

Mucin-Type O-Glycans: Barrier, Microbiota, and Immune Anchors in Inflammatory Bowel Disease

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Abstract: Inflammatory bowel disease (IBD), which affects about 7 million people globally, is a chronic inflammatory condition of the gastrointestinal tract caused by gut microbiota alterations, immune dysregulation, and genetic and environmental factors. The association of microbial and immune molecules with mucin-type O-glycans has been increasingly noticed by researchers. Mucin is the main component of mucus, which forms a protective barrier between the microbiota and immune cells in the colon. Mucin-type O-glycans alter the diversity of gastrointestinal microorganisms, which in turn increases the level of O-glycosylation of host intestinal proteins via the utilization of glycans. Additionally, alterations in mucin-type O-glycans not only increase the activity and stability of immune cells but are also involved in the maintenance of intestinal mucosal immune tolerance. Although there is accumulating evidence indicating that mucin-type O-glycans play an important role in IBD, there is limited literature that integrates available data to present a complete picture of exactly how O-glycans affect IBD. This review emphasizes the roles of the mucin-type O-glycans in IBD. This seeks to provide a better understanding and encourages future studies on IBD glycosylation and the design of novel glycan-inspired therapies for IBD.

Keywords: glycans, inflammation, intestinal mucus, MUC2, microbiota, immunity

Introduction

IBD is a complex chronic inflammation of the gut, including Crohn's disease (CD) and ulcerative colitis (UC). In recent years, IBD has become a crucial yet difficult field of study among digestive diseases because of high incidence, difficulty of complete remission, and high risk of developing cancer in patients with a long history of the disease.¹ The etiological factor and pathogenesis of IBD are still unclear. However, its progression is associated with altered interactions between gut microbes and the intestinal immune system,^{2,3} where these factors result in an uncontrolled inflammatory response that ultimately culminates in irreversible tissue damage.

The body can protect itself from the threat of harmful microorganisms, auto-immune disorders, and intestinal inflammation through a variety of mechanisms. An important factor in these mechanisms is the mucus layer covering the intestinal epithelium, which not only provides lubrication for food passage but also protects the epithelial cells beneath it from microorganisms, and establishing a physical barrier against invasion of pathogens, toxins, and other environmental irritants.⁴⁻⁶ Defects in the structure of the mucus layer before disease progression have been reported in mouse models of IBD.⁷ Kirk et al redefined the colonic mucus system as

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a biological system composed of a loosely “niche” layer, which provides symbiotic bacterial growth, and the tightly “inner” layer, which acts as a barrier between bacteria and intestinal epithelium.⁸ Johansson et al found that the mucus layer of dextran sulfate sodium(DSS)-induced colitis mice allowed bacteria to pass through.⁹ In IL-10^{-/-} mice, a widely used mouse colitis model, it has been found that bacteria could pass through the mucus layer without reducing its thickness.¹⁰ These observations suggest that variation in the “inner” layer may be an initial event in the development of colitis.

The difference in the structure of the two mucus layers mainly lies in mucin, which is produced by surface goblet cells and is released steadily without any stimulation.¹¹ Mucins maintain a certain amount, mainly due to the special glycosylated-connectives on the surface,¹² because the gut itself does not produce any enzymes that can break these connections.¹³ These specific glycosylated links are mucin-types O-glycans, which account for up to 80% of the mass of the mucin molecules. O-glycans in the “niche” layer are loosely arranged, providing potential binding sites to support the growth of symbiotic and pathogenic

bacteria (Figure 1A).^{14,15} In addition, bacteria with mucolytic activity can release monosaccharides from mucin-O-glycans and metabolize them, thereby using O-glycans as a source of carbon and energy. The “inner” layer generated by the nets formed by O-glycans staggered on top of one another that adheres firmly to the cell and does not allow bacteria to penetrate keeps the commensal bacteria at bay, but when this first line of defense fails, bacteria come in contact with the epithelium.^{16,17} This event is likely the first in the inflammatory disease UC.^{10,18}

O-Glycans as the First Line of Defense in the Colon

O-glycans of the gastrointestinal tract are the first line of defense against external stimuli. Since O-glycans have been linked to the development of inflammatory colitis, there is the need to pay closer attention to this knowledge in the light of further research exploration. Here, we discuss the functions of the O-glycans in IBD by focusing on the structure of O-glycans, as well as the synthesis of Mucin-type O-glycans.

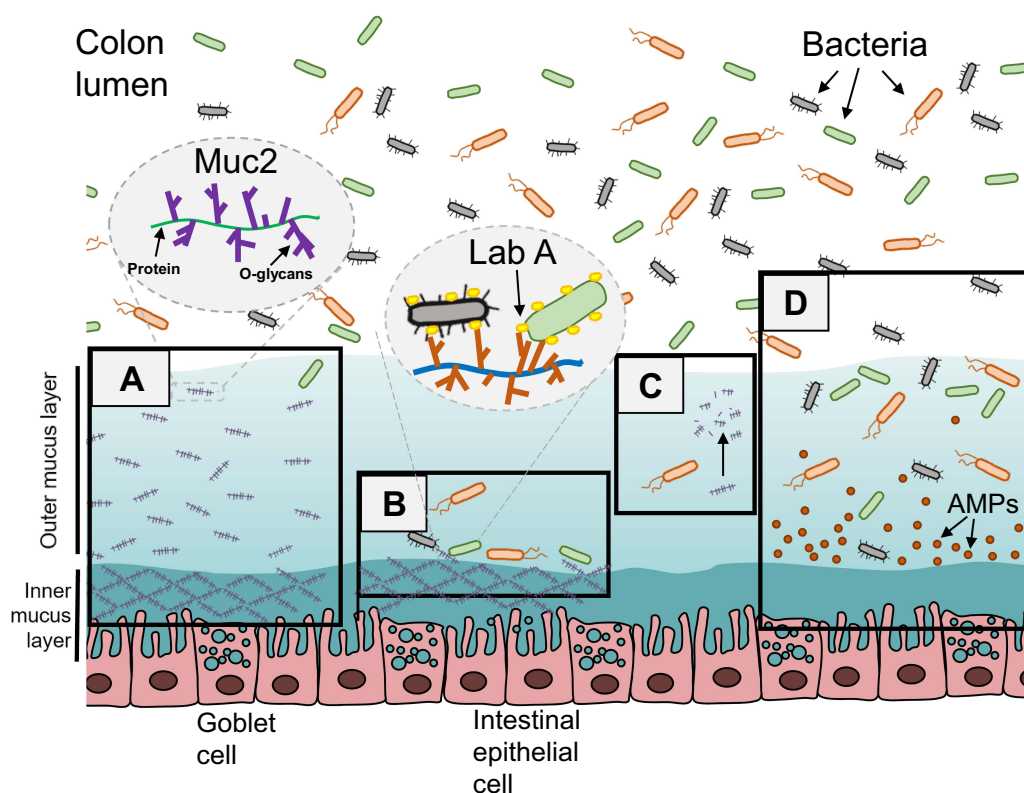


Figure 1 Interactions between mucin-type O-glycans and intestinal flora. (A) The glycan distribution of the outer mucus is loose, and the glycan of the inner mucus interweaves into a network to act as the mucous membrane barrier; (B) LabA interacts with a lactiNAC structure (GalNAcβ1-4GlcNAc) present on the mucin MUC5AC in the gastric mucosal layer; (C) Bacteria decompose glycans into oligosaccharides under the action of glycosidase and use it; (D) the antimicrobial peptides (AMPs) in the mucus are distributed in step concentration and inhibit bacteria pathogens in the mucus.

Synthesis of Mucin-Type O-Glycans

The synthesis of mucin-type O-glycans was shown to have significant associations with the glycosylation process, which is the most complex known post-translational modification of proteins. It has been discovered that the change of intestinal mucus is related to the change of this post-translational modification of mucin rather than the expression of mucus.¹⁹ Defects in O-glycosylation can lead to impaired mucin expression and mucosal barrier destruction, which in turn leads to microbial activation of inflammasomes such as caspase 1, interleukin (IL)1 β , and IL18, then drive inflammation and lead to severe spontaneous bacteria-dependent colitis.^{20,21} The glycosylation of proteins in mammals is mainly classified into three types: O-glycosylation, N-glycosylation, and glycosylphosphatidylinositol (GPI) anchors. O-glycosylation is the main form of glycosylation characterized by the ligation of oligosaccharides including mannose, xylose, and N-acetylgalactosamine (GalNAc) with hydroxyl groups of serine or threonine residues of polypeptide chains to form O-linked glycoproteins.^{22,23} O-linked N-acetylgalactosamine (O-GalNAc) glycan is particularly associated with mucosal sites such as the respiratory tract, urogenital tract, and gastrointestinal tract.^{24,25}

The first step of mucin-type O-glycosylation is to transfer N-acetylgalactosamine (GalNAc) to the hydroxyl group of Ser or Thr in the peptide sequence under the action of polypeptide GalNAc transferases (ppGalNAcTs), forming the Tn antigen structure, which is the substrate of glycosyltransferase.²⁶ Tn antigen can be extended to produce eight different core structures (Figure 2), and Core 1–4 are the four main core structures. Core 1 structure is formed by adding galactose to the Tn antigen. The addition of an N-acetylglucosamine to the GalNAc of Core 1 structure forms Core 2 structure. Core 3 structure is produced by the addition of an N-acetylglucosamine (GlcNAc) to the Tn antigen. Core 3 structure can be extended by the addition of a branching GlcNAc at the C6 hydroxyl group of GalNAc to form core 4 structure. Core 1 and 3 structures eventually form most mucin-type O-glycans.²⁷ The enzymes involved in core 1-derived O-glycans biosynthesis is core 1 β 1,3-galactosyltransferase (C1GalT1, or T-synthase), and loss of C1GalT1 efficiently removes all core 1-derived O-glycans in intestinal epithelial cells. Mice with intestinal epithelial-specific defects of core 1-derived

O-glycans show destruction of the inner mucus layer and develop spontaneous colitis, mainly in the distal colon.²⁸ These data indicate that the abnormal synthesis of mucosal O-glycan plays an important role in the pathogenesis of colitis, suggesting that it may be possible to treat inflammatory enteritis by repairing glycosylated synthesis.

Mucin-Type O-Glycans Disruption and IBD

Most structural investigations on O-glycans have been carried out on mucin-type O-glycans, which have been shown to have a great impact on human health and disease, particularly in IBD. To date, 21 different mucins have been identified, many of which are expressed in the gastrointestinal tract and can be broadly classified into three categories: secretory gel-forming (MUC2, MUC5AC, MUC5B, MUC6), secretory non-gel-forming (MUC7), and membrane-bound (eg, MUC1, MUC3, MUC4, MUC12, MUC13, MUC17).^{26,29–31} Studies have shown different patterns of O-glycosylation profile of MUC2 mucins in patients with UC and controls. Patients with active colitis have a decrease in several complex glycans and an increase in a subset of the smaller glycans. Remarkably, patients with strong alterations in the glycans pattern tended to have a more severe disease course.^{29,32–35} strikingly, MUC2 O-glycosylation distribution returns to normal after remission, demonstrating the potential of glycosylation profiling for predicting disease progression.²¹ Studies found that the loss of core 1-derived O-glycans seriously impairs the formation of the mucus layer, leading to severe spontaneous colitis in mice after 2 weeks of birth, and with time, the severity of the disease is gradually aggravated.^{28,35,36} Importantly, mucus depletion was observed before the onset of colitis in mice, supporting the etiological role of core 1-derived O-glycosylation deficiency and mucus barrier function in the pathogenesis of IBD.

A similar deficiency in mucus layer function has recently been found in human UC tissues.⁹ Interestingly, the loss of core 3-derived O-glycans resulted in increased intestinal mucosal permeability, elevated bacterial levels in the colon, and susceptibility to IBD, but no spontaneous disease was observed in C3GnT knockout mice.³⁷ Like C1GalT1, C3GnT is the only enzyme catalyzing the biosynthesis of core 3-derived O glycan in humans.³⁷ Different from humans, the core 3-derived glycans account for only 1% of the total amount of MUC2

Glycoprotein

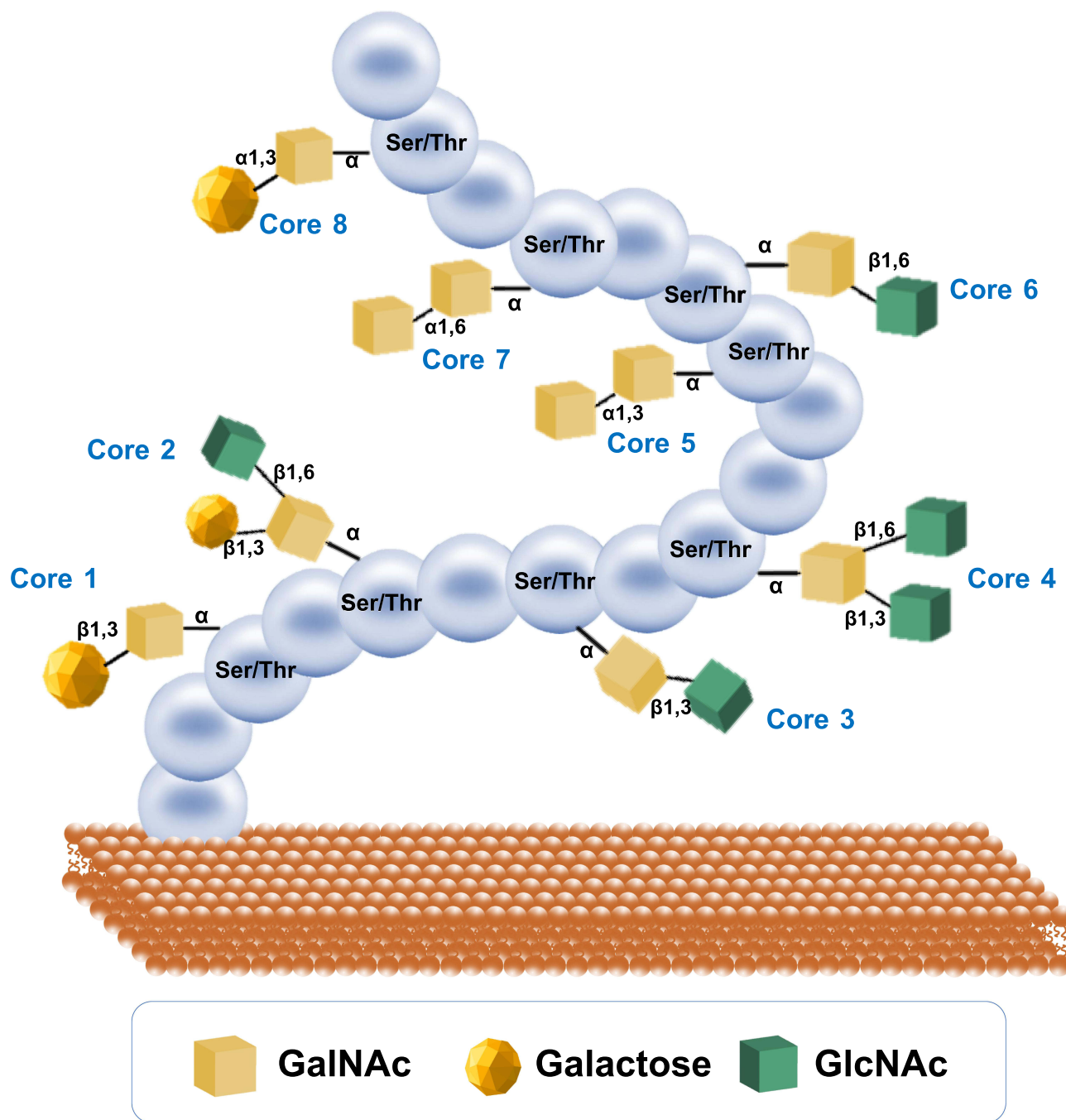


Figure 2 O-glycans structure core 1–8. Core 1 and core 2 structures are the most abundant out of the eight identified mammalian core structures. Core 1 structure is composed of galactose and is attached to the base GalNAc. Core 2 structure utilizes the core 1 structure complex with an addition of GlcNAc. Core 3 structure is produced by the addition of a GlcNAc to the TN antigen. Core 3 structure can be extended by the addition of a branching GlcNAc at the C6 hydroxyl group of GalNAc to form core 4 structure.

Abbreviations: GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine.

O-glycans in mice,³⁸ which may explain why the mice did not develop the spontaneous disease in the absence or loss of core 3-derived O-glycans.²¹ Core 1 and 3 derived

O-glycans in mucus have the same function, that is, to protect the gut from microbes and thus suppress inflammation.

Based on current studies, it is known that mucin-type O-glycans are the main factor that plays a protective role in the mucosal barrier; however, there are still many unanswered questions about how O-glycans interact with bacteria specifically to promote the integrity of the mucus barrier and intestinal flora homeostasis.

Interactions Between Mucin-Type O-Glycans and Microorganisms

Compared with healthy individuals, IBD patients have an unstable microbiota and develop significant bacterial malnutrition. In other words, patients with IBD have fewer anti-inflammatory bacteria and more bacteria with pro-inflammatory properties.³⁹ Remarkably, dysregulation of the microbiota is critical for IBD, because the intestinal habitat of the host can shape microbial community structure. In a study by John Rawls, the performance of reciprocal microbiota transplantations in germ-free zebrafish and mice resulted in a different microbial community structure between the zebrafish and mice due to different selective pressures applied within the gut habitat of each host.⁴⁰ Moreover, the differences between mice and human commensal bacteria support the idea that hosts can influence their bacteria population and diversity.^{41,42} Host factors significantly influence the selection of microbes in the gut. Therefore, the question that remains to be explored is how the host “dictates” the microbe’s population and diversity in the gut.

The mechanism that influences or participates in selecting the host’s bacteria remains unknown but might involve mucin-type O-glycans. Mucin, especially intestinal mucin MUC2 O-glycans, is probably helpful in selecting colonic flora of a particular species.³⁶ Additionally, specific structures on mucin-type O-glycans that bind to bacterial agglutinin-like adhesins probably also influence the composition of intestinal colonies.^{43,44}

Mucin-Type O-Glycans Regulate Microbial Composition

Intestinal flora disturbance can break the ecological balance between normal cells of the small intestine and various microorganisms, including probiotics and pathogenic bacteria, and is one of the leading causes of IBD. Microorganism species also change in mice with O-glycan modification, as shown in mice with Core-1 glycan deficiency in the small intestine.⁴⁵ These mice have higher levels of *Bacteroidetes* and lower levels of

Firmicutes than wild-type mice. This suggests that O-glycans have become increasingly important in shaping the composition of the microbial community in the gut. This has been demonstrated in mice lacking β 1, 4-N-acetylgalactosamine transferase 2 (B4galnt2), which catalyzes GalNAc formation to SDA^{-/-} Cad antigen.⁴⁶ Changes in intestinal bacterial community structure of B4galnt2-deficient mice were determined by 16S rRNA pyrosequencing.⁴⁶ It is reasonable to speculate that O-glycans may shape intestinal microbiota by forming other structures that “control” intestinal microbiota through glycosyltransferases. It is noteworthy that, glycan-deficient mice exhibit loss of colonic mucosa-associated commensal microorganisms without affecting the composition of microbial communities in the small intestine, resulting in spontaneous colitis.⁴⁷

O-glycans in mucus may affect the ability of *Escherichia coli* to thrive after they colonize.⁴⁸ Direct antimicrobial activity has been demonstrated in the gastric mucosa. Studies have shown that the barrier layer of the gastric mucosa is mainly composed of MUC6. MUC6 has been shown to carry α 1-4 linked N-acetylglucosamine (α 1-4 GlcNAc), with residues attached to core 2 branched O-glycans.⁴⁹ These glycans act as a natural antibiotic by inhibiting the growth of *Helicobacter pylori* via reducing the formation of cholesterol- α -D-glucopyranoside, an essential component of *Helicobacter pylori* cell wall.⁵⁰ It is plausible that similar antimicrobial O-glycans can exist in the gut. These results suggest that subtle differences in the structure of O-glycans can influence the composition of microbial species, which may influence susceptibility to intestinal diseases.

A growing body of evidence suggests that mucin glycosylation is critical to the biological and physical role in the gut through its regulation of the composition of mucus-associated microbiota.⁵¹ Interestingly, a recent publication by Bergstrom et al reports that mucus exhibits another functional aspect, showing that proximally derived O-glycosylated mucus encapsulates the fecal and microbiota to regulate the structure and function of the microbiota, as well as transcription in the colonic mucosa.⁵²

As the main bearer of intestinal mucus biological function, MUC2 O-glycans may be important for the selection of typical bacterial composition.³⁶ To this end, certain questions still linger: Does MUC2 glycan participate in the selection of symbiotic flora? How does the mucus lining of the colon relate to colitis and the human disease ulcerative colitis? All these queries require a more detailed

molecular exploration to enhance understanding of the structure and processing of MUC2 mucin and its related proteins that make up the colonic mucus layer.

Mucin-Type O-Glycans Promote Bacterial Adhesion

Pathogenic microorganisms breaking through the intestinal mucus barrier and adhering to the intestinal epithelium is considered to be the first step of pathogenic bacterial infection leading to inflammatory enteritis. Therefore, adhesion is closely related to the biological function of intestinal mucus. It has been shown that C1GalT1 deficient mice cause the loss of core 1-derived O-glycans to disrupt the attachment site of the intestinal flora and alter the composition of the microbiota.⁵³ Mucus serves a dual purpose for the microbiota. On one hand, it can encapsulate the gut microbes, limiting bacterial invasion to the deep intestinal mucosa. On the other hand, the carbohydrate structure of mucins, O-glycans, can also provide an initial site of attachment for bacteria including specific pathogens (Figure 1B).⁵⁴ Studies have shown that MUC1, a glycoprotein found in human breast milk, can be attached to some bacteria, such as *Campylobacter* and enterotoxigenic *Escherichia coli* (ETEC), and interferes with the colonization of these pathogens in the gut of infants.^{55,56} Glycans may play a major role in the increased ability of pathogenic bacteria to adhere to the intestine. Lectins that mediate the binding of bacteria to host cells are proteins that not only bind glycans on the cell surface but also bind free carbohydrates. *Campylobacter jejuni* can bind MUC2 through carbohydrate-lectin.^{57,58} Thus, it can be hypothesized that the binding site of *Campylobacter* to mucin may be O-glycans. In addition to *Campylobacter jejuni*, *Escherichia coli* can also bind MUC2.⁵⁹ Moreover, mucin-type O-glycans have been shown to inhibit EHEC adhesion to epithelial cells.⁶⁰ There are few studies on the specific sites of bacterial-binding mucosa, but some studies have emphasized the importance of O-glycans in bacterial adhesion. The 16S rDNA of colonic bacteria in mice deficient in core 3-derived O-glycans was found to be increased,³⁷ suggesting that the bacteria may increase mucosal adhesion and transfer through core 3-derived O-glycans.

Unlike pathogenic bacteria, commensal bacteria adopt a different strategy to adhere to mucus. Adherence of commensal bacteria to the intestinal mucus layer and occupation of the area can reduce the likelihood of adhesion of pathogenic microorganisms. Interestingly, studies have shown that the associated mucins are resistant to adhesion on the cell

surface. Sumiyoshi et al used chemical primers benzyl- α -GalNAc to disrupt mucin O-glycosylation and observed enhanced adhesion properties on the surface of differentiated corneal epithelial cells.^{61,62} Current studies have shown that symbiotic bacteria mainly promote their adhesion by secreting mucus-binding proteins.¹⁵ The specific mechanism of mucus-binding proteins is still unclear. It can be speculated that this process may be related to O-glycans.

Mucin-Type O-Glycans Inhibit the Virulence of Bacterial

Mucus simultaneously hosts trillions of microbes, but these microbes rarely cause infection in healthy mucus, suggesting that there is a mechanism in the mucus layer that regulates virulence. Changes in the glycosylation pattern of mucin in disease can trigger changes in bacterial phenotypes and alter the binding properties of mucin to microorganisms and their protective functions.⁶³ In addition to acting as a carbon source, mucin-type O-glycans can affect the expression of different genes involved in pathogenicity.⁶⁴ Recently, a study has shown that mucin-type O-glycans attenuate the virulence of *Pseudomonas aeruginosa* in infection by inhibiting the expression of virulence genes in *Pseudomonas aeruginosa*.⁶⁵

Similar observations were seen in *Campylobacter jejuni*, which can also use MUC2 as a signal to regulate the expression of genes associated with motility, adhesion, invasiveness, and toxin formation.⁶⁶ Furthermore, in the gastrointestinal tract, O-glycans downregulate the Hcp secretion island I-encoded type VI secretion system (H1-T6SS), which is associated with *Pseudomonas aeruginosa* chronic infections.⁶⁷ These studies provide new and exciting insights into the role of mucus and its derived O-glycans as a promising prophylaxis factor against bacterial virulence. Thus, mucin-type O-glycans are an effective host signal that can reduce the harm of microorganisms to the host by regulating its toxicity. These studies also demonstrate the potential of mucin-type O-glycan to regulate microbial virulence and provided a theoretical basis for further studies on the prevention of inflammatory enteritis by mucin-type O-glycans.

Bacteria Degrade Glycans as a Source of Nutrients

The permanent renewal of the mucus layer in the gut represents an important ecological niche that is rich in nutrients and provides a particularly beneficial environment for commensal bacteria. Commensal bacteria are

typically characterized by abundant glycosylating enzymes, such as glycosidases. When dietary glycans are scarce, bacteria with mucolytic activity can degrade mucin-type O-glycans and metabolize them (Figure 1C),⁶⁸ thus this greatly increases the energy extracted from the food. Moreover, these sugars can also be used by nearby bacteria.¹⁵ By analyzing the mucin-type O-glycans in the feces of patients with UC, researchers found that the ratio of mucin-type O-glycans in the feces of patients with UC was significantly higher than that of healthy people, suggesting that the utilization of O-glycans by intestinal flora in patients with UC was impaired.⁶⁹ Indeed, various anaerobic bacteria species of gut microbiota, such as *Akkermansia muciniphila*,^{70,71} *Bacteroides thetaiotaomicron*,^{72,73} *Bifidobacterium bifidum*,^{74–76} *Bacteroides fragilis*,^{77–79} *Ruminococcus gnavus*,^{74,80} and *Ruminococcus torques* are now known as mucin-degrading specialists (Table 1).⁷⁴ *Bacteroidetes* are one of the most abundant symbiotic genera in the human colon.⁸¹ *Bacteroides* play an important role in the balance of intestinal flora in inflammatory enteritis, colorectal cancer, and other intestinal diseases.^{82,83} The monosaccharides released during the degradation of mucin-type O-glycans can also serve as chemical cues to help pathogens sense their environment and adapt accordingly.

Bacteria recognize dense mucin O-glycans structures and degrade individual glycan to produce short-chain fatty acids (SCFA), which diffuse through the internal mucus layer and provide an energy source for intestinal epithelial cells.⁸⁴ The harvesting of degraded glycans for their metabolism provides colonization advantages for bacteria. At the same time, this glycan degradation allows oligosaccharides to be provided to non-mucin-degrading bacteria as part of the microbial food chain, thus sustaining the entire intestinal flora.^{85,86}

Bacteria Promote O-Glycans Synthesis and Influence Its Composition

The symbiotic bacteria can affect the composition of goblet cells in the small intestine and their secreted product mucin, thereby increasing the resistance of Intestinal to pathogenic bacteria. Transcriptions of glycosyltransferases involved in core 2 O-glycan biosynthesis were observed during experimental *mycobacterium tuberculosis* infection.⁸⁷ A further notice of the regulatory role of glycosyltransferases in bacterial infection highlights the importance of glycans and its biosynthetic pathway as a possible new target for the regulation of IBD. A study has shown that differences in mucin composition are also influenced by the presence or absence of microbial flora in the intestine.⁸⁸ *B. thetaiotaomicron* metabolizes acetate and enhances goblet cell differentiation, resulting in

Table 1 Glycan-Degrading Bacteria Colonizing the Human GI Tract

Bacterial Species	Mucin Tested	Enzymatic Activities
<i>Akkermansia muciniphila</i> ^{70,71}	pPGM type III	α -Galactosidase; β -galactosidase; α -L-fucosidase; β -glucosidase; α -mannosidase; α -galactosidase; β -D-fucosidase; α -N-acetylgalactosaminidase; β -N-acetylgalactosaminidase; β -N-acetylglucosaminidase
<i>Bacteroides thetaiotaomicron</i> ^{74–76}	Purified O-glycans from PGM type III	α -L-fucosidase; endo- β -N-acetylglucosaminidase; endo- β -galactosidase; α -mannosidase
<i>Bifidobacterium bifidum</i> ^{75–77}	PGM type III	Endo- α -N-acetylgalactosaminidase; α 1,2-L-fucosidase
	pPGM*	Blood group H-degrading activity; sialidase; β -galactosidase; β -N-acetylgalactosaminidase; β -N-acetylglucosaminidase; sialate O-acetyltransferase; glycosulfatase
<i>Bacteroides fragilis</i> ^{77–79}	PGM type III	endo- α -N-acetylgalactosaminidase; α 1,2-L-fucosidase
<i>Ruminococcus gnavus</i> ^{74,80}	pPGM type III pPGM*	α -L-fucosidase; α 2,3-sialidase Blood group B-degrading activity; blood group H-degrading activity; sialidase; β -galactosidase; sialate O-acetyltransferase
<i>Ruminococcus torques</i> ⁷⁴	pPGM*	Unknown

Abbreviations: PGM, pig gastric mucin; PGM*, pig gastric mucin; pPGM, purified PGM, purified according to Miller and Hoskins' method (Miller and Hoskins, 1981).

increased goblet cell number and mucin gene expression in rat colon. In addition, mucin glycosylation is substantially changed after colonization by *B. thetaiotaomicron*, that is, the content of N-acetylglucosamine modified glycan decreased.⁷³ It can be hypothesized that there is a mechanism by which the bacterium may require low levels of N-acetylglucosamine modified glycosylation to attach and colonize the gut. *F. prausnitzii* consumes the acetate produced by *B. thetaiotaomicron* to produce butyrate.⁷³ SCFA, especially butyrate, has an inducer effect on mucin synthesis in vitro.^{89–91} Bacteria not only indirectly affect mucin-type O-glycans by affecting goblet cell secretion and mucin synthesis but can also directly change the diversity and abundance of O-glycans. Gastritis induced by *Helicobacter pylori* is accompanied by a sharp but transient decrease in the diversity and relative abundance of O-glycans in the mucosa of rhesus monkeys.⁹² Porcine dysenteric spirochete infection can regulate mucin glycosylation and induce increased Core 2-derived O-glycans expression in the porcine colon. In addition, one of the prominent glycosylation changes observed in pigs infected with *Pterospira dysenteriae* was an increase in the abundance of shorter glycans.⁹³ Patients with active UC also show elevated levels of small glycan subsets, which are thought to be associated with inflammation and disease severity.²¹ These results suggest that gut bacteria not only sense the composition of mucus and passively adapt to it but also modify mucin-type O-glycans to make the gut more suitable for their own survival.

Mucin-Type O-Glycans and Immune Response

People with immune deficiency or abnormalities have a higher incidence of IBD and higher levels of intestinal inflammation.⁹⁴ Both the innate and adaptive immune responses play an indispensable role in the immune mechanism of intestinal inflammation caused by O-glycan abnormalities. Intestinal mucus is the first line of defense against pathogens and plays an important role in regulating the homeostasis of microbial flora, clarifying pathogens, and maintaining immune tolerance to foreign food antigens.^{95,96} Mucus is a complex fluid that is rich in mucin glycoproteins, which work together with antimicrobial epithelial AMPs to create a gradient of antimicrobial mucus that prevents bacteria from reaching the epithelial cells (Figure 1D).

The glycans are also inseparable from the host's second line of defense against pathogens, which is the immune cells in the intestinal mucosa that recognize “good” and “bad” bacteria. Mucin-type O-glycans can bind to microorganisms, and the structure and negative charge of glycoproteins are conducive to encapsulating bacteria, thus enhancing host interaction as observed in the early stages of IBD.^{97,98} This indicates that O-glycans can affect the intestinal immune system's recognition of intestinal “non-self” substances. In addition to encapsulating bacteria, mucinous O-glycans also play an important role in the recognition of microorganisms by host immune cells. This is confirmed in a study of MUC1, where an increase in glycosylated MUC1 could lead to continuous activation and accumulation of various cells in the innate immune system, resulting in chronic inflammation.⁹⁹ M cells in the small intestine, specialized cells that recognize antigens, are characterized by a mucin-free layer and a thin glycocalyx. O-glycans play an indirect role in the interaction between M cells and intestinal antigen by regulating the volume of glycocalyx.¹⁰⁰ Low glycosylated MUC1 not only provides neoantigen determinants to B cells but also can be processed and presented to T cells more efficiently. This may promote the movement of adaptive immune cells to the inflammatory site.⁹⁹ In turn, intestinal inflammation also promotes the glycosylation of MUC1 in colonic epithelial cells.¹⁰¹ The cause of glycosylation of MUC1 is unknown but may be the result of glycosyltransferase action.

Intestine-related lymphoid tissues directly participate in and regulate intestinal mucosal immune regulation, and are also a predominant part of the body's immune system, including a large number of macrophages, monocytes, natural killer cells (NK cells), dendritic cells (DCs), T cells, and B cells (Table 2). O-glycans of MUC1 sialyl-Tn antigen may affect the immune response by inhibiting the maturation of DCs and the activity of NK cells.^{102,103} Removal of a glycan from the surface of DCs results in increased endocytosis.¹⁰⁴ Altered genes expression associated with neutrophils, macrophages, mast cells, eosinophils, NK cells, and DCs are observed in Muc2 knockout mice,¹⁰⁵ suggesting that these immune cells are probably associated with mucin-type O-glycans.

Cells of the Innate Immune System

Macrophages and monocytes play an important role in the innate immune response to the pathogenesis of IBD. Macrophages have the ability to phagocytosis and digest

Table 2 Intestinal Immune Cells Associated with O-Glycan

Type of Cell	Cell Function	Alter of Glycan	Immune Association
M cells ⁹⁹	Take up antigens from the symbiotic bacteria to prevent abnormal immune activation against parasites	A relatively thin glycocalyx compared with enterocytes	Glycans promote the contact of M cells with antigens
Macrophages ^{28,107}	Phagocytosis and digestion of “bad” foreign substances	Core 1-derived O-glycans deficiency mice	Infiltration of macrophages in the colon tissues; interfere with the recognition of macrophages to apoptotic cells
NK Cells ^{102,100}	Neutrophil recruitment	MUC2 deficiency mice	The activity of NK cells
DCs ^{106,104}	Presentation of antigens and initiation of specific immune responses	Cell surface glycan deficiency	Inhibition of DC maturation; increased endocytosis
T cells ^{28,109,110}	Two subtypes: pro-inflammatory and anti-inflammatory	Core 1-derived O-glycans maturation disorder	Pro-inflammatory T cell proliferation; increased pro-inflammatory factors;

“bad” foreign substances and express pro-inflammatory (M1-like) or anti-inflammatory (M2-like) phenotypes by sensing the microenvironment.¹⁰⁶ Patients with CD have increased numbers of M1 macrophages and monocytes. Other studies have also found increased expression of macrophage-related cytokines in the inflammatory colon tissue.¹⁰⁷ In the mouse model of colitis, there is an inextricable relationship between macrophages and O-glycans. Studies have shown that the number and function of macrophages and monocytes are altered in colitis in mice with core 1-derived O-glycans deficiency. Significant infiltration of monocytes and macrophages in the colon tissues of core 1-derived O-glycans deficient mice is observed following the onset of colitis. Moreover, there is a significant increase in macrophages in mice with core 1-derived O-glycans deficiency.²⁸ This suggests that these cells are the primary cell types capable of sensing early microbial invasion and triggering inflammation. By knocking out core 1 β -1,3-galactosyltransferase-specific molecular chaperone (Cosmc) in macrophages, which is essential for the synthesis of mucin-type O-glycans. It was found that O-glycans can interfere with the recognition of macrophages’ mucin domain-containing molecule 4 to apoptotic cells, thus affecting the phagocytic function of macrophages, leading to incomplete clearance of apoptotic cells by macrophages.¹⁰⁸

Macrophages can in turn influence mucin glycosylation. For example, O-glycans alterations of MUC1 are driven by M2 macrophages through overexpression of glycosyltransferase ST6GALNAC1. The modified enzyme

added sialic acid to the O-linked GalNAC residues to form the tumor-associated sialic acid Tn O-glycans, and this abnormal glycosylation of MUC1 occurs in chronic inflammation including UC.¹⁰⁷ Moreover, antigen-presenting cells (APCs), DCs, and macrophages can promote the differentiation of CD4⁺T cells into helper T(Th)2 cells by phagocytosis and processing antigen, and the cytokine IL-13 secreted by these cells drives intestinal goblet cell proliferation and increases the levels of mucins Muc2 and Muc5a.^{109,110} The close interaction between macrophages and monocytes and mucosal O-glycan suggests that innate immune responses are involved in O-glycan-induced colitis.

Cells of the Adaptive Immune System

T cells play a vital role in the pathogenesis of IBD. The imbalance between pro-and anti-inflammatory T cells may be responsible for the initiation and aggravation of the inflammatory process in patients with IBD.¹¹¹ In patients with active IBD, pro-inflammatory T cell infiltration is increased in intestinal inflammatory tissues, while anti-inflammatory T regulatory (Treg) cell activity is impaired.¹¹¹ Both CD and UC are characterized by significant expansion of inflammatory memory CD4⁺ T cells in the inflamed intestine.^{112,113} In the process of intestinal inflammation, Core-2 β 1, 6-N-acetylglucosamine aminotransferase (C2GNT) 1 is down-regulated, resulting in Core 1-derived O-glycans maturation disorder on the surface of CD4⁺ T cells. Immature Core 1-derived O-glycans bind to galectin-4, a member of the endogenous lectin

family, which then binds to the glycan structure and controls immune cellular processes, leading to CD4⁺ T cell expansion and more severe intestinal inflammation.¹¹⁴ Interestingly, another paper found that a reduction in Core-1 O-glycosylation leads to an increase in the Foxp3⁺ Treg cell, which is microbiota-induced T(regs) cells, that inhibits immune-inflammatory responses.^{115,116} There is a significant increase in microbiota-induced Treg cells (Figure 3) during the disease onset phase (Week 3) of colitis mice specifically deficient in colonic epithelial core 1-derived O-glycans.^{28,117} There is also no significant increase in the number of NK cells in mice with colonic epithelial core 1-derived O-glycans specific deletion.²⁸ During the progression of colitis to the relapse stage (Week 9), symptoms in mice with combined deficiency

of O-glycans and lymphocytes of core 1 source are milder than those in mice with O-glycans deficiency of core 1 source only,²⁸ and pro-inflammatory T cell subsets proliferate significantly.¹¹⁷ This implies that lymphocytes may play an important role in the later stages of colitis.

Although colitis caused by core 1-derived O-glycans deficiency in mice is an inflammatory response driven by innate immunity, not adaptive immunity,²⁸ many changes in Th1 and Th2 cells have been observed in glycan knock-out mouse enteritis models. Researchers created C3GnT^{-/-} mice that lack core 3-derived O-glycans, reduced colon-specific MUC2 protein, and are highly sensitive to colitis. In the absence of DSS, the pro-inflammatory cytokines IL-2, IFN- γ , and tumor necrosis factor (TNF)- α , which are mainly secreted by Th1 cells, are slightly

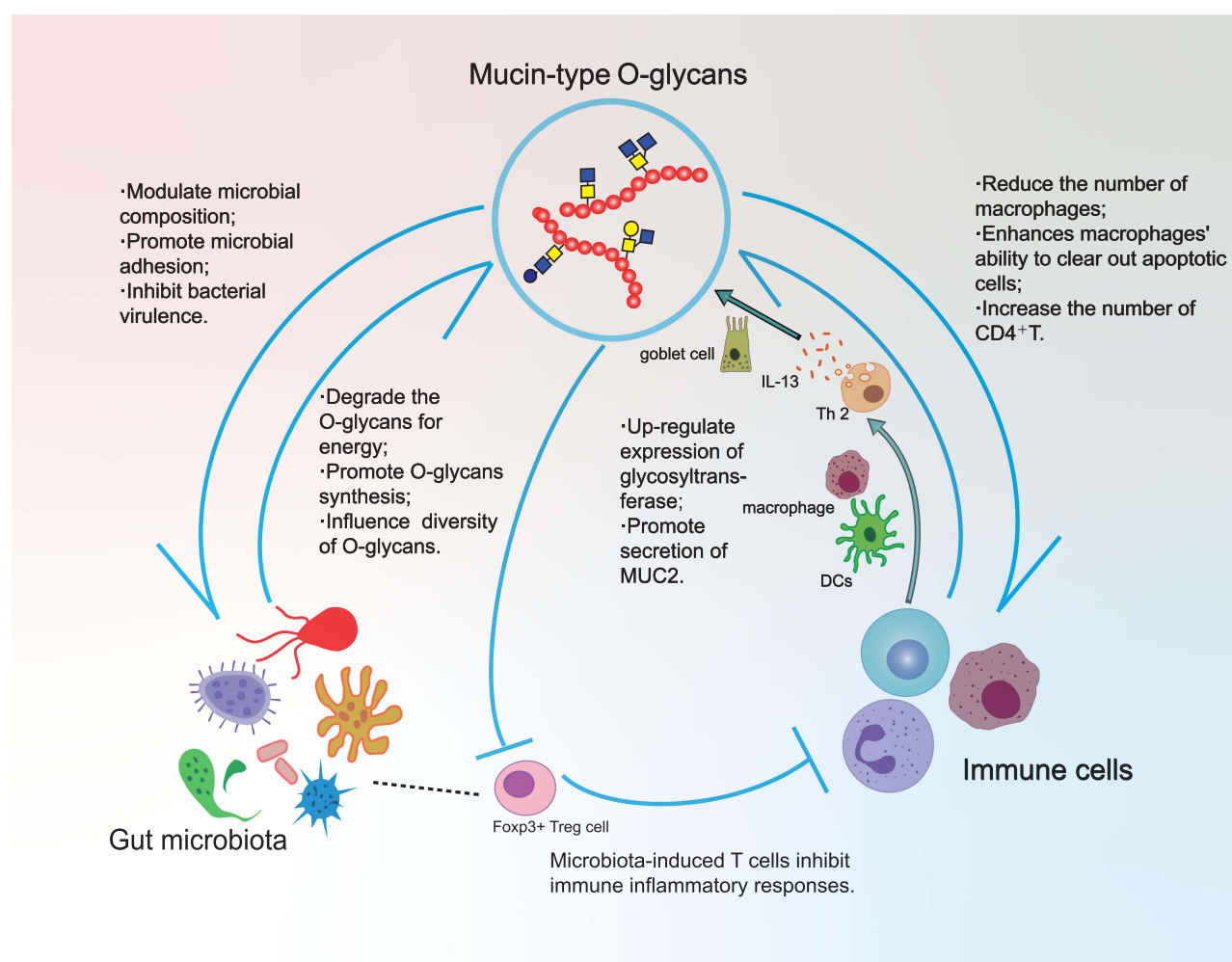


Figure 3 Association between mucin-Type O-glycans, microbiota, and immune cells. O-glycans regulate microbial composition, promote microbial adhesion, and inhibit bacterial virulence. Conversely, intestinal flora can degrade glycan for energy and also affect the composition of glycan. O-Glycans not only affect the number of various immune cells in the body, such as macrophages and CD4⁺T cells but also enhance the ability of macrophages to remove apoptotic cells. Immune cells mainly secrete inflammatory factors to promote mucin glycosylation. Lack of glycosylation can also affect microbial-related regulatory T cells, thereby inhibiting immune responses.

increased in mucosal lymphocytes of C3GnT^{-/-} mice, but are far from developing into Th17 or Th2 immune colitis.³⁷ This suggests that the interaction between O-glycans and lymphocytes is not the main cause of immune colitis. Again, the assertion that the adaptive immune response does not play an important role in the process of colitis caused by O-glycans deficiency is demonstrated.

The glycosylation of the lymphocytes themselves is also associated with colitis. Human glycosyltransferase 1,2-fucosyltransferase (FUT1) transgenic mice are an IBD mouse model with severe glycosylation abnormalities on the lymphocyte surface. A significant reduction in lymphocytes is found in FUT1 transgenic mice, particularly CD4+T cells (reduced by 90%) and CD8+T cells. In addition to the decrease in the number of T cells, the nature of T cells also changes. CD3 is reduced on the surface of T cells, and a large number of T cells show both CD4 and CD8 positive markers.¹¹⁸ This phenomenon indicates that abnormal glycosylation of lymphocytes can lead to T lymphocyte depletion and maturation disorders, leading to colitis. In addition, mice with a specific loss of lymphocyte O-glycans had reduced thymus mass, abnormal thymus structure, and bone marrow hypoplasia.¹¹⁸ Although current data shows that O-glycan-induced colitis is not adaptive immune-driven, O-glycans is one of the important factors affecting the change of T cell subsets.

Conclusions

Several studies have shown that O-glycan is involved in the pathogenesis and development of IBD. Variable O-glycosylation of mucins by large amounts of glycosyltransferases is important for the maintenance of intestinal mucosal barrier function, bacterial-binding capacity, and immune homeostasis. In future research, more attention should be given to the changes in mucosal O-glycan in the mechanism of intestinal flora and immune dysregulation. The intensity of glycosylation changes correlates with the degree of inflammation, suggesting the potential of the glycosylation spectrum to predict IBD progression. In addition, there is the need to develop better experimental animal models that truly reflect human conditions. This means the models should carry human mucins with human glycosylation patterns, which requires some genetic modification in mice. Future explorations should also include the investigation of the association between O-glycan and the microbiota

through appropriate, well-designed, and targeted clinical studies.

Acknowledgments

This research was funded by the National Natural Science Foundation of China (grant no. 32000903), and the Scientific Research Foundation of Jiangsu University for Senior Professional Talents (20JDG48).

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest in this work.

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