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Goblet cells: guardians of gut immunity and their role in gastrointestinal diseases

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ABSTRACT

Goblet cells (GCs) are specialised guardians lining the intestine. They play a critical role in gut defence and immune regulation. GCs continuously secrete mucus creating a physical barrier to protect from pathogens while harbouring symbiotic gut bacteria adapted to live within the mucus. GCs also form specialised GC-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 GC function, highlighting the intricate interplay between these components of a healthy gut. Indeed, imbalances in the gut microbiome can disrupt GC 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 GCs and the immune system. We delve into the underlying mechanisms by which GC dysfunction contributes to the development and progression of gastrointestinal diseases. Finally, we examine current and potential treatments that target GCs and represent promising avenues for further investigation.

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 specialised intestinal epithelial cells (IECs) that play a crucial role in gut defence. 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 characterised 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 (labelled 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 use 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).[1] The role of this activation will be elaborated on in the following sections of this manuscript. GCs also secrete a diverse plethora of interleukins (IL) such as IL-25, IL-18, IL-17, IL-15, IL-13, IL-7 and IL-6 and chemokines such as chemokine exotoxin, chemokine C-C motif ligand (CCL)6, CCL9 and CCL20 which are signalling molecules that further modulate the immune system[11] (figure 1). By combining these functions, GCs play a vital role in maintaining a healthy gut environment and preventing disease. Beyond

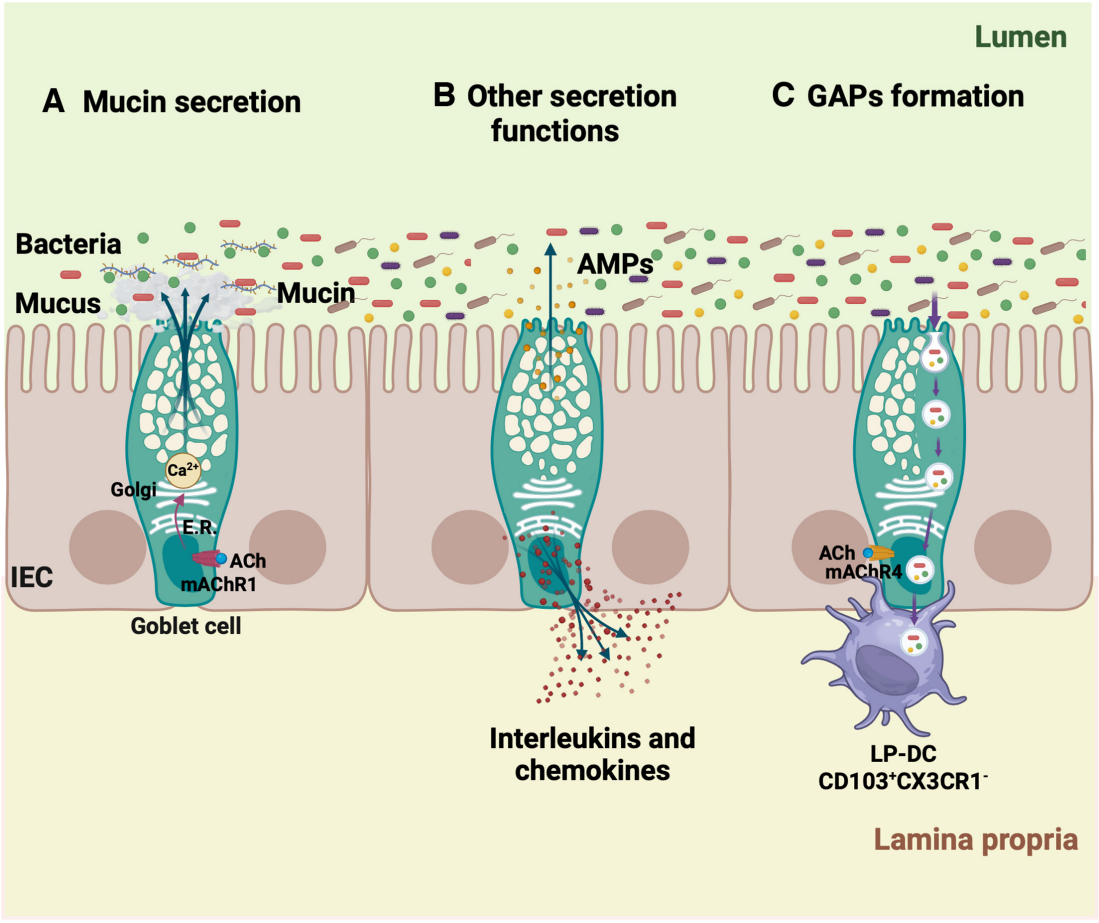

Figure 1 Goblet cells functions. Goblet cells (GCs) play a multifaceted role in the mucosal immune system, including (A) Mucin secretion: GCs constantly produce mucins forming a protective gel layer on the surface of the intestine. This mucus barrier acts as a first line of defence, trapping pathogens 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, GCs 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. On acetylcholine (ACh) exposure, the activation of muscarinic ACh receptor 1 (mAChR1) also triggers the mobilisation of Ca^{2+} from intracellular reserves contributing to mucus secretion and effectively displacing pathogens from the gut lining. (B) 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, regenerating islet-derived 3 proteins and trefoil factor which effectively eliminate commensal bacteria and pathogens that breach the mucus layer. (C) GC-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. E.R., endoplasmic reticulum; IEC, intestinal epithelial cells.

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 specialised structures known as GAPs, which transfer luminal antigens to

antigen-presenting cells (APCs), particularly mononuclear phagocytes (MNP) like 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.[12] This ensures that GAP activity is tailored to the specific needs of each intestinal segment. ACh also stimulates the release of calcium ions (Ca^{2+}) 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 13]

ACh originates from various sources including enteric neurons, fibroblasts, IECs and immune cells.[14] 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.[15–18] SCFAs are synthesised 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.[15] On their production, SCFAs trigger the release of epithelial ACh prompting anion chloride secretion by IECs.[15] 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.[19 20]

When two ACh molecules bind to nicotinic ACh receptors, they induce a conformational change in the pentameric structure forming a transmembrane pore.[21] This pore permits the passage of sodium, potassium and Ca^{2+} resulting in cell depolarisation 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.[21] 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.[16] In addition, lactic acid has also been associated with the inhibition of acetylcholinesterase and butyrylcholinesterase.[22]

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.[18] These cells play a pivotal role in mucosal immunity and interactions with commensal microbes by synthesising 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 defences.[18] 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.[18] These functions collectively contribute to maintaining a balance between the host and commensal microbiota while restricting pathogen invasion.[23]

Enterotoxins such as cholera toxin, produced by *Vibrio cholerae*[24] or those generated by enterotoxigenic *Escherichia coli* (*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 diarrhoea observed in bacterial infections.[24 25]

Several bacterial strains including *Lactobacillus plantarum*, *L. rhamnosus*, *L. fermentum*, *Bacillus subtilis* (*B. subtilis*), *E. coli* and *Staphylococcus aureus* (*S. aureus*) exhibit the capability to produce ACh.[26] 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.[27] This interplay ultimately leads to differential expression of ACh between the small intestine and the colon.[18 19]

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.[28] 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 leading to their phosphorylation.[13] The proximal colon hosts a higher bacterial density compared with the small intestine and features a thinner mucus layer than the distal colon.[13] 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.[28]

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.[29] Additionally, commensal and pathogenic bacteria and their metabolites can trigger MyD88 signalling via Toll-like receptors (TLRs) on the cell surface further impacting EGFR activity.[29] 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.[30] 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 signalling, mirroring the differences observed in GAP formation between these regions.[30]

GAP formation has also been characterised 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 multivesicular bodies and lysosomes. The process is reliant on phosphoinositide 3-kinase, actin polymerisation and microtubule transport for its execution.[1] 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.[31] This is where GAPs take centre stage.[13] 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.[13] These antigens are primarily presented to CD103$^+$ DCs in the small intestine (SI). These DCs, equipped with retinaldehyde dehydrogenase for generating all-trans retinoic acid, 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 with CD11b$^+$CD103$^-$CX3CR1$^+$ APCs may be attributed to their superior migration ability, response to inflammatory factors and T-cell stimulation capabilities.[32] Additionally, this phenomenon is influenced by the location of DCs, where conventional DCs type 2 (cDC2s) are more abundant in the small intestine compared with the colon, while cDC1s are more prevalent in the colon.[33 34] The CD103$^-$CX3CR1$^+$ APCs, on the other hand, are crucial for T helper (Th)17 T-cell formation and tumour necrosis factor (TNF)-α production.[32] 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 MNP is associated with improved Treg cell induction and promotes the development of a tolerogenic MNP phenotype.[35] 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.[35] This orchestrated response not only enables a balanced immune reaction against pathogens but also facilitates the promotion of tolerance towards beneficial gut microbes.[36]

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.[37] 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.[38] 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 defence 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 neutralise pathogens and protect the gut from harmful invaders.[39]

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.[40] 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 characterised 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 defence mechanisms.[41]

Zymogen granule protein 16 (ZG16) plays a crucial role in maintaining epithelial integrity by regulating cell proliferation and differentiation.[42] 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.[43] Additionally, it binds to peptidoglycans in gram-positive bacteria,

forming aggregates that cannot easily penetrate the mucus layer.[44] Interestingly, ZG16 expression decreases in precancerous lesions and colorectal cancer suggesting its potential role as a tumour suppressor.[44]

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.[45 46] Reduced Lypd8 expression in precancerous lesions and colorectal cancer, coupled with its inhibitory effect on cancer cell proliferation and migration on overexpression, implies its therapeutic potential for colon cancer.[45 46]

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.[47] 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.[48] 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 defence.[47] RELM-β, TFF3, Lypd8 and sIgA induce the secretion of antimicrobial peptides by various IECs, including GCs and Paneth cells.[49] Antimicrobial peptides like regenerating islet-derived 3 (REG3) act as a first line of defence 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[49] causing cytoderm destruction and leading to their death.[50]

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.[51] The microbiota impacts GC function by stimulating mucin expression and promoting their appropriate differentiation.[52] 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.[53] Additionally, serotonin plays a crucial role in intestinal mucosal health and turnover.[54] Research indicates that commensal microbes can trigger serotonin secretion through activation of the receptor 5-HT4 on GCs, promoting the release of MUC2.[54] 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 present in these cells.[55] SCFAs can upregulate mucin production.[56] Furthermore, commensal mucolytic bacteria such as *Akkermansia muciniphila* (*A. muciniphila*), *Bifidobacterium 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 favouring a habitable environment and serve as a source of energy for some bacterial species.[58] 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 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 characterised 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 tight junctions by regulating the mRNA expression of zonula occludens-1, occludin, claudin-1, and E-cadherin.[60]

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 characterised by an elevated total bacterial load, particularly enriched in mucin-degrading bacteria.[61] Notably, *Ruminococcus torques* and *R. gnavus* have been consistently observed to be abundant in patients with IBD whereas *A. muciniphila* is notably diminished.[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,* a key butyrate-producing bacterium, accompanied by a decline in the *Clostridium leptum* and *Prevotella nigrescens* subgroups.[64 65]

Dysbiosis of the mucin-associated microbiome has also been implicated in colorectal cancer (CRC). These patients commonly harbour predominant pathogenic bacteria such as *Fusobacterium nucleatum, E. coli* and *B. fragilis*, a bacterium with pro-carcinogenic properties in their intestines.[66] On the other hand, *A. muciniphila* is selectively decreased in the faecal 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] Children with CF exhibit lower alpha diversity and delayed microbiome maturation compared with healthy counterparts. Patients with CF display 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.

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 with the normal state.[69 70] 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.[71 72] 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.[73] Interestingly, germ-free mice exhibit adherent mucus in the small intestine and permeable mucus in the colon.[74]

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 13] Notably, the presentation of luminal antigens by LP-DCs derived from germ-free mice exhibited superior luminal antigen presentation capabilities compared with 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 with $CD103^-$ 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.[70] The development of colonic GAPs in germ-free mice was suppressed by mAChR4 antagonists unlike conventional mice.[13] 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.[28 75]

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

IMPACT OF GASTROINTESTINAL CONDITIONS ON GOBLET CELL FUNCTION

GC dysfunction, characterised 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. Unravelling the mechanisms underlying these disruptions will aid in the development of targeted therapies aimed at restoring GC function and improving gut health.

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 with 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.[76 77] 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.[76 77] There is a shift towards pro-inflammatory mucins, further fuelling the inflammatory response. Importantly, the expression of MUC2, MUC5AC, MUC5B and MUC7 is often reduced in patients with IBD. Even in non-inflamed areas of patients with CD, some transmembrane and secreted mucins like MUC3, MUC4 and MUC5B are also downregulated.[78] Research suggests this decrease in GC products like FCGBP, CLCA1 and ZG16 in patients with UC might be independent of local inflammation but is linked to increased bacterial infiltration and activation of IL-18.[79] 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.[79]

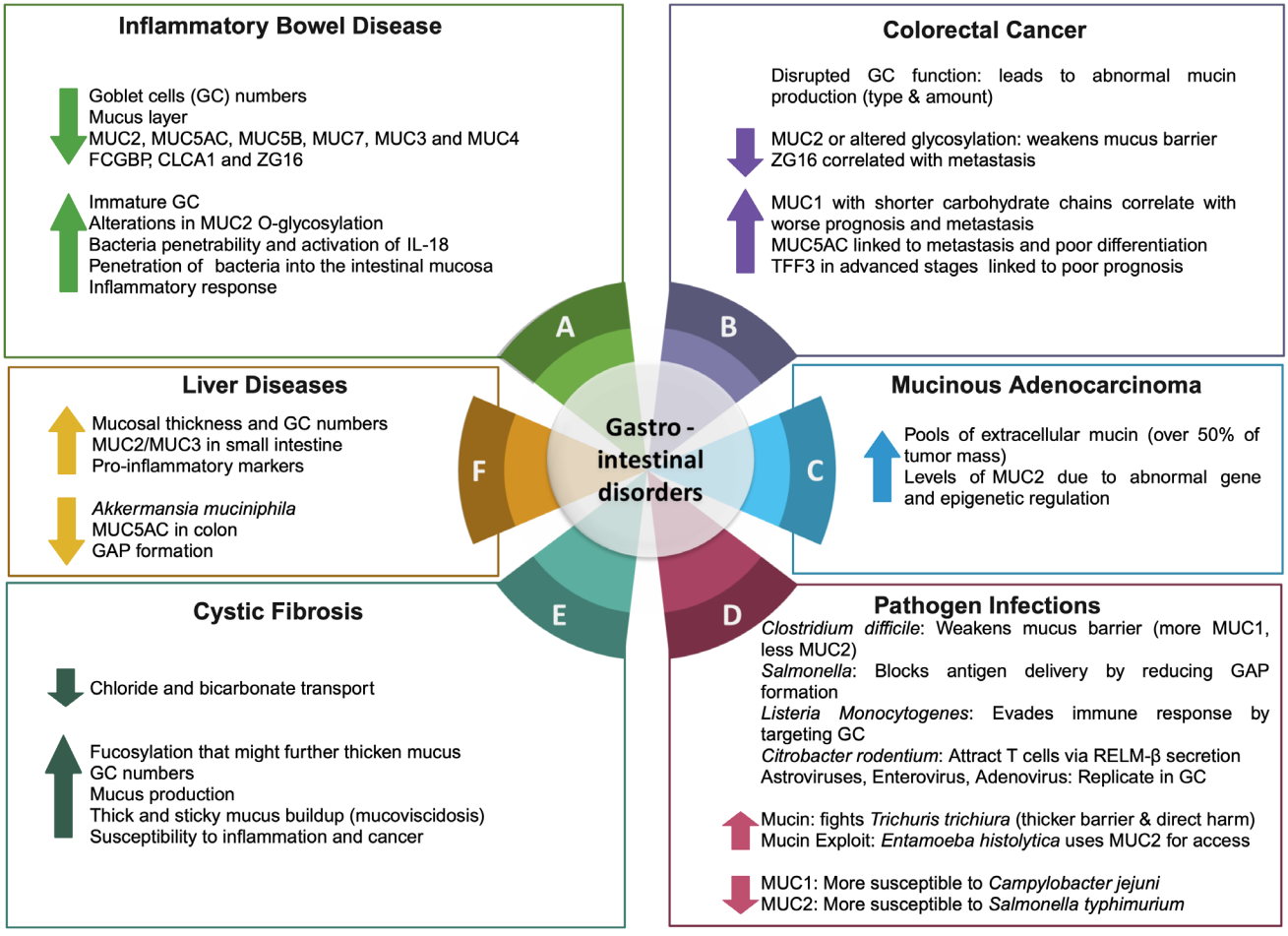

Figure 2 Gastrointestinal disorders impacting goblet cell (GC) function. The malfunction of GCs, marked by changes in numbers, abnormal differentiation and modified mucin production, plays a substantial role in the onset and advancement of various gastrointestinal disorders. These include inflammatory bowel disease, 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. CLCA1, calcium-activated chloride channel regulator 1; FCGBP, Fc-binding protein; GAP, GC-associated antigen passage; IL-18, interleukin 18; MUC2, mucin 2; RELM-β, resistin-like molecule β; TFF3, trefoil factor 3; ZG16, Zymogen granule protein 16.

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.[80] 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.[80] Furthermore, the elevation of negatively charged sialic acid residues on MUC1 could potentially advance metastasis progression by disrupting cell–cell adhesion.[80] 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 tumour stage and poor prognosis when comparing healthy mucosa to patients with CRC.[81] In addition, MUC5AC promotes tumorigenesis through the CD44-Src-integrin axis in mice.[82]

Other mucin components are also altered in CRC. TFF3 expression is significantly higher compared with healthy tissues and is associated with advanced stages of the disease and invasion of blood vessels or nerves.[38] 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 through signal transducer and activator of transcription 3 (STAT3) activation.[83] 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.[84] 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).[85] 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 on the expression of NK group 2 member D. These findings suggest that ZG16 may block tumour cell immune escape and be a potential target for immunotherapy.[85] In addition, the altered composition of mucins also influences the interaction between tumour 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 recognising other signals by receptor masking or by directly reducing their ability to attack invaders inhibiting their cytolytic activity. This impacts the tumour microenvironment and the body's anti-tumour response.[86–88] Furthermore, MUC1 interactions with innate immune cells hinder the cross-presentation of processed antigens on major histocompatibility complex class I molecules.[86–88] MUC1 and MUC16 interact with siglecs on DCs, masking TLRs and promoting an immature DC phenotype, subsequently diminishing T cell effector functions.[86–88] Mucins also interact with or form aggregates with neutrophils, macrophages and platelets, providing protection to cancer cells during haematological dissemination and facilitating their spread and colonisation to metastatic sites.[89]

Mucinous adenocarcinoma

Mucinous adenocarcinoma is an uncommon type of CRC characterised by pools of extracellular mucin comprising more than 50% of the tumour mass.[90] 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.[91] Investigating the crosstalk between GAPs and immune checkpoint pathways, such as programmed cell death protein 1/PD-L1 and cytotoxic T-lymphocyte associated protein 4, could offer insights into mechanisms of immune evasion in CRC.

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*, 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.[92] *Entamoeba histolytica* is a protozoan parasite that infects humans and exploits MUC2, binding to it for access and stimulating hypersecretion. Amoebic 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.[93]

Bacterial infections also alter the mucin composition. For example, *Clostridium difficile* (*C. difficile*) is a spore-forming bacterium known for triggering diarrhoea and weight loss contributing to global epidemics with substantial mortality rates. *C. difficile* infection favours acidic mucus rich in MUC1 while reducing levels of MUC2, thus compromising the protective barrier.[94] Additionally, *C. difficile* infection elevates levels of N-acetylglucosamine and galactose alongside decreased levels of N-acetylgalactosamine.[95]

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* and MUC2 deficiency enhanced susceptibility to *Salmonella typhimurium*.[96] 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.[29] *Listeria monocytogenes*, a bacterium notorious for causing one of the most severe foodborne illnesses known as Listeriosis, can bind to GCs. It uses these cells to traverse the epithelial barrier and evade immune defenses thereby establishing infection more effectively.[29] Bacterial pathogens found in food and water, such as enterohemorrhagic *E. coli*, target the IECs leading to inflammation and diarrhoea. 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.[97] These T lymphocytes then produced IL-22, a cytokine that directly stimulated epithelial cell proliferation. These findings emphasise the crucial role of epithelial/GCs in coordinating the host response to intestinal pathogens.[97]

GCs also serve as targets for several human and mouse viruses. Astroviruses, a major cause of childhood diarrhoea, primarily infect and replicate within actively secreting GCs in mice.[98] Similarly, Enterovirus 71 and adenovirus HAdV-5p referentially infect and replicate in GCs within human epithelial cultures.[99 100] Recent studies indicate that GCs are susceptible to SARS-CoV-2 infection.[101 102] The virus predominantly infects GCs in the bronchial airway because they harbour elevated levels of angiotensin-converting enzyme 2 and transmembrane protease serine 2 compared with ciliated cells.[103] Animal studies suggest that angiotensin-converting enzyme 2

expression levels influence gut permeability either mitigating or exacerbating leaky gut.[104] 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.[105] Additionally, intestinal inflammation can further harm the mucosal barrier and perpetuate the cytokine storm through the actions of lymphocytes, DCs and macrophages.[105]

Cystic fibrosis

CF results from genetic mutations in the CF transmembrane conductance regulator (CFTR) gene which codes for an anion channel crucial for chloride and bicarbonate secretion across epithelial surfaces.[106] 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.[107] 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.[107] A prominent feature of intestinal mucoviscidosis is GC hyperplasia characterised by increased GC numbers, faulty degranulation and the production of thick mucus on the epithelial surface.[108] 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.[107] 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 signalling triggers GC hyperplasia which is secondary to reduced Notch signalling. This hyperplasia aligns with a terminal GC differentiation programme 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 along with decreased Neurog3 expression.[107] In GCs, mature mucin polymers are compacted due to the neutralisation of repulsive forces by H^+ and Ca^{2+} ions. On 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.[109] Enhanced fucosylation of mucin glycans prompted by the activation of fucosyl α1–2 glycosyltransferase (FUT2) might additionally elevate mucin viscosity.[110] 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 metalloendopeptidase meprin β leading to the subsequent release of mucus from the mucosal surface of the intestine.[111] Mucin secretion in the colon of animal models exhibiting CF is contingent on the expression of CFTR and CLCA1.[112] Experiments have shown that reduced expression of CLCA1 in CF mice correlates with thickened and obstructed intestinal mucus in the colon.[113] Recent studies have highlighted gut microbiome changes in CF individuals correlated with increased inflammation, maldigestion, malabsorption, intestinal lesions and poor linear growth.[68 114 115]

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.[116–118] Translocated bacteria can reach the liver via the portal vein promoting hepatic inflammation and exacerbating liver diseases.[116–118] 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.[116–118] The thickening of the gut mucosa and the rise in GC numbers due to chronic ethanol exposure entail reductions in canonical Notch signalling within the gut.[118] This results in a relative increase in genes associated with GCs specification, such as ATOH1, CAMP responsive element binding protein 3 like 1 and SPDEF, which are typically suppressed by Notch 1.[118] Interestingly, despite the increase in GC numbers, ethanol intake led to significant decreases in gut levels of Kruppel-like factor 4, a factor involved along with SPDEF in promoting the terminal differentiation of GCs.[118] Additionally, mice lacking MUC2 are protected against alcohol-related disruptions to the gut barrier and the development of ALD.[116] Furthermore, patients with alcohol use disorder showed a decrease in intestinal α1–2-fucosylation.[119] FUT2 deficient mice, lacking this fucosylation, experience heightened ethanol-induced liver injury, steatosis and inflammation. Furthermore, α1–2-fucosylation diminishes colonisation of cytolysin-positive *E. faecalis* in the intestines of ethanol-fed mice.[119] These findings underscore the promising therapeutic potential of 2'-fucosyllactose (2FL) 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.[120 121] 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.[122] Single nuclear RNA sequencing of the terminal ileum in patients with cirrhosis has provided valuable insights into the dynamics of GCs throughout different disease stages.[123] Advanced decompensation is marked by a notable decrease in GC numbers compared with healthy individuals whereas compensated cirrhosis shows an increased abundance of GCs compared with controls.[123] 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.[123] Moreover, advanced decompensated patients display elevated expression of inflammatory mediators such as STAT1, interferon-alpha 2, interferon-gamma and interferon regulatory factors indicating heightened immune activation. However, all patients with cirrhosis exhibit lower eukaryotic initiation factor 2 signalling levels and increased expression of the transcription factor forkhead box O3 compared with healthy controls suggesting dysregulated cellular responses in cirrhosis.[123] 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.[124]

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[125 126] and colon.[127] MUC2-deficient mice displayed better glucose control, reduced inflammation and increased gene expression involved in fat burning within fat tissue.[128] 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.[128] However, FUT2-deficient mice, despite consuming more calories, are protected from MASLD exhibiting increased energy expenditure and thermogenesis.[129] This protection can be transferred to wild-type mice via microbiota exchange and is reduced with antibiotic treatment.[129] FUT2 deficiency attenuates diet-induced bile acid accumulation and enhances intestinal farnesoid X receptor/fibroblast growth factor 15 signalling, 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.[129]

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. Online supplemental file 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.[130–133]

Notch receptors play a crucial role in regulating the differentiation of colonic GC and stem cells.[134] Dysregulated activation of Notch 1 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 Notch 1 activation in CRC[135] reducing the migration and invasive capacity of CRC cells in vitro and decreasing tumour burden in vivo, but it also increases intestinal GCs.[136] The systemic use of currently available γ-secretase inhibitors is associated with various adverse effects including massive diarrhoea due to increased GC differentiation.[137] 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.[138] However, further investigation in this field is warranted.

Mucolytics like bromelain and N-acetylcysteine break down the mucus layer surrounding cancer cells enhancing the delivery and effectiveness of chemotherapy in CRC[139 140] and help removing intestinal obstructions in CF.[141] Probiotics and faecal microbiota transplantation can boost beneficial mucin-associated bacteria, such as Bifidobacteria or *A. muciniphila* reducing intestinal inflammation, regulating immunity and strengthening the gut barrier.[142–148] Moreover, studies have revealed that the consumption of the prebiotic inulin initiates a notable remodelling of the epithelium in the mouse colon.[149] This remodelling is marked by heightened proliferation of intestinal stem cells and augmented differentiation of GCs. Notably, these effects are contingent on the presence

of the gut microbiota, the activity of γδ T lymphocytes and the availability of IL-22.[149] The impact of other prebiotics like 2FL on GI diseases remains unclear. While restoring gut fucosylation with 2FL improves ALD in mice,[119] it paradoxically worsens liver disease and promotes hepatic steatosis in a MASLD model.[129] A promising new therapeutic approach for ALD is VU0467154, a positive allosteric modulator of the mAChR4.[124] 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 encompassing 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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