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Goblet Cells: Guardians of Gut Immunity and Their Role in Gastrointestinal Disease

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Abbreviations:

2'-fucosyllactose, 2FL; acetylcholine, Ach; acetylcholinesterase, AChE; *Akkermansia muciniphila, A. muciniphila*; alcohol-associated liver disease, ALD; all-trans retinoic acid, ATRA; angiotensin-converting enzyme 2, ACE2; antigen-presenting cells, APCs; antimicrobial peptides, AMPs; atonal homolog 1, ATOH1; *Bacillus subtilis, B.* subtilis; *Bacteroides fragilis, B. fragilis*; *Bifidobacterium bifidum, B. bifidum*; butyrylcholinesterase, BuChE; calcium-activated chloride channel regulator 1, CLCA1; calcium ions, Ca^{2+}; CAMP responsive element binding protein 3 like 1, CREB3l1; *Campylobacter jejuni, C. jejuni*; chemokine C-C motif ligand, CCL; Choline acetyltransferase, ChAT; *Citrobacter rodentium*, *C. rodentium*; *Clostridium difficile, C. difficile*; colorectal cancer, CRC; Crohn's disease, CD; cyclic adenosine monophosphate, cAMP; cystic fibrosis, CF; cystic fibrosis transmembrane conductance regulator, CFTR; cytotoxic T-lymphocyte associated protein 4, CTLA-4; dendritic cells, DCs; dendritic cells type 2, cDC2; *Entamoeba histolytica*, *E. histolytica*; enterohemorrhagic *Escherichia coli*, EHEC; Enterotoxigenic *Escherichia coli;* ETEC; epidermal growth factor receptor, EGFR; *Escherichia coli, E. coli*;

eukaryotic initiation factor 2, EIF2; *Faecalibacterium prausnitzii*, *F. prausnitzii*; forkhead box O3, FOXO3; fucosyl α1-2 glycosyltransferase, FUT2; *Fusobacterium nucleatum, F. nucleatum*: G protein-coupled receptors, GPR; gastrointestinal, GI; GC-associated antigen passages, GAPs; Goblet cells, GCs; growth factor independence 1, GFI1; immunoglobulin G Fc-binding protein, FCGBP; immunoglobulin, Ig; inflammatory bowel disease, IBD; interferon alpha 2, IFNA2; interferon gamma, IFNG; Interferon regulatory factors, IRF; interleukin, IL; intestinal epithelial cells, IECs; Janus kinase, JAK; Kruppel-like factor 4, KLF4; *Lactobacillus plantarum*, *L. plantarum*; Lamina propria, LP; lamina propria dendritic cells, LP-DCs; lipopolysaccharide, LPS; *Listeria monocytogenes*, *L. monocytogenes*; Ly6/PLAUR domain containing 8, Lypd8; major histocompatibility complex, MHC; messenger ribonucleic acid, mRNA; metabolic dysfunction-associated steatotic liver disease, MASLD; metalloendopeptidase meprin β, MEP1B; mitogen-activated protein kinase, MAPK; mononuclear phagocytes, MNPs; muscarinic acetylcholine receptor 1, mAChR1; myeloid differentiation primary response 88, Myd88; N-acetylgalactosamine, GalNAc; N-Acetylglucosamine, GlcNAc; natural killer, NK; natural killer group 2 member D, NKG2D; neurogenic locus notch homolog protein 1, Notch 1; peripheral T-regulatory cells, nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB); pTregs; phosphoinositide 3-kinase, PI3K; *prevotella nigrescens, P. nigrescens*; programmed cell death protein 1, PD-1; programmed death-ligand 1, PD-L1; prostaglandin E receptor subtype 4, EP4; protein arginine methyltransferase 5, PRMT5; protein atonal homolog 1, ATOH1; regenerating islet-derived 3, REG3; regenerating islet-derived 3 beta, REG3B; regenerating islet-derived 3 gamma, REG3G; resistin-like molecule, RELM-β; retinaldehyde dehydrogenase, ALDH1; *Ruminococcus gnavus, R. gnavus*; *Ruminococcus torques, R. torques*; *Salmonella typhimurium, S. typhimurium*; SAM pointed domain-containing Ets transcription factor, SPDEF; Secretory immunoglobulin A, sIgA; Short-chain fatty acids, SCFAs; sialyl-Tn antigen, sTn; signal transducer and activator of transcription 3, STAT3; small intestine, SI; Specific-pathogen-free, SPF; *Staphylococcus aureus, S. aureus*; T helper, Th; Thomsen-nouvelle, Tn; tight junction, TJ; Toll-like receptors, TLRs; transforming growth factor, TGF-β; transmembrane protease serine 2, TMPRSS2; trefoil factor 3, TFF3; *Trichuris trichiura, T. trichiura*; tumor necrosis factor, TNF; ulcerative colitis, UC; *Vibrio cholerae, V. cholerae*; zonula occludens-1, Zo-1; zymogen granule protein 16, ZG16.

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Author Contributions

CL conceptualized the article; FRT drafted the original manuscript, AE helped drafting the article and approved the final version; CL edited the original draft.

Conflicts of interest

None.

ABSTRACT

Goblet cells are specialized guardians lining the intestine. They play a critical role in gut defense and immune regulation. Goblet cells continuously secrete mucus creating a physical barrier to protect from pathogens while harboring symbiotic gut bacteria adapted to live within the mucus. Goblet cells also form specialized goblet cell-associated passages, in a dynamic and regulated manner, to deliver luminal antigens to immune cells, promoting gut tolerance and preventing inflammation. The composition of gut bacteria directly influences goblet cell function, highlighting the intricate interplay between these components of a healthy gut. Indeed, imbalances in the gut microbiome can disrupt goblet cell function, contributing to various gastrointestinal diseases like colorectal cancer, inflammatory bowel disease, cystic fibrosis, pathogen infections, and liver diseases. This review explores the interplay between goblet cells and the immune system. We delve into the underlying mechanisms by which goblet cell dysfunction contributes to the development and progression of gastrointestinal diseases. Finally, we examine current and potential treatments that target goblet cells and represent promising avenues for further investigation.

Keywords: Intestinal immune system, goblet cells, mucin, goblet cell-associated antigen passages (GAPs), microbiota, mucosa-associated bacteria, gastrointestinal disease, therapeutic strategies

INTRODUCTION

The gastrointestinal (GI) tract presents a unique challenge for the immune system. Its extensive surface, lined by a simple columnar epithelium, faces a constant barrage of dietary components and potentially harmful microbes (1). Beneath this epithelium lies the largest concentration of immune cells in the body. A healthy state requires that intestinal immune cells efficiently distinguish between harmless dietary substances and invaders (2). This distinction allows the immune system to develop tolerance towards the former, a hallmark mediated by tolerogenic dendritic cells (DCs) and antigen-specific T regulatory cells (Tregs) (3-5).

Goblet cells (GCs) are specialized intestinal epithelial cells (IECs), essential for gut defense. They continuously secrete and renew the mucus layer, physically pushing away pathogens from the gut lining (Figure 1). Mucins within the mucus also have binding sites for bacteria, further hindering their invasion (6). Some bacterial species in the gut utilize components of the mucus layer as an energy source, influencing both mucus production and the overall gut microbiome

composition (7). They also secrete a diverse plethora of interleukins such as (IL)-25, IL18, IL17, IL15, IL13, IL7, and IL6, and chemokines such as chemokine exotoxin, chemokine C-C motif ligand (CCL)6, CCL9, and CCL20, which are signaling molecules that further modulate the immune system (8) (Figure 1). By combining these functions, GCs play a vital role in maintaining a healthy gut environment and preventing disease.

GCs play a crucial role in maintaining gut health by continuously secreting mucins, which are stored in granules within the cell. These mucins create a protective layer on the surface of the gut (9). When the gut encounters challenges such as microbes or harmful antigens, GCs are triggered to release mucins at an accelerated rate. Various factors, such as neuropeptides, cytokines, and lipids induce mucin secretion (9). A key factor in this process is the activation of muscarinic acetylcholine receptor 1 (mAChR1) (10). Mucins secreted by GCs quickly absorb water, forming a dense and viscous mucus layer that serves as a formidable barrier against potential threats (9). There are over 20 identified mucins (labeled MUC1 to MUC21), each with slightly different structures and functions (11). In the intestine, the predominant mucin is MUC2. Deficiency in MUC2 leads to inflammation and increased susceptibility to infection in mice, highlighting its importance in the gut health (12).

Beyond their well-documented role in mucin production, recent research suggests GCs play a more multifaceted role in immune regulation through the formation of GC-associated antigen passages (GAPs) (Figure 1) (5). This function will be explored in detail throughout this review.

GOBLET CELL-ASSOCIATED ANTIGEN PASSAGES: MOLECULAR PATHWAYS AND IMMUNE RESPONSE

As mentioned earlier, GCs play a role beyond mucus production. They also dynamically create gaps known as GAPs, which transfer luminal antigens to antigen-presenting cells (APCs), particularly mononuclear phagocytes (MNPs) like dendritic cells (DCs) located in the lamina propria (LP). This mechanism is essential for maintaining gut immune tolerance and suppressing inflammatory responses (5). The neurotransmitter ACh acts as the master conductor, directing both mucus secretion and GAP formation. When stimulated, ACh activates different muscarinic receptors on GCs, depending on the location in the gut. In the small intestine and proximal colon, mAChR4 orchestrates GAP formation, while mAChR3 takes over this role in the distal colon (13). This ensures that GAP activity is tailored to the specific needs of each intestinal segment. ACh also stimulates the release of calcium ions, facilitating the fusion of vesicles containing mucin and endocytosed luminal content with the cell surface. This dual action allows GCs to

simultaneously build and maintain the protective mucus barrier while sampling the luminal environment for potential antigens (1, 14).

ACh originates from various sources including enteric neurons, fibroblasts, IECs, and immune cells (15). A complex interplay of factors further influences its secretion into the intestinal lumen. These encompass dietary components, such as short-chain fatty acids (SCFAs) and vegetable glucosides, as well as chemical stimuli like acids and ions, and even microbial pathogens (16-19). SCFAs, such as butyrate, propionate, and acetate, are synthesized within the gut lumen through the microbial fermentation of indigestible carbohydrates that contain β-glycosidic bonds between glucose monomers, which remain inaccessible to mammalian enzymes (16). Upon their production, SCFAs trigger the release of epithelial ACh prompting anion chloride (Cl^-) secretion by IECs (16). Specifically, in the intestinal epithelium, propionate binds to the SCFA G protein-coupled receptors (GPR)41 (FFA3) and/or GPR43 (FFA2). This binding triggers the release of ACh from the surface of IECs. Subsequently, ACh binds to epithelial ACh receptors, thereby initiating anion secretion (18). In addition, vegetable glucosides, compounds found in various plant-based foods, have also demonstrated the ability to influence ACh secretion in the intestine (20). The mechanism through which vegetable glucosides stimulate ACh secretion involves their interaction with the gastrointestinal system, particularly with enteroendocrine cells and the enteric nervous system. For instance, paeoniflorin, a principal bioactive component of *Paeonia lactiflora Pall*, and quercetin, a flavonoid commonly found in fruits and vegetables, proved to inhibit acetylcholinesterase (AChE) activity and promote the expression of serotonin, thereby contributing to gastric motility and the release of ACh in rats (20, 21).

Nicotinic ACh receptors are ligand-gated ion channels consisting of five subunits, typically including two or more α subunits, and possibly β, δ, and γ subunits (22). When two ACh molecules bind to these receptors, they induce a conformational change in the pentameric structure, forming a transmembrane pore (22). This pore permits the passage of sodium, potassium, and calcium ions, resulting in cell depolarization and ACh release. This process enhances smooth muscle contraction and gastrointestinal motility, with potential modifications to neuronal excitability and neurotransmitter release due to ion-level fluctuations (22). Organic acids, such as lactic and butyric acids, produced during fermentation by gut bacteria, have been implicated in stimulating enteroendocrine cells or directly affecting enteric neurons, leading to the release of ACh (17). In addition, lactic acid has also been associated with the inhibition of AChE and butyrylcholinesterase (BuChE) (23).

Pathogen infections can markedly affect ACh secretion, thereby influencing diverse functions in the gastrointestinal tract. For instance, in infections induced by *Citrobacter rodentium* (*C. rodentium*), choline acetyltransferase (ChAT)$^{+}$ T cells migrate to the colon (19). These specialized T-cells play a pivotal role in mucosal immunity and interactions with commensal microbes by synthesizing and releasing ACh. Studies have shown that conditional removal of ChAT in T-cells leads to a significant escalation in *C. rodentium* burden within the colon. This underscores the critical role of ACh synthesized by these specialized T-cells in bolstering mucosal defenses against pathogens (19). ACh also plays a critical role in regulating the release of mucus and antimicrobial peptides, as well as modulating ion and fluid secretion in IECs (19). These functions collectively contribute to maintaining a balance between the host and commensal microbiota while restricting pathogen invasion (24).

Enterotoxins such as cholera toxin, produced by *Vibrio cholerae* (*V. cholerae*) (25) or those generated by enterotoxigenic *E. coli* (ETEC), which produce heat-labile and/or heat-stable enterotoxins, profoundly affect enterocytes in the gut. These toxins act by increasing intracellular levels of cyclic adenosine monophosphate (cAMP) in enterocytes. This can stimulate ACh secretion from enteric neurons, leading to hypersecretion of fluid and electrolytes into the gut lumen contributing to the characteristic watery diarrhea observed in bacterial infections (25, 26).

Several bacterial strains, including *Lactobacillus plantarum* (*L. plantarum*)*, L. rhamnosus*, *L. fermentum*, *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus)* exhibit the capability to produce ACh (27). Notably, *B. subtilis* surpasses *E. coli* and *S. aureus* in the quantity of ACh it produces. On the other hand, while gastrointestinal cells express a spectrum of enzymes and proteins, there is currently limited evidence suggesting the expression of AChE by enteric GCs (28). However, recent findings have unveiled the expression of other enzymes implicated in ACh metabolism within GCs, such as butyrylcholinesterase (BuChE) (29). Despite exhibiting lower efficiency compared to AChE, BuChE contributes to ACh breakdown. This interplay ultimately leads to differential expression of ACh between the SI and the colon (19, 20).

The frequency of GAPs is not uniform throughout the intestine in mice. While approximately 4 - 6 GAPs are found per villus in the SI of healthy adult wild-type mice, a more dynamic and transient pattern emerges in the colon. In the latest, GAPs first appear in the second week of life, peaking around weaning and then declining in adulthood (30). The development of proximal colon GAPs is suppressed by the intrinsic microbial sensing of GCs. Colon microbes impede the

formation of GAPs in a process reliant on myeloid differentiation primary response 88 (Myd88), which activates epidermal growth factor receptor (EGFR) and p42/p44 mitogen-activated protein kinase (MAPK), leading to their phosphorylation (14). The proximal colon hosts a higher bacterial density compared to the SI and features a thinner mucus layer than the distal colon (14). Through the suppression of microbial sensing, the immune system of the proximal colon is protected from exposure to luminal bacteria, thus averting inflammatory reactions. This temporal regulation plays a pivotal role in shaping the gut immune system during development (30).

Similarly, IL-1β can also regulate GC responsiveness to ACh by binding to its receptor on the apical surface of GCs, activating MyD88, and subsequently transactivating EGFR (31). Additionally, commensal and pathogenic bacteria, along with their metabolites, can trigger MyD88 signaling via Toll-like receptors (TLRs) on the cell surface, further impacting EGFR activity (31). Interestingly, GCs express different TLRs depending on their location. All GCs express TLRs 1-5, but small intestinal GCs have slightly higher levels of TLR3, while colonic GCs express significantly higher levels of TLRs 1, 2, 4, and 5 (32). This variation reflects the changing bacterial environment from the SI to the colon, where immune surveillance is also heightened. Consequently, SI and colonic GCs exhibit distinct sensitivities and responses to TLR signaling, mirroring the differences observed in GAPs formation between these regions (32).

GAP formation has also been characterized as an ACh-dependent endocytic process. This mechanism suggests the GAPs are formed by the recovery of secretory granule membranes which traffic fluid-phase cargo to the trans-Golgi network and across the cell by transcytosis as well as the transport of fluid-phase cargo by endosomes to multi-vesicular bodies and lysosomes. The process is reliant on phosphoinositide 3-kinase (PI3K), actin polymerization, and microtubule transport for its execution (10). This specialized endocytic pathway of GCs facilitates the delivery of luminal antigens to the immune system while maintaining the mucus barrier (10). For that reason, and as mentioned before, GAPs play a crucial role in the tolerogenic system. Under normal conditions, LP Foxp3$^+$ peripheral Tregs (pTregs) in the small intestine and distal colon control tolerance to external antigens. These pTregs inhibit CD4$^+$ and CD8$^+$ T cell activation, modulate gut mast cell function, and redirect B cell immunoglobulin (Ig) E secretion. However, the continued presence of their specific antigen is vital for the survival of SI Tregs. Depriving them of this crucial interaction leads to their depletion, ultimately compromising the entire intestinal tolerogenic system (33). This is where GAPs take center stage (14). These transient structures transport dietary and luminal antigens (≤0.02 μm) alongside autocrine

factors like mucins and integrin αvβ6, which induce tolerogenic responses by promoting transforming growth factor (TGF)-β upregulation (14). These antigens are primarily presented to $CD103^+$ DCs in the SI. These DCs, equipped with retinaldehyde dehydrogenase (ALDH1) for generating all-trans retinoic acid (ATRA), stimulate T cell proliferation, induce adaptive immune responses, and promote mucosal immune functions like IgA responses and gut-homing lymphocytes (5). Interestingly, the more frequent interaction between $CD103^+$ APCs and GAPs compared to $CD11b^+CD103^-CX3CR1^+$ APCs may be attributed to their superior migration ability, response to inflammatory factors, and T cell stimulation capabilities (34). Additionally, this phenomenon is influenced by the location of DCs, where conventional DCs type 2 (cDC2) are more abundant in the SI compared to the colon, while cDC1 are more prevalent in the colon (35, 36). The $CD103^-CX3CR1^+$ APCs, on the other hand, are crucial for T helper (Th)17 T cell formation, and tumor necrosis factor (TNF)-α production (34). GCs, through GAPs, deliver not only antigens but also imprint APCs with tolerogenic properties. This includes stimulating IL-10 production by macrophages and enhancing retinoic acid activity in DCs, both contributing to an anti-inflammatory environment. Furthermore, the sampling of the endogenous GC protein Muc2 by MNPs is associated with improved Treg cell induction and promotes the development of a tolerogenic MNP phenotype (37). These diverse interactions highlight the remarkable interplay between GCs and the immune system. Unveiling the intricate mechanisms of this interplay holds immense potential for developing novel therapeutic strategies for gut-related diseases.

OTHER GOBLET CELL-SECRETED FACTORS SHAPING THE IMMUNE RESPONSE

GCs also release a tailored mix of proteins, cytokines, and chemokines, guided by signals from antigen-encountered APCs. These signals encompass cytokines such as IL-10 and TGF-β and other immune-modulating molecules (37). This orchestrated response not only enables a balanced immune reaction against pathogens but also facilitates the promotion of tolerance towards beneficial gut microbes(38). For instance, various cytokines, such as IL-6, IL-7, IL-13, IL-15, IL-17, IL-18, and IL-25, and chemokines like CCL6, CCL9, and CCL20 that attract APCs to the epithelium are secreted by GCs (8).

Furthermore, GCs basolaterally secrete resistin-like molecule (RELM-β) a protein with direct bactericidal properties against commensals and pathogens, while also fostering Treg proliferation and differentiation to support immune tolerance. Furthermore, RELM-β serves as a chemoattractant, recruiting $CD4^+$ T cells to the colon and enhancing IL-22 production for tissue repair (39). Trefoil factor 3 (TFF3), like RELM-β, supports Treg development, fights pathogens,

aids tissue repair, promotes epithelial cell adhesion, regulates cell migration, promotes tight junction (TJ) for gut barrier strength, and exhibits anti-inflammatory effects (40). IgG Fc-binding protein (FCGBP), a protein secreted by colon GCs, forms a heterodimer with TFF3. This collaboration enhances microbial clearance and protects the mucus barrier's structural integrity. FCGBP plays a critical role in the gut's immune defense by facilitating the efficient delivery of antibodies to the gut lumen. This protein binds to the Fc portion of antibodies, enabling their transport across epithelial layers, where they can neutralize pathogens and protect the gut from harmful invaders (41).

Protein arginine methyltransferase 5 (PRMT5) modifies other proteins through arginine methylation, regulates genes essential for GCs function, impacting both mucus production and assembly. Mice deficient in adequate PRMT5 expression display compromised mucus production and an altered inner mucus structure within the colon. This underscores the critical role of PRMT5 in preserving a robust gut barrier. Interestingly, PRMT5 regulates calcium-activated chloride channel regulator 1 (CLCA1), a key mucus assembly factor, through its methyltransferase activity. However, its regulation of other structural proteins like FCGBP and MUC2 occurs independently of this activity (42). As a key part of intestinal mucus, CLCA1 contributes to its robust viscoelastic properties, ensuring a strong barrier against luminal insults. Through proteolytic activity, it cleaves mucus strands, facilitating smoother mucus flow and preventing stagnation, characterized by the accumulation and lack of movement of mucus. CLCA1 interacts with MUC2, enhancing the formation of a physical barrier against pathogens. In addition, it regulates TJ protein expression, and displays anti-inflammatory activity, reinforcing gut defense mechanisms (43).

Zymogen granule protein 16 (ZG16), like CLCA1, plays a crucial role in maintaining epithelial integrity by regulating cell proliferation and differentiation (44). It also exhibits antimicrobial activity, protecting the gut lining from harmful invaders. Notably, ZG16 specifically binds to mannan on the cell walls of certain fungi, potentially triggering an immune response against these pathogens (45). Additionally, it binds to peptidoglycans in gram-positive bacteria, forming aggregates that cannot easily penetrate the mucus layer (46). Interestingly, ZG16 expression decreases in precancerous lesions and colorectal cancer, suggesting its potential role as a tumor suppressor (47).

Ly6/PLAUR domain containing 8 (Lypd8), vital within GCs, binds to harmful bacteria's flagella, hindering their movement and preventing gut epithelium invasion. Lypd8 deficiency increases susceptibility to intestinal inflammation and bacterial overgrowth, underscoring its role in

maintaining the gut barrier (48, 49). Reduced Lypd8 expression in precancerous lesions and colorectal cancer, coupled with its inhibitory effect on cancer cell proliferation and migration upon overexpression, implies its therapeutic potential for colon cancer (48, 49).

Secreted by plasma cells and transported across the epithelium by IECs, secretory immunoglobulin A (sIgA) directly binds to pathogens, inhibiting their movement and adhesion to the gut lining (50). It appears that GCs may also facilitate the transcytosis of IgA from the interstitial space into the lumen of the intestine, respiratory tract, or other ducts, although this process has not been fully elucidated (51). Additionally, sIgA forms immune complexes with invading bacteria, facilitating their clearance through phagocytosis or expulsion. Recent studies reveal that gut microbiota can influence the production of sIgA, highlighting the intricate interplay between the gut ecosystem and immune defense (50). RELM-β, TFF3, Lypd8, and sIgA induce the secretion of antimicrobial peptides (AMPs) by various IECs, including GCs and Paneth cells (52). AMPs like regenerating islet-derived 3 (REG3) act as a first line of defense against invading pathogens directly killing bacteria, disrupting their cell membranes, and inhibiting their growth. AMPs also act as immune regulators, presenting signals that activate immune responses and promote mucosal repair. Importantly, REG3 selectively binds to bacteria (52), causing cytoderm destruction and leading to their death (53).

These components, along with GAP formation and the well-studied mucins, contribute significantly to the complex functions of GCs. By understanding their individual roles and synergistic effects, we can gain a deeper appreciation for the intricate mechanisms that maintain gut health and develop novel therapeutic strategies for various gut-related diseases.

GOBLET CELLS AND THE MICROBIOTA

The interplay between GCs, mucin, and the microbiota is multifaceted. GCs actively sense and respond to the presence of the microbiota, adjusting mucin production accordingly. Mucins, in turn, create a hospitable environment for the microbiota while forming a formidable barrier against potential pathogens. This delicate balance is crucial for maintaining immune tolerance and preventing inappropriate immune responses to harmless commensal microbes (54). The microbiota impacts GCs function by stimulating mucin expression and promoting their appropriate differentiation (55) . SCFAs, generated through bacterial fermentation of fibers, can upregulate mucin production (56). Furthermore, commensal mucolytic bacteria such as *Akkermansia muciniphila* (*A. muciniphila*), *Bifidobacterium bifidum* (*B. bifidum*), *Bacteroides fragilis (B. fragilis), Bacteroides thetaiotaomicron* and *Ruminococcus gnavus* (*R. gnavus*), play a

role in maintaining the optimal turnover of the outer mucus layer, providing a competitive advantage to the host by excluding pathogens (57). In return, mucins offer attachment sites favoring a habitable environment and serve as a source of energy for some bacterial species (58). Consequently, the O-glycans within mucins can influence the composition of the microbiota, fostering a mutually beneficial relationship (59). This symbiotic interaction contributes to the overall health of the gut and is vital for preventing inflammatory responses triggered by pathobionts (59).

In GI diseases, alterations in the mucin-associated microbiome and mucin-degrading bacteria can have significant implications for gut health due to their close proximity to IECs and the immune system. Research has revealed that certain commensal mucin-degrading bacteria, including *Bacteroides spp., Parabacteroides spp., A. muciniphila*, and *Bifidobacterium dentium*, can elicit a mild inflammatory response characterized by low levels of IL-8 and TNF-α (60). Interestingly, these bacteria also exhibit a suppressive effect on the inflammatory response induced by *E. coli*, achieved through the downregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (60). Moreover, the presence of gut commensals has demonstrated potential in enhancing the function of the epithelial TJs by regulating the mRNA expression of TJ protein genes such as *zonula occludens-1* (*Zo-1*), *occludin (Ocln)*, *claudin-1 (Cldn1)*, and *E-cadherin (Cdh1)* (60). These findings underscore the anti-inflammatory effects of commensals against pathogenic infections and their capacity to modulate epithelial barrier function.

Conversely, an overabundance of mucin degradation may undermine the integrity of the mucosal layer, potentially permitting luminal bacteria and antigens to infiltrate IECs, translocate and reach the immune system, thereby triggering or exacerbating inflammatory diseases. For example, inflammatory bowel disease (IBD) is characterized by an imbalance in the mucosa-associated bacterial community. In IBD, patients exhibit an elevated total bacterial load, particularly enriched in mucin-degrading bacteria (61). Notably, *Ruminococcus torques* (*R. torques*) and *R. gnavus* have been consistently observed to be abundant in IBD patients whereas *A. muciniphila* is notably diminished, often by several folds, in these patients (62, 63). Furthermore, in the ileum of patients diagnosed with Crohn's disease (CD), an increased presence of *R. gnavus* appears to coincide with a decreased abundance of *Faecalibacterium prausnitzii* (*F. prausnitzii*), a key butyrate-producing bacterium, accompanied by a decline in the *Clostridium leptum* (*C. leptum*) and *Prevotella nigrescens* (*P. nigrescens*) subgroups (64, 65). These alterations in the microbial composition may play a significant role in the pathogenesis

and progression of IBD, highlighting the complex interplay between the gut microbiota and intestinal inflammation.

Dysbiosis of the mucin-associated microbiome has also been implicated in colorectal cancer (CRC). Research has demonstrated that CRC patients commonly harbor predominant pathogenic bacteria such as *Fusobacterium nucleatum* (*F. nucleatum*), *E. coli*, and *B. fragilis*, a mucin-degrading bacterium with pro-carcinogenic properties, in their intestines (66). On the other hand, *A. muciniphila* is selectively decreased in the fecal microbiota of patients with CRC (67).

Moreover, in patients with cystic fibrosis (CF), gut microbiome dysbiosis begins early in life and persists through adolescence and adulthood (68). Infants and children with CF exhibit lower alpha diversity and delayed microbiome maturation compared to healthy counterparts. Furthermore, CF patients display microbiome alterations, including elevated levels of *Veillonella* and *E. coli*, and reduced levels *of Bacteroides, Faecalibacterium,* and *Akkermansia* (68). Understanding these changes may contribute to elucidating the mechanisms that initiate and perpetuate gut inflammation, and drive the progression of these diseases.

In summary, intestinal dysbiosis in the aforementioned GI pathologies disrupts intestinal homeostasis leading to inflammation, epithelial barrier dysfunction, and alterations in GCs function and composition (61-63, 69). Thus, alterations in GCs can compromise the microbiota composition and the integrity of the epithelial barrier, allowing luminal antigens and pathogens to penetrate the mucosal barrier and trigger immune responses. Furthermore, changes in GC composition affect the quality and quantity of mucins produced, further compromising mucosal protection (70). These alterations in the epithelial barrier and GC function have significant implications in GI diseases, contributing to disease progression and exacerbation of symptoms, and will be further discussed in the following sections.

The fate of GCs in the absence of gut microbiota is a question worth exploring. Valuable insights can be gained by studying germ-free mice, which are raised in sterile environments devoid of microbes. In germ-free environments, there is a reduction in the number of GCs both in the SI and the colon, accompanied by reduced storage of mucin granules compared to the normal state (71, 72). The absence of microbial signals deprives GCs of their usual regulatory cues, impacting their secretory function. Furthermore, there is a decrease in the expression of certain antimicrobial molecules, such as angiogenin 4 and REG 3 gamma (REG3G), and a lack of expansion in the $CD4^+$ T-cell population (73, 74). The mucin glycosylation pattern, denoting the specific glycans arrangement on the protein backbone, is altered in germ-free mice. These

alterations entail decreased levels of specific glycosyltransferases responsible for elongating O-glycans, leading to the development of shorter Muc2 O-glycans. This occurrence is intricately associated with the absence of microbial metabolites such as acetate and can impact the overall functionality of the mucus layer, affecting its protective properties (70). Interestingly, germ-free mice exhibit adherent mucus in the SI and permeable mucus in the colon (75).

Further investigation using germ-free mice has provided insight into the role of GAPs. Unlike conventional mice, small intestinal and colonic GAPs are open in germ-free mice, through which $CD103^+$ LP-DCs can uptake antigens from the intestinal lumen under steady-state conditions (5, 14). Notably, the presentation of luminal antigens by LP-DCs derived from germ-free mice exhibited superior luminal antigen presentation capabilities compared to LP-DCs from mice housed under specific-pathogen-free (SPF) conditions. Specifically, in the small intestine, $CD103^+$ LP-DCs demonstrated superior luminal antigen presentation capabilities compared to $CD103^-$ LP-DCs among germ-free mice (5). This preferential targeting of antigens to DCs with tolerogenic properties suggests a pivotal role in maintaining intestinal immune homeostasis by GAPs (5). While colonic GCs showed a slight rise in germ-free mice, this uptick alone cannot elucidate the significant emergence of colonic GAPs in these mice. Moreover, GCs did not show an increase in antibiotic-treated mice, despite these mice displaying a comparable significant rise in GAPs (72). The development of colonic GAPs in germ-free mice was suppressed by mAChR4 antagonists unlike in conventional mice (14). However, microbiota transplantation and bacterial components such as lipopolysaccharide (LPS) prompted a swift decline in colonic GAPs, indicating that this pathway may significantly contribute to the absence of proximal colonic GAPs (30, 76).

Investigating GCs in germ-free mice underscores the essential role of gut bacteria in ensuring their optimal function, emphasizing the host's dependence on microbial signals for maintaining a healthy gut.

IMPACT OF GASTROINTESTINAL CONDITIONS ON GOBLET CELL FUNCTION

Dysfunction of GCs, characterized by altered numbers, abnormal differentiation, and altered mucin production, is a significant contributor to the development and progression of diverse gastrointestinal diseases (Figure 2). Unraveling the mechanisms underlying these disruptions is crucial for developing targeted therapies that aim to restore GCs function and promote overall gut health.

A. **Inflammatory Bowel Disease:** IBD, including CD and ulcerative colitis (UC), disrupts the function of GCs in the gut lining. Studies show a decrease in GC numbers, especially during active disease flares compared to remission. Furthermore, IBD disrupts GC maturation, leading to the production of less functional immature cells. These cells produce less mucus which results in a thinner mucus layer and weakens the mucus barrier's protective properties (77, 78). Along with a change in the amount of mucus produced, the type of mucus itself is altered in IBD with alterations in MUC2 O-glycosylation, particularly affecting sialylation and sulfation. This results in an increase in certain smaller glycans and a reduction in several complex glycans (77, 78). There is a shift towards pro-inflammatory mucins, further fueling the inflammatory response. Importantly, the expression of MUC2, MUC5AC, MUC5B, and MUC7 is often reduced in IBD patients. Even in non-inflamed areas of CD patients, some transmembrane and secreted mucins like MUC3, MUC4, and MUC5B are also downregulated (79). Research suggests this decrease in GC products like FCGBP, CLCA1, and ZG16 in UC patients might be independent of local inflammation but is linked to increased bacterial infiltration and activation IL-18 (80). This impaired mucus barrier allows bacteria and antigens from the gut lumen to penetrate the intestinal lining, triggering and perpetuating the inflammatory response seen in IBD (80). Consequently, current research explores targeting various aspects of GC function to promote healing and restore gut health in IBD patients. This includes stimulating GC proliferation, modulating mucin expression, and enhancing the mucus barrier's protective properties (81, 82).

B. **Colorectal Cancer:** CRC is one of the leading causes of cancer-related death worldwide. In CRC, GCs function and differentiation are disrupted, leading to abnormal mucin profiles with changes in type and amount produced. In the healthy colon, the monoclonal antibodies detect little to no presence of the polypeptide backbone of MUC1, as it is concealed by a dense layer of long and intricate mucin-type O-glycan chains. However, in CRC cells, MUC1 showcases markedly shortened carbohydrate side chains, including Thomsen-nouvelle (Tn) and sialyl-Tn antigen (sTn), which facilitate its immunodetection. MUC1 upregulation is associated with a worse prognosis and a higher risk of metastasis (83). This is attributed to MUC1's hindrance of T-cell proliferation, impairing the efficient elimination of cancer cells by cytotoxic lymphocytes and thus facilitating evasion from immune detection (83). Furthermore, the elevation of negatively charged sialic acid residues on MUC1 could potentially advance metastasis progression by disrupting cell-cell adhesion. (83). Notably, overexpression of MUC5AC, a mucin normally found in the stomach, and reduced MUC2 expression or altered glycosylation impact the mucus layer's integrity and was strongly associated with lymph node metastasis, poor cellular differentiation, advanced tumor stage,

and poor prognosis when comparing healthy mucosa to CRC patients (84). In addition, MUC5AC promotes tumorigenesis through the CD44-Src-integrin axis in mice (85).

Other mucin components are also altered in CRC. TFF3 expression is significantly higher compared to healthy tissues and is associated with advanced stages of the disease, and invasion of blood vessels or nerves (40). Furthermore, TFF3 is implicated in poor prognosis due to its role in promoting the clonogenic survival of colorectal cancer cells by upregulating prostaglandin E receptor subtype 4 (EP4) through signal transducer and activator of transcription 3 (STAT3) activation (86). A recent study demonstrated that, unlike healthy colons where MUC2 and TFF3 are always expressed together, some colorectal cancer cell lines lack MUC2 while expressing TFF3. This unique subset presents an intriguing area for further investigation (87). CRC tissues exhibit a deficiency in the ZG16 protein, a feature that aligns with negative correlations observed in clinical studies regarding distant metastasis and lymphatic invasion. Moreover, ZG16 plays a pivotal role in shaping the immune response within CRC by actively inhibiting the expression of programmed death-ligand 1 (PD-L1) (88). Co-cultivation of natural killer (NK) cells with medium derived from ZG16-overexpressing cells effectively enhanced both the survival and proliferation of NK cells, with this effect being contingent upon the expression of natural killer group 2 member D (NKG2D). These findings suggest that ZG16 may block tumor cell immune escape and be a potential target for immunotherapy (88). In addition, the altered composition of mucins not only facilitates metastasis but also influences the interaction between tumor cells and the immune system. Mucin-associated sTn antigens bind to receptors on macrophages, NK cells, and DCs, suppressing the immune system. This can happen in two ways: either by blocking the cells from recognizing other signals by receptor masking or by directly reducing their ability to attack invaders inhibiting their cytolytic activity. This impacts the tumor microenvironment and the body's anti-tumor response (89-91). Furthermore, MUC1 interactions with innate immune cells hinder the cross-presentation of processed antigens on major histocompatibility complex (MHC) class I molecules. (89-91). MUC1 and MUC16 interact with siglecs on DCs, masking TLRs and promoting an immature DC phenotype, subsequently diminishing T cell effector functions (89-91). Mucins also interact with or form aggregates with neutrophils, macrophages, and platelets, providing protection to cancer cells during hematological dissemination and facilitating their spread and colonization to metastatic sites (92).

C. **Mucinous Adenocarcinoma:** Mucinous adenocarcinoma is an uncommon type of CRC and is characterized by pools of extracellular mucin, comprising more than 50% of the tumor mass (93). Unlike other types of colorectal cancer, mucinous carcinoma exhibits elevated

expression levels of MUC2, attributed to dysregulated epigenetic and genetic mechanisms. These include promoter hypomethylation of MUC2 and heightened binding of the GCs lineage-associated transcription factor, protein atonal homolog 1 (ATOH1), to the MUC2 promoter (94). Understanding these alterations provide not only insights into the mechanisms driving CRC but also holds promise for developing diagnostic markers and therapeutic strategies to restore normal mucosal function and impede tumor growth. Furthermore, exploring GAPs in CRC presents a promising avenue for understanding the intricate interplay between tumorigenic pathways and immune responses. Investigating the crosstalk between GAPs and immune checkpoint pathways, such as programmed cell death protein 1 (PD-1)/PD-L1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), could offer insights into mechanisms of immune evasion in CRC. Targeting these interactions could enhance anti-tumor immune responses and improve treatment outcomes for CRC patients.

D. **Pathogen Infections:** When pathogens breach the delicate intestinal barrier, GCs become the frontline soldiers, orchestrating a complex and dynamic response. Mucins play a key role in fighting parasitic infections. *Trichuris trichiura* (*T. trichiura*), a soil-transmitted helminth, affects millions worldwide, with children particularly vulnerable (95). This infection heightens mucin production, resulting in a thicker barrier that defends against worm invasion. Additionally, MUC5AC directly harms worms, facilitating their expulsion. (96). *Entamoeba histolytica* (*E. histolytica*) is a protozoan parasite that infects humans and leads to amebiasis (97). *E. histolytica* exploits MUC2, binding to it for access and stimulating hypersecretion. Amebic colitis results in the destruction of cellular layers in the colon's mucosa, enabling the parasites to spread to the liver via the bloodstream, causing amoebic liver abscesses, or to other soft organs such as the brain and lungs. (98).

Bacterial infections also alter the mucin composition. For example, *Clostridium difficile* (*C. difficile*) is a spore-forming bacterium known for triggering an acute inflammatory response marked by heightened neutrophil levels (99). This leads to symptoms such as diarrhea and weight loss, contributing to global epidemics with substantial mortality rates. *C. difficile* infection disrupts mucus composition, favoring acidic mucus rich in MUC1 while reducing levels of MUC2, thus compromising the protective barrier (99). Additionally, individuals with *C. difficile* infection demonstrated changes in mucus composition, including elevated levels of N-Acetylglucosamine (GlcNAc) and galactose, alongside decreased levels of N-acetylgalactosamine (GalNAc) (100).

On the other hand, deficiencies in mucins increase susceptibility to intestinal pathogens, which are major causes of gastroenteritis in humans. For instance, MUC1 deficiency increased susceptibility to *Campylobacter jejuni* (*C. jejuni*), and MUC2 deficiency enhanced

susceptibility to *Salmonella typhimurium* (*S. typhimurium)* (101). Moreover, during *Salmonella* infections, GAP formation in the SI is inhibited, stopping antigen delivery while the gut is under attack. This requires the Myd88-activated EGFR pathway, via IL-1β acting on the IL-1 receptor. This coordinated reaction not only hinders bacterial spread to lymph nodes but also facilitates evasion of immune defenses (31). *Listeria monocytogenes (L. monocytogenes*), a bacterium notorious for causing one of the most severe foodborne illnesses known as Listeriosis, has the ability to bind to GCs. It utilizes these cells to traverse the epithelial barrier and evade immune defenses, thereby establishing infection more effectively (31). Bacterial pathogens found in food and water, such as enterohemorrhagic *Escherichia coli* (EHEC), target the IECs, leading to inflammation and diarrhea. In a study involving mice infected with *C. rodentium*, a relative of EHEC, increased expression and secretion of RELM-β by GCs is necessary to attract T lymphocytes to the infected intestine (102). These T lymphocytes then produced IL-22, a cytokine that directly stimulated epithelial cell proliferation. These findings emphasize the crucial role of epithelial/GCs in coordinating the host response to intestinal pathogens (102).

Emerging research suggests that GCs also serve as targets for several human and mouse viruses. Astroviruses, a major cause of childhood diarrhea, primarily infect and replicate within actively secreting GCs in mice (103). Similarly, Enterovirus 71 and adenovirus HAdV-5p referentially infect and replicate in GCs within human epithelial cultures (104, 105). Recent studies indicate that GCs are susceptible to SARS-CoV-2 infection (106, 107). The virus predominantly infects GCs in the bronchial airway because they harbor elevated levels of angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) compared to ciliated cells (108). Animal studies suggest that ACE2 expression levels influence gut permeability, either mitigating or exacerbating leaky gut (109). SARS-CoV-2 interaction with ACE2 in the GI tract can impair barrier function by disrupting proteins like ZO-1, occludin, and claudins, leading to increased inflammatory cytokine production (110). Additionally, intestinal inflammation can further harm the mucosal barrier and perpetuate the cytokine storm through the actions of lymphocytes, DCs, and macrophages (110). Further research is needed to understand how different pathogens interact with GCs and develop strategies to prevent this invasion, which could lead to novel therapeutic approaches for treating infectious diseases.

D. Cystic Fibrosis: CF results from genetic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which codes for an anion channel crucial for chloride and bicarbonate secretion across epithelial surfaces (111). Dysfunction in CFTR function leads to the accumulation of dehydrated, sticky mucus that plugs ducts and glands of

epithelia-lined organs like the lungs and intestines, a condition termed mucoviscidosis (112). This pathologic mucus buildup causes luminal acidification, disrupts intestinal motility, and can result in blockages within the SI. These alterations not only disturb the normal balance of gut microbes but also hinder the proliferation and differentiation of IECs, contributing to gut dysbiosis, inflammation, compromised barrier integrity, and elevated susceptibility to GI disorders, including cancer (112). A prominent feature of intestinal mucoviscidosis is GCs hyperplasia, characterized by increased GCs numbers, faulty degranulation, and the production of thick mucus on the epithelial surface (113). A recent study presents evidence suggesting that GCs hyperplasia in the SI of CFTR-deficient mice is not directly caused by impaired CFTR activity in the epithelium, but rather appears to be a consequence of the intestinal environment characteristic of CF (112). Within this environment, the upregulation of TLR2 and TLR4 likely plays crucial roles in modulating inflammation and maintaining intestinal homeostasis. It seems that TLR2-dependent signaling triggers GCs hyperplasia, which is secondary to reduced Notch signaling. This hyperplasia aligns with a terminal GC differentiation program involving changes in the expression of key transcription factors, including increased ATOH1, SAM pointed domain-containing Ets transcription factor (SPDEF), and growth factor independence 1 (GFI1), along with decreased Neurog3 expression (112). In GCs, mature mucin polymers are compacted due to the neutralization of repulsive forces by H^+ and Ca^{2+} ions. Upon exocytosis, extracellular HCO_3^- removes these ions, causing rapid expansion of mucin polymers into mucus gels. CFTR loss in CF reduces Cl^- and HCO_3^- transport, critical for mucus gel formation (114). Enhanced fucosylation of mucin glycans, prompted by the activation of fucosyl α1-2 glycosyltransferase (FUT2), might additionally elevate mucin viscosity (115). Furthermore, studies in the ileum of CF mice demonstrated that an elevated luminal concentration of HCO_3^- facilitates the unfolding of MUC2, which is probably essential for cleavage by the brush border metallo-endopeptidase meprin β, leading to the subsequent release of mucus from the mucosal surface of the intestine (116). Mucin secretion in the colon of animal models exhibiting CF is contingent upon the expression of CFTR and CLCA1 (117). Experiments have shown that reduced expression of CLCA1 in CF mice correlates with thickened and obstructed intestinal mucus in the colon (118). Recent studies have highlighted gut microbiome changes in CF individuals, with associations to health outcomes as mentioned in the previous section. These alterations correlate with significant clinical outcomes, including increased inflammation, maldigestion, malabsorption, intestinal lesions, and poor linear growth (68, 119, 120). Understanding the intricate relationship between GCs, mucin alterations, and the

pathogenesis of CF in the intestines holds promise for developing novel therapeutic interventions and improving the quality of life for individuals affected by this condition**.**

E. Liver diseases: While GCs and their secreted mucins diligently shield the intestinal barrier, their roles become significantly more complex in the context of liver diseases. These conditions can disrupt the delicate balance in the intestine, leading to intestinal bacterial overgrowth, increased intestinal permeability, bacterial translocation, intestinal inflammation, and a cascade of other complications (121-123). Translocated bacteria can reach the liver via the portal vein promoting hepatic inflammation and exacerbating liver diseases (121-123). For instance, in alcohol-associated liver disease (ALD), in both humans and mice, due to factors that are not fully understood, alcohol consumption leads to changes in gut mucin composition and an increase in mucosal thickness (121-123). The thickening of the gut mucosa and the rise in GCs numbers due to chronic ethanol exposure entail reductions in canonical Notch signaling within the gut (123). This results in a relative increase in genes associated with GCs specification, such as ATOH1, CAMP responsive element binding protein 3 like 1 (CREB3l1), and SPDEF, which are typically suppressed by Notch 1 (123). Interestingly, despite the increase in GCs numbers, ethanol intake led to significant decreases in gut levels of Kruppel-like factor 4 (KLF4), a factor involved along with SPDEF in promoting the terminal differentiation of GCs (123). Additionally, mice lacking MUC2 are protected against alcohol-related disruptions to the gut barrier and the development of ALD (121). These mice demonstrated decreased alcohol-induced liver injury, steatosis, and plasma LPS levels compared to wild-type mice. Additionally, they exhibited higher expression levels of REG3B and REG3G in the jejunum, leading to improved elimination of commensal bacteria and prevention of intestinal bacterial overgrowth (121). Furthermore, patients with alcohol use disorder showed a decrease in intestinal α1-2-fucosylation (124). Fut2 deficient mice, lacking this fucosylation, experience heightened ethanol-induced liver injury, steatosis, and inflammation. Furthermore, α1-2-fucosylation diminishes colonization of cytolysin-positive *E. faecalis* in the intestines of ethanol-fed mice (124). These findings underscore the promising therapeutic potential of 2'-fucosyllactose for alcohol-associated liver disease. Excessive ethanol consumption can also result in decreased levels of *A. muciniphila* in patients. This reduction is associated with disruptions in microbial metabolite production, compromised intestinal permeability, the onset of chronic inflammation, and the release of cytokines (125, 126). In liver cirrhosis, the gut experiences a paradoxical phenomenon. In the small intestine increased MUC2 and MUC3 mRNA expression has been found in the ileum of rats with liver cirrhosis while MUC5AC production often decreases in the colon, contributing to the overall weakening of the gut barrier. This imbalance disrupts

the protective mucus layer, leaving the gut vulnerable to increased bacterial access and the inflammatory response that ensues. Additionally, the composition of mucins changes, with altered glycosylation patterns weakening their ability to defend against invaders. This combination of factors creates a perfect storm for bacterial translocation, immune activation, and systemic inflammation, further exacerbating the underlying liver disease (127). Single nuclear RNA sequencing of the terminal ileum in cirrhosis patients has provided valuable insights into the dynamics of GCs throughout different disease stages (128). Advanced decompensation is marked by a notable decrease in GCs numbers compared to healthy individuals, whereas compensated cirrhosis shows an increased abundance of GCs compared to controls (128). Furthermore, analysis of gene expression patterns reveals significant upregulation of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-related genes in GCs, particularly in advanced decompensation cases. Interestingly, within the advanced decompensation group, there is a decrease in the expression of GCs differentiation markers FCGBP, CLCA1, and SPDEF, alongside heightened expression of MUC2, which facilitates mucin production (128). Moreover, advanced decompensated patients display elevated expression of inflammatory mediators such as STAT1, interferon-alpha 2 (IFNA2), interferon-gamma (IFNG), and interferon regulatory factors (IRF), indicating heightened immune activation. However, all cirrhosis patients exhibit lower eukaryotic initiation factor 2 (EIF2) signaling levels and increased expression of the transcription factor forkhead box O3 (FOXO3) compared to healthy controls, suggesting dysregulated cellular responses in cirrhosis (128). The inhibition of small intestinal GAP is intricately linked to the development of ALD. Despite chronic alcohol consumption leading to an increase in both SI and colonic GCs, along with heightened protective mucin secretion in mice, an intriguing trade-off emerges: this augmentation occurs at the expense of SI GAP formation, thereby suppressing SI GAPs. This phenomenon can be attributed to the downregulation of the *Chrm4* gene, responsible for encoding mAChR4. Upon ligand recognition, particularly ACh, mAChR4 orchestrates GAP formation. Consequently, the decreased expression of mAChR4 culminates in a diminished population of tolerogenic DCs and Tregs. This inflammatory milieu consequently facilitates bacterial translocation, facilitating bacterial infiltration into the liver and exacerbating the onset of ethanol-induced steatohepatitis (129).

On the other hand, in metabolic dysfunction-associated steatotic liver disease (MASLD), preclinical studies have revealed a decrease in the number of GCs observed in the ileal crypts (130, 131) and colon (132). Muc2-deficient mice, displayed better glucose control, reduced inflammation, and increased gene expression involved in fat burning within fat tissue (133). Additionally, they exhibited higher levels of IL-22 and its target genes associated with gut

protection. The findings suggest that the absence of the mucus barrier activates the immune system, leading to IL-22 production which helps protect against the metabolic effects of a high-fat diet (133). However, Fut2-deficient mice, despite consuming more calories, are protected from MASLD, exhibiting increased energy expenditure and thermogenesis (134). This protection can be transferred to wild-type mice via microbiota exchange and is reduced with antibiotic treatment (134). Fut2 deficiency attenuates diet-induced bile acid accumulation and enhances intestinal farnesoid X receptor/fibroblast growth factor 15 signaling, inhibiting hepatic bile acid synthesis. Dietary supplementation of α1-2-fucosylated glycans reverses the protective effects of Fut2 deficiency indicating the critical role of intestinal α1-2-fucosylation in obesity and steatohepatitis pathogenesis (134).

Taken together, these findings suggest that the roles of intestinal GCs and GAPs extend beyond their immediate function in the gut. These components may play a role in the development of certain diseases in distant organs, highlighting their broader impact on overall health. This highlights that, in addition to their *in-situ* roles, intestinal GCs and GAPs may contribute to the development of certain distant organic diseases. This may occur through a bacteria-regulated mechanism or other currently unknown pathways.

ADVANCING THERAPEUTIC STRATEGIES TARGETING GOBLET CELLS AND MUCIN-ASSOCIATED MICROBIOME

The alterations observed in the mucosa-attached microbiome and GC profile during GI pathologies suggest novel treatment strategies focusing on these interactions. Interventions targeting GC function to modulate mucin production and secretion, thereby reinforcing the protective barrier of the intestinal epithelium, are imperative for advancing current treatments. Table 1 provides an overview of recent efforts to develop therapies based on these strategies. Briefly, Janus kinase (JAK) inhibitors block JAK protein activity, thus preventing the STAT pathway from triggering inflammation. This pathway typically regulates the production of proteins that can damage gut tissues. JAK inhibitors increase the number of GCs and TNF-α, MyD88, and NF-κB2 levels, promoting mucosal healing (135-138).

Notch receptors play a crucial role in regulating the differentiation of colonic GC and stem cells, as well as directing the differentiation of gut progenitor cells (139). Inhibiting the Notch signaling pathway triggers the transcriptional activation of ATOH1 and the expression of MUC2 (140). Dysregulated activation of Notch1 is implicated in the severity of GI diseases such as CRC, IBD, and MASLD. Small molecule inhibitors targeting γ-secretase, which mediates the final cleavage

step of Notch receptors, can block Notch1 activation in CRC (141). However, many inhibitors lack selectivity and cause severe toxicity. Recent research has shown that inactivating Notch signaling reduces the migration and invasive capacity of CRC cells in vitro and decreases tumor burden in vivo, but it also increases intestinal GCs (142). The systemic use of currently available γ-secretase inhibitors is associated with various adverse effects, including massive diarrhea due to increased GC differentiation (143). Achieving precise drug delivery without toxicity holds promise for treating GI diseases. A nanoparticle-mediated delivery system targeting γ-secretase inhibitors in the liver has been developed, avoiding GCs metaplasia caused by intestinal Notch inhibition and reducing hepatic fibrosis and inflammation (144). However, further investigation in this field is warranted.

Mucolytics like bromelain (BRO) and N-acetylcysteine (NAC) break down the mucus layer surrounding cancer cells, enhancing the delivery and effectiveness of chemotherapy in CRC (145, 146) and help removing intestinal obstructions in CF (147).

Probiotics and fecal microbiota transplantation (FMT) can boost beneficial mucin-associated bacteria, such as *Bifidobacteria*, reducing intestinal inflammation, regulating immunity, and strengthening the gut barrier (148-154). Recent studies suggest that the mucin-degrading bacterium *A. muciniphila* plays a significant role in maintaining host barrier function and immune response (155, 156). Reduced intestinal colonization of *A. muciniphila* has been associated with the development and progression of GI diseases (157, 158). These findings highlight the potential of *A. muciniphila* as a therapeutic target and a promising strategy for intervention in gastrointestinal disorders (Table 1). Moreover, studies have revealed that the consumption of the prebiotic inulin initiates a notable remodeling of the epithelium in the mouse colon (159). This remodeling is marked by heightened proliferation of intestinal stem cells and augmented differentiation of GCs. Notably, these effects are contingent upon the presence of the gut microbiota, the activity of γδ T lymphocytes, and the availability of IL-22 (159). The impact of other prebiotics like 2'-fucosyllactose (2FL) on GI diseases remains unclear. While restoring gut fucosylation with 2FL improves ALD in mice (124), it paradoxically worsens liver disease and promotes hepatic steatosis in a MASLD model (134). A promising new therapeutic approach for ALD is VU0467154, a positive allosteric modulator of the mAChR4 (129). Preclinical studies suggest it induces GAPs, which may be linked to several beneficial effects such as modulation of immune cells, production of Reg3 lectins, reduced bacterial translocation, and overall improvement of ALD. Further insights into the regulatory mechanisms governing mucin alterations are essential. It is crucial to identify specific epitopes in mucin glycoproteins that

serve as binding sites for both commensal and pathogenic microbes. Analysis of the impact of native and altered mucins on the balance of protective and pathogenic commensal microbes in the intestine is necessary. Additionally, understanding the impact of colonic and small intestinal GAP formation is vital. These efforts are fundamental for advancing novel therapeutic approaches in managing intestinal diseases, marking a promising avenue for exploration.

Table 1. Therapies targeting goblet cells and mucin-associated microbiome in GI diseases.

Treatment	Mechanism of action	Current state	Ref.
Inflammatory Bowel Disease			
Tofacitinib	Prevents the phosphorylation of JAK proteins, which prevents the triggering of the STAT pathway and downstream signaling of cytokines and the synthesis of pro-inflammatory proteins that are implicated in mucosal inflammation. JAK inhibitors increase the number of goblet cells (GC) and TNF-α, MyD88, and NF-κB2 levels, thereby promoting mucosal healing	Approved by the National Institute for Health and Care Excellence (NICE) for use in moderately to severely active ulcerative colitis (UC)	(136)
Filgotinib	Oral small molecule that selectively inhibits JAK1 promoting mucosal healing	Approved by European Medicines Agency for the treatment of UC and ongoing studies are evaluating its efficacy and safety Crohn's Disease (CD)	(135), FITZROY study, NCT03046056, NCT03077412
Ustekinumab, Infliximab, Risankizumab	GC proliferation and mucosal healing were facilitated via the inhibition of IL-12 and IL-23	Clinical study	(160-162)
Atractylodin, Honokiol, Thymoquinone	Dietary bioactives that stimulate mucus secretion by targeting PPAR-γ signaling pathway	Preclinical study	(163-165)
Anti-IL-13Rα2 (therapeutic antibody specifically targeting IL-13Rα2)	Promotes GC regeneration and mucus secretion	Preclinical study	(166)

The aromatic hydrocarbon receptor agonist 6-formylindolo (3,2-b) carbazole (also known as FICZ)	Inhibits the Notch pathway, increases the Muc2 expression and the number of GCs and reduces bacterial infiltration to ameliorate colitis	Preclinical study	(167)
Probiotic treatment with *Bifidobacterium breve* Bif 195 (Bif195)	Aim to restore the levels of mucosa-associated *Bifidobacteria* to alleviate mucosal inflammation and ulcers	Ongoing Clinical study	NCT04842149
Bacillus subtilis RZ001	Alleviates colitis by inhibiting the Notch signalling pathway and the depletion of GC	Preclinical study	(168)
Akkermansia muciniphila	Alleviated colitis, improving weight, colon length, and inflammation. GCs, mucin production increased, while pro-inflammatory cytokines decreased	Preclinical study	(169)
Prebiotic treatment with Inulin	This study aimed to assess how the prebiotic inulin modifies the gut mucin-associated microbiome of children and young adults with inflammatory bowel disease (IBD) and its potential to decrease disease activity	Completed clinical study	NCT03653481
Fecal microbiota transplantation	Aim to restore balance in the mucin-associated microbiota	Clinical trials	(148, 151) NCT05321745, NCT04637438, NCT04521205
Colorectal Cancer			
Janus kinase inhibitors (JAKi)	Inhibition of JAK/STAT3 pathway promoting mucosal healing	Preclinical study	(137)
Sodium/calcium exchanger (NCX) blockers	Reduces mucin secretion providing a means to control the chemoresistance of mucinous colorectal cancer cells	Preclinical study	(170)
LY3039478, an oral Notch signaling inhibitor	LY3039478 shows promising safety profiles and initial antitumor efficacy as a standalone but is associated with	Clinical study	(141)

	GC hyperplasia and a mucoid enteropathy affecting the small and large intestine		
Mucolytics: bromelain (BRO) and N-acetylcysteine (NAC)	Lysis of extracellular mucus removes the protective mucinous coating surrounding cancer cells and improves chemotherapeutic drug delivery/efficacy in cancer cells	Preclinical study	(145, 146)
Lactobacillus and *Bifidobacterium*	Probiotics exert a protective effect against colorectal cancer by competing with pro-carcinogenic microbiota, modulating host immunity, enhancing the intestinal barrier and restoring balance of the mucin-associated microbiota	Clinical trials	(150) NCT05592886, NCT03782428
Inteleukin-2 and *Akkermansia muciniphila*	Combined treatment showed a stronger antitumor efficacy by protecting gut barrier function and maintaining intestinal structure and GC number	Preclinical study	(171)
galacto-oligosaccharides (GOS)	Prebiotics modulate gut microbiota and mucus layer function	Preclinical study	(149)
Fecal microbiota transplantation	Inhibits colorectal cancer progression by restoring mucin associated bacteria balance and reversing intestinal microbial dysbiosis to enhance anti-cancer immune responses	Preclinical study	(154)
Pathogen infections			
Genistein, one of the active ingredients of soybean isoflavones	Inhibits the GCs loss caused by *Salmonella* infection by regulating the gut bacteria and intestinal stem cell development.	Preclinical study	(172)

Dietary iron	Regulates intestinal GC regeneration, mucin layer function and alleviates *S. typhimurium* invasion	Preclinical study	(173)
Akkermansia muciniphila	Alleviated *Citrobacter rodentium* induced colitis by promoting GCs induction, mucin production, and epithelial antimicrobial peptides	Preclinical study	(174)
Lactobacillus acidophilus	Regenerate GC by inhibiting Notch transcriptional program factors to alleviate *Salmonella* induced colitis	Preclinical study	(175)
Recombinant *L. paracasei* engineered to express *Listeria* adhesion protein (LAP)	Prevents *L. monocytogenes* from causing intestinal barrier loss by maintaining mucus-producing GCs and limiting epithelial apoptotic and proliferative cells	Preclinical study	(176)
Fecal microbiota transplantation for *C. difficile*	Restores the healthy gut microbiome and reestablishes balance in the mucin-associated microbiota	Clinical trials	(152, 153), NCT02134392, NCT03562741, NCT03712722
Cystic fibrosis			
N-acetylcysteine and polyethylene glycol	Successful treatment of distal intestinal obstruction syndrome via colonoscopy by lysis of extracellular mucus	Case report	(147)
Ivacaftor, a CFTR potentiator	Reverses some of the dysbiosis with a significant increment of the mucin-degrading bacteria *Akkermansia*	Clinical trial	(177)
Multistrain Probiotics	Aim to evaluate if probiotics improve GI health in children	Ongoing clinical study	NCT06284577
Lactobacillus rhamnosus GG	Enrichment of gut *Bifidobacteria* (mucin-associated bacteria) correlates with clinical improvements in children	Clinical trial	(178)
Liver diseases			
MASLD			

Lubiprostone	Improved intestinal permeability through the development of colonic mucus and repressed the development of MASLD	Preclinical study	(179)
DPP-4 inhibitor linagliptin and PPAR-alpha agonist WY14643	Restored *Bacteroidetes/Firmicutes* ratio, rescued endotoxemia due to increased tight junction gene expression, mucin production, and numerical density of GCs in intestinal crypts	Preclinical study	(180)
Diammonium glycyrrhizinate (DG), the main component of licorice root extracts	Improved the microbiota composition the expression of tight junction proteins, the GC number, and mucin secretion, and enhanced the function of the intestinal barrier	Preclinical study	(180)
Nanoparticle-mediated delivery system to target γ-secretase inhibitor to liver	Avoids GC metaplasia caused by intestinal Notch inhibition and reduces hepatic fibrosis and inflammation	Preclinical study	(144)
Akkermansia muciniphila	Treatment reduced liver inflammation and hepatocyte damage while enhancing gut health through increased GCs, thickened epithelial and mucosal layers, and improved intestinal integrity	Preclinical study	(181)
Different probiotic mixtures including *Lactobacillus, Bifidobacterium Lactococcus,* etc	Reduced serum levels of ALT, AST, cholesterol, triglycerides, and LDL and reestablishes balance in the mucin-associated microbiota	Clinical trials	(182-186)
Inulin	Inulin regulated the gut microbiota composition increasing the abundance of *Bifidobacterium* and enhanced	Preclinical study	(187)

	intestinal barrier integrity and function by decreasing the presence of inflammatory cells, thickening the mucosal layer, and promoting the elongation of villi with a regular arrangement		
2'-fucosyllactose (2FL)	Increases body and liver weight, more liver injury, and hepatic steatosis. This raises the possibility that the down-regulation of α1-2-fucosylation in MASLD mice is a protective mechanism	Preclinical study	(124)
Fructo - oligosaccharides	Attenuated MASLD by remodeling gut microbiota, preventing the GCs loss, and improving lipid metabolism	Preclinical study	(132)
Fecal microbiota transplantation	Improved balance in the mucin-associated microbiota, intestinal permeability, and hepatic steatosis	Clinical study	(13, 188)
ALCOHOL-ASSOCIATED LIVER DISEASE			
Fenretinide	Reduced alcohol-associated increases in ileal and colonic mucosal thickening, ileal *Muc2*, colonic *Muc2*, *Muc5ac* and *Muc6* mRNAs, and GCs numbers	Preclinical study	(123)
Akkermansia muciniphila and inosine	Enhanced the gut ecosystem, improved intestinal barrier function, upregulated A2AR, CD73, and CD39 expression, modulated Treg cells functionality, and regulated the imbalance of Treg/Th17/Th1 cells and modulates the mucin-associated microbiota	Preclinical study	(189)
Inulin	Modulates the mucin-associated microbiota	Clinical study	(190, 191)

2'-fucosyllactose (2'-FL)	Restoration of intestinal α1-2-fucosylation ameliorates ethanol-induced liver disease	Preclinical study	(124)
VU0467154 (mAChR4 positive allosteric modulator)	Induces small intestinal GAPs which was associated with modulation of antigen-presenting cells, induction of Reg3 lectins, prevention of bacterial translocation, and amelioration of alcohol-associated liver disease	Preclinical study	(129)
Probiotics including *Lactobacillus, Bifidobacterium Streptococcus*, etc	Restoration of the mucin-associated microbiota and reduction of liver injury	Clinical study	(192-197)
Fecal microbiota transplantation	Improved mucin-associated microbiota diversity, antimicrobial peptides expression, and liver markers of disease	Clinical study	(198-203)

CONCLUSION:

The intricate interplay between GCs, the mucus layer, and the immune system is a crucial determinant of gut health, safeguarding against a range of diseases, and encompasses the involvement of GAPs, goblet-secreted factors, and the mucus layer composition. Abundant evidence from both patient studies and animal models reveals that alterations in the mucus layer, abnormal protein modifications after synthesis, and variations in crucial mucin production heavily influence the development and severity of various conditions. Whether addressing intestinal infections, CRC, IBD, or liver disease, maintenance of balanced and healthy mucin levels emerges as a critical factor. Investigating the complex relationship between GCs, the microbiome, GAPs, and the immune system holds immense potential for developing novel therapeutic strategies for various gut diseases.

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Figure legends:

Figure 1: Goblet cells functions. Goblet cells (GCs) play a multifaceted role in the mucosal immune system, including: **1. Mucin secretion:** Goblet cells constantly produce mucins, forming a protective gel layer on the surface of the intestine. This mucus barrier acts as a first line of defense, trapping pathogens and preventing them from reaching the underlying tissues. Under normal circumstances, the thickness of this gel remains upheld through continous mucin secretion. Nevertheless, when the gut faces challenges such as microbial intrusion or harsh stimuli, goblet cells undergo stimulation to accelerate mucin release. Both, physiological or pathological stimuli, result in a marked increase in intracellular calcium ions (Ca^{2+}) -triggered stimulated mucus secretion. Various factors like neuropeptides, cytokines, and lipids further influence the stimulated mucin release. Upon acetylcholine (ACh) exposure, the activation of muscarinic ACh receptor 1 (mAChR1) also triggers the mobilization of Ca^{2+} from intracellular reserves, contributing to mucus secretion and effectively displacing pathogens from the gut lining. **2. Other secretory functions:** The release of chemokines and cytokines initiates and strengthens Th2 responses, facilitating tissue repair and attracting effector cells that perform functions crucial to innate immunity, extending beyond mere barrier maintenance. GCs also discharge antimicrobial peptides (AMPs), including resistin-like molecule ß (RELM-ß), regenerating islet-derived 3 proteins (REG3) and trefoil factor (TFF), which effectively eliminate commensal bacteria and pathogens that breach the mucus layer. **3. Goblet Cell-Associated Antigen Passages (GAPs):** Activation of mAChR4 by ACh initiates a process termed fluid-phase

bulk endocytosis, culminating in the formation of GAPs in the small intestine. This mechanism facilitates the transportation of small soluble luminal antigens and bacteria to the lamina propria dendritic cells (LP-DC). The main LP-DCs subset subadjacent to GAPs is the $CD103^{+}CX3CR1^{-}$ subset and possesses preferential tolerogenic properties. Created with BioRender.com

Figure 2: Gastrointestinal disorders impacting goblet cell function. The malfunction of goblet cells (GC), marked by changes in numbers, abnormal differentiation, and modified mucin production, plays a substantial role in the onset and advancement of various gastrointestinal disorders. These include Inflammatory Bowel Disease (IBD), colorectal cancer, mucinous adenocarcinoma, pathogen infections, cystic fibrosis, and liver diseases. Understanding the mechanisms behind these disruptions is essential for devising targeted therapies aimed at reinstating GC function and enhancing overall gut health. Created with BioRender.com

Figure 1

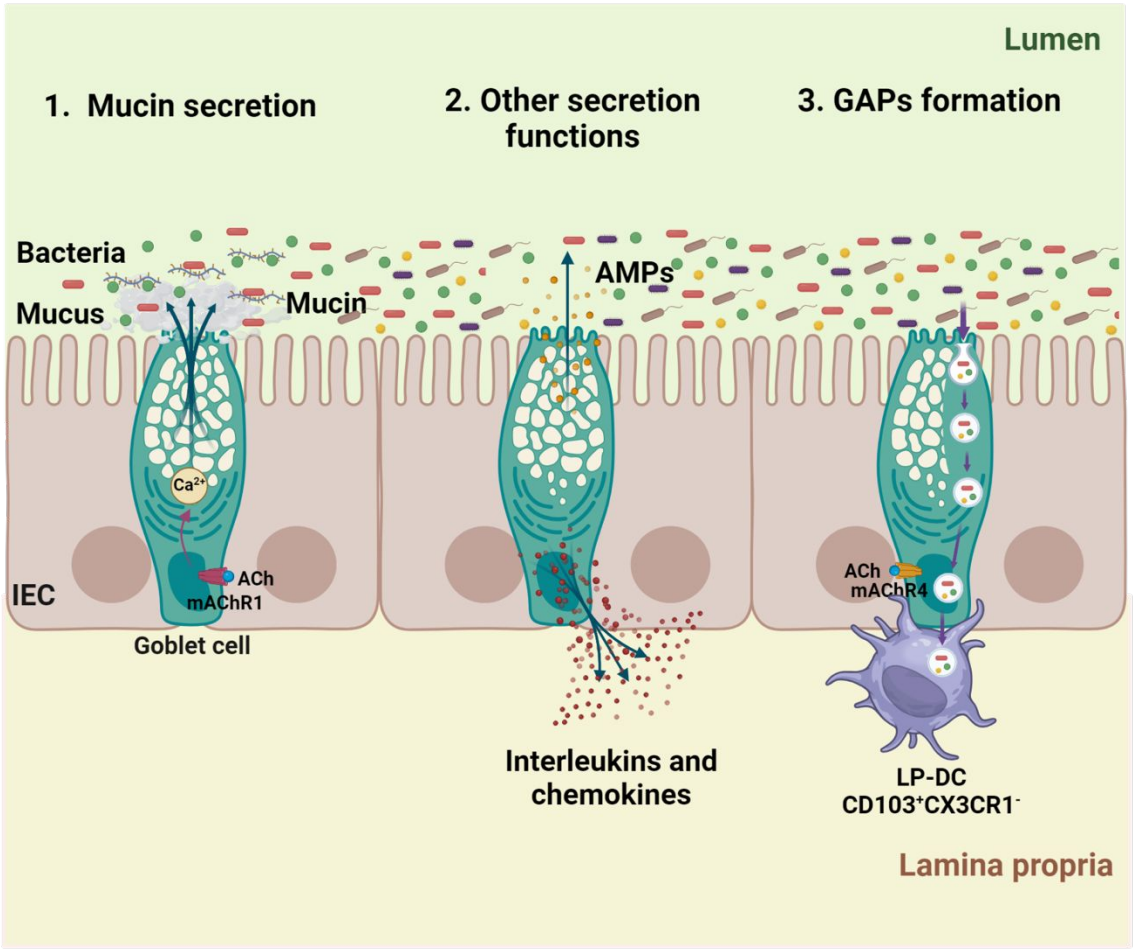
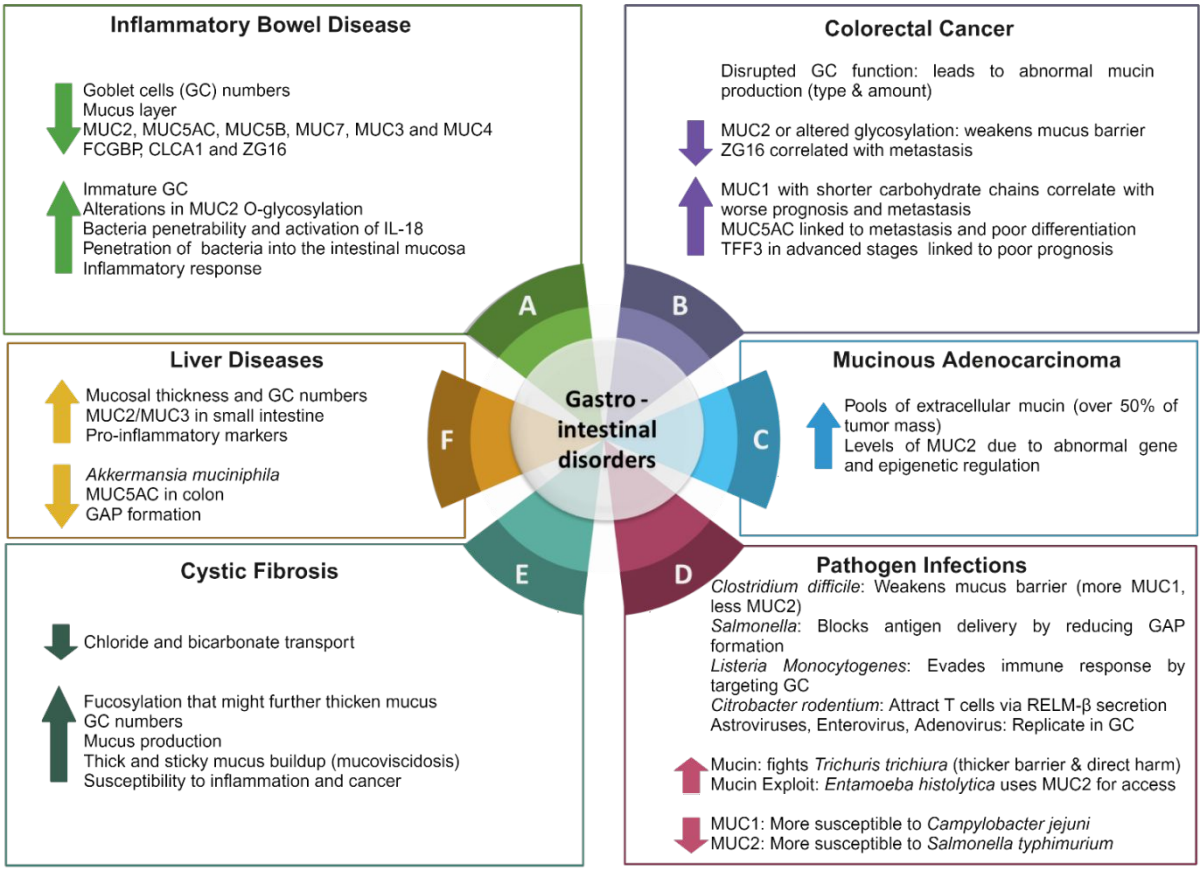
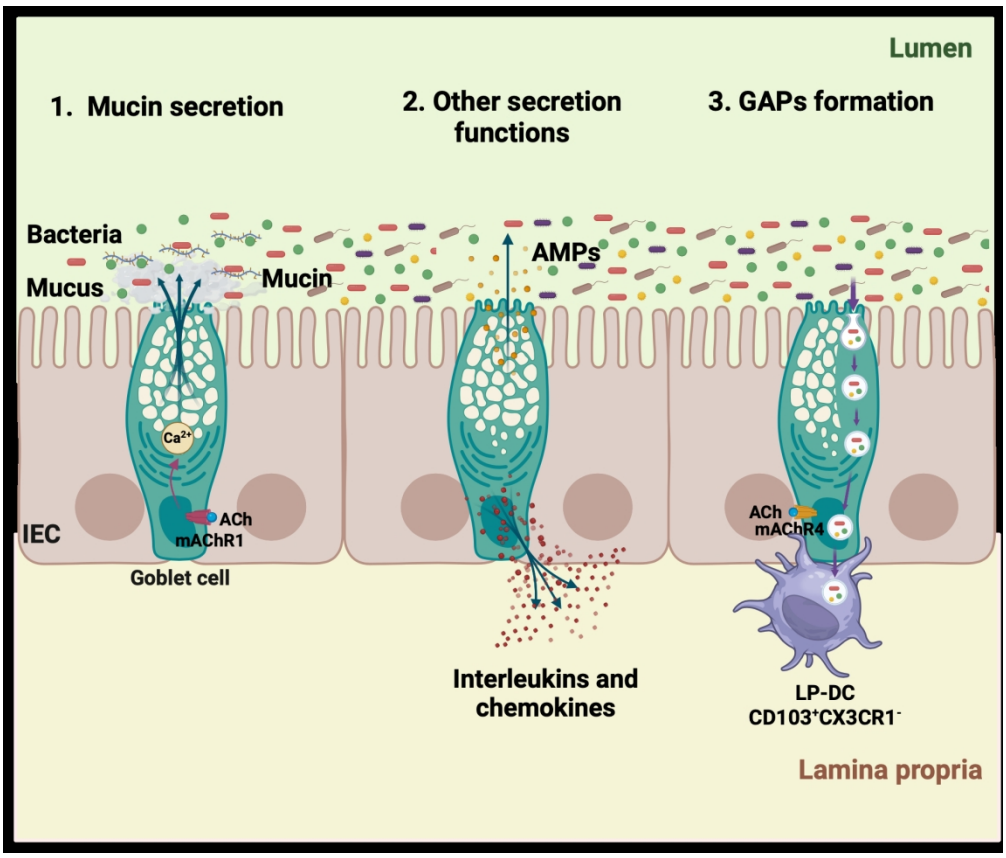


Figure 2


Inflammatory Bowel Disease
Goblet cells (GC) numbers
Mucus layer
MUC2, MUC5AC, MUC5B, MUC7, MUC3 and MUC4
FCGBP, CLCA1 and ZG16
Immature GC
Alterations in MUC2 O-glycosylation
Bacteria penetrability and activation of IL-18
Penetration of bacteria into the intestinal mucosa
Inflammatory response
Colorectal Cancer
Disrupted GC function: leads to abnormal mucin production (type & amount)
MUC2 or altered glycosylation: weakens mucus barrier
ZG16 correlated with metastasis
MUC1 with shorter carbohydrate chains correlate with worse prognosis and metastasis
MUC5AC linked to metastasis and poor differentiation
TFF3 in advanced stages linked to poor prognosis
A
B
Liver Diseases
Mucosal thickness and GC numbers
MUC2/MUC3 in small intestine
Pro-inflammatory markers
Akkermansia muciniphila
MUC5AC in colon
GAP formation
F
Gastro - intestinal disorders
C
Mucinous Adenocarcinoma
Pools of extracellular mucin (over 50% of tumor mass)
Levels of MUC2 due to abnormal gene and epigenetic regulation
Cystic Fibrosis
Chloride and bicarbonate transport
Fucosylation that might further thicken mucus
GC numbers
Mucus production
Thick and sticky mucus buildup (mucoviscidosis)
Susceptibility to inflammation and cancer
E
D
Pathogen Infections
Clostridium difficile: Weakens mucus barrier (more MUC1, less MUC2)
Salmonella: Blocks antigen delivery by reducing GAP formation
Listeria Monocytogenes: Evades immune response by targeting GC
Citrobacter rodentium: Attract T cells via RELM-β secretion
Astroviruses, Enterovirus, Adenovirus: Replicate in GC
Mucin: fights Trichuris trichiura (thicker barrier & direct harm)
Mucin Exploit: Entamoeba histolytica uses MUC2 for access
MUC1: More susceptible to Campylobacter jejuni
MUC2: More susceptible to Salmonella typhimurium



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Goblet Cells: Guardians of Gut Immunity and Their Role in Gastrointestinal Disease

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Abbreviations:

2'-fucosyllactose, 2FL; 5-hydroxytryptamine, 5-HT; acetylcholine, Ach; *Akkermansia muciniphila, A. muciniphila*; alcohol-associated liver disease, ALD; all-trans retinoic acid, ATRA; antigen-presenting cells, APCs; atonal homolog 1, ATOH1; *Bacillus subtilis, B.* subtilis; *Bacteroides fragilis, B. fragilis*; *Bifidobacterium bifidum, B. bifidum*; calcium-activated chloride channel regulator 1, CLCA1; calcium ions, Ca^{2+}; CAMP responsive element binding protein 3 like 1, CREB3l1; *Campylobacter jejuni, C. jejuni*; chemokine C-C motif ligand, CCL; Choline acetyltransferase, ChAT; *Citrobacter rodentium*, *C. rodentium*; *Clostridium difficile, C. difficile*; colorectal cancer, CRC; Crohn's disease, CD; cyclic adenosine monophosphate, cAMP; cystic fibrosis, CF; cystic fibrosis transmembrane conductance regulator, CFTR; cytotoxic T-lymphocyte associated protein 4, CTLA-4; dendritic cells, DCs; dendritic cells type 2, cDC2; *Entamoeba histolytica*, *E. histolytica*; epidermal growth factor receptor, EGFR; *Escherichia coli, E. coli*; eukaryotic initiation factor 2, EIF2; *Faecalibacterium prausnitzii*, *F. prausnitzii*; forkhead box O3, FOXO3; fucosyl α1-2 glycosyltransferase, FUT2; *Fusobacterium nucleatum, F. nucleatum*: G protein-coupled receptors, GPR; gastrointestinal, GI; GC-associated antigen passages, GAPs; Goblet cells, GCs; growth factor independence 1, GFI1; immunoglobulin G Fc-binding protein, FCGBP; immunoglobulin, Ig; inflammatory bowel disease, IBD; interferon alpha 2, IFNA2; interferon gamma, IFNG; Interferon regulatory factors, IRF; interleukin, IL; intestinal epithelial cells, IECs; Janus kinase, JAK; Kruppel-like factor 4, KLF4; *Lactobacillus plantarum*, *L. plantarum*; Lamina propria, LP; lamina propria dendritic cells, LP-DCs; *Listeria monocytogenes*, *L. monocytogenes*; Ly6/PLAUR domain containing 8, Lypd8; messenger ribonucleic acid, mRNA; metabolic

dysfunction-associated steatotic liver disease, MASLD; metalloendopeptidase meprin β, MEP1B; mitogen-activated protein kinase, MAPK; muscarinic acetylcholine receptor 1, mAChR1; myeloid differentiation primary response 88, Myd88; natural killer, NK; natural killer group 2 member D, NKG2D; neurogenic locus notch homolog protein 1, Notch 1; peripheral T-regulatory cells, nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB; pTregs; phosphoinositide 3-kinase, PI3K; *Prevotella nigrescens, P. nigrescens*; programmed cell death protein 1, PD-1; programmed death-ligand 1, PD-L1; prostaglandin E receptor subtype 4, EP4; protein arginine methyltransferase 5, PRMT5; protein atonal homolog 1, ATOH1; regenerating islet-derived 3, REG3; regenerating islet-derived 3 beta, REG3B; regenerating islet-derived 3 gamma, REG3G; resistin-like molecule, RELM-β; retinaldehyde dehydrogenase, ALDH1; *Ruminococcus gnavus, R. gnavus*; *Ruminococcus torques, R. torques*; *Salmonella typhimurium, S. typhimurium*; SAM pointed domain-containing Ets transcription factor, SPDEF; Secretory immunoglobulin A, sIgA; serotonin transporter, SERT; short-chain fatty acids, SCFAs; sialyl-Tn antigen, sTn; signal transducer and activator of transcription 3, STAT3; Specific-pathogen-free, SPF; *Staphylococcus aureus, S. aureus*; T helper, Th; Thomsen-nouvelle, Tn; Toll-like receptors, TLRs; transforming growth factor, TGF-β; transmembrane protease serine 2, TMPRSS2; trefoil factor 3, TFF3; *Trichuris trichiura, T. trichiura*; tumor necrosis factor, TNF; ulcerative colitis, UC; *Vibrio cholerae, V. cholerae*; zymogen granule protein 16, ZG16.

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ABSTRACT

Goblet cells are specialized guardians lining the intestine. They play a critical role in gut defense and immune regulation. Goblet cells continuously secrete mucus creating a physical barrier to protect from pathogens while harboring symbiotic gut bacteria adapted to live within the mucus. Goblet cells also form specialized goblet cell-associated passages, in a dynamic and regulated manner, to deliver luminal antigens to immune cells, promoting gut tolerance and preventing inflammation. The composition of gut bacteria directly influences goblet cell function, highlighting the intricate interplay between these components of a healthy gut. Indeed, imbalances in the gut microbiome can disrupt goblet cell function, contributing to various gastrointestinal diseases like colorectal cancer, inflammatory bowel disease, cystic fibrosis, pathogen infections, and liver diseases. This review explores the interplay between goblet cells and the immune system. We delve into the underlying mechanisms by which goblet cell dysfunction contributes to the development and progression of gastrointestinal diseases. Finally, we examine current and potential treatments that target goblet cells and represent promising avenues for further investigation.

Keywords: Intestinal immune system, goblet cells, mucin, goblet cell-associated antigen passages (GAPs), microbiota, mucosa-associated bacteria, gastrointestinal disease, therapeutic strategies

INTRODUCTION

The gastrointestinal (GI) tract presents a unique challenge for the immune system. Its extensive surface, lined by a simple columnar epithelium, faces a constant barrage of dietary components and potentially harmful microbes (1). Beneath this epithelium lies the largest concentration of immune cells in the body. A healthy state requires that intestinal immune cells efficiently distinguish between harmless dietary substances and invaders (2). This distinction allows the immune system to develop tolerance towards the former, a hallmark mediated by tolerogenic dendritic cells (DCs) and antigen-specific T regulatory cells (Tregs) (3-5).

Goblet cells (GCs) are specialized intestinal epithelial cells (IECs) that play a crucial role in gut defense. They are distributed throughout the epithelial lining of both the small and large intestines, with a notable abundance in the colon, where a robust mucus barrier is particularly necessary (6). The apical surface of GCs is characterized by microvilli, which significantly increase the surface area available for mucin secretion into the intestinal lumen. These cells are equipped

with a well-developed endoplasmic reticulum and Golgi apparatus, which are vital for the synthesis, modification, and packaging of mucins. Their cytoplasm is distinguished by numerous secretory granules containing mucin precursors, highlighting their role in mucin production and secretion. They continuously secrete and renew the mucus layer, physically pushing away pathogens from the gut lining (Figure 1). There are over 20 identified mucins (labeled MUC1 to MUC21), each with slightly different structures and functions (7). In the intestine, the predominant mucin is MUC2. Deficiency in MUC2 leads to inflammation and increased susceptibility to infection in mice, highlighting its importance in gut health (8). Mucins also have binding sites for bacteria, further hindering their invasion (6). Some bacterial species in the gut utilize components of the mucus layer as an energy source, influencing both mucus production and the overall gut microbiome composition (9).

When the gut encounters challenges such as microbes or harmful antigens, GCs are triggered to release mucins at an accelerated rate. Various factors, such as neuropeptides, cytokines, and lipids induce mucin secretion (10). A key factor in mucin secretion is the activation of muscarinic acetylcholine receptor 1 (mAChR1) (11). The role of this activation will be elaborated upon in the following sections of this manuscript. GCs also secrete a diverse plethora of interleukins such as (IL)-25, IL18, IL17, IL15, IL13, IL7, and IL6, and chemokines such as chemokine exotoxin, chemokine C-C motif ligand (CCL)6, CCL9, and CCL20, which are signaling molecules that further modulate the immune system (12) (Figure 1). By combining these functions, GCs play a vital role in maintaining a healthy gut environment and preventing disease. Beyond their well-documented role in mucin production, recent research suggests GCs play a more multifaceted role in immune regulation through the formation of GC-associated antigen passages (GAPs) (Figure 1) (5). In this review, we will focus on this critical function and the secretion of antimicrobial peptides and proteins that enhance the protective barrier function and contribute to the immune response. Furthermore, we examine the intricate interplay between GCs and the commensal microbiota and we also explore the underlying mechanisms by which GCs dysfunction promotes the development and progression of gastrointestinal diseases. Finally, the review examines current and potential therapeutic strategies that target GCs. These promising avenues offer exciting possibilities for future research and development of novel gut disease treatments.

GOBLET CELL-ASSOCIATED ANTIGEN PASSAGES: MOLECULAR PATHWAYS AND IMMUNE RESPONSE

GCs dynamically create specialized structures known as GAPs, which transfer luminal antigens to antigen-presenting cells (APCs), particularly mononuclear phagocytes like dendritic cells (DCs) located in the lamina propria (LP). This mechanism is essential for maintaining gut immune tolerance and suppressing inflammatory responses (5). The neurotransmitter ACh acts as the master conductor, directing both mucus secretion and GAP formation. ACh activates different muscarinic receptors on GCs, depending on the location in the gut. In the small intestine and proximal colon, mAChR4 orchestrates GAP formation, while mAChR3 takes over this role in the distal colon (13). This ensures that GAP activity is tailored to the specific needs of each intestinal segment. ACh also stimulates the release of calcium ions, facilitating the fusion of vesicles containing mucin and endocytosed luminal content with the cell surface. This dual action allows GCs to simultaneously build and maintain the protective mucus barrier while sampling the luminal environment for potential antigens (1, 14).

ACh originates from various sources including enteric neurons, fibroblasts, IECs, and immune cells (15). A complex interplay of factors further influences its secretion into the intestinal lumen. These encompass dietary components, such as short-chain fatty acids (SCFAs) and vegetable glucosides, as well as chemical stimuli like acids and ions, and even microbial pathogens (16-19). SCFAs are synthesized within the gut lumen through the microbial fermentation of indigestible carbohydrates that contain β-glycosidic bonds between glucose monomers, which remain inaccessible to mammalian enzymes (16). Upon their production, SCFAs trigger the release of epithelial ACh prompting anion chloride (Cl^-) secretion by IECs (16). In addition, vegetable glucosides like paeoniflorin, a principal bioactive component of *Paeonia lactiflora Pall*, and quercetin, a flavonoid commonly found in fruits and vegetables, proved to inhibit acetylcholinesterase activity and promote the expression of serotonin, thereby contributing to gastric motility and the release of ACh in rats (20, 21).

When two ACh molecules bind to nicotinic ACh receptors, they induce a conformational change in the pentameric structure, forming a transmembrane pore (22). This pore permits the passage of sodium, potassium, and calcium ions, resulting in cell depolarization and ACh release. This process enhances smooth muscle contraction and gastrointestinal motility, with potential modifications to neuronal excitability and neurotransmitter release due to ion-level fluctuations (22). Organic acids, such as lactic and butyric acids, produced during fermentation by gut bacteria, have been implicated in stimulating enteroendocrine cells or directly affecting enteric neurons, leading to the release of ACh (17). In addition, lactic acid has also been associated with the inhibition of acetylcholinesterase and butyrylcholinesterase (23).

In addition, pathogen infections can markedly affect ACh secretion. For instance, during *Citrobacter rodentium* (*C. rodentium*) infections, choline acetyltransferase (ChAT)$^{+}$ T cells migrate to the colon (19). These cells play a pivotal role in mucosal immunity and interactions with commensal microbes by synthesizing and releasing ACh. Conditional removal of ChAT in T-cells leads to a significant escalation in *C. rodentium* burden within the colon highlighting the critical role of ACh in bolstering mucosal defenses (19). ACh also plays a critical role in regulating the release of mucus and antimicrobial peptides, as well as modulating ion and fluid secretion in IECs (19). These functions collectively contribute to maintaining a balance between the host and commensal microbiota while restricting pathogen invasion (24).

Enterotoxins such as cholera toxin, produced by *Vibrio cholerae* (*V. cholerae*) (25) or those generated by enterotoxigenic *E. coli*, increase intracellular levels of cyclic adenosine monophosphate (cAMP) in enterocytes. This stimulates ACh secretion from enteric neurons, leading to hypersecretion of fluid and electrolytes into the gut lumen contributing to the characteristic watery diarrhea observed in bacterial infections (25, 26).

Several bacterial strains, including *Lactobacillus plantarum* (*L. plantarum*), *L. rhamnosus*, *L. fermentum*, *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus)* exhibit the capability to produce ACh (27). Notably, *B. subtilis* surpasses *E. coli* and *S. aureus* in the quantity of ACh it produces. Although the expression of acetylcholinesterase in enteric GCs remains unclear, recent studies have identified the presence of butyrylcholinesterase within GCs. While less efficient, butyrylcholinesterase can still contribute to ACh breakdown (28). This interplay ultimately leads to differential expression of ACh between the small intestine and the colon (19, 20).

The frequency of GAPs is not uniform throughout the intestine in mice. While approximately 4 - 6 GAPs are found per villus in the small intestine of healthy adult wild-type mice, a more dynamic and transient pattern emerges in the colon. In the latest, GAPs first appear in the second week of life, peaking around weaning and then declining in adulthood (29). Colon microbes impede the formation of GAPs in a process reliant on myeloid differentiation primary response 88 (Myd88), which activates epidermal growth factor receptor (EGFR) and p42/p44 mitogen-activated protein kinase (MAPK), leading to their phosphorylation (14). The proximal colon hosts a higher bacterial density compared to the small intestine and features a thinner mucus layer than the distal colon (14). Through the suppression of microbial sensing, the immune system of the proximal colon is protected from exposure to luminal bacteria, thus averting inflammatory

reactions. This temporal regulation plays a pivotal role in shaping the gut immune system during development (29).

Similarly, IL-1β can also regulate GC responsiveness to ACh by binding to its receptor on the apical surface of GCs, activating MyD88, and subsequently transactivating EGFR (30). Additionally, commensal and pathogenic bacteria, and their metabolites, can trigger MyD88 signaling via Toll-like receptors (TLRs) on the cell surface, further impacting EGFR activity (30). Interestingly, GCs express different TLRs depending on their location. All GCs express TLRs 1-5, but small intestinal GCs have slightly higher levels of TLR3, while colonic GCs express significantly higher levels of TLRs 1, 2, 4, and 5 (31). This variation reflects the changing bacterial environment from the small intestine to the colon, where immune surveillance is also heightened. Consequently, small intestine and colonic GCs exhibit distinct sensitivities and responses to TLR signaling, mirroring the differences observed in GAP formation between these regions (31).

GAP formation has also been characterized as an ACh-dependent endocytic process. This mechanism suggests the GAPs are formed by the recovery of secretory granule membranes which traffic fluid-phase cargo to the trans-Golgi network and across the cell by transcytosis as well as the transport of fluid-phase cargo by endosomes to multi-vesicular bodies and lysosomes. The process is reliant on phosphoinositide 3-kinase (PI3K), actin polymerization, and microtubule transport for its execution (11). Under normal conditions, LP Foxp3$^+$ peripheral Tregs (pTregs) in the small intestine and distal colon control tolerance to external antigens. These pTregs inhibit CD4$^+$ and CD8$^+$ T cell activation, modulate gut mast cell function, and redirect B cell immunoglobulin (Ig) E secretion. However, the continued presence of their specific antigen is vital for the survival of small intestine Tregs (32). This is where GAPs take center stage (14). These transient structures transport dietary and luminal antigens (≤0.02 μm) alongside autocrine factors like mucins and integrin αvβ6, which induce tolerogenic responses by promoting transforming growth factor (TGF)-β upregulation (14). These antigens are primarily presented to CD103$^+$ DCs in the SI. These DCs, equipped with retinaldehyde dehydrogenase (ALDH1) for generating all-trans retinoic acid (ATRA), stimulate T cell proliferation, induce adaptive immune responses, and promote mucosal immune functions like IgA responses and gut-homing lymphocytes (5). Interestingly, the more frequent interaction between CD103$^+$ APCs and GAPs compared to CD11b$^+$CD103$^-$CX3CR1$^+$ APCs may be attributed to their superior migration ability, response to inflammatory factors, and T cell stimulation capabilities (33). Additionally, this phenomenon is influenced by the location of DCs, where conventional DCs type 2 (cDC2s) are more abundant in the small intestine compared to the

colon, while cDC1s are more prevalent in the colon (34, 35). The $CD103^{-}CX3CR1^{+}$ APCs, on the other hand, are crucial for T helper (Th)17 T cell formation, and tumor necrosis factor (TNF)-α production (33). GCs, through GAPs, deliver not only antigens but also imprint APCs with tolerogenic properties. This includes stimulating IL-10 production by macrophages and enhancing retinoic acid activity in DCs, both contributing to an anti-inflammatory environment. Furthermore, the sampling of the endogenous GC protein Muc2 by mononuclear phagocytes is associated with improved Treg cell induction and promotes the development of a tolerogenic MNP phenotype (36). These diverse interactions highlight the remarkable interplay between GCs and the immune system. Unveiling the intricate mechanisms of this interplay holds immense potential for developing novel therapeutic strategies for gut-related diseases.

OTHER GOBLET CELL-SECRETED FACTORS SHAPING THE IMMUNE RESPONSE

GCs also release a tailored mix of proteins, cytokines, and chemokines, guided by signals from antigen-encountered APCs. These signals encompass recognition of microbial patterns, cytokines such as IL-10 and TGF-β, and contributions from Tregs and other immune-modulating molecules (36). This orchestrated response not only enables a balanced immune reaction against pathogens but also facilitates the promotion of tolerance towards beneficial gut microbes (37).

Furthermore, GCs basolaterally secrete resistin-like molecule (RELM-β) a protein with direct bactericidal properties against commensals and pathogens, while also fostering Treg proliferation and differentiation to support immune tolerance. RELM-β serves as a chemoattractant, recruiting $CD4^{+}$ T cells to the colon and enhancing IL-22 production for tissue repair (38). Trefoil factor 3 (TFF3) supports Treg development, fights pathogens, aids tissue repair, promotes epithelial cell adhesion, regulates cell migration, promotes tight junction for gut barrier strength, and exhibits anti-inflammatory effects (39). IgG Fc-binding protein (FCGBP), a protein secreted by colon GCs, forms a heterodimer with TFF3. This collaboration enhances microbial clearance and protects the mucus barrier's structural integrity. FCGBP plays a critical role in the gut's immune defense by facilitating the efficient delivery of antibodies to the gut lumen. This protein binds to the Fc portion of antibodies, enabling their transport across epithelial layers, where they can neutralize pathogens and protect the gut from harmful invaders (40).

Protein arginine methyltransferase 5 (PRMT5) modifies other proteins through arginine methylation and regulates genes essential for GCs function, impacting mucus production and

assembly. Interestingly, PRMT5 regulates calcium-activated chloride channel regulator 1 (CLCA1), a key mucus assembly factor, through its methyltransferase activity. However, its regulation of other structural proteins like FCGBP and MUC2 occurs independently of this activity (41). As a key part of intestinal mucus, CLCA1 contributes to its robust viscoelastic properties, ensuring a strong barrier against luminal insults. Through proteolytic activity, it cleaves mucus strands, facilitating smoother mucus flow and preventing stagnation, characterized by the accumulation and lack of movement of mucus. CLCA1 interacts with MUC2, enhancing the formation of a physical barrier against pathogens. In addition, it regulates tight junction protein expression, and displays anti-inflammatory activity, reinforcing gut defense mechanisms (42).

Zymogen granule protein 16 (ZG16) plays a crucial role in maintaining epithelial integrity by regulating cell proliferation and differentiation (43). It also exhibits antimicrobial activity, protecting the gut lining from harmful invaders. Notably, ZG16 specifically binds to mannan on the cell walls of certain fungi, potentially triggering an immune response against these pathogens (44). Additionally, it binds to peptidoglycans in gram-positive bacteria, forming aggregates that cannot easily penetrate the mucus layer (45). Interestingly, ZG16 expression decreases in precancerous lesions and colorectal cancer, suggesting its potential role as a tumor suppressor (46).

Ly6/PLAUR domain containing 8 (Lypd8), vital within GCs, binds to harmful bacteria's flagella, hindering their movement and preventing gut epithelium invasion. Lypd8 deficiency increases susceptibility to intestinal inflammation and bacterial overgrowth, underscoring its role in maintaining the gut barrier (47, 48). Reduced Lypd8 expression in precancerous lesions and colorectal cancer, coupled with its inhibitory effect on cancer cell proliferation and migration upon overexpression, implies its therapeutic potential for colon cancer (47, 48).

Secreted by plasma cells and transported across the epithelium by IECs, secretory immunoglobulin A (sIgA) directly binds to pathogens, inhibiting their movement and adhesion to the gut lining (49). It appears that GCs may also facilitate the transcytosis of IgA from the interstitial space into the lumen of the intestine, respiratory tract, or other ducts, although this process has not been fully elucidated (50). Additionally, sIgA forms immune complexes with invading bacteria, facilitating their clearance through phagocytosis or expulsion. Recent studies reveal that gut microbiota can influence the production of sIgA, highlighting the intricate interplay between the gut ecosystem and immune defense (49). RELM-β, TFF3, Lypd8, and sIgA induce the secretion of antimicrobial peptides by various IECs, including GCs and Paneth cells

(51). Antimicrobial peptides like regenerating islet-derived 3 (REG3) act as a first line of defense against invading pathogens directly killing bacteria, disrupting their cell membranes, and inhibiting their growth. They also act as immune regulators, presenting signals that activate immune responses and promote mucosal repair. Importantly, REG3 selectively binds to bacteria (51), causing cytoderm destruction and leading to their death (52).

These components, along with GAP formation and the well-studied mucins, contribute significantly to the complex functions of GCs. By understanding their individual roles and synergistic effects, we can gain a deeper appreciation for the intricate mechanisms that maintain gut health and develop novel therapeutic strategies for various gut-related diseases.

GOBLET CELLS AND THE MICROBIOTA

The interplay between GCs, mucin, and the microbiota is multifaceted and crucial for maintaining immune tolerance (53). The microbiota impacts GC function by stimulating mucin expression and promoting their appropriate differentiation (54). Serotonin, primarily produced by enterochromaffin cells in the gastrointestinal tract, acts on GCs via receptors like 5-hydroxytryptamine (5-HT) 3 and 5-HT4. This interaction stimulates GCs to secrete mucus (55). Additionally, serotonin plays a crucial role in intestinal mucosal health and turnover (56). Research indicates that commensal microbes can trigger serotonin secretion through activation of the receptor 5-HT4 on GCs, promoting the release of MUC2 (56). Recent studies have observed that under normal conditions, both MUC2 and serotonin are found in the cytoplasm of GCs, with serotonin's presence facilitated by the serotonin transporter (SERT) present in these cells (57). SCFAs can upregulate mucin production (58). Furthermore, commensal mucolytic bacteria such as *Akkermansia muciniphila* (*A. muciniphila*), *Bifidobacterium bifidum* (*B. bifidum*), *Bacteroides fragilis (B. fragilis), Bacteroides thetaiotaomicron* and *Ruminococcus gnavus* (*R. gnavus*), play a role in maintaining the optimal turnover of the outer mucus layer, providing a competitive advantage to the host by excluding pathogens (59). In return, mucins offer attachment sites favoring a habitable environment and serve as a source of energy for some bacterial species (60). This symbiotic interaction contributes to the overall health of the gut and is vital for preventing inflammatory responses triggered by pathobionts (61).

In GI diseases, alterations in the mucin-associated microbiome and mucin-degrading bacteria can have significant implications for gut health due to their proximity to IECs and the immune system. Certain commensal mucin-degrading bacteria, including *Bacteroides spp., Parabacteroides spp., A. muciniphila*, and *Bifidobacterium dentium*, can elicit a mild

inflammatory response characterized by low levels of IL-8 and TNF-α (62). Interestingly, these bacteria also exhibit a suppressive effect on the inflammatory response induced by *E. coli*, achieved through the downregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (62). Moreover, the presence of gut commensals has demonstrated potential in enhancing the function of the epithelial tight junctions by regulating the mRNA expression of *zonula occludens-1*, *occludin*, *claudin-1*, and *E-cadherin* (62).

Conversely, an overabundance of mucin degradation may undermine the integrity of the mucosal layer, potentially permitting luminal bacteria and antigens to infiltrate IECs, and reach the immune system, thereby triggering inflammatory diseases. For example, inflammatory bowel disease (IBD) is characterized by an elevated total bacterial load, particularly enriched in mucin-degrading bacteria (63). Notably, *Ruminococcus torques* (*R. torques*) and *R. gnavus* have been consistently observed to be abundant in IBD patients whereas *A. muciniphila* is notably diminished (64, 65). Furthermore, in the ileum of patients diagnosed with Crohn's disease (CD), an increased presence of *R. gnavus* appears to coincide with a decreased abundance of *Faecalibacterium prausnitzii* (*F. prausnitzii*), a key butyrate-producing bacterium, accompanied by a decline in the *Clostridium leptum* (*C. leptum*) and *Prevotella nigrescens* (*P. nigrescens*) subgroups (66, 67).

Dysbiosis of the mucin-associated microbiome has also been implicated in colorectal cancer (CRC). These patients commonly harbor predominant pathogenic bacteria such as *Fusobacterium nucleatum* (*F. nucleatum*), *E. coli*, and *B. fragilis*, a bacterium with pro-carcinogenic properties, in their intestines (68). On the other hand, *A. muciniphila* is selectively decreased in the fecal microbiota of patients with CRC (69).

Moreover, in patients with cystic fibrosis (CF), gut microbiome dysbiosis begins early in life and persists through adolescence and adulthood (70). Children with CF exhibit lower alpha diversity and delayed microbiome maturation compared to healthy counterparts. CF patients display elevated levels of *Veillonella* and *E. coli*, and reduced levels *of Bacteroides, Faecalibacterium,* and *Akkermansia* (70). Understanding these changes may contribute to elucidating the mechanisms that initiate and perpetuate gut inflammation, and drive the progression of these diseases.

The fate of GCs in the absence of gut microbiota is a question worth exploring. In germ-free environments, there is a reduction in the number of GCs both in the small intestine and the colon, accompanied by reduced storage of mucin granules compared to the normal state (71,

72). The absence of microbial signals deprives GCs of their usual regulatory cues, impacting their secretory function. Furthermore, there is a decrease in the expression of certain antimicrobial molecules, such as angiogenin 4 and REG 3 gamma (REG3G), and a lack of expansion in the $CD4^+$ T-cell population (73, 74). The mucin glycosylation pattern, denoting the specific glycans arrangement on the protein backbone, is altered in germ-free mice. These alterations entail decreased levels of specific glycosyltransferases responsible for elongating O-glycans, leading to the development of shorter Muc2 O-glycans. This occurrence is intricately associated with the absence of microbial metabolites such as acetate and can impact the overall functionality of the mucus layer, affecting its protective properties (75). Interestingly, germ-free mice exhibit adherent mucus in the small intestine and permeable mucus in the colon (76).

Further investigation using germ-free mice has provided insight into the role of GAPs. Unlike conventional mice, small intestinal and colonic GAPs are open in germ-free mice, through which $CD103^+$ LP-DCs can uptake antigens from the intestinal lumen under steady-state conditions (5, 14). Notably, the presentation of luminal antigens by LP-DCs derived from germ-free mice exhibited superior luminal antigen presentation capabilities compared to LP-DCs from mice housed under specific-pathogen-free (SPF) conditions. Specifically, in the SI, $CD103^+$ LP-DCs demonstrated superior luminal antigen presentation capabilities compared to $CD103^-$ LP-DCs among germ-free mice (5). This preferential targeting of antigens to DCs with tolerogenic properties suggests a pivotal role in maintaining intestinal immune homeostasis by GAPs (5). While colonic GCs showed a slight rise in germ-free mice, this uptick alone cannot elucidate the significant emergence of colonic GAPs in these mice. Moreover, GCs did not show an increase in antibiotic-treated mice, despite these mice displaying a comparable significant rise in GAPs (72). The development of colonic GAPs in germ-free mice was suppressed by mAChR4 antagonists unlike conventional mice (14). However, microbiota transplantation and bacterial components such as lipopolysaccharide prompted a swift decline in colonic GAPs, indicating that this pathway may significantly contribute to the absence of proximal colonic GAPs (29, 77).

Investigating GCs in germ-free mice underscores the essential role of gut bacteria in ensuring their optimal function, emphasizing the host's dependence on microbial signals for maintaining a healthy gut.

IMPACT OF GASTROINTESTINAL CONDITIONS ON GOBLET CELL FUNCTION

GC dysfunction, characterized by altered numbers, abnormal differentiation, and disrupted mucin production, significantly contributes to the development and progression of various

gastrointestinal diseases. Chronic inflammation within the mucosa disrupts GC function and alters mucin production, while microbial infections can directly damage GCs or modify their secretory function. Dysregulation of mucin production, resulting from imbalances in synthesis and secretion pathways, also leads to pathological changes in GCs. Genetic mutations affecting GC differentiation, function, or survival can predispose individuals to GC-related disorders. Environmental factors, such as exposure to toxins, pollutants, or dietary components, may further impact GC health and function (Figure 2). Understanding these processes is essential for developing effective strategies to manage and treat conditions involving GC pathology. Unraveling the mechanisms underlying these disruptions will aid in the development of targeted therapies aimed at restoring GC function and improving gut health.

A. **Inflammatory Bowel Disease:** IBD, including CD and ulcerative colitis (UC), disrupts the function of GCs in the gut lining. Studies show a decrease in GC numbers, especially during active disease flares compared to remission. Furthermore, IBD disrupts GC maturation, leading to the production of less functional immature cells. These cells produce less mucus which results in a thinner mucus layer and weakens the mucus barrier's protective properties (78, 79). The type of mucus itself is altered in IBD with alterations in MUC2 O-glycosylation, particularly affecting sialylation and sulfation. This results in an increase in certain smaller glycans and a reduction in several complex glycans (78, 79). There is a shift towards pro-inflammatory mucins, further fueling the inflammatory response. Importantly, the expression of MUC2, MUC5AC, MUC5B, and MUC7 is often reduced in IBD patients. Even in non-inflamed areas of CD patients, some transmembrane and secreted mucins like MUC3, MUC4, and MUC5B are also downregulated (80). Research suggests this decrease in GC products like FCGBP, CLCA1, and ZG16 in UC patients might be independent of local inflammation but is linked to increased bacterial infiltration and activation of IL-18 (81). This impaired mucus barrier allows bacteria and antigens from the gut lumen to penetrate the intestinal lining, triggering and perpetuating the inflammatory response seen in IBD (81).

B. **Colorectal Cancer:** CRC is one of the leading causes of cancer-related death worldwide. In CRC, GC function and differentiation are disrupted, leading to abnormal mucin profiles with changes in type and amount produced. MUC1 showcases markedly shortened carbohydrate side chains, including Thomsen-nouvelle (Tn) and sialyl-Tn antigen (sTn), which facilitate its immunodetection. MUC1 upregulation is associated with a worse prognosis and a higher risk of metastasis (82). This is attributed to MUC1's hindrance of T-cell proliferation, impairing the efficient elimination of cancer cells by cytotoxic lymphocytes and thus facilitating evasion from immune detection (82). Furthermore, the elevation of negatively

charged sialic acid residues on MUC1 could potentially advance metastasis progression by disrupting cell-cell adhesion. (82). Notably, overexpression of MUC5AC, a mucin normally found in the stomach, and reduced MUC2 expression or altered glycosylation impact the mucus layer's integrity and was strongly associated with lymph node metastasis, poor cellular differentiation, advanced tumor stage, and poor prognosis when comparing healthy mucosa to CRC patients (83). In addition, MUC5AC promotes tumorigenesis through the CD44-Src-integrin axis in mice (84).

Other mucin components are also altered in CRC. TFF3 expression is significantly higher compared to healthy tissues and is associated with advanced stages of the disease, and invasion of blood vessels or nerves (39). Furthermore, TFF3 is implicated in poor prognosis due to its role in promoting the clonogenic survival of CRC cells by upregulating prostaglandin E receptor subtype 4 (EP4) through signal transducer and activator of transcription 3 (STAT3) activation (85). A recent study demonstrated that, unlike healthy colons where MUC2 and TFF3 are always expressed together, some colorectal cancer cell lines lack MUC2 while expressing TFF3 (86). CRC tissues exhibit a deficiency in the ZG16 protein, a feature that aligns with negative correlations observed in clinical studies regarding distant metastasis and lymphatic invasion. Moreover, ZG16 plays a pivotal role in shaping the immune response within CRC by actively inhibiting the expression of programmed death-ligand 1 (PD-L1) (87). Co-cultivation of natural killer (NK) cells with a medium derived from ZG16-overexpressing cells effectively enhanced both the survival and proliferation of NK cells, with this effect being contingent upon the expression of natural killer group 2 member D (NKG2D). These findings suggest that ZG16 may block tumor cell immune escape and be a potential target for immunotherapy (87). In addition, the altered composition of mucins also influences the interaction between tumor cells and the immune system. Mucin-associated sTn antigens bind to receptors on macrophages, NK cells, and DCs, suppressing the immune system. This can happen in two ways: either by blocking the cells from recognizing other signals by receptor masking or by directly reducing their ability to attack invaders inhibiting their cytolytic activity. This impacts the tumor microenvironment and the body's anti-tumor response (88-90). Furthermore, MUC1 interactions with innate immune cells hinder the cross-presentation of processed antigens on major histocompatibility complex class I molecules. (88-90). MUC1 and MUC16 interact with siglecs on DCs, masking TLRs and promoting an immature DC phenotype, subsequently diminishing T cell effector functions (88-90). Mucins also interact with or form aggregates with neutrophils, macrophages, and platelets, providing protection to cancer cells during hematological dissemination and facilitating their spread and colonization to metastatic sites (91).

C. Mucinous Adenocarcinoma: Mucinous adenocarcinoma is an uncommon type of CRC characterized by pools of extracellular mucin, comprising more than 50% of the tumor mass (92). Unlike other types of colorectal cancer, mucinous carcinoma exhibits elevated expression levels of MUC2, attributed to dysregulated epigenetic and genetic mechanisms. These include promoter hypomethylation of MUC2 and heightened binding of the GCs lineage-associated transcription factor, protein atonal homolog 1 (ATOH1), to the MUC2 promoter (93). Investigating the crosstalk between GAPs and immune checkpoint pathways, such as programmed cell death protein 1 (PD-1)/PD-L1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), could offer insights into mechanisms of immune evasion in CRC.

D. Pathogen Infections: When pathogens breach the delicate intestinal barrier, GCs become the frontline soldiers, orchestrating a complex and dynamic response. Mucins play a key role in fighting parasitic infections. *Trichuris trichiura* (*T. trichiura*), a soil-transmitted helminth, heightens mucin production, resulting in a thicker barrier that defends against worm invasion. Additionally, MUC5AC directly harms worms, facilitating their expulsion (94). *Entamoeba histolytica* (*E. histolytica*) is a protozoan parasite that infects humans and exploits MUC2, binding to it for access and stimulating hypersecretion. Amebic colitis destroys cellular layers in the colon's mucosa, enabling the parasites to spread to the liver via the bloodstream or to other soft organs such as the brain and lungs (95).

Bacterial infections also alter the mucin composition. For example, *Clostridium difficile* (*C. difficile*) is a spore-forming bacterium known for triggering diarrhea and weight loss, contributing to global epidemics with substantial mortality rates. *C. difficile* infection favors acidic mucus rich in MUC1 while reducing levels of MUC2, thus compromising the protective barrier (96). Additionally, *C. difficile* infection elevates levels of N-acetylglucosamine and galactose, alongside decreased levels of N-acetylgalactosamine (97).

On the other hand, deficiencies in mucins increase susceptibility to intestinal pathogens, which are major causes of gastroenteritis in humans. For instance, MUC1 deficiency increased susceptibility to *Campylobacter jejuni* (*C. jejuni*), and MUC2 deficiency enhanced susceptibility to *Salmonella typhimurium* (*S. typhimurium)* (98). Moreover, during *Salmonella* infections, GAP formation in the small intestine is inhibited, stopping antigen delivery while the gut is under attack. This requires the Myd88-activated EGFR pathway, via IL-1β acting on the IL-1 receptor. This coordinated reaction not only hinders bacterial spread to lymph nodes but also facilitates evasion of immune defenses (30). *Listeria monocytogenes (L. monocytogenes*), a bacterium notorious for causing one of the most severe foodborne illnesses known as Listeriosis, can bind to GCs. It utilizes these cells to

traverse the epithelial barrier and evade immune defenses, thereby establishing infection more effectively (30). Bacterial pathogens found in food and water, such as enterohemorrhagic *Escherichia coli*, target the IECs, leading to inflammation and diarrhea. In a study involving mice infected with *C. rodentium*, a relative of enterohemorrhagic *E. coli*, increased expression and secretion of RELM-β by GCs is necessary to attract T lymphocytes to the infected intestine (99). These T lymphocytes then produced IL-22, a cytokine that directly stimulated epithelial cell proliferation. These findings emphasize the crucial role of epithelial/GCs in coordinating the host response to intestinal pathogens (99).

GCs also serve as targets for several human and mouse viruses. Astroviruses, a major cause of childhood diarrhea, primarily infect and replicate within actively secreting GCs in mice (100). Similarly, Enterovirus 71 and adenovirus HAdV-5p referentially infect and replicate in GCs within human epithelial cultures (101, 102). Recent studies indicate that GCs are susceptible to SARS-CoV-2 infection (103, 104). The virus predominantly infects GCs in the bronchial airway because they harbor elevated levels of angiotensin-converting enzyme 2 and transmembrane protease serine 2 (TMPRSS2) compared to ciliated cells (105). Animal studies suggest that angiotensin-converting enzyme 2 expression levels influence gut permeability, either mitigating or exacerbating leaky gut (106). SARS-CoV-2 interaction with angiotensin-converting enzyme 2 in the GI tract can impair barrier function by disrupting proteins like zonula occludens-1, occludin, and claudins, leading to increased inflammatory cytokine production (107). Additionally, intestinal inflammation can further harm the mucosal barrier and perpetuate the cytokine storm through the actions of lymphocytes, DCs, and macrophages (107).

E. Cystic Fibrosis: CF results from genetic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which codes for an anion channel crucial for chloride and bicarbonate secretion across epithelial surfaces (108). Dysfunction in CFTR function leads to the accumulation of dehydrated, sticky mucus that plugs ducts and glands of epithelia-lined organs like the lungs and intestines, a condition termed mucoviscidosis (109). This pathologic mucus buildup causes luminal acidification, disrupts intestinal motility, and can result in blockages within the SI. These alterations not only disturb the normal balance of gut microbes but also hinder the proliferation and differentiation of IECs, contributing to gut dysbiosis, inflammation, compromised barrier integrity, and elevated susceptibility to GI disorders, including cancer (109). A prominent feature of intestinal mucoviscidosis is GC hyperplasia, characterized by increased GC numbers, faulty degranulation, and the production of thick mucus on the epithelial surface (110). A recent study presents evidence suggesting that GC hyperplasia in the small intestine of CFTR-deficient mice is not directly

caused by impaired CFTR activity in the epithelium but rather appears to be a consequence of the intestinal environment characteristic of CF (109). Within this environment, the upregulation of TLR2 and TLR4 likely plays crucial roles in modulating inflammation and maintaining intestinal homeostasis. It seems that TLR2-dependent signaling triggers GC hyperplasia, which is secondary to reduced Notch signaling. This hyperplasia aligns with a terminal GC differentiation program involving changes in the expression of key transcription factors, including increased ATOH1, SAM pointed domain-containing Ets transcription factor (SPDEF), and growth factor independence 1 (GFI1), along with decreased Neurog3 expression (109). In GCs, mature mucin polymers are compacted due to the neutralization of repulsive forces by H^+ and Ca^{2+} ions. Upon exocytosis, extracellular HCO_3^- removes these ions, causing rapid expansion of mucin polymers into mucus gels. CFTR loss in CF reduces Cl^- and HCO_3^- transport, critical for mucus gel formation (111). Enhanced fucosylation of mucin glycans, prompted by the activation of fucosyl α1-2 glycosyltransferase (FUT2), might additionally elevate mucin viscosity (112). Furthermore, studies in the ileum of CF mice demonstrated that an elevated luminal concentration of HCO_3^- facilitates the unfolding of MUC2, which is probably essential for cleavage by the brush border metallo-endopeptidase meprin β, leading to the subsequent release of mucus from the mucosal surface of the intestine (113). Mucin secretion in the colon of animal models exhibiting CF is contingent upon the expression of CFTR and CLCA1 (114). Experiments have shown that reduced expression of CLCA1 in CF mice correlates with thickened and obstructed intestinal mucus in the colon (115). Recent studies have highlighted gut microbiome changes in CF individuals correlated with increased inflammation, maldigestion, malabsorption, intestinal lesions, and poor linear growth (70, 116, 117).

F. Liver diseases: While GCs and their secreted mucins diligently shield the intestinal barrier, their roles become significantly more complex in the context of liver diseases. These conditions can disrupt the delicate balance in the intestine, leading to intestinal bacterial overgrowth, increased intestinal permeability, bacterial translocation, intestinal inflammation, and a cascade of other complications (118-120). Translocated bacteria can reach the liver via the portal vein promoting hepatic inflammation and exacerbating liver diseases (118-120). For instance, in alcohol-associated liver disease (ALD), in both humans and mice, due to factors that are not fully understood, alcohol consumption leads to changes in gut mucin composition and an increase in mucosal thickness (118-120). The thickening of the gut mucosa and the rise in GC numbers due to chronic ethanol exposure entail reductions in canonical Notch signaling within the gut (120). This results in a relative increase in genes associated with GCs specification, such as ATOH1, CAMP responsive element

binding protein 3 like 1 (CREB3l1), and SPDEF, which are typically suppressed by Notch 1 (120). Interestingly, despite the increase in GC numbers, ethanol intake led to significant decreases in gut levels of Kruppel-like factor 4 (KLF4), a factor involved along with SPDEF in promoting the terminal differentiation of GCs (120). Additionally, mice lacking MUC2 are protected against alcohol-related disruptions to the gut barrier and the development of ALD (118). Furthermore, patients with alcohol use disorder showed a decrease in intestinal α1-2-fucosylation (121). Fut2 deficient mice, lacking this fucosylation, experience heightened ethanol-induced liver injury, steatosis, and inflammation. Furthermore, α1-2-fucosylation diminishes colonization of cytolysin-positive *E. faecalis* in the intestines of ethanol-fed mice (121). These findings underscore the promising therapeutic potential of 2'-fucosyllactose for alcohol-associated liver disease. Excessive ethanol consumption can also result in decreased levels of *A. muciniphila* in patients. This reduction is associated with disruptions in microbial metabolite production, compromised intestinal permeability, the onset of chronic inflammation, and the release of cytokines (122, 123). In liver cirrhosis, the gut experiences a paradoxical phenomenon. Increased MUC2 and MUC3 mRNA expression has been found in the ileum of rats while MUC5AC production often decreases in the colon, contributing to the overall weakening of the gut barrier. Additionally, the composition of mucins changes, with altered glycosylation patterns weakening their ability to defend against invaders. This combination of factors creates a perfect storm for bacterial translocation, immune activation, and systemic inflammation, further exacerbating the underlying liver disease (124). Single nuclear RNA sequencing of the terminal ileum in cirrhosis patients has provided valuable insights into the dynamics of GCs throughout different disease stages (125). Advanced decompensation is marked by a notable decrease in GC numbers compared to healthy individuals, whereas compensated cirrhosis shows an increased abundance of GCs compared to controls (125). Furthermore, analysis of gene expression patterns reveals significant upregulation of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-related genes in GCs, particularly in advanced decompensation cases. Interestingly, within the advanced decompensation group, there is a decrease in the expression of GCs differentiation markers FCGBP, CLCA1, and SPDEF, alongside heightened expression of MUC2, which facilitates mucin production (125). Moreover, advanced decompensated patients display elevated expression of inflammatory mediators such as STAT1, interferon-alpha 2 (IFNA2), interferon-gamma (IFNG), and interferon regulatory factors (IRF), indicating heightened immune activation. However, all cirrhosis patients exhibit lower eukaryotic initiation factor 2 (EIF2) signaling levels and increased expression of the transcription factor forkhead box O3 (FOXO3) compared to healthy controls, suggesting dysregulated cellular

responses in cirrhosis (125). The inhibition of small intestinal GAP is intricately linked to the development of ALD. Despite chronic alcohol consumption leading to an increase in both small intestinal and colonic GCs, along with heightened protective mucin secretion in mice, an intriguing trade-off emerges: this augmentation occurs at the expense of small intestinal GAP formation, thereby suppressing small intestinal GAPs. This phenomenon can be attributed to the downregulation of the *Chrm4* gene, responsible for encoding mAChR4. Consequently, the decreased expression of mAChR4 culminates in a diminished population of tolerogenic DCs and Tregs. This inflammatory milieu consequently facilitates bacterial infiltration into the liver exacerbating the onset of ethanol-induced steatohepatitis (126).

On the other hand, in metabolic dysfunction-associated steatotic liver disease (MASLD), preclinical studies have revealed a decrease in the number of GCs observed in the ileal crypts (127, 128) and colon (129). Muc2-deficient mice, displayed better glucose control, reduced inflammation, and increased gene expression involved in fat burning within fat tissue (130). Additionally, they exhibited higher levels of IL-22 and its target genes associated with gut protection. The findings suggest that the absence of the mucus barrier activates the immune system, leading to IL-22 production which helps protect against the metabolic effects of a high-fat diet (130). However, Fut2-deficient mice, despite consuming more calories, are protected from MASLD, exhibiting increased energy expenditure and thermogenesis (131). This protection can be transferred to wild-type mice via microbiota exchange and is reduced with antibiotic treatment (131). Fut2 deficiency attenuates diet-induced bile acid accumulation and enhances intestinal farnesoid X receptor/fibroblast growth factor 15 signaling, inhibiting hepatic bile acid synthesis. Dietary supplementation of α1-2-fucosylated glycans reverses the protective effects of Fut2 deficiency indicating the critical role of intestinal α1-2-fucosylation in obesity and steatohepatitis pathogenesis (131).

Taken together, these findings suggest that the roles of intestinal GCs and GAPs extend beyond their immediate function in the gut.

ADVANCING THERAPEUTIC STRATEGIES TARGETING GOBLET CELLS AND MUCIN-ASSOCIATED MICROBIOME

Interventions targeting GC function to modulate mucin production and secretion, thereby reinforcing the protective barrier of the intestinal epithelium, are imperative for advancing current treatments of GI pathologies. Table 1 (Supplementary 1) overviews recent efforts to develop therapies based on these strategies. Briefly, Janus kinase (JAK) inhibitors block JAK protein activity, thus preventing the STAT pathway from triggering inflammation. JAK inhibitors

increase the number of GCs and TNF-α, MyD88, and NF-κB2 levels, promoting mucosal healing (132-135).

Notch receptors play a crucial role in regulating the differentiation of colonic GC and stem cells,(136). Dysregulated activation of Notch1 is implicated in the severity of GI diseases such as CRC, IBD, and MASLD. Small molecule inhibitors targeting γ-secretase, which mediates the final cleavage step of Notch receptors, can block Notch1 activation in CRC (137) reducing the migration and invasive capacity of CRC cells *in vitro* and decreasing tumor burden *in vivo*, but it also increases intestinal GCs (138). The systemic use of currently available γ-secretase inhibitors is associated with various adverse effects, including massive diarrhea due to increased GC differentiation (139). A nanoparticle-mediated delivery system targeting γ-secretase inhibitors in the liver has been developed, avoiding GCs metaplasia caused by intestinal Notch inhibition and reducing hepatic fibrosis and inflammation (140). However, further investigation in this field is warranted.

Mucolytics like bromelain (BRO) and N-acetylcysteine (NAC) break down the mucus layer surrounding cancer cells, enhancing the delivery and effectiveness of chemotherapy in CRC (141, 142) and help removing intestinal obstructions in CF (143). Probiotics and fecal microbiota transplantation (FMT) can boost beneficial mucin-associated bacteria, such as *Bifidobacteria* or *A. muciniphila*, reducing intestinal inflammation, regulating immunity, and strengthening the gut barrier (144-150). Moreover, studies have revealed that the consumption of the prebiotic inulin initiates a notable remodeling of the epithelium in the mouse colon (151). This remodeling is marked by heightened proliferation of intestinal stem cells and augmented differentiation of GCs. Notably, these effects are contingent upon the presence of the gut microbiota, the activity of γδ T lymphocytes, and the availability of IL-22 (151). The impact of other prebiotics like 2'-fucosyllactose (2FL) on GI diseases remains unclear. While restoring gut fucosylation with 2FL improves ALD in mice (121), it paradoxically worsens liver disease and promotes hepatic steatosis in a MASLD model (131). A promising new therapeutic approach for ALD is VU0467154, a positive allosteric modulator of the mAChR4 (126). Preclinical studies suggest it induces GAPs, which may be linked to several beneficial effects such as modulation of immune cells, production of Reg3 lectins, reduced bacterial translocation, and overall improvement of ALD. Further insights into the regulatory mechanisms governing mucin alterations are essential. Additionally, understanding the impact of colonic and small intestinal GAP formation is vital. These efforts are fundamental for advancing novel therapeutic approaches in managing intestinal diseases, marking a promising avenue for exploration.

CONCLUSION:

The intricate interplay between GCs, the mucus layer, and the immune system is a crucial determinant of gut health, safeguarding against a range of diseases, and encompasses the involvement of GAPs, goblet-secreted factors, and the mucus layer composition. Abundant evidence from both patient studies and animal models reveals that alterations in the mucus layer, abnormal protein modifications after synthesis, and variations in crucial mucin production heavily influence the development and severity of various conditions. Whether addressing intestinal infections, CRC, IBD, or liver disease, maintenance of balanced and healthy mucin levels emerges as a critical factor. Investigating the complex relationship between GCs, the microbiome, GAPs, and the immune system holds immense potential for developing novel therapeutic strategies for various gut diseases.

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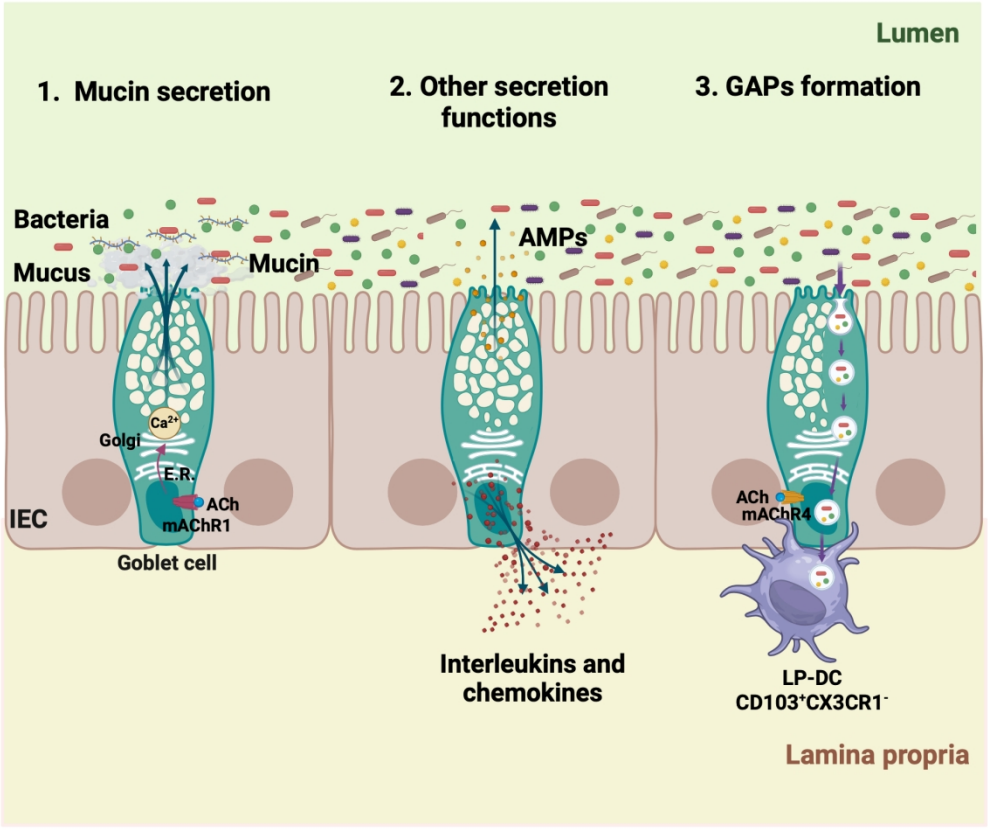
Figure legends:

Figure 1: Goblet cells functions. Goblet cells (GCs) play a multifaceted role in the mucosal immune system, including **1. Mucin secretion:** Goblet cells constantly produce mucins, forming a protective gel layer on the surface of the intestine. This mucus barrier acts as a first line of defense, trapping pathogens and preventing them from reaching the underlying tissues. Under normal circumstances, the thickness of this gel remains upheld through continuous mucin secretion. Nevertheless, when the gut faces challenges such as microbial intrusion or harsh stimuli, goblet cells undergo stimulation to accelerate mucin release. Both, physiological or pathological stimuli, result in a marked increase in intracellular calcium ions (Ca^{2+}) -triggered stimulated mucus secretion. Various factors like neuropeptides, cytokines, and lipids further influence the stimulated mucin release. Upon acetylcholine (ACh) exposure, the activation of muscarinic ACh receptor 1 (mAChR1) also triggers the mobilization of Ca^{2+} from intracellular reserves, contributing to mucus secretion and effectively displacing pathogens from the gut lining. **2. Other secretory functions:** The release of chemokines and cytokines initiates and strengthens Th2 responses, facilitating tissue repair and attracting effector cells that perform functions crucial to innate immunity, extending beyond mere barrier maintenance. GCs also discharge antimicrobial peptides (AMPs), including resistin-like molecule ß (RELM-ß), regenerating islet-derived 3 proteins (REG3) and trefoil factor (TFF), which effectively eliminate commensal bacteria and pathogens that breach the mucus layer. **3. Goblet Cell-Associated Antigen Passages (GAPs):** Activation of mAChR4 by ACh initiates a process termed fluid-phase

bulk endocytosis, culminating in the formation of GAPs in the small intestine. Endocytic vesicles containing luminal fluid-phase cargo are transported through the cell for degradation, membrane recycling, and transcytosis. This allows the cargo to be acquired by lamina propria dendritic cells (LP-DCs). The main LP-DCs subset subadjacent to GAPs is the $CD103^{+}CX3CR1^{-}$ subset and possesses preferential tolerogenic properties. Created with BioRender.com

Figure 2: Gastrointestinal Disorders Impacting Goblet Cell Function. The malfunction of goblet cells (GC), marked by changes in numbers, abnormal differentiation, and modified mucin production, plays a substantial role in the onset and advancement of various gastrointestinal disorders. These include Inflammatory Bowel Disease (IBD), colorectal cancer, mucinous adenocarcinoma, pathogen infections, cystic fibrosis, and liver diseases. Understanding the mechanisms behind these disruptions is essential for devising targeted therapies aimed at reinstating GC function and enhancing overall gut health. Created with BioRender.com



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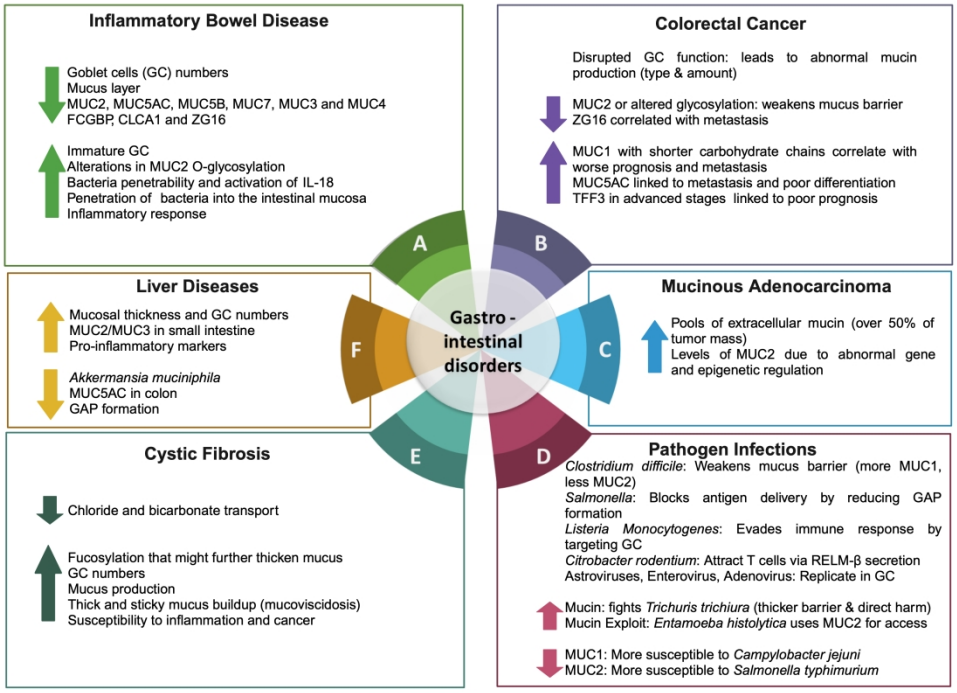

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Table 1. Therapies targeting goblet cells and mucin-associated microbiome in GI diseases.

Treatment	Mechanism of action	Current state	Ref.
Inflammatory Bowel Disease			
Tofacitinib	Prevents the phosphorylation of Janus kinase (JAK) proteins, which prevents the triggering of the signal transducer and activator of transcription (STAT) pathway and downstream signaling of cytokines and the synthesis of pro-inflammatory proteins that are implicated in mucosal inflammation. JAK inhibitors increase the number of goblet cells (GCs) and tumor necrosis factor alpha (TNF-α), myeloid differentiation primary response 88 (MyD88), and nuclear factor kappa-light-chain-enhancer of activated B subunit 2 (NF-κB2) levels, thereby promoting mucosal healing	Approved by the National Institute for Health and Care Excellence (NICE) for use in moderately to severely active ulcerative colitis (UC)	(133)
Filgotinib	Oral small molecule that selectively inhibits JAK1 promoting mucosal healing	Approved by European Medicines Agency for the treatment of UC and ongoing studies are evaluating its efficacy and safety Crohn's Disease (CD)	(132), FITZROY study, NCT03046056, NCT03077412
Ustekinumab, Infliximab, Risankizumab	GC proliferation and mucosal healing were facilitated via the inhibition of interleukin (IL)-12 and IL-23	Clinical study	(152-154)
Atractylodin, Honokiol, Thymoquinone	Dietary bioactives that stimulate mucus secretion by targeting peroxisome proliferator-activated	Preclinical study	(155-157)

	receptor gamma (PPAR-γ) signaling pathway		
Anti-IL-13Rα2 (therapeutic antibody specifically targeting IL-13Rα2)	Promotes GC regeneration and mucus secretion	Preclinical study	(158)
The aromatic hydrocarbon receptor (AhR) agonist 6-formylindolo (3,2-b) carbazole (also known as 6-formylindolo[3,2-*b*]carbazole (FICZ))	Inhibits the Notch pathway, increases the Mucin 2 (Muc2) expression and the number of GCs and reduces bacterial infiltration to ameliorate colitis	Preclinical study	(159)
Probiotic treatment with *Bifidobacterium breve* Bif 195 (Bif195)	Aim to restore the levels of mucosa-associated *Bifidobacteria* to alleviate mucosal inflammation and ulcers	Ongoing Clinical study	NCT04842149
Bacillus subtilis RZ001	Alleviates colitis by inhibiting the Notch signalling pathway and the depletion of GCs	Preclinical study	(160)
Akkermansia muciniphila	Alleviated colitis, improving weight, colon length, and inflammation. GCs number and mucin production increased, while pro-inflammatory cytokines decreased	Preclinical study	(161)
Prebiotic treatment with Inulin	This study aimed to assess how the prebiotic inulin modifies the gut mucin-associated microbiome of children and young adults with inflammatory bowel disease (IBD) and its potential to decrease disease activity	Completed clinical study	NCT03653481

Fecal microbiota transplantation	Aim to restore balance in the mucin-associated microbiota	Clinical trials	(144, 147) NCT05321745, NCT04637438, NCT04521205
Colorectal Cancer			
Janus kinase inhibitors (JAKi)	Inhibition of JAK/STAT3 pathway promoting mucosal healing	Preclinical study	(134)
Sodium/calcium exchanger (NCX) blockers	Reduces mucin secretion providing a means to control the chemoresistance of mucinous colorectal cancer cells	Preclinical study	(162)
LY3039478, an oral Notch signaling inhibitor	LY3039478 shows promising safety profiles and initial antitumor efficacy as a standalone but is associated with GC hyperplasia and a mucoid enteropathy affecting the small and large intestine	Clinical study	(137)
Mucolytics: bromelain (BRO) and N-acetylcysteine (NAC)	Lysis of extracellular mucus removes the protective mucinous coating surrounding cancer cells and improves chemotherapeutic drug delivery/efficacy in cancer cells	Preclinical study	(141, 142)
Lactobacillus and *Bifidobacterium*	Probiotics exert a protective effect against colorectal cancer by competing with pro-carcinogenic microbiota, modulating host immunity, enhancing the intestinal barrier and restoring balance of the mucin-associated microbiota	Clinical trials	(146) NCT05592886, NCT03782428
Inteleukin-2 and *Akkermansia muciniphila*	Combined treatment showed a stronger antitumor efficacy by protecting gut barrier function and	Preclinical study	(163)

	maintaining intestinal structure and GC number		
Galacto-oligosaccharides (GOS)	Prebiotics modulate gut microbiota and mucus layer function	Preclinical study	(145)
Fecal microbiota transplantation	Inhibits colorectal cancer progression by restoring mucin associated bacteria balance and reversing intestinal microbial dysbiosis to enhance anti-cancer immune responses	Preclinical study	(150)
Pathogen infections			
Genistein, one of the active ingredients of soybean isoflavones	Inhibits the GCs loss caused by *Salmonella* infection by regulating the gut bacteria and intestinal stem cell development.	Preclinical study	(164)
Dietary iron	Regulates intestinal GC regeneration, mucin layer function and alleviates *Salmonella typhimurium (S. typhimurium* invasion)	Preclinical study	(165)
Akkermansia muciniphila	Alleviated *Citrobacter rodentium* induced colitis by promoting GCs induction, mucin production, and epithelial antimicrobial peptides	Preclinical study	(166)
Lactobacillus acidophilus	Regenerate GC by inhibiting Notch transcriptional program factors to alleviate *Salmonella* induced colitis	Preclinical study	(167)
Recombinant *Lactobacillus paracasei (L. paracasei)* engineered to express *Listeria* adhesion protein (LAP)	Prevents *Listeria monocytogene (L. monocytogenes*) from causing intestinal barrier loss by maintaining mucus-producing GCs and limiting epithelial apoptotic and proliferative cells	Preclinical study	(168)

Fecal microbiota transplantation for *Clostridium difficile* (*C. difficile*)	Restores the healthy gut microbiome and reestablishes balance in the mucin-associated microbiota	Clinical trials	(148, 149), NCT02134392, NCT03562741, NCT03712722
Cystic fibrosis			
NAC and polyethylene glycol	Successful treatment of distal intestinal obstruction syndrome via colonoscopy by lysis of extracellular mucus	Case report	(143)
Ivacaftor, a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator	Reverses some of the dysbiosis with a significant increment of the mucin-degrading bacteria *Akkermansia*	Clinical trial	(169)
Multistrain probiotics	Aim to evaluate if probiotics improve gastrointestinal health in children	Ongoing clinical study	NCT06284577
Lactobacillus rhamnosus GG	Enrichment of gut *Bifidobacteria* (mucin-associated bacteria) correlates with clinical improvements in children	Clinical trial	(170)
Liver diseases			
MASLD			
Lubiprostone	Improved intestinal permeability through the development of colonic mucus and repressed the development of metabolic dysfunction-associated steatotic liver disease (MASLD)	Preclinical study	(171)
Dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin and PPAR-alpha agonist WY14643	Restored *Bacteroidetes/Firmicutes* ratio, rescued endotoxemia due to increased tight junction gene expression, mucin production, and	Preclinical study	(172)

	numerical density of GCs in intestinal crypts		
Diammonium glycyrrhizinate (DG), the main component of licorice root extracts	Improved the microbiota composition the expression of tight junction proteins, the GC number, and mucin secretion, and enhanced the function of the intestinal barrier	Preclinical study	(172)
Nanoparticle-mediated delivery system to target γ-secretase inhibitor to liver	Avoids GC metaplasia caused by intestinal Notch inhibition and reduces hepatic fibrosis and inflammation	Preclinical study	(140)
Akkermansia muciniphila	Treatment reduced liver inflammation and hepatocyte damage while enhancing gut health through increased GCs, thickened epithelial and mucosal layers, and improved intestinal integrity	Preclinical study	(173)
Different probiotic mixtures including *Lactobacillus, Bifidobacterium Lactococcus,* etc	Reduced serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides (TGs), and low-density lipoprotein (LDL) and reestablishes balance in the mucin-associated microbiota	Clinical trials	(174-178)
Inulin	Inulin regulated the gut microbiota composition increasing the abundance of *Bifidobacterium* and enhanced intestinal barrier integrity and function by decreasing the presence of inflammatory cells, thickening the	Preclinical study	(179)

	mucosal layer, and promoting the elongation of villi with a regular arrangement		
2'-fucosyllactose (2FL)	Increases body and liver weight, more liver injury, and hepatic steatosis. This raises the possibility that the down-regulation of α1-2-fucosylation in MASLD mice is a protective mechanism	Preclinical study	(121)
Fructo - oligosaccharides	Attenuated MASLD by remodeling gut microbiota, preventing the GCs loss, and improving lipid metabolism	Preclinical study	(129)
Fecal microbiota transplantation	Improved balance in the mucin-associated microbiota, intestinal permeability, and hepatic steatosis	Clinical study	(13, 180)
ALCOHOL-ASSOCIATED LIVER DISEASE			
Fenretinide	Reduced alcohol-associated increases in ileal and colonic mucosal thickening, ileal *Muc2*, colonic *Muc2*, *Muc5ac* and *Muc6* mRNAs, and GCs numbers	Preclinical study	(120)
Akkermansia muciniphila and inosine	Enhanced the gut ecosystem, improved intestinal barrier function, upregulated A2AR, CD73, and CD39 expression, modulated Treg cells functionality, and regulated the imbalance of Treg/Th17/Th1 cells and modulates the mucin-associated microbiota	Preclinical study	(181)
Inulin	Modulates the mucin-associated microbiota	Clinical study	(182, 183)

2'-fucosyllactose (2'-FL)	Restoration of intestinal α1-2-fucosylation ameliorates ethanol-induced liver disease	Preclinical study	(121)
VU0467154, a muscarinic acetylcholine receptor 4 (mAChR4) positive allosteric modulator	Induces small intestinal GC-associated antigen passages (GAPs) which was associated with modulation of antigen-presenting cells, induction of regenerating islet-derived 3 (Reg3 lectins), prevention of bacterial translocation, and amelioration of alcohol-associated liver disease	Preclinical study	(126)
Probiotics including *Lactobacillus, Bifidobacterium Streptococcus*, etc	Restoration of the mucin-associated microbiota and reduction of liver injury	Clinical study	(184-189)
Fecal microbiota transplantation	Improved mucin-associated microbiota diversity, antimicrobial peptides expression, and liver markers of disease	Clinical study	(190-195)

Table abbreviations: 2'-fucosyllactose, 2FL; 6-formylindolo[3,2-*b*]carbazole, FICZ; activator of transcription, STAT; alanine aminotransferase, ALT; aromatic hydrocarbon receptor, AhR; aspartate aminotransferase, AST; bromelain, BRO; *Clostridium difficile*, *C. difficile*; Crohn's Disease, CD; cystic fibrosis transmembrane conductance regulator, CFTR; diammonium glycyrrhizinate, DG; dipeptidyl peptidase-4, DPP-4; galacto-oligosaccharides, GOS; GC-associated antigen passages, GAPs; goblet cells, GCs; inflammatory bowel disease, IBD; interleukin, IL; Janus kinase inhibitors, JAKi; Janus kinase, JAK; *Lactobacillus paracasei, L. paracasei; Listeria* adhesion protein, LAP; *Listeria monocytogenes, L. monocytogenes;* low-density lipoprotein, LDL; metabolic dysfunction-associated steatotic liver disease, MASLD; Mucin 2, Muc2; muscarinic acetylcholine receptor 4, mAChR4; myeloid differentiation primary response 88, MyD88; N-acetylcysteine, NAC; National Institute for Health and Care Excellence, NICE; nuclear factor kappa-light-chain-enhancer of activated B subunit 2, NF-κB2; peroxisome proliferator-activated receptor gamma, PPAR-γ; regenerating islet-derived 3, Reg3 lectins; *Salmonella typhimurium, S. typhimurium* invasion; Sodium/calcium exchanger, NCX; triglycerides; tumor necrosis factor alpha, TNF-α; ulcerative colitis, UC.