations in the chemical composition of the produced mucins. During maturation, secreted mucins become more acidic and develop more sites for N- and O-glycosylation (14, 48).

After synthesis, mucin-packed granules are transported to the apical cell surface via secretory vesicles and are released into the lumen by the processes of basal secretion or compound exocytosis/regulated secretion (49). Basal secretion, occurring under normal physiological conditions, involves the continuous fusion and release of single mucin granules into the gut

lumen. This steady and unstimulated release maintains the thickness of the mucus barrier and protects the underlying epithelia from the constant threat of luminal contents (50). Compound exocytosis, on the other hand, is a process induced or stimulated by inciting factors such as microbial products, hormones, inflammatory cytokines and neurotransmitters such as acetylcholine (26, 49, 51–53). Here, centrally stored mucin vesicles rapidly fuse and empty their contents in response to these secretagogues (54). Since the 1980s, the morphological process, molecular mechanism, and relationship between immune and non-immune regulators of compound exocytosis have been active areas of research.

IMMUNOLOGICAL REGULATION OF MUCIN PRODUCTION

The immune response can be broadly divided into the innate and adaptive systems. The innate system relies on evolutionarily conserved, somatically-encoded receptors to recognize molecular patterns on microbes (55, 56). In contrast, the adaptive immune response relies on the specific recognition of pathogenic antigens for initiation of said immune response (57).

Innate Immunological Regulation

Within the innate domain, pattern recognition receptors (PRRs) such as TLRs and cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) play an essential role in mucin synthesis (58). TLRs are a family of 11 evolutionarily conserved transmembrane receptors which are located on the cell surface or on intracellular endosomes. These receptors are activated by pathogen-associated molecular patterns (PAMPs). This activation terminates with the induction of the NF- κ B family of transcription factors and the upregulation of the immune response (25, 59).

Expression profiles of TLRs differ throughout the GI tract. Recently, by utilizing five strains of TLR reporter mice, the spatial expression of TLRs in intestinal epithelial tissue was visualized. TLR2, 4, 5, 7, and 9 were found to be minimally or not expressed in the small intestinal epithelium while TLR2, 4, and 5 expression levels were substantially higher in the colon (60). Further, TLR3 was expressed at similar levels in both small intestinal and colonic epithelial cells (60). Only TLR5 was expressed by goblet cells in the small intestine while colonic goblet cells were found to express TLR1, 2, 4, and 5 (61) suggesting intrinsic TLR-mediated mucin regulation in these cells.

Through their activation of TLRs, ligands such as lipopolysaccharide (LPS) found on the outer membrane of most Gram-negative bacteria, lipoteichoic acid (LTA) on the cell wall of Gram-positive bacteria, and flagellin found in bacterial flagella are all potent activators of MUC2 expression (25, 62). For instance, upon stimulation by their respective ligands TLR2/1, TLR4, and TLR5, promote the downstream activation of the NLRP6 inflammasome in subpopulations of sentinel goblet cells located at the entrance of colonic crypts. This inflammasome, a multiprotein complex located in the cytoplasm of these cells, functions as a sensor for cellular stresses and plays a key role in

intestinal barrier maintenance, infection defense and mucosal renewal (54, 63). These TLR-initiated cascades stimulate the compound exocytosis of MUC2 and trigger mucin secretion from adjacent goblet cells through intercellular gap junction signals. This subsequent increased MUC2 secretion can thus aid in the expulsion of bacteria from the upper part of the crypts (54). Recent studies have shown that TLR4 activation is important in goblet cell response during *Citrobacter rodentium* infection (64) and can regulate the differentiation of goblet cells in intestinal organoids (65). The activation of TLR4, involving the binding of lipid A moiety of LPS to the LPS binding protein (LBP), can upregulate the expression of MUC2 through the Ras-MEK1/2-Erk1/2 and NF- κ B pathways (66). Establishing whether the particular crypt location of the goblet cells is a determining factor in mucin production in response to various TLR ligands will be a worthwhile direction for future research.

Further evidence gleaned from genetic knockout models have helped highlight the differential effects of TLRs on mucin regulation. For instance, naïve *Tlr1*^{-/-} mice have defective production and/or secretion of MUC2 in the colon leading to a patchy and significantly depleted mucus layer (67). *Tlr5*^{-/-} mice display a mosaic phenotype; a subset of these mice develop spontaneous colitis while the majority do not. Interestingly, compared to wild-type mice, colitic *Tlr5*^{-/-} mice do not display the normal dual-layer of colonic mucus; only a disorganized and largely penetrable layer is present. In contrast, non-colitic *Tlr5*^{-/-} mice had a normal though slightly thinner mucus layer (68).

Scant research exists involving the interaction between TLRs, goblet cell function, and mucin regulation in intestinal parasitic infection *in vivo* and this area remains largely unexplored. *In vitro*, however, antigens from the intestinal trematode *Gymnophalloides seoi* have been found to induce the expression of both TLR2 and MUC2 in HT-29 cells in an IFN γ -dependent manner. Further, co-stimulation with *G. seoi* antigen and antibodies against both TLR2 and TLR4 have been shown to diminish MUC2 expression in HT-29 cells compared to those cells treated with the antigen only. Thus, the authors of this study hypothesize that the induction of MUC2 expression as an antiparasitic response in human IECs, may, at least in part, be a result of TLR activation (69). Additional *in vivo* and *in vitro* research will provide valuable insights into the interaction between TLRs, goblet cell function and mucin regulation in parasitic infection.

In contrast to the transmembrane TLRs, NLRs are a family of innate intracellular receptors (70). However, similar to TLR signaling, activation of NLRs such as NOD1 and NOD2 by intracellular ligands (i.e., bacterial peptidoglycans) ultimately results in the activation of important transcription factors, such as NF- κ B, to induce immune responses (71). An enteric infection model using the helminth, *Trichuris muris*, has revealed that NOD1 and NOD2 receptors are necessary for MUC2 synthesis and parasitic expulsion; knocking out both of these genes (Nod-DKO) result in infected mice with lower goblet cell numbers and decreased MUC2 expression (72).

Other receptors can also impact mucin production via the innate response. LTA from Gram-positive bacteria can upregulate

MUC2 expression by acting on platelet-activating factor receptor (PAFR) and, through a multistep process, activate the Ras-MEK1/2-Erk1/2 and NF- κ B pathways (25). Moreover, flagellin signals through the glycolipid receptor asialoGM1 (ASGM1) ultimately lead, again, to upregulated expression of MUC2 (73). ASGM1-mediated upregulation of MUC2 involves the sequential activation of phospholipase C, an increase in calcium ion levels as well as ERK1/2 and NF- κ B activation (73).

Dendritic Cells and Macrophages

In the local draining lymph node, antigen presenting cells (APCs) such as dendritic cells and macrophages present the phagocytosed and processed antigen to aid in the differentiation of naïve CD4⁺ T cells to Th2 cells. These cells then secrete effector cytokines such as IL-13 and, thusly, contribute to mucus production and goblet cell hyperplasia (74). In addition, macrophages, primed by the canonical type 2 cytokines IL-4 and IL-13, can transition into alternatively activated or M2 macrophages (75). Moreover, alternatively activated macrophages can be generated by IL-33 and polarization was associated with increased induction of IL-13 (76), suggesting these APCs may also play a more direct role in the regulation of mucin via this cytokine.

Innate Lymphoid Cells (ILCs)

ILCs are a recently discovered group of innate immune cells that play an essential role in host immunity, tissue protection, and adaptive immune regulation, particularly within the intestinal mucosal barrier (77–79). ILCs bridge the gap between the innate and adaptive immune responses by producing immune-regulatory cytokines. Based on the effector cytokines that ILCs secrete, the transcription factors that regulate their development, and markers dotting their cell surfaces, this family of cells can be subdivided into three groups: ILC1, ILC2, and ILC3 (77, 78).

ILC1

T-bet, a transcription factor expressed by ILC1s, is widely known as an important regulator for type 1 immunity. However, it has also been shown to protect against intracellular pathogens, such as *Salmonella enterica*. During *Salmonella* infection, ILC1s play an important role by producing IFN- γ and, thus, driving the secretion of mucus-forming glycoproteins (80).

ILC2

ILCs bridge the gap between the innate and adaptive immune responses by producing immune-regulatory cytokines. It is becoming increasingly apparent that ILCs, particularly ILC2, have emerged as a crucial innate immune cell critical for the production of mucin through T helper 2 (Th2) immune responses. ILC2s arise from common lymphoid progenitor (CLP) cells (81) and express the transcription factors, retinoic acid receptor-related orphan receptor α (ROR α) and GATA binding protein 3 (GATA3) (82). Mature ILC2s respond to epithelial cell-derived cytokines including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) to produce Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13 (83–86). These effector cytokines support the development of type 2 inflammation as well as mucin production

in the context of parasitic immunity and allergic diseases (82). Recently, the function of these cells in helminth infection resistance has been demonstrated, particularly with regards to the impact of IL-13-secreting ILC2s on mucin-producing goblet cells. IL-33 has been shown to indirectly induce intestinal goblet cell differentiation and MUC2 expression via IL-13-secreting ILC2s (87). Moreover, IL-33-deficient (*Il33*^{-/-}) mice fail to expel *Nippostrongylus brasiliensis* worms due to impairment of ILC2 (88), further demonstrating the essential role of ILC2s in helminth infection immunity.

ILC3

ILC3s are also implicated in the maintenance of gut homeostasis. ILC3s express the transcription factor, ROR γ t, and IL-22, one of the effector cytokines secreted by ILC3s (89). Upon binding to its receptors, IL-22R1 and IL-10R2, on the intestinal epithelial cells, IL-22 induces mucin generation and goblet cell hyperplasia (90, 91). In addition, IL-22 promotes the activation of NOD signaling which leads to mucin secretion by goblet cells (92).

Adaptive Immunological Regulation

Unlike the innate immune system which relies on germ-line encoded PRRs, the adaptive immune system generates specific receptors to recognize the substantial diversity of harmful antigens through a process called somatic recombination (93). The principal cell types of the adaptive immune system are T and B lymphocytes which are vital in maintaining gut homeostasis as well as host protection in GI diseases (94). Consequently, T lymphocytes play an important role in the regulation of mucin release by goblet cells (95). Initial studies demonstrated that during *N. brasiliensis* infection, anti-CD4 antibody treatment in mice prevented spontaneous recovery. These mice also displayed T-helper cell depletion along with a reduction in mucin levels, despite unchanged goblet cell counts (96).

The immune response to intestinal helminth infection is characterized as a Th2-dominant response accompanied by the upregulation of cytokines, such as interleukin (IL)-4, IL-5, and IL-13 (97, 98). Animal models of enteric infections including *N. brasiliensis* (99), *Strongyloides ratti* (100), *T. muris* (98), and *Trichinella spiralis* (101) have shown that these helminth infections are also accompanied by goblet cell hyperplasia and an increase in mucin production and secretion that aid in worm expulsion (102). It is postulated that these changes in mucin production and goblet cell hyperplasia are at least partially the result of the Th2-mediated immune response (103). As an activator of Th2 immune responses, signal transducer and activator of transcription factor 6 (Stat6) has been identified as a critical inducer of goblet cell hyperplasia (3, 104). This factor's crucial role is neatly illustrated in *Hymenolepis diminuta* infection; STAT-6 knockout mice infected with this tapeworm were unable to clear this infection due, in part, to diminished goblet cell response, unlike their IL-13 and IL-4 deficient counterparts at 12 days post-infection (105). Interestingly, it has been shown that goblet cell hyperplasia may also occur in a Th2-independent manner without the influence of IL-4 and IL-13 in some parasitic infections, including *Schistosoma mansoni* (106). It should also be noted that with the discovery

of ILC2s, our understanding of the type 2 immune response has expanded. Both ILC2 and Th2 cells can be activated by IL-33, IL-25, and TSLP, and release type 2 effector cytokines (e.g., IL-5 and IL-13) during parasite infections, and thus, contribute to type 2 immunopathology. Further, Th2 cells respond to antigen stimulation by dendritic cells which also receive signals from cytokines released by ILC2s (107). These findings suggest that Th2 immune responses may be initiated by ILC2 since the activation of ILC2s occurs during the early phase of type 2 immune responses (108, 109).

Moreover, emerging evidence suggests a novel role for other cytokines in mucus production. Previously considered a non-factor in mediating parasitic expulsion, IL-22, a member of the IL-10 family of cytokines, has been shown to induce goblet cell hyperplasia and mucin release. IL-22-deficient (*Il22^{-/-}*) mice have defective goblet cell responses during *N. brasiliensis* infection despite strong Th2 cytokine induction (110). Furthermore, evidence suggests that, following infection with *T. trichiura*, the human gut accumulates IL-22-producing Th cells within the intestinal mucosa and the resultant increase in IL-22 production and Th2 cytokines promotes goblet cell hyperplasia and mucus production (111). Similarly, humans infected with *Necator americanus*, a parasitic hookworm, showed upregulated IL-22 production as well as a robust systemic and mucosal Th2 and T-regulatory (T_{reg}) response, ultimately promoting goblet cell hyperplasia and worm expulsion (112). In addition to quantitative changes, cytokines can also regulate the quality and composition of mucins. A recent study found that administration of IL-10 reduces endoplasmic reticulum (ER) stress and prevents the misfolding of MUC2 in an *in vitro* model (LS174T) of intestinal goblet cells (113). Consequently, T lymphocytes play an essential role in controlling the release of mucins through signaling by Th2 cytokines (e.g., IL-4, IL-5, and IL-13) and other mediators such as IL-22.

In addition to Th2 cytokines, several Th1 cytokines have been shown to regulate mucin biosynthesis. In the intestinal cancer cell line, LS180, it was observed that the pro-inflammatory cytokines, including IL-1, IL-6, and TNF- α , increased the expression of MUC2 mRNA (95). Due to the influx of Th1 cytokines, reduced mucin glycosylation via incomplete processing of N-glycans was also present (95). In addition, several *in vitro* and *in vivo* models have demonstrated the relationship between downregulated MUC2 expression and increased IL-6 in the context of colon cancer. Interestingly, this inverse relationship tends to be associated with liver metastasis and the promotion of tumor growth in mice (114, 115). Further, in BALB/c duodenal explants, macrophage-derived IL-1 and human rIL-1 β have the ability to induce mucin secretion from goblet cells (116). The authors suggest that this dose-dependent process may act as a protective mechanism by aiding in the clearance of toxic substances from the gut in periods of mucosal inflammation (116). In contrast, in a murine model of *C. rodentium* infection, the Th1 cytokines, IFN- γ , and TNF- α , decreased intestinal mucin production and its speed of transport from the Golgi to secretory vesicles. Lending further support to this finding, *in vitro* treatment of infected and non-infected intestinal mucosal surfaces with IFN- γ and TNF- α decreased the number of goblet

cells, mucus thickness and transport (117). The contrasting data in the above paragraph implies that the effect of Th1 cytokines on mucin synthesis, transport, and secretion depends critically on, not only, the type of cytokine but also the pathological process in question.

Though extensive, the presented results highlight the need for further exploration of mucin's diverse immunological functions and the impact that the immune system itself has on mucin production. Indeed, these findings establish the crucial role mucin plays in barrier and immune function as well as the importance of immune-associated signaling on the regulation of mucin composition and function. **Figure 2** highlights various immunological regulations that affect mucin production and goblet cell function within the gut.

Enteric Nervous System Regulation

It is increasingly apparent that the enteric nervous system (ENS) also contributes to mucin production. Recent studies have shown that mucosal neurons, which are situated in close proximity to lamina propria immune cells (e.g., APCs and ILCs), interact with epithelial cells and act as key regulators of mucin production at intestinal mucosal surfaces. Acetylcholine (ACh) is a primary parasympathetic neurotransmitter that is released by preganglionic nerve fibers and the vagus nerve (118). Previously, it has been established that ACh is important for goblet cell degranulation and can induce mucin secretion (54, 119, 120).

Muscarinic ACh receptors (MRs) are one of the two types of cholinergic receptors along with nicotinic ACh receptors (NRs) (121). Of increasing interest, type 3 muscarinic receptors (M3Rs) have received significant attention for their key role in maintaining mucosal barrier function (122) and in regulating mucin production and secretion within the GI tract (123). In addition, M3Rs contribute to host defense against *N. brasiliensis* (124, 125) and *C. rodentium* (121). Despite *N. brasiliensis* infection, mice deficient in the muscarinic acetylcholine receptor M3 (*Chrm3^{-/-}*) show an absence of typical goblet cell expansion (125). These mice also display impaired immunity to *C. rodentium* along with decreased goblet cell number and MUC2 gene expression in the colon compared with WT mice on day 13 post-infection (121).

Similar to MRs, vasoactive intestinal peptide (VIP) also participates in regulating intestinal goblet cell numbers and function at baseline; VIP-deficient mice show impaired goblet cell development and reduced expression of MUC2 (126, 127). VIP is a 28-amino-acid peptide secreted by enteric neurons and regulates gut motility (126). There are three known receptors for VIP: the higher affinity receptors, VIP receptor 1 (VPAC1) and VPAC2, and the lower affinity receptor PAC1 (128). Among these, VPAC1 mRNA is the most highly expressed on the apical membrane of the intestinal epithelium and receives signals from VIP fibers (129). Although VPACs are expressed on airways (130) and ocular mucosa (131), there is no direct evidence that intestinal goblet cells express VIP receptors. However, a recent study has reported that *ex vivo* treatment with VPAC antagonists resulted in a substantial decrease in goblet cell counts in the mouse ileum indicating potential ongoing VIP regulation of goblet cell production (126). Furthermore, lamina propria ILC2

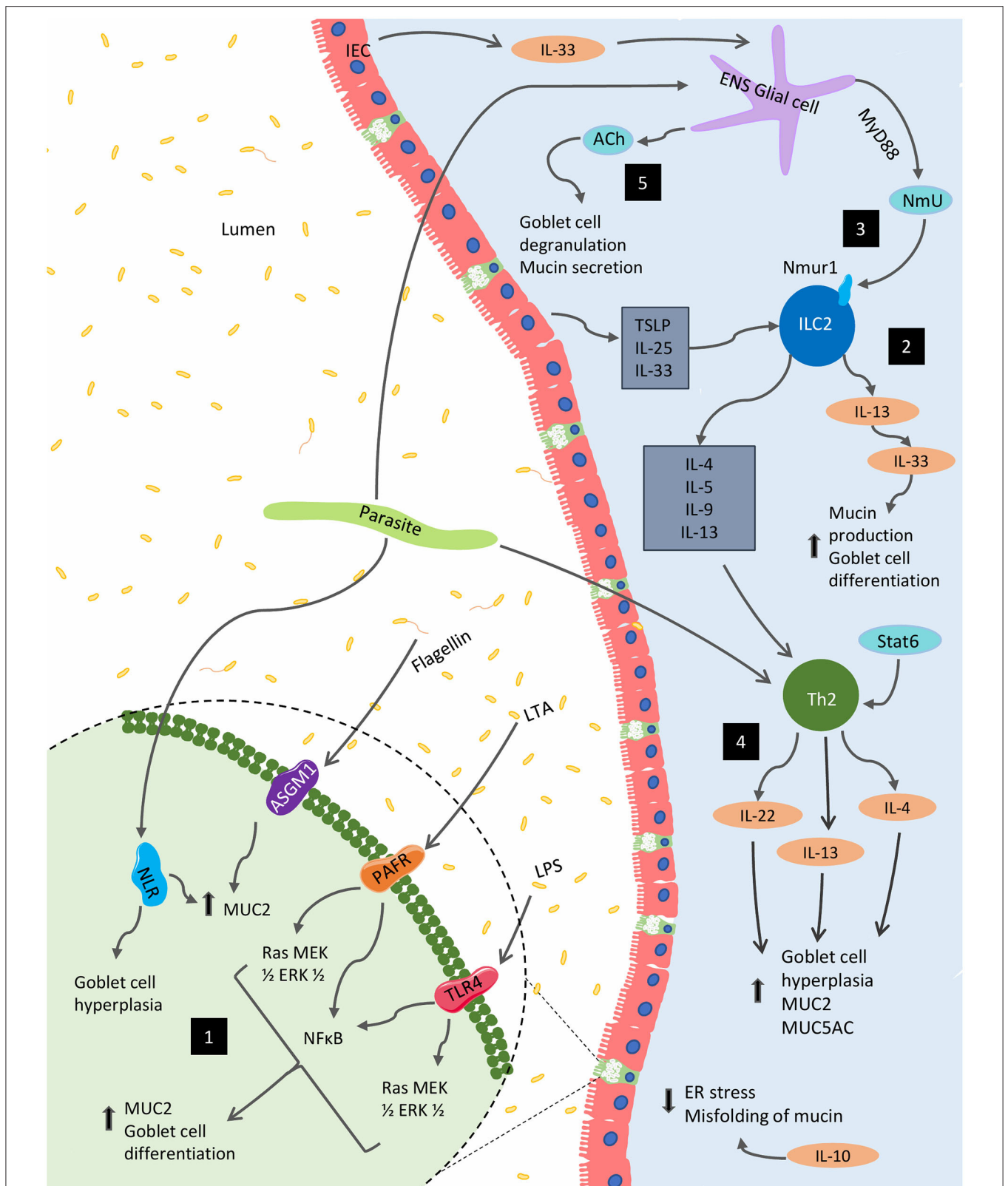


FIGURE 2 | Immunological regulation of mucin production and goblet function within the GI tract. Several immunological factors regulate and alter the production of mucins and the goblet cell function within the gut. (1) Bacterial components, including lipopolysaccharide (LPS), lipoteichoic acids (LTA) and flagellin, are potent activators of MUC2 expression via Toll-like receptors (TLRs). The latter two of these components can also stimulate MUC2 expression via platelet-activating factor (Continued)

FIGURE 2 | receptor (PAFR) and glycolipid receptor, asialoGM1 (ASGM1), respectively. In parasitic models, NOD-like receptors (NLRs) are necessary for MUC2 synthesis and parasitic expulsion. (2) In the context of parasitic infection, mature ILC2s respond to epithelial cell-derived cytokines including IL-25, IL-33, and TSLP to produce IL-4, IL-5, IL-9, and IL-13; supporting the development of type 2 inflammation, as well as, mucin production. (3) These cells are also impacted by NmU derived from glial cells stimulated by the ESPs of certain parasites as well as IEC-derived IL-33. (4) Enteric parasitic models also incite Th2 cytokines, IL-4, and IL-13 promote goblet cell hyperplasia and mucus production. (5) From the enteric nervous system (ENS), acetylcholine (ACh) plays a role in for goblet cell degranulation and can induce mucin secretion. Several Th1 cytokines such as IL-1 β , IFN- γ , and TNF- α can also regulate mucin biosynthesis (not shown).

function is also regulated by VIP via VPAC2 during parasite infection (132).

Interestingly, recent studies have also indicated indirect regulation of mucin production by a neuropeptide called neuromedin U (NmU) via ILC2 (89, 133, 134). During *N. brasiliensis* infection, glial cells sense intestinal epithelial cell-derived IL-33 and *N. brasiliensis* excretory/secretory products (ESPs) and trigger NmU production in a MyD88-dependent pathway (133). NmU not only induces smooth muscle contractions but also binds to NmU receptor 1 (Nmur1) on ILC2s promoting the secretion of IL-13, and, potentially impacting mucin secretion (135).

MUCIN PRODUCTION IN INTESTINAL INFLAMMATION: EVIDENCE FROM CLINICAL STUDIES

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an umbrella term which includes chronic inflammatory conditions of the gastrointestinal tract such as ulcerative colitis (UC) and Crohn's disease (CD). UC is characterized by superficial inflammation radiating proximally from the rectum, whereas CD is characterized by zones of deeply inflamed and non-inflamed tissue that can extend throughout the GI tract (136). These conditions affect millions worldwide, and the incidence is increasing globally (137).

Early studies suggested that single nucleotide polymorphisms in certain mucin genes including MUC3A, MUC3B, MUC12, and MUC17, predisposed individuals to CD and UC, however, these findings have not held up in more recent genome-wide association studies (GWAS) (138). Meta-analyses of several GWAS have more recently identified the gene encoding MUC1 (139) and the locus of leucine-rich repeat kinase 2 (LRRK2) which contains the MUC19 gene, (140) to have significant associations with CD.

Both CD and UC are accompanied by dysregulation of mucin synthesis and altered post-translational modification leading to barrier dysfunction (138). In UC, changes including reduced glycosylation and sulphation (141) as well as increased sialylation alter the efficacy of the mucins present and hinder their ability to maintain effective intestinal barrier function (20) particularly with regards to bacterial penetration (142, 143). Healthy colonic mucus is often heavily sulphated with increasing levels of sulphation extending from proximal to distal regions and conferring increasing resistance to bacterial enzymatic degradation. In both CD and UC, however, this phenomena is muted (20, 141). In inflamed tissue, goblet cell depletion is present in both UC and CD compared with controls (144)

and altered mucus layer thickness has also been found in both conditions (145).

UC is associated with a relatively thin and discontinuous mucus layer, goblet cell depletion and reduced MUC2 synthesis (145–147). Reduced sulfate content of MUC2 has also been noted, though compensatory and preferential secretion of this mucin in active disease results in overall unaltered sulfate levels in the colon of UC patients (147). Interestingly, MUC5AC, a mucin not normally present in the colon, has been found in UC patients undergoing surgery (148). In addition, site-specific increases in MUC1 expression and decreases in MUC2 expression were observed in UC patients at the site of crypt abscesses and adjacent to ulceration, respectively (149). Reduction in MUC9 (150) and MUC20 (151) gene expression was also noted in both active and quiescent UC compared with healthy controls. Increased gene expression of MUC16 has also been described in UC patients with active disease as well as those in remission compared to healthy controls (151). Sialylation also plays a similar role to sulphation, increasing the resistance to enzymatic degradation. In rectal biopsies of UC patients an increased average extent of sialylation per mucin oligosaccharide was noted rather than a simple increase in oligosaccharide chains (152). Intriguingly, glycan “profiles” have been established whereby healthy controls and those UC patients with inactive disease displayed similar glycan/glycosylation patterns; abundant levels of complex and larger glycans and relatively small amounts of shorter glycans. In opposition, those with active UC displayed increased presence of shorter glycans and a marked decrease in several complex glycans (153). Furthermore, these aberrant glycosylation profiles were associated with the degree of inflammation and severity of disease (153). Similarly, in a large-scale proteomics study, van der Post et al. established a core colonic mucus proteome, a set of 29 core secreted and transmembrane proteins that form the mucus barrier in healthy controls and UC patients in remission. Several of these proteins were found to be reduced in active UC patients including core structural components, MUC2 and IgGFc-binding protein (FCGBP), and other goblet cell products including calcium-activated chloride channel regulator 1 (CLCA1) and zymogen granule protein 16 (ZG16). Interestingly, this trend occurred independent of local inflammation and was associated with increased bacteria penetrability and activation of IL-18 (143). In active UC, contributing to the thinner and penetrable mucus layer, insufficient replenishment or exhaustion of sentinel goblet cells in response to successive microbial challenges has been noted and may precede the activation of disease and/or local inflammation (142, 143).

While the relationship between UC and mucus thickness is relatively well-established, the relationship regarding CD and

mucus thickness remain under dispute. Early findings suggested CD patients displayed increased mucus layer thickness compared to healthy controls (146), however, more recent work has indicated that mucus layer thickness in CD patients was not significantly different compared with healthy controls (145). In seeming contradiction, a recent systematic review and meta-analysis concluded that, on average, patients with CD have a 34% reduction in total mucin levels due to significantly decreased levels of MUC5AC, MUC5B, and MUC7 (154). Similar to UC, ileal CD has also been linked with aberrant protein expression of MUC5AC and decreased expression of MUC2 (155). Aberrant expression of MUC6 has also been noted in ileal CD (156). Moreover, in the ileum of CD patients, a marked decrease in MUC1 mRNA was also observed in inflamed vs. non-inflamed tissue from the same patient (156). Interestingly, reduced gene expression of the transmembrane mucins, MUC3 and MUC4, as well as the secreted mucin MUC5B have been noted in non-inflamed tissue sections of CD patients compared with healthy controls (156) suggesting local inflammation is not necessarily a precursor for these alterations. Alterations in oligosaccharide length have also been noted in CD patients and although it has been established that levels of sulphation remain unchanged compared with healthy controls, changes in glycosylation levels in CD patients have not been thoroughly explored (20, 141, 147, 152).

From the above evidence, it is clear that alterations in mucin expression and function play a unique role in IBD. However, because of significant dispute in the literature, it is challenging to characterize distinct mucin expression profiles associated with IBD, and it should be noted that significant heterogeneity exists.

Colorectal Cancer

Colorectal cancer is the third most common cancer in the world and accounts for substantial mortality every year (157). The expression of secreted and transmembrane mucins is altered in colorectal cancer patients. MUC1 expression was found to be increased in colon cancer patients and was correlated with poor prognosis and metastasis (158). Though MUC1 is usually undetectable with tandem repeat peptide antibodies in healthy colons due to heavy glycosylation, this post-translational modification is reduced in colorectal cancer patients which may account for the observed differences (159, 160). In addition, MUC2 expression was found to be decreased in colorectal cancer instances, other than in mucinous adenocarcinomas (12, 161). Moreover, MUC5AC, a normal component of gastric mucus which is usually absent from the colon, was shown to be expressed *de novo* in colorectal cancer (162, 163).

MUCIN PRODUCTION IN INTESTINAL INFLAMMATION: EVIDENCE FROM ANIMAL EXPERIMENTS

Chemical Colitis

Since the 1990s, the dextran sulfate sodium (DSS) model has been used extensively in rodents to understand the pathophysiology of colitis (164). The cytokine profile associated with this chemical

model is predominantly within the Th1 immune response with the upregulation of the cytokines, IL-12, IFN- γ , and TNF- α (165). Animal studies using DSS have highlighted the important and diverse roles of mucins in modulating intestinal inflammation.

As previously mentioned, MUC2 is a secreted gel-forming mucin and the main structural component of intestinal mucus which contributes significantly to host protection in the context of intestinal inflammation. *In vivo* results show that rats placed on DSS regimen undergo goblet cell depletion; however, depending on the observed location within the colon, the expression of MUC2 was unaltered or increased (166). Under a different DSS regimen, mucin expression was shown to vary with the progression of colitis. Compared with controls, DSS-treated rats showed initial upregulation of MUC2 and MUC3, followed by a rapid reduction in expression over time (167). Within 12 h of DSS administration, decreased mucus thickness and increased mucus permeability in the colon allows commensal microbes to penetrate the inner mucus layer and reach the intestinal epithelial cells. It is thought that this early bacterial invasion plays a critical role in eliciting the infiltration of immune cells and driving the development of colitis (168).

Despite the lack of consistent expression patterns in the above results, it is evident that MUC2 is an important component of the protective mucus layer of the intestine. In fact, MUC2-deficient (*Muc2*^{-/-}) mice develop spontaneous colonic inflammation characterized by weight loss, changes in stool consistency, and increased expression of pro-inflammatory cytokines (169). *Muc2*^{-/-} mice also show increased severity of inflammation and disease activity when exposed to DSS (169). Therefore, it is clear that MUC2 offers significant protection against colonic inflammation and that a well-maintained colonic mucus layer is vital in preventing murine colitis. In contrast to this distinct protective role of MUC2, not all mucins have shown to have protective functions in a DSS-induced colitis model.

Unlike MUC2, MUC4 is a transmembrane mucin and a component of the glycocalyx found in the intestine. Mice deficient in MUC4 show resistance to DSS colitis and have reduced levels of pro-inflammatory cytokines compared to wild-type animals (170). Although the mechanism behind the protective role of MUC4 deletion is unknown, it is possible that *Muc4*^{-/-} mice respond by upregulating protective mucins in a compensatory manner to resist DSS-induced colitis. In fact, *Muc4*^{-/-} mice challenged with DSS colitis have been found to have higher expression of both MUC2 and MUC3 compared to wild-type mice (170). Similarly, mice deficient in MUC1, another transmembrane mucin, develop less severe colitis accompanied by a reduction in T cell infiltration (171). The lower severity of colitis in *Muc1*^{-/-} mice may also be attributed to compensatory increases in the expression of MUC2 and MUC3 (171, 172).

Genetic Models

Besides chemical models, genetically modified animals are also commonly utilized to study intestinal inflammation and mucin production. One such model is the IL-10 knockout murine model (173). If kept in non-germ-free housing, mice deficient in IL-10 spontaneously develop colitis. This unprompted inflammation is accompanied by a reduction in the number of mucin-producing

goblet cells in the intestine (173). In contrast, mice lacking the *IL-1rn* gene, coding for the IL-1 receptor antagonist, spontaneously developed IBD-like abnormalities including increased immune cell infiltration and secretion of pro-inflammatory cytokines, and had an increased number of goblet cells in the jejunum and ileum compared to wild-type mice. Dosh et al. reasoned that this rise in goblet cell number was due to increased expression of the transcription factors, Hath1 and Kruppel-like factor 4 (KLF4) in the inflammatory process (174). The contradictory findings of goblet cell number in IL-10 and IL-1rn deficient mice demonstrate the implications and importance of studying various genetic models reflective of the pathogenesis of intestinal inflammation. Furthermore, genetically altered mice with tamoxifen-induced villin-Cre-dependent intestinal deletion of kindlin 1 and 2 manifest UC-like features due to modified mucus composition and hydrophobicity. These mutant mice develop mucosal colonic inflammation secondary to defective tight junction morphology and extended paracellular space in the mucosal barrier. Here, defective tight junctions prevent the paracellular transport of phosphatidylcholine (PC) causing reduced mucus PC content and a >50% reduction in mucus hydrophobicity. Consequently, the mucosa was predisposed to microbial invasion and subsequent inflammation (175). This finding highlights the role of altered mucus composition as a driving event in UC and not merely a secondary result of inflammation.

In addition to immune markers, recently, significant emphasis has been placed on studying the role of autophagy in mediating intestinal inflammation, and genetically modified animal models have been created to study this phenomenon. Autophagy is an evolutionarily conserved, catabolic cellular mechanism whereby cytoplasmic contents are delivered to, and degraded in, the lysosome (176, 177). A link between autophagy and intestinal inflammation has been proposed, and genome-wide association studies have identified the gene encoding ATG16L1, an autophagy-related protein, as a susceptibility locus for CD (178). Further, mice deficient in the autophagy-related protein, ATG7 in the intestinal epithelial cells (*Atg7^{ΔIEC}*), have been shown to develop more severe symptoms of colitis when placed on a DSS regimen compared to wild-type mice (179). These mice also had reduced expression of anti-microbial and anti-parasitic peptides, and an increased abundance of gut microbial content. Moreover, evidence suggests that *Atg7^{ΔIEC}* mice also have diminished release of intestinal mucins, particularly MUC2, and a less thick mucus layer. These findings indicate that the altered abundance, as well as the increased severity of colitis in *Atg7^{ΔIEC}* mice, could at least be partially caused by the reduced levels of intestinal mucins released from goblet cells (179).

Lack of proper glycosylation has been illustrated in UC patients (20, 141, 180) and several genetic models regarding this process provide insight into the implications of altered glycosylation in intestinal inflammation. As mentioned previously, O-linked oligosaccharides, in particular core 1- and core 3- derived mucin type O-glycans, are crucial components in the maintenance and stability of the colonic mucus layer, helping to prevent the penetration of this layer by microbial species via protease degradation and to avert

unwarranted activation of the immune response (28, 29, 181). Found throughout the colon, core 1 β1,3-galactosyltransferase (C1GalT1) controls the synthesis of core 1 O-glycans (181). Due to the impaired glycosylation and disrupted mucus integrity found when knocking out this protein's corresponding gene, mice with intestinal epithelium-specific deficiency of core 1 derived O-glycans (*C1galt1^{ΔIEC}*) develop spontaneous colitis in the distal regions of the colon (181) characterized by myeloid cell infiltration, crypt abscess, epithelial ulceration, goblet cell loss, reduced mucin levels, disrupted mucus layer, and increased epithelial-microbial interaction (153, 180). In contrast to C1GalT1, core 3 β1,3-N-acetylglucosaminyltransferase (C3GnT) expression, which regulates core 3 O-glycan formation, is more localized to the proximal colon (181). *C3Gnt^{-/-}* mice show increased susceptibility to colitis and colorectal cancer (182–184). Loss of both intestinal core 1- and 3-derived O-glycans generate mice that develop colitis ranging from the proximal to distal regions of the colon and display earlier onset and more severe intestinal inflammation when compared to *C1galt1^{ΔIEC}* mice and *C3Gnt^{-/-}* mice suggesting the loss of these vital components confers compounding deleterious effects (182, 184). Further, the above phenomena with regards to impaired glycosylation of the mucins and its effects on intestinal inflammation seem to be dependent, at least somewhat, on the presence of the resident microbiota; several studies have shown that antibiotic treatment lessens the severity of colitis and boosted mucus layer integrity in these models (180, 181, 183). Thus, evidence from the *C1galt1^{ΔIEC}*, *C3Gnt^{-/-}*, and double knockout models suggest proper glycosylation is crucial in maintaining the integrity of the colonic mucus layers, and suggests these O-glycans are a key factor in protecting the underlying epithelium from abnormal microbial interaction and preventing unsolicited intestinal inflammation.

From the above evidence, it is clear that within different physiological environments, different mucins, and different alterations in those mucins can greatly affect the host susceptibility to intestinal inflammation. These findings highlight the essential role of mucin in the pathogenesis of intestinal inflammation and exemplify the importance of maintaining mucin levels within a healthy homeostatic range. Because of this complexity, further research must be carried out to clearly elucidate the various roles of mucins in IBD.

Enteric Infection

Trichuris trichiura is a soil-transmitted helminth which affects millions of people, particularly children, around the world (185). To gain a more comprehensive understanding of the immune response against *T. trichiura*, enteric parasitic animal models have been developed. One such model which has been extensively used in both our laboratory and others is the murine infection, *T. muris*. Resistant mice or those that are able to expel worms successfully display a characteristic Th2 immune response with the upregulation of IL-4 and IL-13. In contrast, susceptible hosts such as AKR mice develop a Th1 immune response associated with chronic infection and increased worm burden (186). Changes in mucin production, both qualitative and quantitative, can affect host protection against parasitic

infections, and thus, the protective role of mucins in *T. muris* infection is increasingly being explored.

There are several methods through which mucins contribute to parasitic clearance. Firstly, it has been shown that some mucins, such as MUC5AC, have direct damaging effects on worms and reduce their viability (31). Thus, it comes as no surprise that mice deficient in MUC2 or MUC5AC show delayed worm expulsion in response to *T. muris* infection (31, 187). Secondly, mucins can create a thick and impermeable physical barrier to protect the underlying epithelial cells from *T. muris* invasion. In fact, *T. muris* infection leads to an increase in the thickness of glycocalyx, particularly due to the upregulation of MUC4, MUC13, and MUC17 proteins (103). Interestingly, the serine proteases secreted by *T. muris* can degrade MUC2, but not MUC5AC, giving this mucin considerable influence on the successful clearance of these invaders (31, 188).

Entamoeba histolytica, a human protozoan parasite, causes amebic colitis, and liver abscess, a condition collectively called amebiasis (189). Mostly present in the developing world, symptoms manifest in 10% of infected individuals possibly due to variation in host immune response (189). Interestingly, MUC2 plays a vital role in the development and progression of amebiasis (189). After ingestion of contaminated food or water, *E. histolytica* colonizes the colonic outer mucus layer by binding to glycans on the MUC2 molecule via its Gal/GalNAc-lectin (190). The protozoa then cleave MUC2 by glycosidases and proteases and subsequently, comes in to contact with intestinal epithelial cells (191, 192). Thus, the strength of the mucin layer is a key player in the physical defense against *E. histolytica*-induced inflammation and epithelial invasion. In addition to MUC2 degradation, altered MUC2 production is found in intestinal amebiasis. *E. histolytica* has been found to bind to $\alpha\beta3$ integrins on goblet cells and stimulate the hypersecretion of mucus by exocytosis (193). Contrasting data suggests, however, that *E. histolytica* impairs the regulation of Math1 transcription factor required for goblet cell differentiation and can actually lead to decreased mucus production (194). In addition, during *E. histolytica* infection, MUC2, possibly by acting either as a cAMP ligand or by activating TLRs, can promote elevated levels of antimicrobial peptides such as cathelicidins (195).

Clostridium difficile is an anaerobic, spore-forming bacterium that causes worldwide epidemics with significant mortality rates (196). Initial infection by this enteric pathogen is characterized by an acute inflammatory response with a substantial influx of neutrophils, diarrhea, and weight loss (197). Fortunately, murine colitis models utilizing *C. difficile* have provided a better understanding of this infection. Previously, it has been shown that patients with *C. difficile* infection secreted acidic mucus primarily composed of MUC1 and have decreased MUC2 expression indicating defective mucosal barrier function (198). Moreover, patients with *C. difficile* infection exhibited altered mucus composition with higher GLcNAc and galactose levels, but lower GalNAc levels (198). Intriguingly, fecal microbiota transplantation (FMT) has received a growing interest as an effective therapeutic strategy to treat this infection. Recently, it has been shown that upon IL-33 treatment, *C. difficile*-infected mice exhibited increased goblet cell number and mucin

production via activation of IL-13-secreting ILC2s and FMT rescued IL-33 expression in the colon after antibiotic-mediated depletion (199). Other enteric bacterial infections have also illustrated the implications of alteration within the mucus layer. For instance, animal studies have shown that deficiency in the transmembrane mucin, MUC1, results in increased susceptibility and more severe intestinal damage in response to infection with *Campylobacter jejuni* (200). Similarly, mice deficient in MUC2, the main ingredient of colonic mucus, had increased intestinal permeability and were more susceptible to *S. enterica* serovar Typhimurium infection (201, 202).



In addition to alteration in quantity, post-translational modifications can also affect the ability of mucin to maintain intestinal barrier integrity. One such modification, the addition of fatty acids called palmitoylation, is particularly important for maintaining this barrier function. Inactivation of the enzyme, fatty acid synthase (FAS) in intestinal epithelial cells prevents palmitoylation of MUC2, and ultimately leads to an increase in intestinal permeability (203, 204). Improper sulfonation, or the addition of sulfate anions to mucins, has similar effects of intestinal permeability in *C. jejuni* infection (205). In addition, barrier function and pathogen adhesion are largely regulated by the diverse glycosylation pattern of mucins. Analysis of gastric mucin glycosylation profiles reveal important roles of terminal $\alpha1,2$ -fucose residues on Lewis-b and H type 1 structures expressed on MUC5AC (206). By utilizing $\alpha1,2$ -fucosyltransferase-deficient (*FUT2*^{-/-}) mice, it has been established that the binding capacity of the infectious agent, *Helicobacter pylori*, to Lewis-b and H-type antigens is impaired due to the loss of gastric MUC5AC fucosylation (207). Similarly, norovirus infection is dependent on the fucosylation status of soluble mucins within the GI tract (206, 208). It is speculated that reduced mucin fucosylation in the GI tract results in decreased mucus thickness and increased intestinal permeability to pathogens (206). Further, inhibition of the enzyme, core 3 $\beta1,3$ -N-acetylglucosaminyltransferase (C3GnT) which plays a crucial role in the addition of O-linked oligosaccharides, can alter the structural and functional features of mucins and has been shown to decrease MUC2 levels and increase intestinal permeability (184, 209). Moreover, goblet cells in mice resistant to chronic *T. muris* infection contain high levels of sulphated mucins in contrast with the goblet cells of susceptible mice which are dominated by sialylated mucins. Consequently, sulphation of mucins promoted by Th2 immune mediators, such as IL-13, augments the protection offered by mucins by aiding in their ability to resist degradation (210).

Table 1 summarizes the various and dynamic changes in mucins and the mucus layer in the context of the aforementioned pathologies and animal models.

DISCUSSION

In the current review, we have highlighted the structural and functional features as well as the immunological regulation of mucins and have examined, within the context of

TABLE 1 | The intestinal mucus layer is a dynamic part of the innate immune system which undergoes quantitative and qualitative changes in response to inflammation. Evidence collected in clinical studies, as well as information gained from animal experiments, have shed light on some of these changes.

	Condition	Effects observed	References
	Crohn's disease	↑Mucus thickness or no change ↓MUC2, MUC3, MUC4, MUC5B, MUC7 MUC5AC and MUC6 present Goblet cell depletion	(145, 146) (155, 156) (155) (144)
	Ulcerative colitis	↓Mucus thickness ↓Glycosylation and sulphation ↑Sialylation ↓MUC2, MUC9, MUC20 ↑MUC1, MUC16 MUC5AC present Goblet cell depletion	(145, 146) (20) (147, 150, 151) (149, 151) (148) (144)
	Colorectal cancer	↑MUC1 ↓MUC2 <i>De novo</i> MUC5AC synthesis	(158) (12, 161) (162, 163)
	Chemical colitis	↑MUC2 (or no change) ↑MUC2 followed by rapid reduction ↑Th1 cytokines (IL-12, TNF- α etc.)	(166) (167) (165)
	Enteric parasitic infection	↑Th2 cytokines (IL-4 and IL-13 etc.) ↑Mucin production/goblet cell hyperplasia ↑Thickness of glycocalyx/MUC4, MUC13, MUC17	(97, 98, 186) (98–103) (103)
	Genetic models:		
	1) <i>Muc4</i> ^{-/-}	↓DSS inflammation severity ↑MUC2 and MUC3	(170)
	2) <i>Muc2</i> ^{-/-}	↑DSS inflammation severity/spontaneous colitis	(169)
3) <i>Atg7</i> ^{ΔIEC}	↑DSS inflammation severity ↓MUC2/mucus layer thickness	(179)	
4) <i>IL-1m</i> ^{-/-}	↑Goblet cell number	(174)	
5) <i>C1galt1</i> ^{ΔIEC}	Spontaneous colitis Microbially breached mucus layer	(153, 180, 181)	

intestinal inflammation, the changes associated in goblet cell biology and mucin production. The collection of evidence herein gives credence not only to the significant role mucins play in barrier function but also to the bidirectional relationship between mucus production and the immune system.

Several inferences can be made from the evidence presented. Firstly, the intestinal mucus layer is a highly dynamic system which responds to various pathological alterations within the GI tract, including changes in enteric infection, colorectal cancer, and IBD. While examining the evidence, it was noted that much of the research investigating the role of mucins in intestinal inflammation focuses on animal models and enteric infection. Hence, further clinical research would promote a better understanding of the role of mucin in these pathogenesis and also potentially open up new avenues of treatment. Secondly, dysfunction, whether qualitative or quantitative, of the mucus layer causes a sharp reduction in its ability to maintain barrier function. The presented evidence has highlighted the critical role of mucins in offering protection in the context of intestinal inflammation. The conflicting results with respect to several gel-forming and transmembrane mucin knockout models such as *Muc2*^{-/-} and *Muc4*^{-/-} illustrate that not

all mucins confer similar effects within the context of colitis. Inability to maintain a delicate balance of the proper ratios and varieties of mucins, thus, can significantly affect the host susceptibility to intestinal inflammation. Due to this complexity, more research must be done to further clarify the various roles of mucin in IBD pathogenesis. Similarly, the above analysis has illustrated that post-translational modifications such as sialylation, sulphation, and O-glycosylation are altered in response to several pathological conditions and can greatly alter the functional properties of the mucus layer. Investigating if reversing or supplementing the effects of these altered post-translational modifications also attenuates disease severity will prove interesting. Thirdly, via TLRs, NLRs, ILCs, and T lymphocytes through several Th2 cytokines, the immune system can significantly influence the production of mucins and the quality and efficacy these mucins display in maintaining the gut barrier. Though not exclusively touched on in this review, the impact pathogenic organisms have on the resident gut microbial community and how these changes influence goblet cell function and mucin production will provide an interesting area for further exploration.

Above all, evidence from clinical and animal models profoundly suggests that alterations in the mucus layer, aberrant

post-translational modifications, and differential expression of key mucins are critical factors in the pathogenesis and severity of several conditions including enteric infection, colorectal cancer, and IBD, further emphasizing the importance of maintaining mucin levels within a healthy homeostatic range. Future studies on the impact of mucins within these conditions can only further our understanding of the immunological regulation and clinical implications of mucins within the GI tract.

AUTHOR CONTRIBUTIONS

JG, YK, PF, and SH reviewed the literature and wrote the manuscript. JG designed and created the figures. JG, YK, and WK edited and revised the manuscript. PF and YK conceived the idea for the article. WK supervised the project. All authors provided critical feedback and shaped the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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