The Immune Interactions of Gut Glycans and Microbiota in Health and Disease

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Abstract

The human digestive system harbors a vast diversity of commensal bacteria and maintains a symbiotic relationship with them. However, imbalances in the gut microbiota, known as dysbiosis, accompany various diseases, such as inflammatory bowel diseases (IBDs) and colorectal cancers (CRCs), which have a significant impact on the well-being of people globally. Glycosylation of the mucus layer is a key factor that plays a critical role in maintaining the homeostatic environment in the gut. This review delves into the ways in which the gut microbiota, gut epithelial barrier, and immune cells work together to establish a balanced gut environment. Specifically, the role of glycosylation in regulating immune cell responses and mucus metabolism in this process is examined. Additionally, the review explores various modulatory approaches used to maintain or restore the functional gut microbiota.

Introduction

The microbiota encompasses a wide range of beneficial bacteria that coexist within the human gastrointestinal (GI) system. These symbiotic microorganisms play a crucial role in maintaining the overall health and wellness of the human body, aiding in digestion, nutrient absorption, and immune system regulation[1]. The study of gut microbiota has increasingly gained attention, particularly in the last two decades. Over time, the terms microbiota and microbiome have been used interchangeably. In this article, we use microbiota to refer to bacterial taxa and microbiome to refer to microbial genome[2].

The human microbiota has a complex network of diverse microbes, including bacteria, fungi, protozoa, archaea, and viruses[3-5]. Through symbiosis, microbiota plays central roles in protection against enteric pathogens, maturation and homeostasis of the immune system, regulation of the immune response, energy metabolism, and production of essential nutrients[6-9]. On the other hand, dysbiosis of the microbiota is associated with numerous conditions, including IBDs, antibiotic therapy-related colitis, and colonic cancer (Table 1)[2,10-13].

Glycans and glycoconjugates (i.e., glycoproteins, glycolipids, proteoglycans, etc.) are found in all living organisms and displayed in diverse and distinct structures, combinations and sizes[14]. They play essential roles in cell signaling, energy metabolism, and structural support[15]. At the GI tract, glycans are primarily found on mucin glycoproteins and dietary fibers, both metabolized by the gut microbiota[15-17]. Therefore, by gaining a deeper comprehension of the relationship between the gut microbiota and host/dietary glycans, we can pinpoint the root pathophysiologic mechanisms of diseases associated with the dysbiosis of the gut microbiota.

This review discusses the complexity and function of gut microbiota from a bacterial perspective. We explore how various elements like the gut microbiota, gut epithelial barrier, and immune cells work together to regulate gut immunity to establish and maintain a homeostatic environment. We also examine the significance

of glycan and mucus metabolism in this process. Lastly, we explore different modulatory approaches for maintaining and restoring functional microbiota in the GI tract.

The Gut Microbiota

The human gut microbiota (hGM), also known as the "forgotten organ" is a highly diverse composition of microbial agents that increase in density and diversity from proximal to the distal gut[1,18,19]. The GI tract is colonized by trillions of bacteria, with more than 1000 different bacterial species[20]. These commensal bacteria play a crucial role in maintaining the homeostatic environment in the gut[21].

Newborns have a functionally and structurally immature GI system[22]. During birth, the GI tract is inoculated with microorganisms, and the mode of delivery affects the microbial composition[23-25]. The gut microbiota takes its rudimentary shape during the first year of life and begins to resemble that of an adult by age one[26]. The mature, healthy gut microbiota is primarily composed of phyla such as Bacteroidetes, Firmicutes, Acinetobacteres, Proteobacteria, and Actinobacteria; Bacteroidetes and Firmicutes being the most dominant phyla[27-29]. These symbiotic bacteria have various roles in metabolism, such as producing essential vitamins, helping digest the polysaccharides that are otherwise indigestible, maintaining tissue homeostasis, and protecting the host against opportunistic pathogens[30,31]. The effects of microbiota greatly depend on its composition which varies due to factors such as age, diet, and antibiotic usage[32-37].

Dysbiosis is associated with diseases, such as IBDs, CRCs, and metabolic syndrome, and is a main causative factor in the course of these diseases[38-40]. The microbiota's composition also changes with the host's physiological state. For example, obese individuals harbor less diverse microbiota compared to lean individuals[41,42]. Improved overall hygiene due to urbanization may lead to a decrease in the diversity of bacterial genera in the gut, especially Bacteroides, Prevotella, and Lactobacilli, as reviewed by Fan and Pedersen[1]. This decreased bacterial diversity and bacterial gene complexity can be linked to insulin resistance, inflammation, and eventually dyslipidemia[40]. Antibiotics, while essential in medical practice, can cause GI side-effects, such as hypersensitivity and antibiotic-associated diarrhea[43]. According to a study, a short course of broad-spectrum antibiotic treatment can lead to dysbiosis and a decrease in some commensal bacteria in adults[44].

Patients with IBDs, such as Crohn's disease, ulcerative colitis (UC), and indeterminate colitis, typically have low microbial diversity in their gut microbiota (Table 1)[45]. Irritable bowel syndrome (IBS), *Clostridium difficile* (*C. difficile*)-associated colitis, and acute diarrhea are also associated with an alteration in fecal microbiota composition[46-48]. Dysbiosis is a well-established phenomenon in CRCs, the second leading cause of cancer-related deaths in the U.S., as demonstrated by large-scale human studies[49,50]. Increased numbers of resident bacteria such as *Bacteroides fragilis* (*B. fragilis*),*Escherichia coli* (*E. coli*), and *Streptococcus gallolyticus* (*S. gallolyticus*) were observed in CRC patients[39,51-53]. In addition, specific pathogenic bacterial populations are found to be enriched in CRC patients, most notably *Fusobacterium nucleatum* (*F. nucleatum*),*Solobacterium moorei* (*S. moorei*), *Peptostreptococcus anaerobius* (*P. anaerobius*), and *Parvimonas micra*(*P. micra*) (Table 1)[39]. Interestingly, dysbiosis is present even in patients with colorectal *adenomas* , resulting in increased numbers of pathogens like *F. nucleatum* and*S. moorei* [54].

The epithelial and the immune cells of the GI tract act as a physical barrier between the host and microbes and mediate mucosal immune responses to regulate its microbiota[55]. The immune responses include mucus composition, IgA, and antimicrobial secretion such as RegIIIγ (an antibacterial lectin produced by enterocytes and Paneth cells) and defensins[56-60]. Another mechanism of bacterial selection in the gut is positive growth selection, whereby the beneficial species outgrow their less beneficial counterparts[61]. This positive growth can be achieved by bacteria feeding on molecules from the host epithelia, such as fucose and bacteria attaching to the gut epithelia (Fig. 1G)[62,63].

Gut immunity is regulated by the gut microbiota, glycans, and the immune system

The GI system has a complex and multifaceted immune regulation. The gut-associated lymphoid tissues (GALTs) develop before birth and include mesenteric lymph nodes (MLNs), appendix, Peyer's patches, and isolated lymphoid follicles[64,65]. The gut microbiota and the immune system work together to establish and

maintain homeostasis (Fig. 1).

Immune regulation via the gut microbiota & lymphatic cells

The microbiota plays an essential role in the immune system maturation and homeostasis since GALT maturation, T-cell activation, and plasma cell recruitment are all dependent on the microbiota-derived signals[30,64,66]. Immune cells in the intestine can be associated with two different functional sites: inductive sites (Peyer's patches, MLNs, and lymphoid follicles) and effector sites (lamina propria and epithelia)[67]. Studies have shown that germ-free mice with immature gut microbiotas are more susceptible to GI infection with pathogenic bacteria due to their smaller MLNs and lamina propria, and reduced levels of T-helper 17 (Th17) and IgA[31,68-70]. However, these abnormalities can be ameliorated with the normal microbiota colonization of the gut[5]. While the commensal bacteria boost the host's digestive system efficiency, colonization with pathogens can lead to inflammation and sepsis[71]. Alterations in the hGM can cause IBDs (Fig. 2)[72]. The intricate interplay between the human immune system and the microbiota has led to the cultivation of the latter by the former for protective purposes, and the evolution of metabolic benefits for both[73]. Our immune system has evolved to maintain a balanced environment by "identifying commensal bacteria and distinguishing them" from the pathogenic ones. Pathogenic organisms are sensed by the pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and Nod-like receptors (NLRs)[74]. PRR activation results in the induction of a cascade of pro-inflammatory responses as a result of the recognition of pathogen-associated molecular patterns (PAMPs)[74]. Pili, flagella, and peptidoglycans are examples of known PAMPs.

Mononuclear phagocytes residing in the lamina propria, such as macrophages and dendritic cells (DCs) can distinguish between beneficial and harmful bacteria. These phagocytes are hyporesponsive to TLR ligands from commensal bacteria, which prevents the production of immune responses like TNF or IL-6[5,75]. However, these same innate immune cells produce pro-inflammatory cytokines such as pro-IL-1-β when exposed to harmful bacteria, which can in turn induce the production of IL1-β through the NLRC4 inflammasome, which does not rely on TLR signaling (Fig. 1F, Fig. 2F)[76]. In addition, commensal microbiota instructs the intestinal immune system to limit responses to luminal antigens by inhibiting the transport of bacteria from the lumen to the mesenteric lymph nodes[77]. In a dysbiotic environment, non-invasive bacteria are trafficked to the CD103+ DCs in the mesenteric lymph nodes by $CX3CR1^{hi}$ mononuclear phagocytes in a CCR7-dependent manner. This results in T-cell activation and increased IgA production due to a lack of commensal bacteria-induced Myd88 activation (Fig. 2C)[77,78].

The commensal microbiota promotes the development of regulatory T cells that play an essential role in immune tolerance[9,38]. In the GI tract, antigen-presenting cells (APCs) process the bacterial antigens and then present them to aid in the naive $CD4^+$ T-cell transformation to a Th2 cell (Fig. 1F)[79]. This process allows Th2 cells to secrete effector cytokines like IL-13[79]. Germ-free mice were shown to have a $CD4^+$ T-cell imbalance with a Th2 bias[80], and mono-colonization of these mice with the commensal bacterium *B. fragilis* can reestablish the Th1 and Th2 balance[80]. Paneth cells in the small intestine secrete α-defensins, an antimicrobial peptide, which are the predominant antibacterial factors against enteric pathogenic bacteria (Fig. 1D)[80]. Recognition of microbial-associated molecular patterns (MAMPs) is another important pathway for immune regulation in the gut as MAMP receptors are expressed by epithelial cells and activate signaling cascades that influence cytokine production, such as IL-10, as well as other immune signaling molecules[38,60].

Immune regulation via the epithelial barrier

The gut epithelial layer is a mechanical and immune barrier in the gut that plays an essential role in immune regulation. It contains goblet cells (produce mucus), M cells (present in Peyer's patches and lymphoid cells, sensing and transporting microbes), enterocytes (absorption), stromal cells (tissue regeneration and wound repair of epithelium), and Paneth cells (produce zinc, anti-microbial molecules like lysozyme, and sense microbial products via Myd88-dependent pathways)[81-84]. Commensal bacteria cause an auto-activation of Myd88, an adaptor protein that plays a central role in TLR activation, which limits bacterial access to the mucosal layer (Fig. 1D)[82,85]. TLRs have a distinct placement in the epithelial layer as they are located on

both apical and basolateral surfaces, and this placement prevents the immune response to the commensal gut bacteria that crosses the mucus layer[86]. The integrity of the epithelial cells is a structural feature of how immune regulation takes place in the gut as the alteration of the tight junctions between the epithelial cells is how pathogens like Rotaviruses, *C. difficile* , *Shigella flexneri* , and*Salmonella typhimurium* cause diseases (Fig 2A)[87-90].

Immune regulation via IgA

IgA is the predominant antibody isotype in the mucosal immunity[91]. IgA can be found in two forms: mucosal IgA and serum IgA. Mucosal IgA has a polymeric structure and is concentrated in the outer layer of mucus. Mucosal IgA is produced by plasma cells within the germinal centers of the Peyer's patches and binds to microbial antigens with a very high affinity[92,93]. Secretory IgA, cleaved from the mucosal polymeric IgA, interacts with many antigens in the lumen of the intestine[94]. IgA has numerous roles in the gut homeostasis, which include entrapping antigens in the mucus, reducing invasive properties of bacteria, excreting antigens from the lamina propria into the intestinal lumen, and reducing bacterial motility[92]. IgA's pro- and anti-inflammatory effects are mediated by its binding to the FcαRI. While the FcαRI binding of soluble IgA mediates the anti-inflammatory responses, binding of the aggregated form mediates the pro-inflammatory responses[95]. IgA helps maintain gut immune regulation in a non-specific fashion via a process called immune exclusion[96]. This process is dependent on IgA's ability to prevent microbial access to the epithelial layer with a chain of events called agglutination (bacterial clump formation), entrapment, and clearance (Fig. 1C, Fig. 2C)[97].

IgA is heavily glycosylated, and variations in its glycosylation have been associated with colorectal cancer (as well as breast and ovarian cancer)[95]. Alterations in the terminal glycan motifs such as high fucosylation and sialylation, are accompanied by malignant transformation[98]. IgA antibodies distinctly recognize tumor-associated cancer antigens (TACAs) (i.e., T, Tn, and sialyl-Tn antigens), and glycosylation alterations in IgA antibodies that bind to TACAs are observed in CRC patients[95,99].

Immune regulation via RegIII

The Reg gene family encodes a diverse group of secreted proteins, which are further classified into subgroups (I, II, III, IV) that contain conserved sequence motifs found in C-type lectin carbohydrate recognition domains (CRDs)[100]. Increased expression of RegIII is dependent on factors such as surgery, nutrition, and inflammation due to bacterial invasion or mucosal damage[100-102]. Additionally, RegIIIγ (the mouse homolog of human REG3α) expression is dependent on the microbiota as a study demonstrated a significant decrease in RegIIIγ expression in germ-free mice compared to wild-type mice[100].

The C-type lectins of the RegIII family, secreted by enterocytes and Paneth cells primarily in the distal ileum, show bactericidal properties by restricting mucosal access of gram-positive bacteria to the small intestinal epithelium and by enabling spatial segregation[59,103]. This protection is achieved through TLRs, which detect the microorganisms and activate Myd88 signaling (Fig. 1D)[59]. Mice lacking Myd88 showed a dramatic increase in the bacterial number in the small intestine, compared to their wild-type littermates[59]. Additionally, in RegIIIγ knockout mice, the number of gram-positive bacteria increased in the small intestine compared to the wild-type littermates, but the gram-negative bacterial loads stayed the same[59].

Immune regulation via lectins

Bacterial glycans can be detected by lectins on immune cell surfaces as a major class of PRRs. C-type lectins, Siglecs, and galectins are three major lectin families[104].

C-type lectin receptors (CLRs) bind carbohydrates in a Ca^{+2}-dependent manner and can generate immune responses to pathogens[105]. In Peyer's patches, macrophage inducible C-type lectin (Mincle) recognizes commensal bacteria in the mucosa[106]. This recognition induces expression of IL-6 and IL-23p19 and thereby regulates Th17 differentiation and IL-17 secretion (Fig. 1E)[106]. The same study demonstrated that Mincle deficient mice develop systemic translocation of the gut microbiota, for instance, Proteobacteria to the liver from the gut[106]. *Helicobacter pylori*(*H . pylori*), a pathogen that colonizes gastric mucosa, was shown to

interact with Mincle through its Lewis antigens of lipopolysaccharides (LPS) and cause an anti-inflammatory response to reside in the host[107]. *H. pylori* can also bind to DC-SIGN, a CLR, to evade immune responses by blocking the maturation of naive T-cells to Th1 cells (Fig. 2E)[108].

Siglecs, Sialic acid-binding immunoglobulin-like lectins, are expressed on nearly all immune cells, and binding of bacterial products to these receptors may create both pro- and anti-inflammatory responses (Fig. 1F, Fig. 2F)[109]. For instance, Siglec-10 recognition of*Campylobacter jejuni* (*C. jejuni*) flagella promotes an anti-inflammatory response, thereby affecting functional properties of DCs and macrophages[104,110].

Galectins, a family of lectins with an affinity for beta-galactosides, are expressed by immune cells, including natural killers (NKs), DCs, macrophages, and activated T- and B-cells[111,112]. Galectins can regulate adaptive immunity by influencing T-cell signaling and activation, and modulating immunosuppressive Treg function[113]. During infections, galectin expression can vary and interactions with bacteria and galectins can affect infection and sepsis[114,115]. Galectin-1 (Gal-1) dampens Th1 and Th17 mediated responses, creating a Th2 dominant immune response[116-118]. This effect is due to Th1 and Th17 cells expressing Gal-1 binding glycans; conversely, Th2 cells display α2,6-sialic acid-capped glycoproteins on their surfaces (Fig. 1F)[119]. A study utilizing a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model in mice demonstrated that treating with recombinant Gal-1 improved the disease outcome[119]. Recombinant Gal-1 diminishes the effects of TNBS related T-cells[120]. Galectin-3 (Gal-3) binds to the O-antigen side chains of *H. pylori* and helps it adhere to the gastric epithelia[121]. This interaction increases Gal-3 expression to allow phagocytic cells to traffic the infection sites[121]. Galectins can also recognize host glycans expressed on vacuoles that harbor intracellular pathogens and then induce autophagy[122]. A mouse study utilizing a Gal-3 knockout (KO) model demonstrated that when colitis was induced via dextran sulfate sodium administration, KO mice developed more severe colitis compared to wild-type littermates[119]. Treating the mice with Gal-3 ameliorated the effects of colitis[119]. A recent study demonstrated that Galectin-4 in intraepithelial lymphocytes coats cytosolic*Salmonella enterica* serovar Worthington, inducing bacterial chain and aggregate formation[123]. This process restricts bacterial motility and helps potentiate the inflammasome activation[123]. Chemotherapy treatments using agents like Fludarabine and Busulfan can lead to intestinal damage and result in increased T-cell activation and migration. Damaged organoids have been shown to possess increased Galectin-9, the key mediator for this chemotherapy-associated T cell activity (Fig. 2F)[124].

Immune regulation of intestinal glycosylation

The host immune system regulates mucin glycosylation. Cytokines such as IL-4, IL-15, and IL-22 regulate mucosal immunity by supporting mucus secretion and IL-10 by preventing MUC2 misfolding in the goblet cells (Fig. 1B)[125-128]. On the other hand, pro-inflammatory cytokines IL-6 and TNF-[?] can modulate the glycosyltransferase activity leading to alterations in mucin glycosylation (Fig. 2B)[129]. As demonstrated in IBD patients, these alterations occur early in the disease course with hypo-sialylated glycans increased and fucosylated glycans decreased in expression[129]. TACAs, like T antigen and sialyl Tn (STn) antigen, were overexpressed on MUC2 in patients with CRC[130].

Role of glycans in microbiota homeostasis

Glycans decorate all cell surfaces, such as mucin glycans and proteoglycans on host epithelial cells or capsular polysaccharides, lipopolysaccharides, or glycoproteins on bacterial cells[131,132]. The glycocalyx (combined coat of glycans on the host cell surfaces, acting as a protective barrier between the epithelial layer and mucus layer) is essential in mediating immune responses in the gut by separating epithelial cells from the microbiota (Fig. 1A)[133].

Intestinal Glycans & Mucus

Mucin Structure and Types

Glycans covalently attach to the polypeptide chains through the amide nitrogen of asparagine side-chain (N-linked), the hydroxyl group of serine/threonine side-chains (O-linked), or the thiol group of cysteine side-chain (S-linked)[15]. Epithelial glycans are major components of the intestinal mucus and regulators of the

interaction between the gut microbiota and the gut epithelia[134]. The mucus is composed of proteins, salts, lipids, immunological factors, and a hydrogel layer made up of mucins (MUCs) [135]. Mucins are glycoproteins produced by goblet cells and can be broadly classified as secreted or membrane mucins[136]. They are heavily O-glycosylated (nearly 80%) by the host Golgi apparatus and contain large peptide domains with repeating proline, threonine, and serine amino acids (the PTS domain) (Fig. 3)[137175]. Mucin glycosylation and the number of repeats depend on the cell lineage, developmental stage, and anatomical section[137-139]. These O-linked oligosaccharides are mainly composed of N-acetyl galactosamine (GalNAc), N-acetyl glucosamine (GlcNAc), galactose (Gal), sialic acid (Sia), and fucose (Fuc)[137,140,141]. The number of O-linked oligosaccharides in mucin is proportional to its molecular weight. Increased glycosylation also leads to increased water absorption and relative expansion of the mucus layer, which leads to a greater barrier[141]. In addition, O-glycosylation of mucins helps protect the mucus layer from bacterial proteases[142].

Transmembrane mucins, for instance, MUC1, MUC3, and MUC4 are major components of the glycocalyx[143]. Secreted mucins form the mucus layer. Mucin 2 (MUC2) glycoprotein mostly forms the secreted mucus layer in the colon and small intestine, while MUC5 and MUC6 can be found in the secreted mucus layer in the stomach[144]. The colonic mucus has two layers (a denser inner layer that is largely free of bacteria and a looser outer layer that contains some bacteria); the small intestine has a mucus structure composed of only one layer (Fig. 1)[145]. The interplay between the gut mucins and the gut microbiota is important to establish a healthy mucus layer as mucin glycosylation shapes the nature and diversity of mucus and is a major contributor to the gut microbiota diversity and function[146]. The mucus layer is a habitat for bacteria since it contains attachment sites for the bacteria[147,148]. The impaired mucus layer grants access to the bacteria to reach the epithelial layer and cause inflammation (Fig. 2A)[149].

Mucin Glycosylation and Dysbiosis/Disease

The glycan expression changes from infancy to adulthood, and similar to how the gut microbiota differs among individuals, mucin glycans also differ[56,150,151]. Genetic factors play a major role in these differences as fucosylation of oligosaccharides is dependent on fucosyltransferase (FUT) secretor status and Lewis genes[150]. FUT is the enzyme that adds Fuc to epithelial glycan chains in conformations like α1-2, α1-3, and α1-6[152]. It has been demonstrated that α1-2 FUT (FUT1 and FUT2) polymorphism is highly associated with IBD susceptibility, and individuals with FUT non-secretor status (inactivating polymorphisms of FUT2) have an increased risk for Crohn's disease as this deficiency inhibits Notch signaling, triggers spontaneous colitis, and possesses different microbiota properties than secreter status individuals[153-155]. The H antigen (Fucα1-2Gal) is formed by the addition of α1-2 Fuc to terminal Gal residues. FUT2 encodes the H antigen on intestinal epithelial cells, which allows for bacterial binding such as *H. pylori* [15,156,157]. Increased epithelial α1-2 Fuc expression also helps promote the colonization of commensal bacteria like Bacteroides and Ruminococcaceae and, at the same time, reduce the colonization of opportunistic gut bacteria like *Enterococcus faecalis* (Fig. 1A)[157]. How fucosylation mediates this homeostatic gut environment has many aspects to it. Epithelial fucosylation can be negatively affected by IL-10-producing $CD4^+$ T-cells (Fig. 2A)[158]. On the other hand, commensal and pathogenic bacteria (and their products like LPS) stimulate group 3 innate lymphoid cells (ILC3s), producing IL-22 and inducing α1-2 fucosylation of intra-epithelial cells[159]. In addition, while it has been demonstrated that loss of function mutations in FUT2 are in the group of IBD-associated genetic factors, increased FUT2 expression is detected in the mucosa of CD patients (Table 1)[153,160].

Mucin glycans provide surfaces for bacteria to anchor themselves[150,151]. Bacterial populations that harbor enzymes such as glycoside hydrolases, sulfatases, and proteases can remove glycans from mucins, giving access to the anchor attachment points[143,150,151,161]. Mucin glycans act like decoys for epithelial surface glycans and confine bacteria to the mucus layer, preventing them from accessing epithelial surface glycans[162]. However, certain bacteria such as *B. fragilis* can directly attach to epithelial mucins, highlighting the significance of glycans in the diversity of gut microbiota[163,164]. Accordingly, the mucus layer in the gut protects the epithelial layer and helps prevent microbial invasion by separating microbes from the intestinal surface (Fig. 1A)[81]. This process helps control immune activation and maintains the balance in the host-microbial relationship[81]. However, pathogens may colonize the GI tract by binding to the fucosylated mucin glycans[165].

For instance, *Bacillus subtilis* YesU and *Salmonella typhimurium* Std fimbriae bind to these glycans to adhere to the gut[166,167].

Mucin production results in a continuous flow of mucus, which can change during inflammation (Table 1)[63,168,169]. While Crohn's disease is associated with increased mucus production, the mucus layer is thinner and discontinuous in ulcerative colitis with mucin glycosylation altered to shorter and less complex glycoforms (Fig. 3B)[137,168]. The properties of MUC2 and other mucins vary with the disease course, activity, and severity[138,170,171]. Impairment of the mucus barrier results in increased permeability, thereby allowing easier access for bacteria to the epithelial layer, and consequently inflammation[168,169]. Decreased O-glycosylation of mucin may cause faster digestion by bacteria. As a consequence, the mucus barrier malfunctions, increasing the susceptibility to diseases like IBDs[172,173]. A healthy gut microbiota helps maintain the integrity of mucus by preventing dysbiosis-induced changes to MUC2 production and thickness[174].

The MUC2 monomer contains more than 5000 amino acids, rich in proline, serine, and threonine (Fig. 3A)[175-177]. It regulates the gut microbiota by providing nutrients, acting as ligands to microbial agents, and mediating host signaling[135,178]. Functional mucus layer is not possible in the absence of MUC2, as demonstrated by the development of bacterial overgrowth, spontaneous colitis, and progressive carcinomas in MUC2 deficient mice[81,179]. Additionally, changes in MUC2 glycosylation are associated with increased inflammation in ulcerative colitis as a result of the disrupted mucus layer, which leads to bacterial perfusion of the epithelial layer[138,180]. Similar to complete loss of MUC2, reduced MUC2 expression or MUC2 mutations have been found to cause spontaneous colitis[179,181]. Bacterial products like LPS, lipoteichoic acids, and flagellin can activate the expression of MUC2 via TLRs and trigger the secretion of mucin from goblet cells[182-184]. Germ-free mice show a decreased MUC2 expression and impaired mucosal layer due to their fewer and smaller goblet cells and less sialylated glycans in the mucus layer (Table 1)[134,185].

The resident microbiota can affect the function of goblet cells and, thereby, the mucus layer properties via the release of bioactive compounds[186]. It has been established that LPS of gram-negative bacteria can stimulate the secretion of MUC5AC and MUC5B[187]. In addition, a gram-positive bacterium,*Lactobacillus plantarum* (*L . plantarum*), has been shown to increase the secretion of MUC2 and MUC3[188]. These commensal bacteria not only stimulate the secretion of different mucin types, but they also play an essential role in preventing pathogenic bacteria from gaining access to the epithelial layer. For instance, increased expression of MUC3 can inhibit the attachment of enteropathogenic *Escherichia coli* (EPEC)[189]. In addition, a combination of probiotic bacteria Lactobacillus and Bifidobacterium spp. attenuates the pathogenicity of *C. jejuni* by stimulating the production of unique (i.e., low luminal pH) mucus layers[144,190].

Mucin properties are also altered in CRC patients, as it was demonstrated that MUC1 expression is increased in these patients (Table 1)[191]. MUC1 is hyperglycosylated and expressed at very low levels in the colonic tissue of healthy individuals (up to 10%); conversely, it is hypoglycosylated and expressed in very high levels in the colonic tissue of CRC patients[191,192]. In addition, increased levels of MUC1 was associated with poor prognosis and metastasis[193]. To add to this, MUC2 expression levels decreased in patients with non-mucinous colon adenocarcinomas[194]. MUC5AC, a mucin that is normally found in gastric mucus and absent in the colon, was found to be expressed in CRCs[195].

Mucins are a major source of sialic acid, and Neu5Ac is the most abundant sialic acid in the GI system. In adults, while the Fuc expression decreases from the proximal to the distal gut, sialic acid expression increases from the ileum to the colon[196,197]. A recent study has demonstrated that terminal sialylation of mucin glycans by ST6GALNAC1 (ST6) plays an important role in the integrity of the mucus layer by preventing excessive bacterial proteolytic degradation[198]. Furthermore, mutations of ST6 cause a defective mucus layer in patients with IBDs[198]. Bacterial sialidases can liberate the sialic acids that cap mucin glycans to be used by the same bacteria, other commensal bacteria, and/or pathogenic bacteria[199]. Besides the sialylation of mucin, sialylation of IgG also plays a part in the IBD pathogenesis as serum IgG sialylation levels decrease in patients with ulcerative colitis and Crohn's disease[200].

Bacterial Processing of Glycans

Dietary carbohydrates are essential for the gut microbiota[201]. Many gut commensals have carbohydrate-active enzymes (CAZymes), which facilitate the processing of a range of glycans (Fig. 1G)[202,203]. CAZymes vary among individuals depending on factors such as age and geography[204]. Differential glycan preferences are exhibited by the various microbes found in the gut, resulting in diverse metabolism[203]. For example, *Bacteroidetes thetaitaomicron, B. fragilis,* and *Ruminococcus torques* can break down molecules such as mucin[163,205,206]. *B .thetaitaomicron* can metabolize L-fucose as an energy source and induce host FUTs to increase mucosal fucosylation, creating a beneficial environment for itself[62,207]. Additionally, *B .thetaitaomicron* can help increase sialic acid-carrying glycan expression[208]. Bacterial exoglycosidases release monosaccharide residues from mucin for the bacteria to use as an energy source under homeostatic conditions (Fig. 1G)[209].

On the other hand, Bifidobacteria can break down human milk oligosaccharides (HMOs) to aid in their digestion, a process important for infants who lack the enzymatic capability to process HMOs[210,211]. Exposure to HMOs in breast milk in infancy helps *Bifidobacteria* to colonize the gut and help the host develop an immune tolerance towards commensal bacteria since HMOs act as a carbon source for the gut microbiota and host[212]. HMO metabolism is a virulence-suppressing process and is required to prevent the adhesion of pathogens to the intestinal epithelia as HMOs resemble epithelial surface glycans, allowing them to act as decoy receptors for bacteria (like *E. coli* and *Vibrio cholerae*), and hence, preventing the attachment of the bacteria to the gut[211,213]. HMOs assert their anti-inflammatory effects by regulating interleukin production and lymphocyte activation. Additionally, sialylated HMOs can help maintain a Th1/Th2 balance[214,215].

The process of dietary fiber fermentation by the gut microbiota starts with the breakdown of complex glycans into simpler sugars, which are then fermented by the intestinal anaerobic microorganisms (Fig. 1G). This fermentation process causes the production of short-chain fatty acids (SCFAs), which are absorbed from the colon to be used in numerous metabolic processes[216,217]. One such SCFA is butyrate. It is the preferred energy source of colonocytes, and it facilitates the maturation of the colonic mucus barrier[218,219]. However, deviations from its physiological concentrations may cause colon cancer[220]. Impaired butyrate oxidation is also evident in patients with ulcerative colitis (Fig. 2G)[221]. A low-fiber diet has been associated with reduced beneficial gut bacteria and increased risk for IBD[222]. Comparably, a high-fiber diet can promote an increase in the Prevotella genus, which has reduced colonization in individuals with low-fiber diets[223,224]. A high-fiber diet stimulates an increase in glycan metabolism by the gut bacteria and helps up-regulate SCFA production, thereby reducing inflammation[225].

Due to its high glycan content, the mucus layer serves as a nutrient source for the inhabitant bacteria[202]. Mucin glycan utilization gives bacteria a consistent nutrient supply and helps the bacteria colonize the mucus layer[226]. In the hGM, due to their diverse CAZymes, Bacteroidetes are general glycan degraders that can use both dietary and host glycans (Fig. 1G)[227]. Bifidobacteria and Firmicutes genera show similar glycan degradation patterns since they both use carbohydrates with low levels of polymerization[227].*Akkermansia muciniphila* is another important bacterial species that degrades mucus glycans into acetate to support butyrate-producing bacteria[228,229]. Conversely, *A.muciniphila* can show mucus thinning effects in low-fiber diets and dysbiosis[230]. Mucolytic bacteria can also generate SCFAs through fermentation. These SCFAs can be utilized by non-mucolytic bacteria or used by the host to recover the energy that was used in the mucin synthesis and secretion[231]. MUC2 expression can also be increased by SCFAs[232].

Mucin degradation by bacteria is an essential process in establishing a stable microbiota[168]. The symbiotic relationship between the host and the microbiota relies on the microbes' capabilities of host glycan digestion and the host's ability to secrete mucin glycans for microbial stimuli[17]. If the dietary polysaccharides were to be depleted from the host's diet, this would result in a significant shift to gut bacterial consumption of host mucus[8]. If the host has a low-fiber diet, this results in decreased microbial diversity and a shift in microbial composition to sole reliance on host mucus[8,149]. Increased reliance on host mucus eventually wears down the mucus barrier and causes an inevitable breach of the mucus layer, as observed in ulcerative colitis patients[149]. This data also supports the finding that the low fiber content in the Western diet leads to increased IBD prevalence[149].

Bacterial Glycans

Bacterial glycans are essential in the colonization of bacteria, invasion of host tissues, and modulation of immune responses in the gut[104]. Glycoconjugates produced by bacteria include LPS, teichoic acids, glycoproteins, glycolipids, peptidoglycans, and capsular polysaccharides[233]. Some species, such as*Pseudomonas aeruginosa* and *Neisseria meningitidis* express O-linked glycoproteins, mainly found in pilin and flagellin subunits, and other species, such as *Haemophilus influenzae* express N-linked glycoproteins[234,235]. *L. plantarum* , a commensal bacterium, expresses an O-glycosylated protein, Acm2, as its major autolysin[236] . The glycosylation machinery in *C. jejuni* mostly relies on oligosaccharyltransferase PglB, which transfers oligosaccharides to proteins[237].*C. jejuni* deficient in PglB have attenuated pathogenesis, highlighting the microbe's reliance on its glycosylation system[238].

Bacterial surface glycans can be recognized by immune cells[239]. One example is when the glycans expressed by bacteria are recognized by host lectins such as CLRs, galectins, and Siglecs. This recognition may result in the engulfment of the microbe by DCs, allowing for the processing and presentation of immunogenic epitopes[103,240,241]. Zwitterionic polysaccharide (ZPS) capsules in bacteria are highly studied immunomodulatory bacterial glycans, which play many important roles in the regulation of intestinal immune homeostasis[242]. For example, Polysaccharide A (PSA) of *B. fragilis* , a ZPS, induces the production of IL-10 through APCs and thereby creates a tolerogenic environment to colonize[243]. It can also skew the T-helper balance in favor of Th1 and help maintain the Th1/Th2 balance in the gut[80]. Bacteria can also mimic host glycan structures (molecular mimicry)[152,244]. Bacteria like *B. fragilis* can use the free fucose on their capsule and imitate the fucosylated epithelial glycans[245]. This process also aids in immune evasion of the bacteria[152].

Apart from expressing glycans on their surface, gut bacteria also produce enzymes that modulate glycan expression on immune cells[103]. For example, immune evasion by*Streptococcus pyogenes* (Group A streptococcus), a pathogen that causes diseases like acute rheumatic fever and post-streptococcal glomerulonephritis, is attained by direct glycan modulation on antibodies[246,247]. A targeted mass spectrometry study has shown that this is achieved by an endoglycosidase, EndoS, that cleaves the conserved N-glycan on IgG antibodies[246].

Concluding Remarks and Future Directions

The human gut is vast and complex, posing a challenge in comprehending the yet-to-be-discovered physiological processes that regulate homeostasis. Ongoing research provides a glimpse into its intricacies. As we continue to understand the hGM, it will lead to new investigation into dysbiosis-associated diseases. Dysbiosis is often found in diseases such as IBDs and CRCs. This raises the question: which comes first, the disease or the dysbiosis? As a way to approach this question, stool samples from patients with CRC were transferred to germ-free or conventional mice, and colonic inflammation occurred[248]. Since most diseases associated with dysbiosis are inflammation-related, in addition to the already established pathophysiologic mechanisms, infestation with pathogenic bacteria (or dysbiosis) may also play an inducing role in diseases like CRCs and IBDs. Various models have been proposed to understand the relationship between dysbiosis and CRCs. The alpha-bug model is based on the Enterotoxigenic *B. fragilis* inducing activation of signal transducer and activator of transcription-3 (STAT-3), which causes colitis through Th17 responses[51,249]. The inflammation caused by this process is postulated to promote colorectal cancer. This alpha-bug can displace commensal bacteria that protect against cancer[249]. The driver-passenger model suggests that the "driver" bacteria initiate the formation of CRC through their products causing epithelial cell damage. This damage allows other commensal "passenger" bacteria to breach the epithelia, further deteriorating the disease prognosis[250]. Although not fully elucidated, these two models may potentially be applicable to IBDs, too.

Aberrant mucin O-glycosylation and overexpression of T, Tn, and STn antigens are observed in both CRC and IBD tissues (Fig. 3B)[152,251]. Although we don't fully understand the mechanisms behind the glycosylation changes seen in ulcerative colitis and CRCs, it's clear that these changes are involved in the development of both diseases. For instance, *F. nucleatum* can be recruited to the tumor tissue through the expression

of T antigen, via fatty-acid binding protein 2[252]. Additionally, these glycosylation changes may potentially explain why we observe specific changes in bacterial taxa in both diseases (Table 1).

There has been a growing interest in modulating the composition of hGM to prevent diseases like IBDs, as it plays an important role in regulating immune responses in the gut directly or through diet-derived metabolites[253]. Current approaches to modulate the gut microbiota include diet modifications and over-the-counter pre-/pro-/syn-/post-biotics, as well as oral and fecal microbiota transplantations. Diet is a major factor regulating the gut microbiota by either fiber or fat content[222]. The hGM is negatively influenced by diets with high saturated or monosaturated fat contents[254]. Conversely, a diet with a high polyunsaturated fat content does not have a negative influence on the hGM[254]. *Faecalibacterium prausnitzii* , Firmicutes with fiber degrading abilities, and fecal SCFA levels were shown to increase in individuals on a Mediterranean diet (high-fiber, low animal protein, low in glycemic index carbohydrates) compared to the individuals on a Western diet[222]. Sialic acid consumption through HMOs or meat-based foods is beneficial for the growth of commensal bacteria that possess sialic acid metabolism[255]. Because HMOs are rich in sialic acid, necrotizing enterocolitis (NEC) was more frequently (6-10 times) observed in formula-fed infants, compared to the breast-fed infants[256]. Thus, supplementing the diet of formula-fed infants with sialic acids may prevent NEC. Probiotics, such as Lactobacillus species, can increase the MUC2 production and mucin secretion, enhancing the pathogenic resistance of the intestine[257]. SCFAs, such as sodium butyrate and propionate, can be used in colitis patients since they enhance the production of MUC2[258]. While prebiotics and probiotics may boost immune responses, their effects are often transient and not significant[259,260]. In addition to these over-the-counter supplements, fecal microbiota transplantation (FMT) is a medical procedure to re-establish the homeostatic environment in the gut. There are two prominent FMT methods: heterologous and autologous. While heterologous FMT is the transfer of fecal material from a healthy donor to a recipient with the purpose of re-establishing or replacing the recipient's gut microbiota, autologous FMT is the transplantation of an individual's own fecal content prior to disease or dysbiosis[261,262]. FMT is considered an effective therapy option for recurrent *C. difficile* infections, but it is yet to be an established treatment method for IBDs, such as Crohn's disease and ulcerative colitis since the data suggests that the success of FMT in IBDs is still uncertain (clinical remission 24% to 50%)[148,263-267]. Recent studies have demonstrated that FMT could be an important tool in the treatment regimen of CRC patients after observing FMT improve refractory immune checkpoint inhibitor-induced colitis[39]. Although both the donors and the donated fecal content are tested for transmissible pathogens, FMT still carries the risk of infectious agent transmission. FMT is an emerging procedure and there is still more progress to be made in eligible patient selection, donor selection, preventing unwanted infections and allergic reactions, and administration methods[268,269]. As a major step forward from using FMT, the FDA recently approved rectally and orally administered microbiota therapeutics that are consortia of defined bacteria for the prevention of recurrent *C. difficile* infections.

To summarize, glycans play crucial roles in regulating immune cells, maintaining mucus structure and integrity, and promoting symbiosis among gut microbes. As a result, it is becoming increasingly apparent that we must uncover the specific mechanisms by which glycans contribute to regulatory processes. By doing so, we can develop effective treatments that modify the gut glycome to promote homeostasis and prevent diseases in the future.

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Table 1: Select Bacterial Taxa and Mucosal Alterations in UC, CD, and CRC

Disease	Bacterial Taxa	Bacterial Taxa	Mucosal Alterations	Mucosal Alterations
	Upregulated	**Downregulated**	**Increased**	**Decreased**
Ulcerative Colitis (UC)	*Bacteroides fragilis*[270] *Clostridium hathewayi*[271] *Clostridium bolteae*[271] *Escherichia coli*[272]	*Akkermansia muciniphila*[272] *Bifidobacterium longum*[270] *Eubacterium rectale*[270] *Eubacterium rectum*[272] *Faecalibacterium prausnitzii*[270]	MUC1, MUC16[141,273] MUC5AC presence[209] Expression of truncated or shortened O-glycans[152]	MUC2, MUC9, MUC20[171,179,274,275] Mucus thickness, sulfation, and sialylation[209] Goblet cell number[209,276]
Crohn's Disease (CD)	Actinomyces[272] *Bacteroides fragilis*[270] *Clostridium hathewayi*[271] *Clostridium bolteae*[271] *Escherichia coli*[272]	*Clostridium leptum*[272] *Eubacterium rectale*[270] *Faecalibacterium prausnitzii*[270]	MUC1 (in recurrent cases)[277] MUC5AC presence[209] Mucus thickness (or no change)[209] Non-sense mutation of FUT2[278]	MUC2, MUC3, MUC4, MUC5B, MUC7[141,277] Goblet cell number[209,276]

Disease	Bacterial Taxa	Bacterial Taxa	Mucosal Alterations	Mucosal Alterations
Colorectal Cancer (CRC)	*Bacteroides fragilis*[270] *Enterococcus faecalis*[279] *Escherichia coli*[280] *Fusobacterium nucleatum*[281] *Streptococcus bovis*[282] *Streptococcus gallolyticus*[283] *Solobacterium moorei*[39] *Parvominas micra*[284] *Peptostreptococcus anaerobius*[39]	Alistipes[279] Eubacterium[279] Parasutterella[279] Roseburia[279]	MUC1[285] MUC5AC (APC pathway)[195]	MUC2[286] MUC5AC (BRAF pathway)[287] MUC4[287] MUC17 (in BRAF pathway)[287]

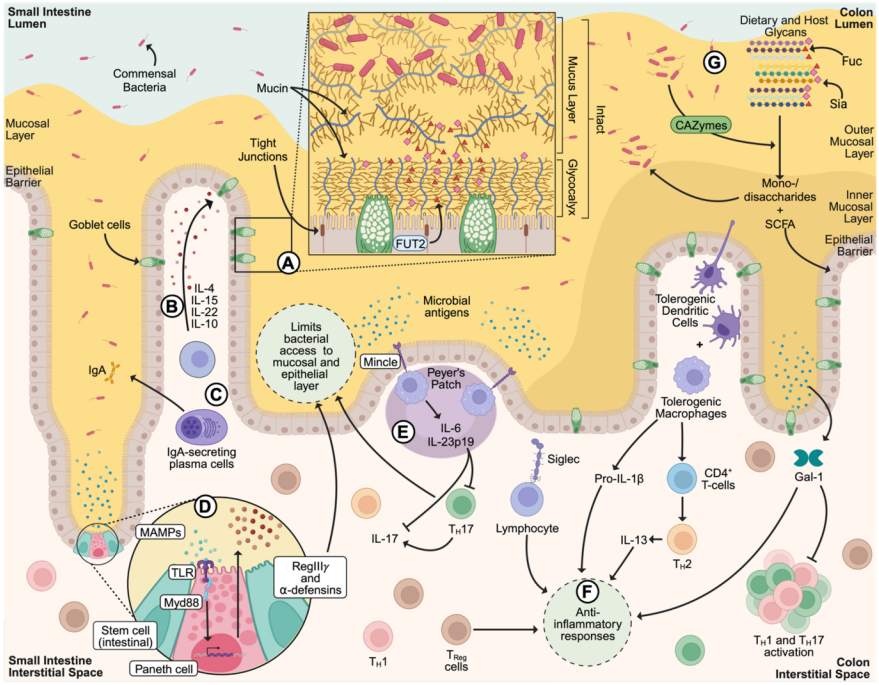

Fig. 1: Diagrammatic representation of the interaction between the immune system, mucus layer, epithelial barrier, and microbiota in a homeostatic gut environment. A. Intact mucus layer, glycocalyx, and epithelial tight junctions restrict bacterial access to the epithelial barrier. Mucin glycans aid in colonization by acting as attachment points and as sources of nutrients. Mucin glycan sialylation protects the integrity of the mucus layer from bacterial proteolytic degradation. Fucosylation of mucin through FUT2 promotes positive growth. **B.**IL-4, IL-15, IL-22 secretion promotes mucus secretion, and IL-10 secretion prevents MUC2 misfolding in goblet cells. **C** . Secretory IgA secreted by IgA-secreting plasma

cells regulates bacterial concentration in the mucosal layer. **D.** MAMPs trigger TLR-Myd88 signaling to induce antimicrobial peptides (i.e., RegIIIγ and α-defensins), which limit bacterial access to mucosal and epithelial layers. **E.** Mincle recognition of commensal bacteria in the gut inhibits IL-17 cytokine and Th17 cell responses through IL-6 and IL-23p19 cytokines. **F.** Anti-inflammatory responses maintain the homeostatic gut environment through Treg cell activity, Siglec activity, tolerogenic dendritic cells and macrophages, and galectin-1 activation.**G.** Commensal bacteria digest dietary (i.e., fiber) and host glycans through CAZymes to generate monosaccharides, disaccharides, and SCFAs, which are subsequently used as a nutrient and energy resource by the microbiota and host.

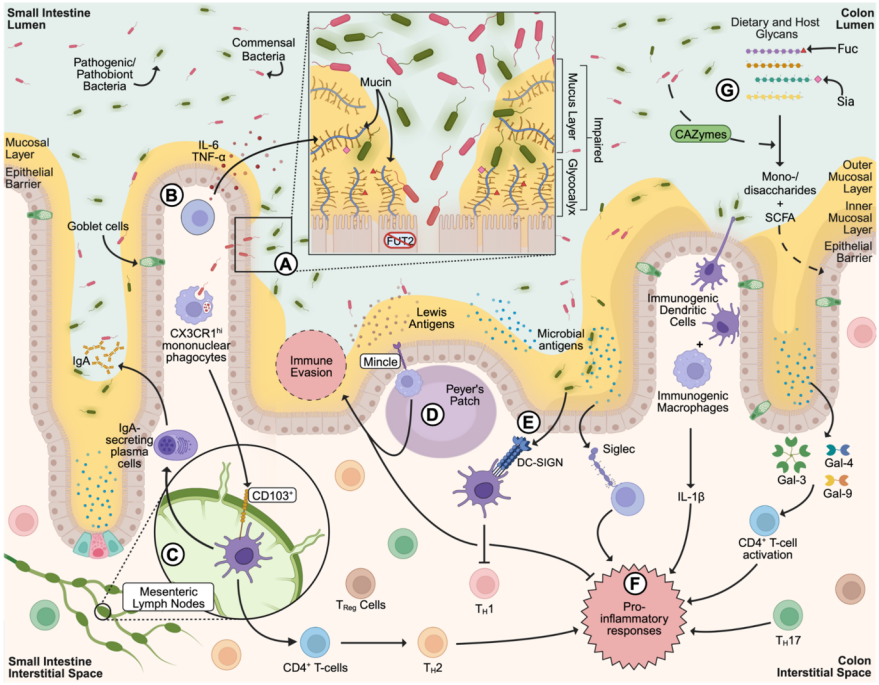

Fig. 2: Diagrammatic representation of the interaction between the immune system, mucus layer, epithelial barrier, and microbiota in a disease/dysbiotic gut environment. A. Impaired/degraded mucus layer, glycocalyx, and epithelial barrier grants access to both commensal and pathogenic/pathobiont bacteria to permeate beyond the barrier. A decreased amount of mucin-producing goblet cells and altered mucin glycosylation and sialylation lead to an impaired mucus layer and glycocalyx. Decreased fucosylation due to downregulated FUT2 activity or CD4+ T-cell pro-inflammatory IL-10 secretion impairs positive growth.**B** . IL-6 and TNF-[?] secretions promote altered mucin glycosylation by modulating glycosyltransferase activity. **C.**CX3CR1hi mononuclear phagocytes transport commensal bacteria to CD103+ dendritic cells in the mesenteric lymph nodes, inducing pro-inflammatory T-cell responses and increased IgA production and aggregation.**D.** Downregulated Mincle levels and Mincle-dependent immune evasion by opportunistic pathobionts. **E.** DC-SIGN-dependent immune evasion enabled by Th1 pro-inflammatory response inhibition after recognition of pathogenic/pathobiont bacteria. **F.** Additional pro-inflammatory responses induce the inflammatory gut environment by Th17 cell activity, Siglec activity, immunogenic dendritic cells and macrophages, and galectin-3,-4, and -9 activity. **G.** Altered digestion of dietary and host glycans leads to altered production of monosaccharides, disaccharides, and SCFA.

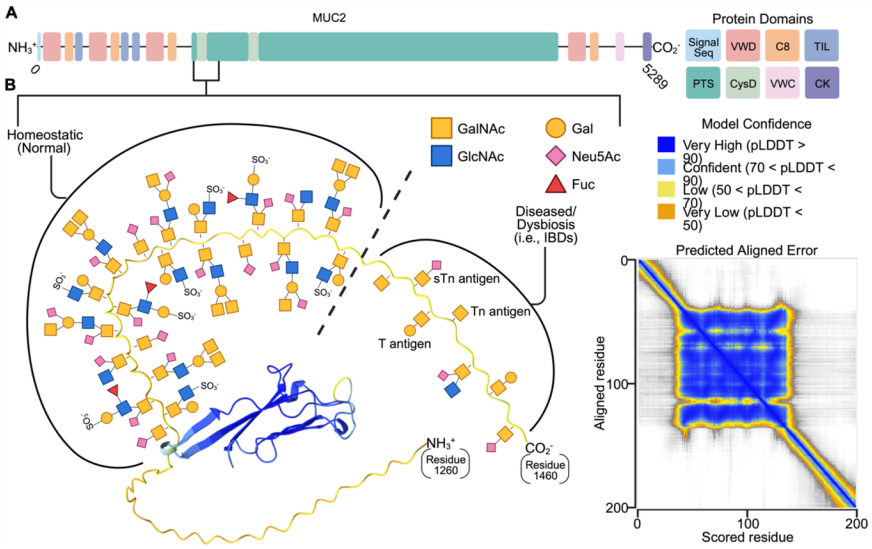

Fig. 3: MUC2 protein glycosylation in homeostatic (normal) gut environment versus disease/dysbiotic gut environment. A. MUC2 protein domain organization. The high density of O-glycosylation takes place in PTS domains. **B.** AlphaFold depiction of MUC2 region from residues 1260 to 1460 (UniProt Primary Accession: Q02817). Protein structure is colored according to model confidence. Visualization of the predicted aligned error plot demonstrates high model confidence between residues 1300-1395, associated with the ordered domain highlighted in dark and light blue. Model confidence falters in regions associated with PTS-repeats, indicative of the inherent disordered state of the protein backbone and displayed in orange and yellow. MUC2 glycans observed in a homeostatic (normal) gut environment are found to be densely packed. Glycan structures are elongated, sulfated, and fucosylated. MUC2 glycans observed in disease/dysbiotic gut environment are found to be sparser. Glycan structures are shorter, altered, and aberrant with increased expression of T antigen, Tn antigen, and STn antigen[134,156,176,177,288,289].