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Intestinal epithelial glycosylation in homeostasis and gut microbiota interactions in IBD

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Abstract

Inflammatory bowel disease (IBD) affects 6.8 million people globally. A variety of factors have been implicated in IBD pathogenesis, including host genetics, immune dysregulation and gut microbiota alterations. Emerging evidence implicates intestinal epithelial glycosylation as an underappreciated process that interfaces with these three factors. IBD is associated with increased expression of truncated O-glycans as well as altered expression of terminal glycan structures. IBD genes, glycosyltransferase mislocalization, altered glycosyltransferase and glycosidase expression and dysbiosis drive changes in the glycome. These glycan changes disrupt the mucus layer, glycan–lectin interactions, host–microbe interactions and mucosal immunity, and ultimately contribute to IBD pathogenesis. Epithelial glycans are especially critical in regulating the gut microbiota through providing bacterial ligands and nutrients and ultimately determining the spatial organization of the gut microbiota. In this Review, we discuss the regulation of intestinal epithelial glycosylation, altered epithelial glycosylation in IBD, and mechanisms for how these alterations contribute to disease pathobiology. We hope that this Review provides a foundation for future studies on IBD glycosylation and the emergence of glycan-inspired therapies for IBD.

Abstract

Intestinal epithelial glycosylation is influenced by host genetics, the environment and the gut microbiota. In this Review, Kudelka et al. describe the functions of epithelial glycans and discuss the role of epithelial glycosylation in Crohn’s disease and ulcerative colitis.

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Author contributions

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Competing interests

The authors declare no competing interests.

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, affects 6.8 million people globally and is thought to be the result of inappropriate immune activation in response to the gut microbiota in susceptible individuals[1–5]. Multiple factors contribute to disease pathogenesis, including genes, the environment, the gut microbiota and the immune system. Although early studies focused on identifying host factors that contribute to IBD pathogenesis, advances in next-generation sequencing have led to an emerging interest in the gut microbiome.

The human gut microbiota consists of 10^{11} bacteria, 10^{8} archaea, 10^{8} viruses and 10^{6} fungi per gram of stool and has diverse roles in regulating metabolism, immunity and health [6–8]. Although many bacterial genes are shared across individuals, gut microbiota composition (such as the relative levels of specific species) is highly diverse between people as well as in different parts of the gut[9,10]. The gut microbiota in human IBD demonstrates reduced diversity, a shift in the abundance of specific taxa, and altered functional capacity[7]. Patients with IBD have a reduction in levels of *Bacteroides*, Firmicutes, Clostridia, Ruminococcaceae, *Bifidobacterium*, *Lactobacillus* and *Faecalibacterium prausnitzii* and an increase in levels of Gammaproteobacteria, *Escherichia coli* and *Fusobacterium* species[7]. Functionally, IBD is associated with loss of protective factors, such as short-chain fatty acids (a major nutrient source for colonocytes), as well as with an increase in pro-inflammatory factors, such as pathways involved in auxotrophy (a feature of pathobionts), sulfate transport, oxidative stress and toxin secretion, in addition to changes in lipopolysaccharide (LPS) structure that are critical for establishing an inflammatory versus tolerogenic milieu[7,11,12].

A major question is how genes, the environment and the gut microbiota interact to contribute to IBD pathogenesis. Epithelial glycans **[G]** are a major component of the intestinal mucosa, facilitate interactions between the gut microbiota and the intestinal epithelia, and are regulated by host genetics and the environment[13–16]. Thus, they are well-placed to integrate host, microbial, and environmental cues. Epithelial glycans provide ligands and nutrients and induce host signalling to regulate the gut microbiota, and are altered in IBD[15,17–27]. These alterations to glycans result in several changes that contribute to IBD pathogenesis, including compromised mucin 2 (MUC2) barrier function, disrupted glycan–lectin **[G]** interactions, pathological host-microorganism interactions and altered mucosal immunity[15,16,19,22,23,28–32]. We review the regulation of epithelial glycans, their contribution to disease pathogenesis, and opportunities to target host glycans in IBD. Specifically, we discuss mechanisms of protein glycosylation in general, the normal glycan structures in the gut and their regulation, as well as glycan alterations in IBD and how those alterations arise. Additionally, we compare glycome alterations in IBD and colorectal cancers and discuss downstream effects of altered glycosylation in IBD, including in mucin biology, glycan–lectin interactions, host–microorganism interactions, and immune regulation. Finally, we discuss translational opportunities of glycobiology in IBD. Although the gut contains a variety of types of microorganisms, we primarily focus on glycan–bacteria interactions.

Protein glycosylation

Glycans, or polysaccharides, are an abundant post-translational modification found on the cell surface, in intracellular membranes, and in the cytosol (Fig. 1, Box 1). In mammals, combinations of ten monosaccharides (galactose (Gal); glucose (Glc); mannose (Man); fucose (Fuc); xylose (Xyl); N-acetylgalactosamine (GalNAc); N-acetylglucosamine (GlcNAc); glucuronic acid (GlcA); iduronic acid (IdoA); and sialic acids (Sia; this is a family of monosaccharides, the most common of which is N-acetylneuraminic acid (Neu5Ac))) are attached in branched and linear patterns with α or β glycosidic linkages to form thousands of unique structures, sometimes called the glycocode[33–35]. In vertebrates, this complex system is regulated by >700 genes (called glycogenes), accounting for 1–2% of the genome, that include those encoding glycosyltransferases, sugar transporters and glycan-binding proteins[36]. In addition, many more genes indirectly regulate glycosylation[37]. Although glycans can form free structures, they are typically attached to other macromolecules to form glycoconjugates, including glycoproteins and glycolipids, which are either membrane-associated (glycolipids and glycoproteins) or cytosolic (glycoproteins)[38] (Fig. 1).

Glycans that are attached to proteins regulate protein stability and oligomerization, and serve as ligands for glycan-binding proteins, including antibodies and lectins. Functionally, glycans have essential roles in a variety of biological processes, such as cell adhesion, cell growth, cell death, cell migration, embryonic development, homeostasis and immunity. Deletion of genes encoding major classes of glycans, such as N-glycans or O-glycans, is typically embryonically lethal, whereas disruption of monosaccharides within terminal epitopes of glycan chains, rather than monosaccharides directly attached to the protein backbone, results in diverse phenotypes[39].

N-linked and O-linked glycans

N-linked and O-linked glycans, a major component of both the cell surface glycocalyx and the secreted glycoproteome[38,40], constitute the two major classes of glycoproteins, and are defined by their glycan–protein linkage. N-linked glycans are linked by the nitrogen of asparagine to form a $Man_3GlcNAc_2Asn$ core in the Asn-X-Ser/Thr sequon **[G]** and are added en bloc to proteins in the endoplasmic reticulum before being processed in the endoplasmic reticulum and Golgi (Supplementary Figure 1). O-linked glycans are linked to the oxygen atom of serine or threonine in glycoproteins. Mucin-type O-glycans, which are attached to mucins (a family of glycoproteins), are the most abundant membrane-associated glycans[41,42]. Unlike N-glycans, mucin-type O-glycans (hereafter referred to as O-glycans unless otherwise specified) do not attach to a conserved sequon; however, machine learning algorithms predict O-glycosylation sites reasonably well[41]. Unlike N-glycan biosynthesis, O-glycans are added progressively as glycoproteins traverse the Golgi (Supplementary Figure 2).

N-glycan biosynthesis occurs in the endoplasmic reticulum and the Golgi apparatus (Supplementary Figure 1). In brief, a 14-sugar precursor glycan, $Glc_3Man_9GlcNAc_2$, is synthesized and transferred to the nascent polypeptide chain and deglucosylated to a monoglucosylated species. The protein folding and quality control machinery evaluates the

protein; properly folded proteins are marked by removal of the last glucose. In one synthetic pathway, mannose residues are removed followed by addition of GlcNAc by GlcNAc-transferase 1 (GnT1) and then eventually by GnT2 to form a biantennary $GlcNAc_2Man_3GlcNAc_2$ structure. This structure is then modified by the addition of a core fucose to the first GlcNAc. This structure can be further branched, modified by addition of a bisecting GlcNAc, and/or modified by extension with galactose, GlcNAc, fucose and/or sialic acid. N-glycans that terminate in mannose (high-mannose), galactose or sialic acid (complex) or some combination (hybrid) can be found on the cell surface.

Mucin-type O-glycan biosynthesis is described in detail in Supplementary Figure 2. Mucin-type O-glycans consist of hundreds to thousands of unique structures built out of nine core structures that are attached to >80% of the cell surface proteins and secreted proteins[33,34,41,43–45] (Fig. 2). Cores 1 – 4 are the most common cores, with expression of core 1 (also known as T antigen and Thomsen–Friedenreich (TF) antigen; Galβ1,3GalNAc) and 2 [GlcNAcβ1,6(Galβ1,3)GalNAc] in all cell types and core 3 (GlcNAcβ1,3GalNAc) and 4 [GlcNAcβ1,6(GlcNAcβ1,3)GalNAc] exclusively in the intestinal epithelium (Supplementary Figure 2). All mucin-type O-glycans are built from a single GalNAc attached via α linkage to serine or threonine in a glycoprotein by a family of 20 polypeptide GalNAc-transferases to form GalNAc-α-Ser/Thr (also known as Tn antigen)[46]. These enzymes have been extensively studied and can be differentiated according to expression patterns (whether their expression is ubiquitous or tissue-specific) and by their ability to add GalNAc to either an unmodified or a Tn-antigen-containing glycopeptide[46]. Although Tn can be expressed in diseased cells, healthy tissues extend Tn beyond a single GalNAc to form the core O-glycans[35,47,48]. T-synthase (encoded by *C1GALT1*), which requires the molecular chaperone COSMC to form a properly folded active glycosyltransferase, extends a single Tn antigen by adding galactose to Tn to form core 1 (Supplementary Figure 2)[49]. Similarly, core 3 β1,3-N-acetylglucosaminyltransferase (C3GnT) adds GlcNAc to Tn to form core 3[50]. Either core 1 or core 3 can be extended by the addition of a branching GlcNAc at the C6 hydroxyl group of GalNAc to form core 2 or core 4 structures, respectively[35]. These structures can then be further modified by addition of galactose, GlcNAc, fucose, sialic acid and sulfate to form glycans of various lengths that are decorated by a variety of terminal epitopes. Although some epitopes are unique to O-glycans (for example, CHO-131 (a core 2 structure terminating in sialyl-Lewis x, a ligand for P-selectin)), many terminal extensions are present on both O-glycans and N-glycans, including blood group antigens and Lewis antigens **[G]** (Supplementary figure 3).

ABO blood group antigens

ABO blood group antigens are formed by modification of terminal galactose residues by addition of fucose in an α1,2 linkage to form Fucα1,2Gal (also known as the H antigen) (Supplementary figure 3a)[51]. Whereas *FUT2* (historically *Se* or *secretor*) encodes the H antigen in epithelial linings of the gastrointestinal tract, *FUT1* encodes these structures on erythrocytes[51,52]. The A and B blood group antigens are formed by addition of GalNAc and Gal in an α1,3-linkage to the galactose of H antigen by the A (A3GALNT) and B (A3GALT1) transferases, respectively. By contrast, individuals with O blood type have a

functional *FUT2* but lack a functional A or B glycosyltransferase. In that case, H antigen is produced, but it is not modified to form A or B antigens.

Lewis antigens

Lewis antigens are present on glycoproteins and glycolipids on red blood cells and gastrointestinal epithelia or their secreted products. These structures are only made in the gastrointestinal tract and are not red blood cell precursors. Thus, gastrointestinal epithelia release glycolipids that are adsorbed on the red blood cell surface from plasma. Although many healthy individuals express Lewis antigens, these structures are often upregulated in inflammatory states[53,54]. Lewis antigens are formed by addition of one or two fucose residues to a terminal Gal-GlcNAc structure with either a β1,3 linkage (Galβ1,3GlcNAc, type 1 chain) or a β1,4 linkage (Galβ1,4GlcNAc, type 2 chain). All Lewis structures have a fucose residue attached to the subterminal GlcNAc. If this residue is the sole fucose, then the structure is called Lewis^a (for type 1 chains) or Lewis^x (for type 2 chains). If a second fucose is also present on the terminal galactose, forming a difucosylated structure, the Lewis structure is called Lewis^b (for type 1 chains) or Lewis^y (for type 2 chains), respectively (Supplementary figure 3b)[51]. If only one fucose is added, it is first attached to the GlcNAc. By contrast, if two fucoses are added, the first fucose is attached to the galactose, rather than to the sub-terminal GlcNAc, forming the H antigen[51,55,56]. Enzymatically, *FUT2* (*Se*), which encodes the H antigen, first synthesizes H (for Lewis^b and Lewis^y). Then, FUT3 adds fucose to the subterminal GlcNAc. Alternative fucosyltransferases are sometimes involved[51,55,56].

The healthy gut glycome

The intestinal epithelial glycome is developmentally and regionally regulated by host and environmental factors[13,57,58]. Mucin-type O-glycans are the major class of glycan in the gut, accounting for 80% of the mass of human MUC2, the most abundant intestinal mucin [59–61]. In humans, the stomach and duodenum contain core 1 and core 2-based structures, the rest of the small intestine contains core 3-based structures, and the colon contains core 3 (primarily in the sigmoid colon; Figure 3a) and core 4-based structures[62]. These structures are further modified by Gal, GlcNAc, GalNAc, fucose, sialic acid and sulfate and regulated by genes implicated in IBD(Fig. 3)[62,63]. Terminal epitopes follow a rostral–caudal gradient, with increasing levels of sialic acid, sulfate and Cad antigen **[G]** and decreasing levels of fucose, blood group antigens and Lewis antigens from the stomach to the rectum[57]. Although this gradient is present across species, the direction appears to vary. In contrast to humans, mice exhibit an increase in fucose and a reduction in charged species from the stomach to the rectum[64]. As Lewis and ABO blood group antigens (which are expressed in the proximal gut) vary across the human population, the proximal gut glycome, including that of the caecum and small intestine, is highly variable across the population, in contrast to the relatively invariant distal gut glycome[63]. The variability in the proximal gut glycome mirrors the variability seen in other secretory materials, such as milk, saliva, lungs and cervix, leading to the hypothesis that the invariant distal gut glycome selects for commensal microorganisms whereas the variable proximal gut glycome and the glycomes of other secretory organs defend against pathogens by providing decoy glycan receptors[63].

Accordingly, pathogens that are able to subvert this system and bind to epithelial-associated rather than secretory glycans in the gut can cause inflammation[19]. This hypothesis is further supported by computational studies that suggest that mucus glycan–microorganism interactions support positive selection of commensal microorganisms during the slow transit through the colon and negative selection of pathogens during the fast transit through the small intestine[65]. Experimental studies involving engineered deletion of mucus glycans support these computational findings[15]. For example, 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 but no inflammation of the small intestine[15]. Such mechanisms could be relevant for ulcerative colitis or Crohn's disease, in both of which the balance between commensal microorganisms, pathobionts, and pathogens influence interactions with the immune system[8,66].

Sulfated motifs on the termini of glycans are one of the structures that most reliably distinguish ileal from colonic glycoproteins by immunohistochemistry: there is increased sulfation in the human colon. Sulfation is likely induced by the gut microbiota: surgical construction of an ileal pouch, which results in a shift to a more colonic morphology and microbiota, results in elevated sulfate[67–69]. Sulfation is likely to be highly relevant in IBD, as patients have reduced levels of sulfate compared with healthy individuals, which is partly due to increased production of bacterial sulfatases[70–72]. Interestingly, the increased sulfatase activity is observed in patients with ulcerative colitis but not those with Crohn's disease[70].

Different glycans are expressed in the human fetal gut than in the adult gut, indicating that epithelial glycosylation is developmentally regulated[58]. In particular, the human fetal gut primarily expresses neutral core 2-based glycans, whereas the adult gut primarily expresses glycans with charged termini (for example, those modified by Neu5Ac and/or sulfate). In addition, Cad antigen is not expressed in the human fetus, and there is an absence of the characteristic gradient of sulfate, sialic acid and fucose seen in the adult gut. Also, some oncofetal antigens are expressed in fetuses but not in adults, such as T antigen (Galβ1,3GalNAc-Ser/Thr) and sialyl-Tn (STn) antigen (Neu5Acα2,6GalNAc-Ser/Thr).

Regulation of epithelial glycosylation

Studies investigating fetal versus adult intestinal glycosylation indicate that the glycome is developmentally regulated. However, whether this regulation depends solely on host cues or also on environmental signals has been a major unanswered question. In contrast to humans, the mouse intestinal glycome primarily consists of core 2-based glycans, and the regional distribution of some terminal epitopes varies compared with humans[13,64]. Nonetheless, both mice and humans express terminal Cad, sialic acid, sulfate and fucose. Thus, mice are an ideal model to study regulation of epithelial glycosylation.

Epithelial fucosylation has been a model epitope to understand regulation of intestinal glycosylation. Fucose can be added to proteins in α1,6, α1,3 and α1,2 linkages. The lectin Ulex europaeus agglutinin I (UEAI) recognizes α1,2-linked fucose, the expression of which depends on two enzymes: FUT1 and FUT2. α1,2-linked fucose is the major class of

fucosylation in the intestinal epithelium in humans and mice [73]. Importantly, in mouse models FUT2 regulates fucosylation in most of the intestinal epithelia, whereas FUT1-dependent fucosylation is restricted to Paneth cells and microfold cells (M cells) in the small intestine[73,74]. Interestingly, preliminary work shows that distinct populations of murine Paneth cells express either FUT1 or both FUT1 and FUT2[75].

One of the first observations that the gut microbiota can induce epithelial glycosylation arose in studies comparing UEAI staining in conventionally housed with germ-free mice[27,76]. These studies demonstrated that conventionally housed mice, but not germ-free mice, express α1,2 fucose in the ileum[27,76] . Further, colonization of germ-free mice with a commensal gut microorganism, *Bacteroides thetaiotaomicron*, also induced α1,2 fucose[76]. Many other microorganisms can also probably induce FUT2 expression, as has been shown for the commensal segmented filamentous bacteria and the pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium[27]. Bacteria are able to metabolize fucose, and, interestingly, deleting genes associated with fucose sensing or metabolism inhibits the ability of *B. thetaiotaomicron* to induce epithelial fucosylation[24,76]. Some bacteria, such as *Bacteroides fragilis*, can salvage free fucose and incorporate it into its own glycoconjugates[77]. This ability provides a competitive advantage in colonization experiments, perhaps by making the bacteria more closely resemble host cells and thus avoid immunological detection, in a process called molecular mimicry **[G]** .

Although initial experiments proposed that bacteria interact directly with the intestinal epithelia to induce fucosylation, later work suggested an indirect mechanism involving immune cells (Fig. 4). Using a variety of null mutant mouse models, Goto et al. and Pham et al. showed that bacteria interact with group 3 innate lymphoid cells (ILC3s) in the gut to induce expression of IL-22, which interacts with IL-22R on intestinal epithelial cells to induce FUT2 expression in the intestinal epithelia[27,78]. In addition to the bacteria-inducible IL-22 signal, ILC3s constitutively express lymphotoxin, which is also required for FUT2 expression. These observations suggest a system in which ILC3s secrete lymphotoxin in a microbiota-independent manner to induce baseline intestinal epithelial fucosylation, which is then supplemented by microbially induced IL-22 that achieves increased intestinal epithelial fucosylation. Other immunological signals might also regulate intestinal epithelial fucosylation. Goto et al. showed that T cell-deficient mice, but not B cell-deficient mice, had elevated intestinal epithelial fucosylation, and that IL-10-producing $CD4^+$ T cells, but not soluble IL-10 alone, were responsible for suppressing epithelial fucosylation via transcriptional regulation of *FUT2*[79]. Thus, components of the innate immune system induce fucosylation whereas components of the adaptive immune system repress it. This microorganism-induced fucosylation seems to be crucial in the small intestine but not in the colon. In contrast to the absence of α1,2 fucose in the ileum of germ-free mice, levels of colonic α1,2 fucose are similar in germ-free and conventionally housed mice[76]. Therefore, distinct mechanisms regulate epithelial glycosylation in different regions of the gut. Importantly, microbiota-induced glycosylation is not limited to fucosylation. Compared with germ-free mice, conventionally raised mice express a more-complex, extended O-glycome throughout the intestine due to increased levels of many, but not all, glycosyltransferases[13].

Epithelial glycosylation in IBD

Alterations in glycosylation in human IBD

Multiple lines of evidence implicate altered epithelial glycosylation in IBD pathogenesis. Such evidence includes the effects of disruption of mucin-type O-glycans, N-glycans and termini common to multiple classes of glycosylation [15,16,20,23,28,80,81]. Genome-wide association studies (GWAS) have identified human genes that directly regulate epithelial glycosylation, such as *FUT2* and *COSMC*, and those that indirectly regulate glycosylation via the microbiota–immune–intestinal epithelia axis, such as interleukin-23 (IL-23) and signal transducer and activator of transcription 3 (STAT3), which help regulate FUT2 expression in the intestinal epithelium (Fig. 3b, Table 1, Supplementary Table 1)[16,73,80,82–89]. In addition to genes associated with O-glycan synthesis, genes that are important for N-glycan synthesis, such as those that encode components of the oligosaccharyltransferase complex[81], are also implicated in IBD. Although most glycan-associated genes are altered in both ulcerative colitis and Crohn's disease (such as *C1GALT1C1* and *TMEM258*), which is consistent with overall disease genetics, a few are specific to either ulcerative colitis (such as *MAN2A1*) or Crohn's disease (such as *FUT2*)[15,16,23,27,79–81,83–85,87–105] (Supplementary Table 1).

In addition to disease genetics, immunohistochemical and structural studies indicate that IBD, including Crohn's disease and ulcerative colitis, is associated with a simplified O-glycome[106], characterized by increased levels of the truncated O-glycans T (in Crohn's disease and ulcerative colitis)[107], Tn (in ulcerative colitis; not well studied in Crohn's disease)[28], and STn (in Crohn's disease more than in ulcerative colitis) antigens[26,108–110]. In addition to class-restricted changes in O-glycans, IBD is associated with alterations in terminal structures that are present on multiple classes of glycosylation, including O-glycans, N-glycans and glycolipids[14,20,26,28,107,110–113]. In particular, IBD is associated with increased terminal sialylation (in ulcerative colitis; not well studied in Crohn's disease)[112,114], fucosylation (in Crohn's disease and ulcerative colitis)[16,107], reduced terminal sulfation (in ulcerative colitis more than in Crohn's disease)[70,113], reduced Sd^a and Cad antigens (in ulcerative colitis; not well studied in Crohn's disease)[64] and either increased or decreased Lewis antigens (depending on the specific structure as described below; in ulcerative colitis and Crohn's disease)[26,64,115,116] (Fig. 3a). Sialyl-$Lewis^a$ (also known as CA19-9) is increased in Crohn's disease and ulcerative colitis and Sulfo-$Lewis^{a/x}$ is decreased in ulcerative colitis, which might simply reflect an increased sialylation-to-sulfation ratio rather than altered Lewis antigen synthesis[26,115,116]. Interestingly, IBD is also associated with distinct changes in serum N-glycans, including an increase in large glycans with a corresponding decrease in hybrid and high-mannose structures, a decrease in fucosylation and galactosylation, and an increase in sialylation (in both Crohn's disease and ulcerative colitis but more so in Crohn's disease)[117]. These identified serum N-glycans can be found on secreted intestinal glycoproteins, immunoglobulins or as acute phase reactants[117]. In addition to changes in cell surface glycosylation, patients with IBD also have a reduction in intracellular O-GlcNAcylation in the intestinal epithelia (in Crohn's disease and ulcerative colitis)[118]. Intriguingly, STn expression in humans seems to predict progression from ulcerative colitis to colitis-associated colorectal cancer [119].

In some cases, changes in glycosylation precede inflammation. Unaffected monozygotic twins of patients with Crohn's disease or ulcerative colitis exhibit elevated T antigen expression in the epithelial cells of the intestinal crypt surface with concomitant elevation of nuclear factor kappa-B (NF-κB) activation in the same cells, which suggests that genetic or possibly environmental factors induce T antigen expression before disease onset and that T antigen expression might lead to increased inflammatory tone[111]. As well as altered glycosylation preceding inflammation, glycan structural alterations have also been shown to occur in response to inflammation. For example, the glycans of patients with ulcerative colitis in remission (n=13) resembled those of control patients (n=25; those with histologically normal colons who underwent colonoscopy for anaemia, rectal bleeding or polyp surveillance), whereas inflammation-associated glycan truncation was observed in patients with ulcerative colitis undergoing a flare (n=15)[26]. Importantly, in patients with active disease the IBD-associated changes in glycosylation normalized within the same individual after resolution of the ulcerative colitis flare, indicating that components of the IBD-associated glycome are reversible within the same individual over time [26]. Thus, it is likely that some alterations in intestinal epithelial glycosylation precede inflammation and others result from inflammation.

Mechanisms of altered glycosylation in IBD

Altered cell surface glycosylation can arise from disrupted glycan synthesis, degradation and substrate expression (that is, expression of proteins or lipids to which the glycans attach). Glycan synthesis is complex and relies on coordinated regulation of synthesis and processing genes, the proteins they encode, and the distribution of glycans in organelles, notably the endoplasmic reticulum and the Golgi apparatus. Hundreds of genes are involved in glycan synthesis and at least twenty of these have been implicated in IBD, including those that encode transcription factors, cytokines, glycosyltransferases and glycosidases (N-linked, O-linked and glycolipids), lectins and ion channels. Whereas a few of these are specific to ulcerative colitis or Crohn's disease, most are shared between the two diseases (Fig. 3b, Supplementary Table 1)[15,16,23,27,79,80,85,86,89–105].

In terms of synthesis, single-nucleotide polymorphisms identified by GWAS affect protein structure, transcriptional regulation and alternative splicing regulation of human genes including glycogenes[120]. Growth factors, such as pro-inflammatory cytokines, activate signal transduction pathways that alter glycosyltransferase expression, such as increasing sialyltransferase and sulfotransferase expression and, subsequently, sialyl-Lewis^x expression [121]. Mutations in genes involved in extension of mucin-type O-glycans have been observed in small cohorts of patients with IBD [28].

Although the precise mechanisms for altered epithelial glycosylation in IBD are unknown, clues can be gained by analysing mechanisms for altered glycosylation in cancer, which exhibits a similar simplification of the glycome[35,122,123]. In cancer cell lines (fibroblast, kidney and breast), growth factors have been shown to mislocalize glycosyltransferases, including from the Golgi back to the endoplasmic reticulum, resulting in increased expression of truncated mucin-type O-glycans[124,125]. Additionally, a wide array of genetic and epigenetic disruptions have been observed in glycogenes in cancer, especially for those

in the mucin-type O-glycosylation pathway[35,126–128], and altered Golgi pH alters Golgi structure, in turn disrupting glycosyltranferase localization and/or the flow of substrates through the secretory apparatus, resulting in expression of truncated glycans such as T antigen[129–131].

Enzymatic and non-enzymatic mechanisms degrade cell surface glycans and might contribute to altered glycosylation in IBD. The gut microbiota produces glycosidases that digest host mucin and non-mucin glycans[132], and intestinal inflammation results in oxidative stress, which in other settings has been shown to chemically release or degrade the glycans of glycoconjugates[133,134]. These mechanisms are likely to be important in IBD pathogenesis but will need to be tested further.

IBD results in altered substrate expression, including altered and ectopic glycoprotein expression as a result of disrupted gene expression and alternative splicing. For example, levels of MUC2 are reduced in both Crohn's disease and ulcerative colitis, whereas levels of MUC1 are decreased in Crohn's disease and increased in ulcerative colitis[135]. The level of substrate expression inversely correlates with glycosite **[G]** density as well as the abundance of specific glycan structures[34,136]. In IBD, ectopic expression of gastric mucins in the intestine, as well as alternative splicing of glycoproteins already present in the intestine such as CD44, introduces novel glycosylation substrates and peptide-driven glycan motifs[89].

Another clue to altered regulation of intestinal epithelial glycosylation comes from examining the glycomes of germ-free mice, which express a simplified glycome that in part mirrors changes observed in IBD, for example increased T antigen expression [13]. Although the mechanisms for this simplification are largely unexplored, a few examples, such as microorganism-induced fucosylation, indicate that specific microbial signals induce intestinal epithelial expression of glycosyltransferases involved in glycan extension through lymphocyte-dependent mechanisms[27]. Thus, disrupted intestinal epithelial–microorganism crosstalk, for example via genetic disruption of microorganism-sensing genes, might contribute to expression of an IBD-associated glycome.

Epithelial glycome: IBD versus CRC

Colorectal cancer results in a number of glycan alterations that are also observed in IBD. These alterations include truncation of mucin-type O-glycans, such as increased Tn, STn and T antigens; altered terminal epitopes across glycan classes, such as increased sialyl-Lewis[a]; increased sialic acid; and reduced sulfate [20,28,35,70,110,112–114,116]. Such alterations differ from changes that only occur in cancer (and not IBD), such as loss of ABO blood groups and increased N-glycan branching[137]. In contrast to increased N-glycan branching in breast and colon cancers, IBD exhibits a reduction in human and mouse T cell N-glycan branching that is pro-inflammatory[138,139]. Similarly, in humans, O-GlcNAcylation **[G]** is increased in colorectal cancer (CRC) but decreased in IBD [118,140,141].

Although the mechanisms underlying these changes are still under investigation, some mechanisms might be shared between colorectal cancer and IBD. Genetic disruptions in human mucin-type O-glycan biosynthesis have been observed in colorectal cancer cells and

IBD tissue[28,142]. Addition of a single galactose to the Tn antigen to form the T antigen, which serves as a platform for the synthesis of all extended core 1 and core 2-based O-glycans, requires the molecular chaperone COSMC[49]. COSMC, in effect, serves as a master regulator of O-glycosylation. Disruption of this chaperone has been observed in cancer and IBD. In human cancer tissues or cancer cells (cervical, leukaemia, colorectal, melanoma and pancreas), mutation, loss of heterozygosity, and epigenetic silencing lead to loss of COSMC activity[35,47,48,142–145], whereas in IBD, inactivating mutations in *COSMC* have been identified in patient biopsy samples, and GWAS studies have identified single-nucleotide polymporphisms in *COSMC* associated with IBD[28,80]. Loss of *Cosmc* in mice and humans leads to O-glycan truncation and expression of Tn antigen or STn antigen, its sialylated counterpart[142,146,147]. STn expression can also arise owing to overexpression of the ST6GalNAc-I sialyl transferase, which modifies Tn[148–150]. However, whether these or other mechanisms are responsible for pathological Tn and STn expression in the gut is still unclear[128,151]. In addition to Tn antigen and STn antigen, T antigen is also overexpressed in IBD and CRC[19,20]. The mechanisms for T antigen overexpression are less clear, but expression likely depends on glycosyltransferase mislocalization, either due to growth factor signalling or to an altered Golgi structure, arising for example from Golgi alkalinization [124,125,131].

IBD increases the risk of CRC in patients with ulcerative colitis more than it does in patients with Crohn's disease[152,153]. Notably, in Crohn's disease the risk of intestinal cancer is mainly elevated in people with colonic disease and not ileal disease[154]. Thus, some of these shared glycan alterations in IBD and CRC might have functional consequences. Indeed, STn predicts an increased rate of malignant progression in patients with ulcerative colitis[109], whereas engineered expression of Tn (and concomitant STn) in the mouse gut results in ulcerative colitis-like pathogenesis and subsequently CRC[15,22,28], with corresponding activation of oncogenic pathways[143].

Food substances and bacteria both interact with these IBD and CRC-associated intestinal epithelial glycans. Plant lectins, such as those contained in peanuts, bind to T antigen and induce mitogenic activity in human cell line models[155–157], whereas the gut microbiota, constituents of which bind to and consume host glycans, exhibits similar alterations in IBD and cancer, which suggests a possible link between IBD, CRC, epithelial glycans and the gut microbiota. In particular, in humans, IBD and CRC both exhibit a reduction in bacterial diversity, a reduction in the genus *Bacteroides*, and an enrichment of specific bacterial species, such as *Fusobacterium nucleatum*, *Campylobacter* species, colibactin-producing polyketide synthase (pks)$^{+}$ *E. coli*, enterotoxigenic *B. fragilis* and *Enterococcus faecalis*[158–162].

Altered glycosylation drives some of these bacterial changes. Engineered expression of Tn and STn and subsequent loss of extended O-glycans in the gut leads to loss of bacterial diversity, reduction of *Bacteroides* species, which consume host carbohydrates, and the emergence of pro-inflammatory *Campylobacter* species in mice[15]. Additionally, in mice, expression of T antigen recruits *F. nucleatum* to the tumour via fatty-acid binding protein 2 (Fap2), a Gal-GalNAc bacterial lectin[19]. These bacteria activate inflammatory cascades

and/or damage DNA[160]. Thus, IBD-associated changes to the epithelial glycome, which persist in CRC, result in recruitment of pro-inflammatory and pro-tumorigenic bacteria.

Effects of altered glycosylation in IBD

Evidence from a variety of mouse models with engineered expression of IBD-associated glycosylation supports a functional role for altered glycosylation in IBD. These models include mice that express truncated Tn and STn antigens through deletion of *Cosmc* (also known as *C1galt1c1*), *C1galt1* (the gene encoding T-synthase), or *B3gnt6* (also known as *core 3 synthase*, which synthesizes Core 3 O-glycan); mice with loss of core 2 and core 2-based extensions as a result of deletion of core 2 GlcNAc-transferase-2 (*Gcnt3*); mice with reduced sulfation as a result of deletion of N-acetylglucosamine 6-O-sulfotransferase 2 (GlcNAc6ST2, *Chst4*) or the Na^+–sulfate cotransporter (*Slc13a1*); and mice with reduced O-GlcNAcylation as a result of deletion of the gene encoding O-GlcNAc transferase (*Ogt*)[15,28–32,118,163]. In conjunction with data from human studies, the mechanisms by which these alterations to glycans contribute to inflammation are detailed in this section, with a focus on mucin biology, glycan–lectin and host–microorganism interactions, and altered immunity (Box 2).

MUC2 synthesis and stability

Epithelial glycosylation contributes to barrier formation, host–microorganism symbiosis and immunity. Thus, it is no surprise that altered glycosylation has a putative role in IBD. Mouse models have demonstrated that glycans are critical for synthesis of the MUC2 mucus layer, which forms a single loose layer in the small intestine and an outer loose layer and inner attached layer in the colon[164]. The loose layers in the small intestine and colon serve as habitats and nutritional substrates for the gut microbiota and are penetrable to bacteria, whereas the inner mucus layer is impenetrable to bacteria and prevents bacterial–epithelial interactions in the distal gut, where bacterial loads can reach 10^{12} bacteria per gram of stool[164] (Fig. 5a).

MUC2 contains hundreds of O-glycans, accounting for ~80% of its mass (Fig. 5b)[165], and ~20 possible N-glycan attachment sites[166]. Nonetheless, both N- and O-glycosylation are important for MUC2 biology (Fig. 5c). MUC2 is translated and dimerized in the endoplasmic reticulum. Then, N-glycosylation in the endoplasmic reticulum is crucial for transfer of MUC2 to the Golgi[167], where MUC2 is highly O-glycosylated and obtains many charged residues such as sialic acid and sulfate[63,165] (Fig. 5c). Each MUC2 protein in the dimer then participates in additional interactions to form trimers, which, along with low pH and Ca^{2+} content in the Golgi, enables dense compaction of the MUC2 network[168]. MUC2 is stored in a specific structure within goblet cells called the theca and is released homeostatically or as a result of environmental triggers, for example by acetylcholine or Toll-like receptor ligands[169,170]. Nearby cystic fibrosis transmembrane conductance regulator (CFTR) channels release bicarbonate into the intestinal lumen, which neutralizes the pH and chelates Ca^{2+}[171], thereby facilitating unfolding of the MUC2 network into stratified sheets (Fig. 5d)[172]. In the small intestine, additional processing by the protease meprin-β releases the attached mucus layer, in a microbiota-dependent fashion, from the

epithelium to form an unattached loose layer[173]. By contrast, expansion of the colonic mucus layer involves the host metalloprotease calcium-activated chloride channel regulator 1 (CLCA1), which cleaves MUC2 independent of the gut microbiota[174]. Accordingly, the colons of germ-free mice have an outer and inner mucus layer, but in explant cultures full mucus expansion does not occur in the presence of protease inhibitors; however, it can be induced by recombinant CLCA1[165,174].

Although N-glycans are less abundant than O-glycans on MUC2, they are crucial for proper protein folding. Pharmacological or genetic disruption of N-glycosylation leads to the unfolded protein response, endoplasmic reticulum stress and subsequent inflammation in mice [81,175].

O-glycans likely have two major roles in MUC2 synthesis. First, O-glycans (as well as N-glycans) contain charged residues that interact with Ca^{2+} in the Golgi and goblet cell theca, thereby facilitating tight packing and storage of MUC2[172]. Although not yet experimentally tested, a loss of charged residues in MUC2 likely results in reduced packing, thereby reducing the amount of MUC2 that can be stored and released. Second, glycans on MUC2 might prevent mucus degradation by bacterial proteases, for example by blocking access to the polypeptide backbone. O-glycan extension blocks the degradation of defined peptides and murine gastrointestinal mucins by the model proteases RgpB (from *Porphyromonas gingivalis*) and pronase, respectively [176,177]; however, whether proteases derived from commensal bacteria are able to access and degrade O-glycan-deficient MUC2 in the inner mucus layer has not been demonstrated. Nonetheless, *T-synthase*-knockout mice and *Cosmc*-knockout mice, which are deficient in O-glycans extended beyond a single GalNAc or their sialylated counterpart, both have loss of the outer and inner mucus layers and increased bacterial–epithelial contact in the distal colon[15,28]. Loss of the mucus layer results in increased gut permeability[28]. However, whether this increased permeability is due to an indirect effect of cytokine-induced remodelling of the tight junctions or due to a direct effect of O-glycans on an adhesion molecule (for example, via disrupted protein stability or disrupted glycan–protein interactions) is not known[178]. An additional mechanism for the loss of O-glycans and the resultant mucus degradation arises from the observation that administration of a Westernized diet (with fewer complex carbohydrates) to mice results in increased host glycan foraging by the gut microbiota and associated mucus thinning and increased permeability compared with standard chow[179]. Although this increased foraging can be driven by changes in diet, variations in the gut microbiota between animals alone (in the absence of dietary changes) can also result in enhanced mucus glycan degradation; when comparing colonies of mice housed in different rooms, differences in the microbiome between mice led to differences in bacterial inner mucus penetrability[180]. Thus, defects in N- and O-glycosylation could affect mucin synthesis and stability upon secretion into the intestinal lumen, leading to compromised barrier function.

Glycan–lectin interactions

Perturbed glycan–lectin interactions can contribute to inflammation. Host lectins from diverse species, including R, L, P, I and S-type lectins and many of the cluster of differentiation markers, interact with intestinal epithelial cell (IEC) glycans (Table 2)[181,182].

R-type lectins include members of the polypeptide GalNAc-transferases, which add GalNAc to serine or threonine in a peptide to form the Tn antigen and are largely conserved between mice and humans[46]. L-type lectins are involved in protein folding quality control (for example, calnexin and calreticulin) and vesicular sorting (for example, in mice and humans, endoplasmic reticulum–Golgi intermediate compartment 53 (ERGIC53), vesicular integral-membrane protein 36 (VIP36) and vesicular integral-membrane protein L (VIPL)) and interact with monoglucosylated or non-glucosylated high-mannose N-glycans, respectively[183]. The P-type lectin mannose-6-phosphate (M6P) receptor (MPR) binds to M6P on N-linked glycans to ensure proper shuttling of enzymes to the lysosome[184,185]. Mice and humans have two MPRs, a cation-independent mannose-6-phosphate receptor (CI-MPR) and a smaller cation-dependent mannose-6-phosphate receptor (CD-MPR), which are both critical for proper lysosomal hydrolase sorting[184,185]. C-type lectins, including dectin-2, the mannose receptor, and macrophage galactose type-lectin (MGL)), recognize high-mannose N-glycans, α-linked mannose and Tn antigen on mucins, respectively[17,186–188]. Interestingly, while human MGL (hMGL) has a single gene, mouse MGL (mMGL) includes two genes (*mMGL1* and *mMGL2*)[189–191]. hMGL and mMGL2 both bind to terminal GalNAc, including Tn antigen, whereas mMGL1 binds to Lewis a/x structures[189–191]. Although selectins are probably the best-studied C-type lectin, they bind to endothelial or leukocyte glycoconjugates rather than to epithelia[192]. I-type lectins include Siglecs, which are sialic-acid-binding proteins that inhibit immune activation[193]. These lectins include human and mouse Siglec-15, human Siglec-5, and human Siglec-14, which bind to IBD and cancer-associated STn antigen[193–196], and human Sigleg-7 and Siglec-9, which block natural killer cell activation[197]. Although sialic acid is increased in IBD, expression of specific Siglec ligands, such as the Siglec-9 ligands sulfo-Lewis antigens, is decreased in in IBD[26,115,116,193]. Thus, loss of Siglec inhibitory signals in human IBD might contribute to immune hyperactivity[193,197–199]. LacNAc (Galβ1,4GlcNAc) is recognized by galectins (previously known as S-type lectins), which regulate immune cells, bacteria and IECs (for example, they have roles in cell turnover, intestinal permeability and tissue repair)[200–209]. Furthermore, galectin expression levels in humans distinguish IBD from non-IBD control tissues (endoscopically examined for abdominal pain, constipation, irritable bowel syndrome or CRC screening) but not ulcerative colitis from Crohn's disease [200–209].

Intestinal epithelial glycans also interact with foreign lectins from plants, viruses, bacteria, toxins and parasites (Table 2). For example, peanuts produce peanut agglutinin (PNA), which binds to GalGalNAc on the intestinal epithelium and leads to human colorectal epithelial cell proliferation [156,157].

These interactions can have pathological consequences. For example, Fap2 on *F. nucleatum* binds to GalGalNAc, leading to inflammation and cancer in mouse models [19,210–212]. Bacterial toxins that bind to IEC glycans include *Pseudomonas aeruginosa* 1 (PA1; binds to Gal; this binding is interferon-γ-inducible, and expression leads to intestinal permeabilization and sepsis) and PA2 (binds to fucose or mannose), *Clostridioides difficile* toxin A (binds to a GalNAc-terminating glycosphingolipid), *E. coli* heat-labile toxin (binds to GM1), *Shigella dysenteriae* Shiga toxin (binds to Galα1,4Galβ), and *Vibrio cholerae* cholera toxin (Galβ1,3GalNAc)[3,213–217].

In addition to toxins, parasite lectins recognize IEC Gal/GalNAc (*Entamoeba histolytica*), sialic acid (*Trypanosoma cruzi*), heparan sulfate (*T. cruzi*), GalGalNAc (*Cryptosporidium parum*), mannose (*Giardia lamblia*) and Neu5Acα2,3Galβ1,4GlcNAc (*Toxoplasma gondii*)[218–220]. Collectively, the diverse interactions of host and foreign lectins with intestinal epithelial glycans influence inflammatory tone and gut homeostasis.

Host–microorganism interactions

Intestinal epithelial O-glycans can directly regulate host–microorganism interactions by providing ligands for bacterial adhesins and nutrients for bacterial metabolism. Indirectly, O-glycans have diverse roles, ranging from controlling epithelial gene and protein expression to regulating inflammatory tone by regulating the mucosal barrier as well as through antigen uptake and immune imprinting. Ultimately, these diverse mechanisms converge to select commensals for long-term colonization in the gut and to facilitate (or inhibit) pathogen binding to intestinal epithelia and subsequent damage.

Microbial host glycan-binding proteins were first studied in pathogens, including bacteria and viruses. Many of these proteins bind to fucose residues that are only present in secretor-positive individuals (that is, the 80% of the population who express FUT2 glycosyltransferase and are therefore able to synthesize blood group antigens on mucosal and salivary secretions in addition to on erythrocytes[221]). These glycan-binding proteins include BabA in *H. pylori* as well as adhesins in norovirus and rotavirus[18,222–224]. *H. pylori* is an interesting example because glycans both facilitate and inhibit binding. The deeper glands of the human stomach, including the fundic and pyloric glands, express terminal α1,4GlcNAc-containing O-glycans that block synthesis of a cholesterol-containing cell wall component by inhibiting cholesterol α-glucosyltransferase from *H. pylori*[225–227]. Thus, in contrast to BabA–glycan interactions, which promote *H. pylori* colonization, α1,4GlcNAc prevents gastric invasion, inflammation and oncogenesis[228].

A number of glycan-binding adhesins of commensal bacteria have also been identified. These adhesins have been expressed by a number of *Lactobacillus* species: *Lactobacillus plantarum*-expressed glyceraldehyde 3-phosphate dehydrogenase (GAPDH) binds to blood group A and B-containing mucins on human colonic cells; *Lactobacillus mucosae* binds to human A and B blood groups through a newly identified adhesin (Lam29) that is expressed by *Lactobacillus mucosae* ME-340 and has homology with ATP-binding cassette (ABC) transporters; *Lactobacillus rhamnosus* GG binds to mucin through the subtilin biosynthesis protein SpaC in a glycan-dependent manner; and *Lactobacillus reuteri* binds to mucin through a family of mucus-binding proteins in a sialic acid-dependent manner[229–234]. In addition to glycans of lactobacilli, a collection of family 1 solute binding proteins (F1SBPs) from *Bifidobacterium longum* subsp. *infantis* bind to various host glycans[235]. In addition, polysaccharide utilization loci **[G]** require glycan-binding proteins to ultimately uptake and degrade extracellular glycans[132]. These glycan-binding proteins are homologous to starch utilization system C (SusC) and SusD molecules from the starch utilization locus and likely facilitate bacterial adhesion to host glycans in addition to aiding glycan metabolism[236].

The small intestine degrades and absorbs proteins and simple sugars, but many complex plant polysaccharides and mucosal glycans are indigestible by the host, thereby providing a

food source for the gut microbiota. The gut microbiota degrades these carbohydrates via a collection of glycosyl hydrolases, sugar transporters, sensors and regulatory proteins that bind to, import and degrade glycans[62]. The genes encoding these molecules are either organized as a single loci that contains all the necessary genes for glycan utilization, such as the polysaccharide utilization loci that are common in *Bacteroides*, or spread across the genome into individual operons dedicated to encoding specific monosaccharides, a strategy commonly used by members of the Firmicutes phylum, such as *Ruminococcus gnavus* and *Ruminococcus torques*, as well as in some *Bifidobacterium* species that lack an outer membrane and classic *Bacteroidetes* polysacchardide utilization loci components[62,237]. *Bacteroides* species contain a broad repertoire of polysaccharide utilization loci, with *B. thetaiotamicron* containing 88 different loci[238]. Studies of this model organism led to the idea that although some bacteria can degrade both dietary and host glycans, dietary polysaccharides are preferentially utilized when both are present[239]. However, recent work questions this idea[15,240]. Kudelka et al. found that host glycans were required for colonization of commensal bacteria in the mucosa of the distal intestine in a mouse model, even in the presence of dietary polysaccharides[15]. Additionally, further analysis revealed that although some bacteria, such as *B. thetaiotomicron*, are able to metabolize multiple classes of glycans, other bacterial species are more limited and preferentially consume either dietary or host glycans[240]. For example, *Bifidobacterium* species and *Akkermansia muciniphilia* contain a more limited repertoire of polysaccharide utilization loci that restricts foraging to host glycans[241], which corresponds to *A. muciniphilia*'s enrichment in the human intestinal mucosa[241].

Another emerging concept is that some microorganisms share resources. This concept was initially observed in pathogens that contain monosaccharide transporters but not the glycosidases needed to release them, and that therefore require commensals for colonization[242,243]. However, resource sharing has since been observed among members of the order Bacteroidales, which suggests that this might be a more common feature among commensals than has previously been believed[244]. Historically, theoretical models to describe selection of commensal bacteria in the intestine predicted that one type of bacteria would eventually dominate a mixed population given sufficient time. This prediction contrasts with the experimental evidence that the human gut microbiota is incredibly diverse and generally stable over time[245]. Resource sharing would explain mutualism and the persistence of two species as well as the idea that the gut contains many distinct ecological niches or biogeographies in both the mucosal–luminal and rostral–caudal axes[10,246]. Multiple studies have shown that bacterial inoculation into animals induces expression of polysaccharide utilization loci and that this expression is critical for colonization, transmission and maintenance of a long-term reservoir (for example, of *Bacteroides* within the crypt channel) following antibiotic or pathogen challenge[25,238,247].

Additionally, microorganisms can reprogramme intestinal epithelial glycosylation. *Salmonella* directly modifies human glycans through bacterial glycosidases and induces host gene expression to alter host glycan biosynthesis[248,249]. These changes can have a major effect on host physiology. In addition to an increase in direct invasion of intestinal epithelial cells, *Salmonella* induced host neuraminidase activity, which led to enhanced intestinal alkaline phosphatase (IAP) turnover in the duodenum of mice [249,250]. As IAP typically

dephosphorylates and detoxifies LPS, these mice developed chronic inflammation even after clearing the initial infection. And as previously discussed, commensals also release host monosaccharides, such as sialic acid, which can facilitate pathogen expansion by providing a nutrient source[242].

Intestinal epithelial glycosylation spatially regulates the gut microbiota and altered glycosylation contributes to the spatial pattern of dysbiosis in IBD[15]. Patients with IBD, including Crohn's disease and ulcerative colitis, and healthy individuals both have spatial regulation of the gut microbiota, with patients with IBD exhibiting more pronounced alterations in the mucosal microbiome than in the luminal microbiome[10,82,246,251]. However, the mechanisms underlying spatial regulation of the microbiome in IBD were until the past few years poorly understood. But in 2014, *COSMC*, which controls the extension of O-glycans beyond a single GalNAc, was identified as an IBD risk gene in a GWAS[80]. As host glycans have a direct interface with the gut microbiota, Kudelka et al. tested the hypothesis that *Cosmc* spatially regulates the gut microbiome. Deletion of *Cosmc* in the mouse intestine led to loss of microbial diversity and emergence of a pathobiont in the mucosa of the distal colon but not in the overlying lumen or in the mucosa of the small intestine, indicating that *Cosmc* and the downstream O-glycans spatially regulate the gut microbiome (Fig. 6)[15]. Further, regional alterations in the microbial ecology led to regional pathology, with the most pronounced inflammation being in the distal colon. Thus, intestinal epithelial glycosylation regulates microbial biogeography in IBD.

Although it is difficult to determine which mechanisms are most important in vivo, especially in patients, these studies suggest that the simplified glycome observed in IBD contributes to an altered gut microbiota and reduced microbial diversity due to disrupted bacterial binding and reduced resource availability, leading to altered spatial geography and disease.

Altered immunity

Epithelial glycans and the mucins they decorate are involved in antigen uptake, immune imprinting and inflammatory cell recruitment. Although glycan-dependent disruption of intestinal immunity in IBD has not been rigorously evaluated, observations from basic immunology suggest that these mechanisms have an important role in disease pathogenesis. Antigen is taken up in the small intestine via multiple routes. First, in mice and humans, the small intestine can take up antigen in the Peyer's patches **[G]** using specialized cells known as M cells[252]. M cells lack a mucin layer and have a relatively thin glycocalyx compared with enterocytes, thereby facilitating interaction of these cells with the luminal contents[253]. Expression of cell surface receptors (such as integrin β1, complement component 5a receptor (C5aR), glycans, glycoprotein 2 (GP2) and a poorly characterized immunoglobulin A receptor (IgAR)) facilitates uptake of microparticles via endocytosis, phagocytosis or micropinocytosis[254,255]. In addition, specialized dendritic cells called lysozyme-expressing dendritic cells can extend dendrites through M-cell pores to sample antigen in the gut lumen[254]. It has been demonstrated in a mouse model that antigen uptake by M cells is critical for the generation of faecal IgA against the commensal microbiota, thereby preventing aberrant immune activation against these resident bacteria[256].

A second route of antigen uptake is CD11c$^+$ dendritic cells in the lamina propria extending dendrites into the intestinal lumen and sampling antigens. During this process, dendritic cells maintain transepithelial resistance by expressing tight junction molecules that intercalate with a reorganized epithelial tight junction[257]. In contrast to the M cells of Peyer's patches, this route occurs in villous epithelia, which therefore substantially expands the potential surface area for antigen sampling. A third route, identified in mice using in vivo imaging and confirmed in humans, uses goblet-associated passages (GAPs) that expand the surface area available for antigen sampling[258]. This route mechanistically differs from antigen sampling by transepithelial dendrites[258]. GAPs form a transepithelial passage, or tunnel, through the goblet cell by which soluble antigens can cross the intestinal epithelium and access CD103$^+$ lamina propria dendritic cells[258,259]. Whereas antigen sampling by transepithelial dendrites likely has an important role during infection, GAPs are involved in steady-state sampling and result in tolerogenic responses[260].

Although the three routes already described can sample antigens not coated with antibodies, a fourth route, involving the human neonatal Fc receptor (FcRn), is involved in uptake of immune complexes[261,262]. Human FcRn transports IgG in the basal to apical direction across the intestinal epithelium and into the faeces[261]. The faecal IgG then binds to antigen to form immune complexes that are then retrotranslocated by FcRn back across the intestinal epithelium to the lamina propria and its associated dendritic cells for processing and subsequent presentation to CD4$^+$ T cells[261]. The translocated antigen is subsequently able to prime CD4$^+$ T cell responses. FcRn mediates this uptake throughout the absorptive cells, or enterocytes, of the intestinal epithelium. The different routes of antigen uptake differ in the type and size of antigens as well as in the quality of immune response, whether it be inflammatory or regulatory. Thus, these routes differentially participate in the uptake of pathogens, commensals and food antigens in the human intestine[254].

The first route, uptake via M cells, depends on intestinal epithelial glycosylation. There are at least five distinct classes of receptor–ligand interactions (integrin β1, C5aR, glycans, GP2 and putative IgAR are all M cell receptors) that mediate antigen uptake, and two of these clearly depend on glycosylation[254]. First, binding of α2,3-linked sialic acid as well as α1,2-linked fucose residues to M cell receptors facilitates antigen uptake[263–265]. Second, the bacterial lectin FimH interacts with GP2 in a carbohydrate-dependent manner[266]. The FimH of a subset of Gram-negative bacteria, such as *E. coli* and *S.* Typhimurium, recognize mannose residues on GP2 likely in the context of the GP2 peptide backbone, as free mannose or deletion of GP2 both abrogate binding and bacterial uptake [267]. It is interesting that human serum antibodies against GP2 are highly expressed in Crohn's disease and positively correlate with antibodies against mannose-rich phosphopeptidomannan (called anti-*Saccharomyces cerevisiae* antibodies (ASCA)), an established serological marker of Crohn's disease[268]. Anti-GP2 human serum antibodies also exhibit a weaker positive correlation with the presence of anti-outer membrane porin C precursor (OmpC; an *E. coli* surface protein) antibodies[269]. Notably, although anti-GP2, ASCA and anti-OmpC antibodies are present in Crohn's disease, they are minimally expressed in ulcerative colitis[268,269]. The co-occurrence of anti-GP2, ASCA and anti-OmpC antibodies in Crohn's disease presents the interesting possibility that M cells with expression of GP2 engulf FimH$^+$ *E. Coli–S. cerevisiae* clusters (via a FimH–mannan interaction), resulting in the generation

of anti-*E. coli* (specifically, anti-OmpC) and anti-yeast (specifically, ASCA) allo-antibodies as well as of anti-GP2 autoantibodies. Although it is unclear whether these specific antibodies trigger dysbiosis or an autoinflammatory reaction in IBD, it is possible that the mannose–FimH axis might be a treatment target in addition to a biomarker in IBD. The increased abundance of ASCA and anti-GP2 antibodies in ileal Crohn's disease compared with ulcerative colitis (or colonic-only Crohn's disease) makes sense considering the location of M cells primarily in the small intestine[268,269]. In addition to these direct roles for glycans in mediating M-cell–antigen interactions, glycans also have an indirect role in antigen uptake via the regulation of glycocalyx bulk — the thin glycocalyx present on M cells facilitates interactions with luminal antigens[253]. Glycans, as well as the glycoconjugates to which they are attached, determine glycocalyx thickness. For example, overexpression of the polypeptide GalNAc transferase 7 (GALNT7), which adds GalNAc to Ser or Thr to initiate mucin-type O-glycosylation, increases glycocalyx bulk[270].

Although not yet experimentally tested, it is likely that both paracellular dendritic cell sampling and GAP uptake depend on glycosylation. Glycans help to maintain important intercellular junctions in the intestinal epithelium, for example via increasing the stability of JAM-A and desmoglein-2 [204,271]. Similar glycan-dependent interactions are also likely important in regulating interactions of epithelial cells and dendritic cells during antigen sampling, as dendrites extend from the basal to the luminal side of the intestinal epithelial sheet[257]. Glycans are also crucial in multiple aspects of MUC2 synthesis and stability in goblet cells and in the mucus layer[28]. Thus, one would predict that disruption in epithelial glycosylation would perturb GAP-dependent uptake, although this will need to be tested.

Intestinal epithelial glycosylation also facilitates tolerogenic imprinting and leukocyte–epithelial interactions. Interactions between MUC2 and dendritic cells in the lamina propria and Peyer's patches in the small intestine suppress inflammatory signals and induce tolerance[21]. This tolerogenic activity of MUC2 depends on its glycosylation. Mechanistically, MUC2 binds to soluble, cell surface galectin-3, which in turn interacts with dectin-1 and FcγRIIB, which are cell-surface receptors on dendritic cells. Deglycosylation of MUC2 or biochemical or genetic disruption of galectin-3, dectin-1 or FcγRIIB impairs the ability of MUC2 to bind to dendritic cells and induce downstream regulatory cytokines. Glycosylated MUC2 activates AKT and glycogen synthase kinase 3β (GSK3β) phosphorylation, thereby facilitating β-catenin nuclear translocation[21], which is crucial for induction of tolerogenic signals in intestinal dendritic cells[272]. Given the role of MUC2 glycosylation in inducing anti-inflammatory signals in the gut, it is possible that the impaired MUC2 glycosylation observed in IBD results in a switch from tolerogenic to inflammatory dendritic cells, leading to immune activation against the commensal microbiota and host molecules[273].

In addition to their crucial role in antigen sampling, intestinal epithelial glycans also directly regulate leukocyte recruitment via receptor–ligand interactions. The best-known example of glycan-mediated leukocyte recruitment is selectin-mediated leukocyte rolling. L-selectin on leukocytes and P-selectin and E-selectin on endothelia bind to cognate glycans to facilitate cell rolling. These interactions are a prerequisite for subsequent integrin-mediated tight binding and diapedesis **[G]**[38,274]. Glycans also mediate interactions between leukocytes and

the epithelia. This role is especially important in IBD, as neutrophil transepithelial migration and accumulation in crypt bases of neutrophils to form crypt abscesses is a hallmark of disease[275]. Multiple steps are involved in the migration of neutrophils from the vasculature to the gut lumen. After extravasating from blood vessels, neutrophils undergo transepithelial migration in the basal to apical direction. Multiple receptors are involved in attaching neutrophils to the apical membrane, including intercellular adhesion molecule 1 (ICAM1), decay-accelerating factor (DAF; also known as CD55) and the CD44 isoform CD44v6[275]. Sialyl-Lewis^a glycans expressed on epithelial-expressed, inflammation-induced CD44v6 are important regulators of neutrophil–epithelia interactions[276]. In particular, an antibody targeting Sialyl-Lewis^a, GM35, blocks transepithelial migration by inhibiting apical detachment of neutrophils into the gut lumen[276,277]. Although the mechanism blocking neutrophil release is not fully clear, it seems to depend in part on GM35-mediated inhibition of ectodomain shedding of CD44[275,276]. Thus, both epithelial and non-epithelial glycans regulate multiple steps in leukocyte recruitment from the blood to the intestinal lumen.

Translational opportunities

Translational glycobiology is still in its infancy; however, clinical trials of glycan mimetics and antibodies that target protein–glycan interactions have generated great excitement, for example in inflammatory diseases such as sickle cell disease and cancer[278–283]. In IBD, a number of approaches targeting intestinal epithelial glycosylation could have a major effect on treatment and diagnosis. These approaches would broadly fall into therapeutics that remodel surface glycans or block protein–glycan interactions and diagnostics that evaluate epithelial glycans or anti-glycan antibodies.

Evidence from the past decade highlights a pathological role for aberrant intestinal epithelial glycosylation in IBD, which suggests that ‘normalizing’ the disease-associated glycome could benefit patients. A number of methods can be used to remodel the intestinal glycome, including direct methods, such as editing glycans with glyosyltransferases and/or glycosidases or their inhibitors or replacing glycans through the addition of antibodies or lipids linked to intestinal glycans[284], and indirect methods, such as normalizing the gut microbiota or reprogramming the glycome using select bacterial products (that is, glycosylation inducers).

In terms of direct methods, one promising approach is developing inhibitors of glycosidases, including bacterial glyosidases involved in glycan simplification or host glycosidases such as O-GlcNAcase, that are involved in pathogenic signalling. Indeed, inhibiting O-GlcNAcase ameliorates gut inflammation in a pre-clinical mouse model[118]. In addition to loss of terminal glycosylation, IBD is also associated with a reduction in mucin sulfation, a post-glyosylational modification that contributes to disease pathogenesis. Reduced sulfation arises in part from increased mucin sulfatase activity. Although the nature (extracellular versus intracellular, host versus bacterial) and identify of this sulfatase is not clear, a number of sulfatase inhibitors have been developed for various applications and some have been tested in patients (although not in IBD)[285–290]. An alternative approach to increase sulfation could be to deliver sulfation precursors such as 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to patients either systemically or locally, although this would need to be tested[291].

In terms of indirect methods, bacterial interactions with the intestinal epithelia result in host signalling that induces gut glycosylation in mice and probably also in humans[76]. Therefore, normalizing the gut microbiota through faecal microbiota transplantation, prebiotics, probiotics and engineered diets might correct components of glycan disruption in IBD. Similarly, in a feed-forward loop, inflammation itself drives pathological changes to the glycome, which in turn disrupts the gut microbiota and promotes recruitment of inflammatory cells in humans[1–3,26]. Given the role of immune cells in regulating the glycome in mice, anti-inflammatory drugs, such as biologics, immunomodulators, steroids and aminosalicylates, might in part act through reprogramming of the glycome; however, this will need to be tested[27,75,79,85]. Although gut microbiota normalization and use of immunomodulators are complex interventions that contribute to disease improvement via multiple mechanisms, it is possible that correcting the glycome is one such mechanism.

Protein–glycan interactions contribute to IBD pathogenesis by recruiting inflammatory cells or pathogenic bacteria and can be blocked through glycome reprogramming or by inhibitors such as antibodies that interrupt protein–glycan interaction or small molecules (for example, glycan mimetics) that target protein–glycan interactions or glycosyltransferases or glycosidases in experimental models[235,276]. Interestingly, dietary substances contain lectin–glycan inhibitors that might ameliorate gut inflammation. For example, ingestion of plant products high in galactose correlates with reduced incidence of CRC (512 CRC cases versus 512 controls; OR 0.67; CI 0.47–0.95)[292], suggesting that galactose interferes with *F. nucleatum* FAP2–GalGalNAc interactions, which are pathogenic in IBD and cancer. This observation lays the groundwork for other dietary substances, such as selected or designed fibre oligosaccharides, to interfere with or promote pathogenic or beneficial host–microorganism interactions, respectively. High fibre intake is associated with reduced IBD incidence and disease severity, and specific dietary interventions such as exclusive enteral nutrition can induce disease remission in select patients with IBD[293,294]. However, such dietary interventions in general have not been designed or evaluated to date for specific glycan content, providing an opportunity for new investigation. Similarly, synthetic inhibitors, for example those that have been developed to block pathogenic *E. coli* FimH lectin activity, could also be useful and have entered clinical trials for Crohn's disease (EB8018 FimH inhibitor)[295,296].

Additionally, glycome simplification and loss of extended glycans unmasks the protein backbone, making mucins more accessible to host and bacterial proteases[176,177]. Loss of this protective mucin layer leads to enhanced bacterial–intestinal epithelia interactions, which in theory can be blocked with appropriate mucin protease inhibitors[22,176,177].

Intestinal epithelial glycomics, serum glycomics, anti-glycan immunohistochemistry and glycan microarrays offer unique diagnostic opportunities. All of these approaches distinguish IBD from healthy or non-IBD controls[26,115,297,298]. However, perhaps the most exciting application of these technologies is in the field of precision medicine. Clinicians could use alterations in the epithelial glycome and the anti-glycome immune response to stratify patients and potentially influence therapeutic selection. In addition, some of these assays might augment current approaches for evaluating treatment response. The potential of

the various approaches to target intestinal epithelial glycosylation to improve IBD is exciting.

Conclusions

IBD is the result of the dysregulation of immune cells, the gut microbiota and the gut epithelia. Current evidence suggests that intestinal epithelial glycosylation plays a central part in all of these processes. Further, studies of patients with IBD indicate that the presence of disease and worse disease activity correlate with the expression of truncated O-glycans, although the mechanisms underlying these alterations are currently unclear. In twin studies, altered glycosylation can in some cases precede disease initiation, whereas other glycan changes emerge during active disease. Glycosylation is crucial for biosynthesis and stability of MUC2, the major glycoprotein in the intestinal mucus layer, and loss of MUC2 results in spontaneous inflammation and CRC[299,300]. The mucus layer is often compromised in IBD, which suggests that altered glycosylation might be one explanation for this. Intestinal epithelial glycosylation and dietary glycans likely synergize to establish microbial networks in the intestine, and alterations in intestinal epithelial glycosylation might explain some aspects of dysbiosis in IBD. Additionally, epithelial glycosylation might be a key part of regulating the intestinal immune system. Glycans are involved in antigen uptake, tolerogenic imprinting and immune cell interactions with the intestinal epithelia. Alterations in the normal glycome might lead to disruptions in these processes that tip the gut from a regulatory to an inflammatory environment. Novel animal models and advances in disease glycomics and genetics will hopefully lead to new approaches to treat IBD (Box 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Lectin
A non-antibody glycan binding protein isolated from animals or plants

Cad antigen
A non-ABO blood group antigen present on red blood cells and secretions. Structurally, Cad antigen consists of a galactose modified by addition of sialic acid and GalNAc at two different positions on the galactose ring: GalNAcβ1,4(Neu5Acα2,3)Galβ-R

Glycan
A carbohydrate consisting of at least two monosaccharides linked together

Sequon

A defined series of amino acids that serve as an attachment site for a glycan

Glycosite
The amino acid position within a protein where a specific glycan is attached

Diapedesis
The process of blood cells travelling between endothelial cells as the blood cells exit the blood vessel and enter the tissue

Polysaccharide utilization locus
A bacterial gene cluster that encodes proteins that bind, degrade and transport extracellular polysaccharides across the bacterial cell membrane and into the bacterial cell

Peyer's patches
An organized collection of immune cells with a specific microarchitecture that is found in the gut, most commonly in the mucosal and submucosal layers of the ileum

Lewis antigens
Lewis (Le) antigens are fucosylated non-ABO blood group antigens. The basic Le structure consists of a terminal galactose attached via β-linkage to a subterminal N-acetylglucosamine (GlcNAc), which is modified by attachment of a fucose in α-linkage. The galactose can be attached to the 3 or 4 position of the GlcNAc and the fucose can be attached to the open 4 or 3 position, depending on which position is available. This basic Le structure (Le^a or Le^x, depending on the exact linkages) can be further modified by additionally adding a fucose to the galactose to form Le^y or Le^b depending on the exact linkages and/or by addition of sulfate or sialic acid to either galactose or GlcNAc. FUT3 attaches fucose to the GlcNAc, whereas FUT2 attaches fucose to the galactose

Molecular mimicry
In glycobiology, this term refers to the process of foreign microorganisms, typically bacteria, expressing glycan structures that resemble host glycan structures. This prevents immune recognition of the bacteria as a foreign invader and is used by pathogens and commensals alike

O-GlcNAcylation
This is a class of nucleocytoplasmic glycosylation defined by β-linkage of unmodified N-acetylglucosamine on serine or threonine on glycoproteins. Unlike the majority of cell surface glycans, which last for the lifespan of the protein, O-GlcNAc is added and removed rapidly throughout the lifespan of a protein. O-GlcNAcylation often occurs at the same site as phosphorylation and similarly regulates cell signalling

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Box 1 | Glycosylation nomenclature

Glycan nomenclature

There are multiple ways to refer to glycans, including by glycan classes (for example, mucin-type O-glycans and N-glycans), subclasses based on common motifs or core structures within a glycan class (such as high-mannose, hybrid or complex N-glycans, and core 1-based, core-2 based and core-3 based mucin-type O-glycans), common epitopes (such as Lewis^a, CHO-131, CAD and ABO), partial structural descriptions (for example, mucin-type core 1 O-glycan (Gal-GalNAc-Ser/Thr)), or complete structural descriptions that include linkage information (for example, mucin-type core 1 O-glycan (Galβ1,3GalNAcα1-Ser/Thr)). In the latter case, β refers to the anomeric linkage (which can be either α or β) of galactose to GalNAc. '1,3' indicates that position 1 on the galactose ring is attached to position 3 on the GalNAc ring. The level of detail used to describe a glycan in general and in this Review often depends on the biological function of the glycan. In some cases, the presence or absence of a glycan class, the specific epitope (that, for example, interacts with a glycan-binding protein) or the core structure that determines the number and type of terminal epitopes (for example, high-mannose N-glycans terminating in mannose versus complex N-glycans that can be decorated with diverse sialylated and fucosylated termini) are most important in defining function. Further, depending on the type of structural analysis performed in general, linkage information is often unavailable, whereas in other cases it is empirically defined. Thus, linkage information is often omitted from the structural description (for example, in GalGalNAc versus Galβ1,3GalNAc), although the linkage information can sometimes be inferred. This is similar to how peptide mass fingerprinting can be used for protein identification in the absence of de novo sequencing.

Glycosyltransferase nomenclature

Glycosyltransferases are divided into families on the basis of amino-acid sequence and named on the basis of enzymatic activity. Sometimes a single glycosyltransferase can have multiple names, in part reflecting the history of its discovery. For example, the enzyme that extends GalNAc-Ser/Thr (also known as Tn antigen) by adding a galactose (at the β1,3 linkage) to form Galβ1,3GalNAc-Ser/Thr (also known as core 1 (or T antigen)) is called core 1 synthase (core 1 β1,3-galactosyltransferase 1 (C1GALT1)) or T-synthase, to reflect the two names used for the glycan (core 1 or T antigen).

Box 2 | Effects of disrupted intestinal epithelial glycosylation in IBD

Altered immunity

- Altered antigen uptake
  - M cells
  - Transepithelial dendrites
  - Goblet-associated passages
- MUC2-dependent tolerogenic imprinting
- Transepithelial leukocyte recruitment to the crypts

Glycan–lectin interactions

- Host lectins
  - Altered glycosyltransferase binding
  - Impaired protein quality control
  - Impaired protein sorting
  - Impaired Siglec immune inhibition
- Foreign lectins
  - Altered binding of plant lectins, viruses, bacteria, toxins and parasites

MUC2 synthesis and stability

- Impaired MUC2 synthesis
- Increased MUC2 degradation
- Increased intestinal permeability

Host–microorganism interactions

- Altered bacteria–intestinal epithelial cell (IEC) binding:
  - Helicobacter pylori
  - Lactobacillus
  - Bifidobacterium
  - Polysaccharide utilization loci
- Altered IEC glycan induction
  - Fucose and N-glycans
- Spatial dysbiosis
- Microorganism nutrient dysregulation

- Bacteroidales

Box 3 | Unanswered questions in IBD glycosylation

- How does inflammatory bowel disease (IBD) genetics contribute to the structural changes in glycans that are observed in patient tissues?
- How do host glycans interact with dietary glycans to regulate the gut microbiota?
- What host factors are able to compensate for changes in glycosylation to maintain homeostasis?
- How does the gut microbiota induce or degrade host glycosylation to regulate gut biology?
- How does compromised intestinal epithelial glycosylation affect the intestinal immune system?
- How do glycans select for or against specific gut bacteria?
- How can we best engineer the epithelial glycome in vivo to repair the pro-inflammatory IBD-associated glycome?
- What protein–glycan interactions would be best to target in IBD to ameliorate inflammation?
- How can we develop better small molecule inhibitors for glycosyltransferases, glycosidases and sulfatases for IBD and other diseases?
- How do current IBD therapies, including immunomodulators, dietary interventions and faecal microbiota transplantation, affect the epithelial glycome?
- Can eating glycans, glycomimetics or lectins reduce inflammation in IBD?
- How can we use next-generation glycomic technologies (for example, tissue or serum glycomics, glycan microarrays and glycan imaging) to improve diagnosis and treatment of IBD?

Key points

- A large set of transcriptional and enzymatic pathways spatially and developmentally regulate glycosylation in the gut.
- Host genetics, environment and the gut microbiota influence intestinal epithelial glycosylation.
- Epithelial glycans have many functions: they act as ligands and nutrient sources, and establish immunological tone, for the gut microbiota.
- Genome-wide association studies and biochemical studies implicate altered intestinal epithelial glycosylation in Crohn’s disease and ulcerative colitis.
- Disrupted glycosylation contributes to inflammation by perturbing intestinal barrier function, glycan–lectin interactions, the gut microbiota and mucosal immunity.
- Targeting epithelial glycans in the intestine provides an opportunity to combat inflammatory bowel disease.

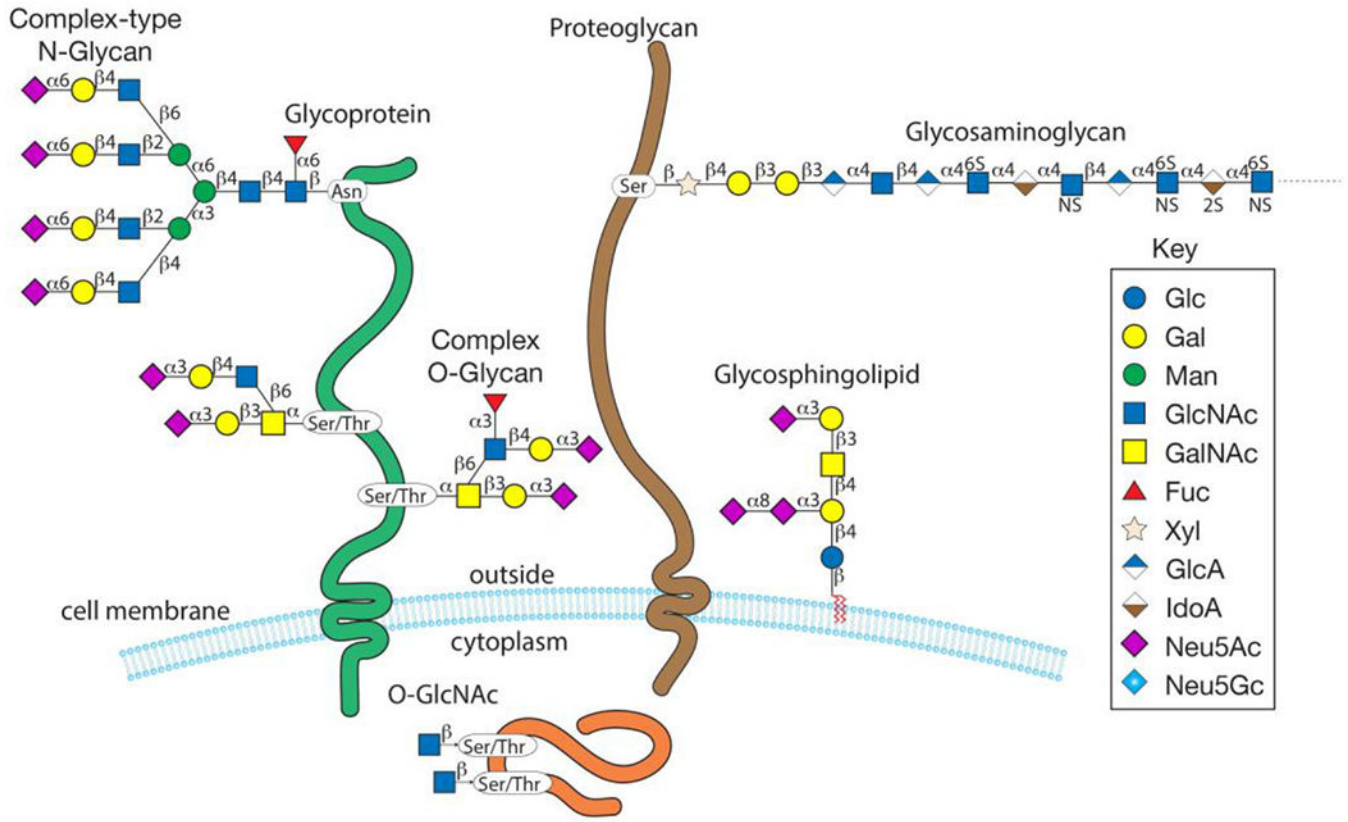

Figure 1. Mammalian glycan classes and monosaccharides.

Cell-surface glycans, including N-glycans, O-glycans, glycosaminoglycans, glycosphingolipids and intracellular O-GlcNAc, are shown. NS, N-sulfated glucosamine ($GlcNSO_3$); 6S, 6-O-sulfate.

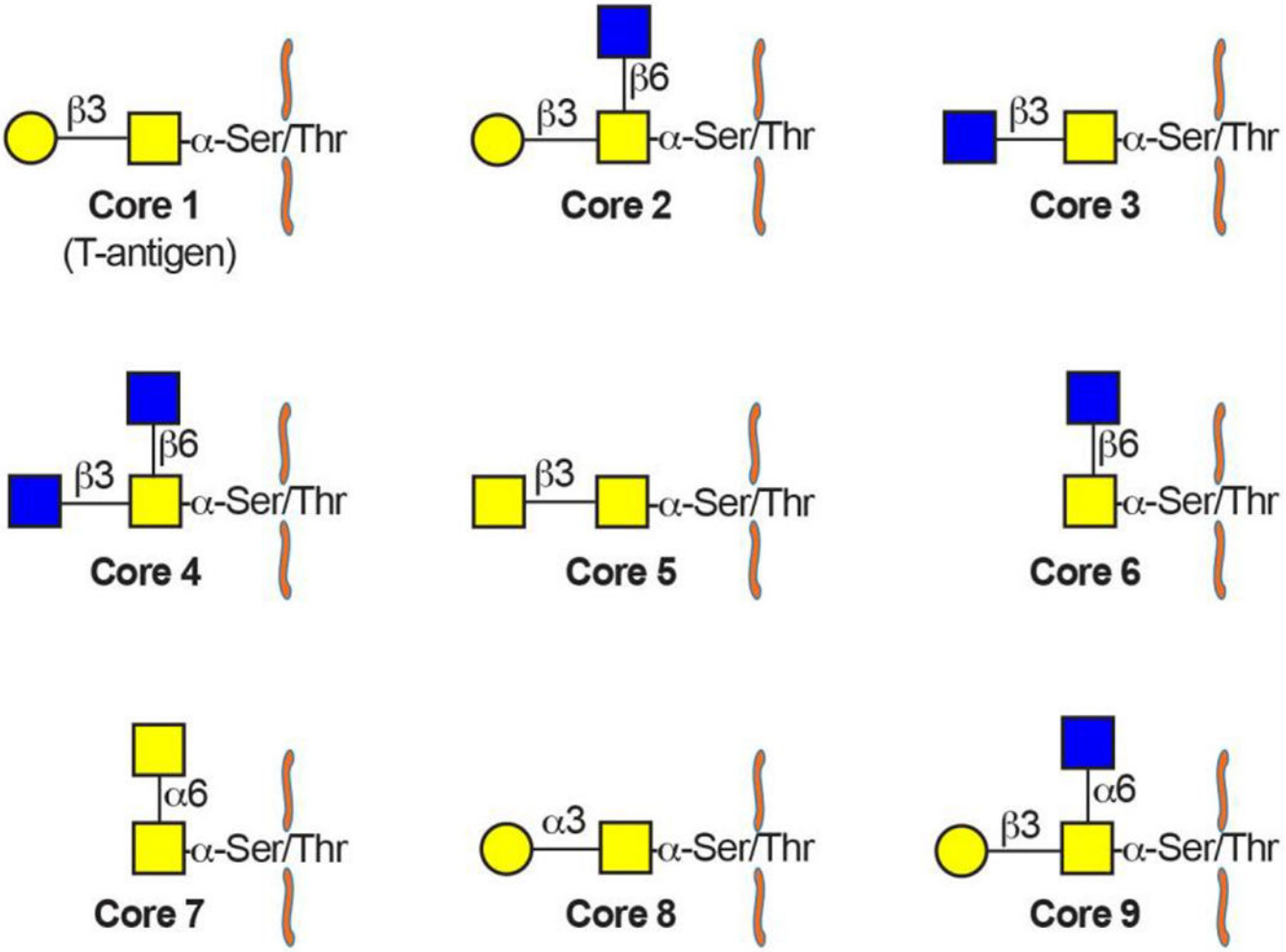

Figure 2. O-glycan core structures.

Cores 1–9 are shown. Core 1–4-based structures constitute the majority of O-glycans, whereas cores 5–9 are generally absent or a relatively minor component. Cores 1 and 2 are ubiquitous whereas cores 3 and 4 are restricted to the gastrointestinal tract.

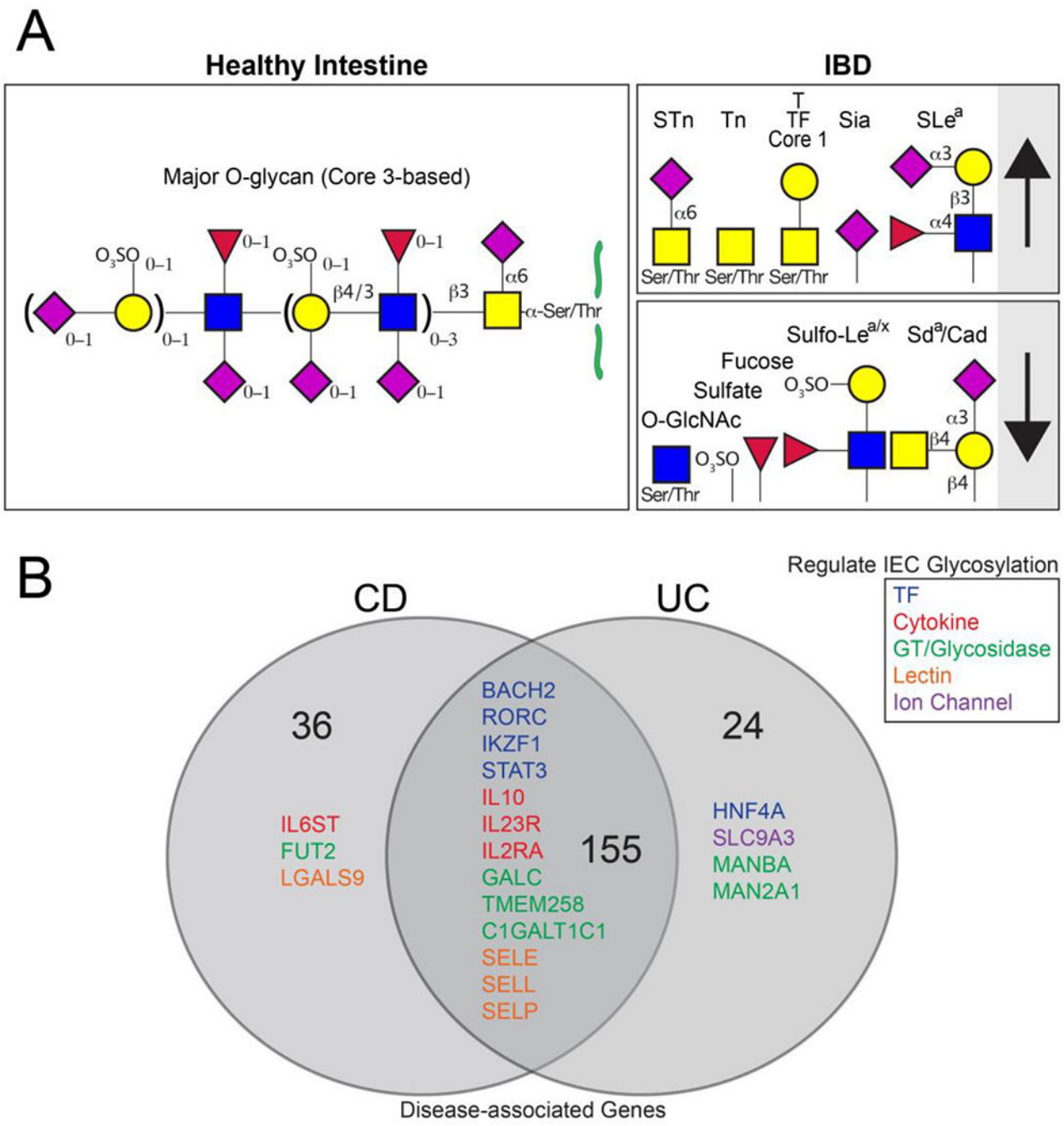

Figure 3. Altered glycan structures and genes in inflammatory bowel disease.

(A) Major O-glycans of the healthy sigmoid colon and alterations in inflammatory bowel disease (IBD) are depicted[20,26,28,63,72,112,113,115]. **(B)** All candidate genes for Crohn's disease, ulcerative colitis or both (IBD) are enumerated, with those implicated in glycosylation listed[80,83–89]. Genes are divided by function as indicated.

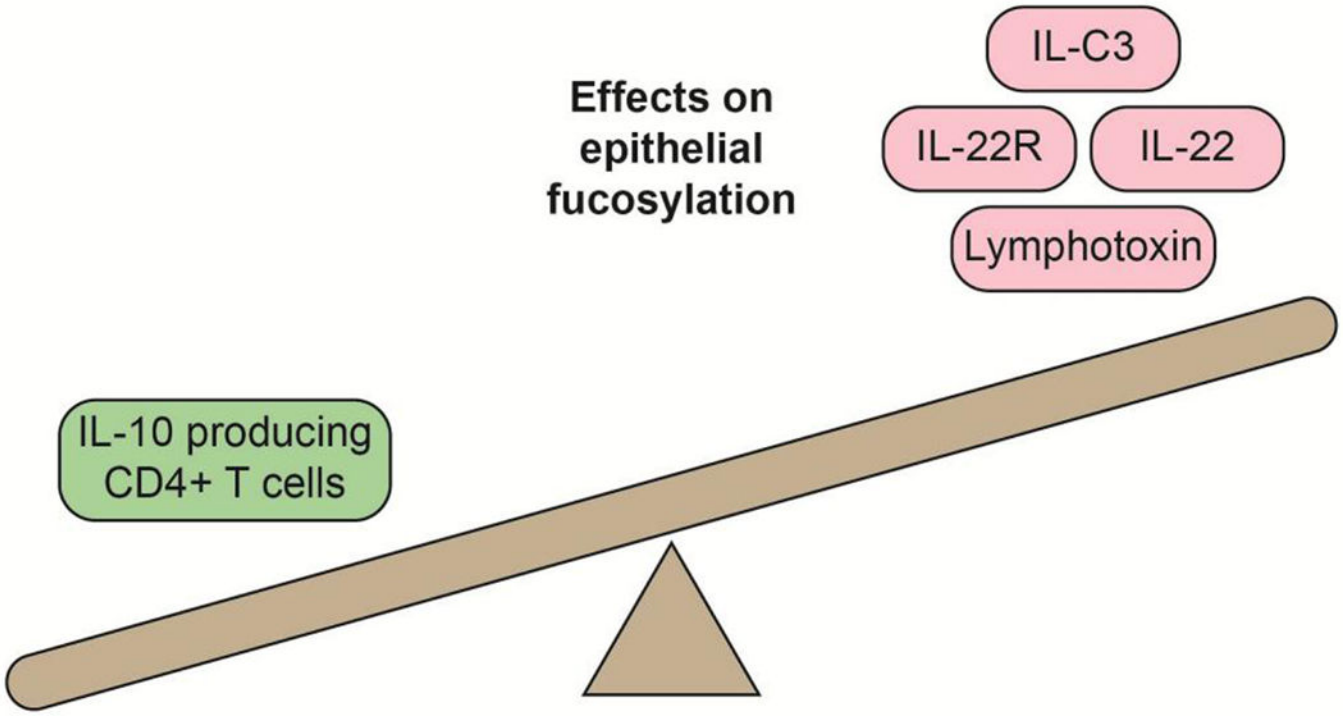

Figure 4. Immune regulation of epithelial fucosylation.

Cytokines, cytokine receptors and immune cells that reduce (left) or increase (right) epithelial fucosylation are depicted.

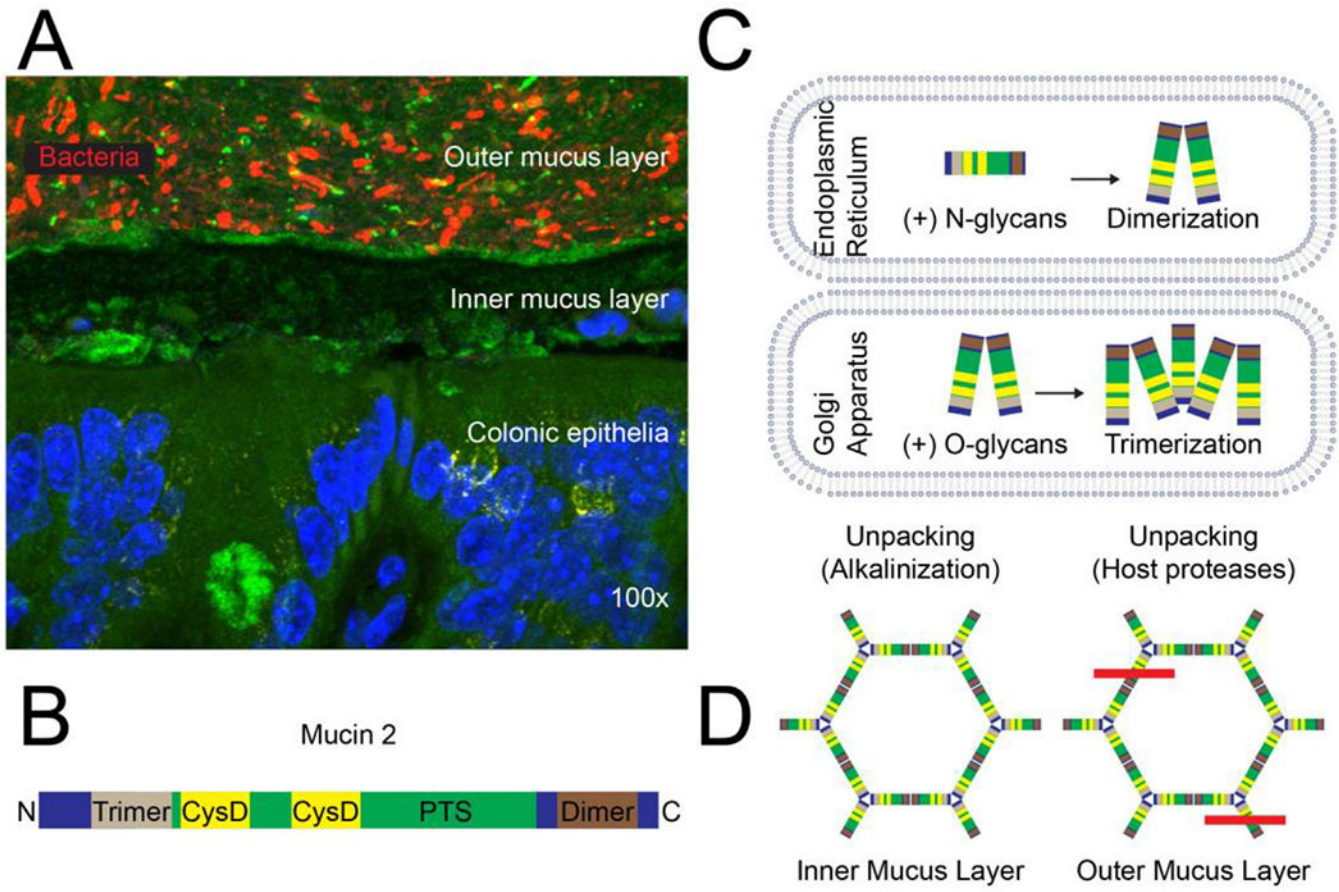

Figure 5. Mucus structure and synthesis.

(A) Topography of the normal colonic mucus layer. Bacteria in the outer mucus layer overlay the inner mucus layer, which is normally impenetrable to bacteria; DAPI (blue), MUC2 (green; sc-15334, H-300), bacteria (16S FISH; red), magnification: 100x[15]. **(B)** The MUC2 glycoprotein has an N-terminal trimerization and a C-terminal dimerization domain with a PTS domain that is rich in proline, threonine, serine in between. The N-terminus contains three von Willebrand D domains and the C-terminus contains one (not shown). The PTS domain is highly O-glycosylated in the Golgi and interspersed by two CysD domains, contributing to intramolecular disulfide bonds and intermolecular non-covalent interactions. **(C)** MUC2 is synthesized in the endoplasmic reticulum, N-glycosylated and dimerized. It is then transported to the Golgi through an N-glycan-dependent mechanism, where it is O-glycosylated and forms trimers. **(D)** MUC2 is released and unpacked in the inner mucus layer through alkalinization and chelation of Ca^{2+} ions and then further unpacked in the outer mucus layer through proteolysis; the red line indicates cleavage by host proteases.

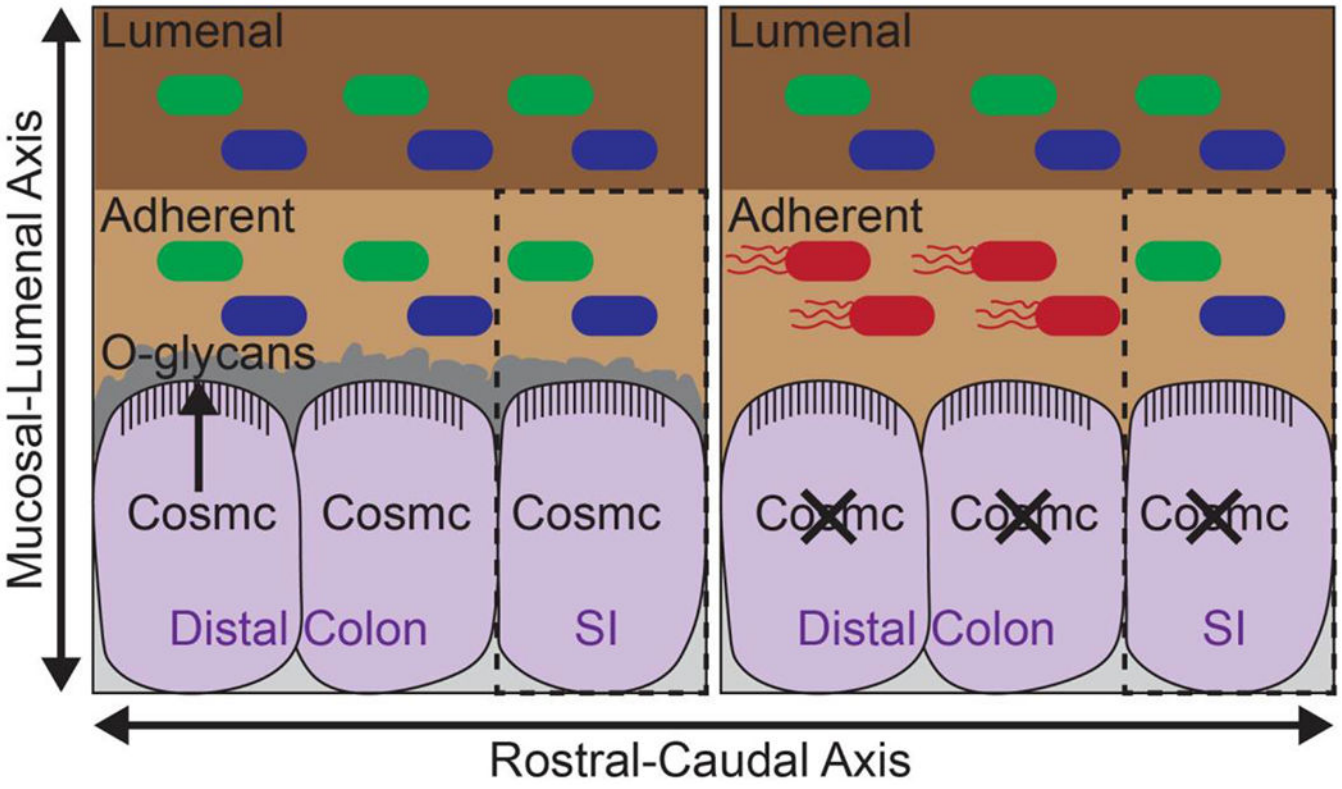

Figure 6. COSMC and O-glycans spatially regulate the gut microbiota.

COSMC regulates extension of O-glycans in intestinal epithelia and formation of the colonic mucus layer, separating the gut microbiota from contact with the intestinal epithelia. In mice, loss of *Cosmc* leads to loss of extended O-glycans and increased bacterial–epithelial contact in the colon. This loss coincides with a loss of bacterial diversity and the emergence of a pro-inflammatory pathobiont in the colonic mucosa but not in the overlying lumen or ileal mucosa[15]. These changes correspond to the spatial dysbiosis observed in patients with inflammatory bowel disease (IBD)[82].

Table 1.

Genes regulating glycosylation that are implicated in inflammatory bowel disease

Gene	Change in IBD[a]	Role in regulating glycosylation[b]	Refs
Cytokines			
IL6ST	SNP/Increased	Increased N-glycan galactose	89,90,93,95
IL10	Decreased	Decreased fucose	79,90,92,93,101
IL23R	SNP	Increased fucose	27,85,90,93,99,104
IL2RA	SNP/Decreased	Decreased N-glycan branching	90,93,97
Lectins			
SELE	Increased	Glycan binding protein	89,90,93
SELL	Increased	Glycan binding protein	89,90,93
SELP	Increased	Glycan binding protein	89,90,93
LGALS9	Increased	Glycan binding protein; immunosuppressive	90,91,93
Glycosyltransferases and glycosidases			
FUT2	Decreased	α1,2-fucosyltransferase	16,23,27,85,90,93
C1GALT1C1	Decreased	Chaperone for T-synthase/core 1 O-glycan synthase	15,80,90,93
GALC	SNP	Lysosomal galactosidase	90,93
TMEM258	SNP/Decreased	Oligosaccharyltransferase complex, N-glycan synthesis	86,90,93
MANBA	SNP	Lysosomal mannosidase	90,93
MAN2A1	SNP/Increased	N-glycan processing and maturation	90,93,105
Transcription factors			
BACH2	Increased	Increased biantennary monogalactosylated N-glycan with core fucose	89,90,93
IKZF1	SNP/Decreased	Decreased fucose, increased bisecting GlcNAc, binds glycosyltransferase promoters	89,90,93,95
RORC	Decreased	Increased fucose, transcription factor for ILC3 and subsequent FUT2 induction	27,90,93
HNF4A	SNP/Decreased	Increased fucose, increased GDP-fucose and fucosyltransferase	89,90,93,98,100,103
STAT3	Increased	Increased FUT2 via immune signalling	27,85,90,93,99,102
Gut pH/ion channel			
SLC9A3	Decreased	Na/H exchanger; gene deletion increases gut lumen pH and bacteria-induced fucose	85,90,93,94,96

[a]Single nucleotide polymorphisms (SNPs) or gene/transcript increase or decrease; SNP/increased or SNP/decreased implies that both SNPs and a change in transcript levels are implicated in IBD.

[b]Increased or decreased implies positive or negative correlation with transcript level; for example, ‘increased’ in this column corresponds to an increase in IBD if the transcript is increased in IBD (see Change in IBD column), but if the transcript is decreased in IBD ‘increased’ in this column would imply a decrease in IBD.

Table 2.

Host and foreign lectins that bind to intestinal epithelial glycans

Lectin type	Lectin	Ligand
Host lectins		
R-type lectin	ppGalNAcT	GalNAcα1-Ser/Thr
L-type lectin	Calnexin and calreticulin	Monoglucosylated high-mannose N-glycans
	ERGIC-53, VIP36, and VIPL	High-mannose N-glycans
P-type lectin	Mannose-6-phosphate receptor	Mannose-6-phosphate on N-glycans
C-type lectin	Dectin-1/2	β-glucan, high-mannose N-glycan
	Mannose receptor	α-linked mannose on microorganisms or mucins
	Macrophage galactose-type lectin (MGL)	GalNAc
	Siglec 5, Siglec 14 and Siglec 15	SialylTn
	Siglec-7	α2,8-linked disialic acid
	Siglec-9	6-sulfo sialyl Lewis x
S-type lectin	Galectins	LacNAc (Galβ1,4GlcNAc)
Foreign lectins		
Plant	Peanut agglutinin	GalGalNAcα1-Ser/Thr
Viruses	Influenza, adenovirus, reovirus, rotavirus, murine respirovirus, polyomavirus	Sialic acid
	VP8 (rotavirus)	Non-sialyl type 1 or 2 chains on O-glycans or N-glycans
	HSV, HIV, dengue, foot and mouth disease	Heparan sulfate
Bacteria	*Vibrio cholerae* sialidase	Sialic acid
	E. coli FimH	Mannose
	Enterotoxigenic *E. coli* F17-G adhesin	GlcNAc
	Bacterioides, *Clostridium*, *E. coli*, *Lactobacillus*	Galabiose (Galα1,4Gal)
	F. nucleatum Fap2	GalGalNAcα1-Ser/Thr
	Borrelia burgdorferi ErpG	Heparan sulfate
	Campylobacter jejuni flagella/LPS	H-antigen (Fucα1,2Galβ1,4GlcNAcβ),
	E. coli K99 fimbriae	GM3(Neu5Gc) (Neu5Gcα2,3Galβ1,4Glc-Cer)
	Helicobacter pylori BabA	Sialyl Lewis x
	Helicobacter pylori SabA	Lewis b
Toxins	*Pseudomonas aeruginosa* PA1	Galactose
	Pseudomonas aeruginosa PA2	Fucose or mannose
	Clostridioides difficile toxin A	GalNAcβ1,3Galβ1,4GlcNAcβ1,3Galβ1,4GlcβCer
	E. coli heat-labile toxin	GM1
	Shigella dysenteriae Shiga toxin	Galα1,4GalβCer or Galα1,4Galβ1,4GlcβCer
	Vibrio cholerae cholera toxin	Galβ1,3GalNAc of GM1
Parasites	*Entamoeba histolytica* 260-kDa lectin	Gal/GalNAc
	Trypanosoma cruzi surface ‘mucins’	NeuAc, heparan sulfate
	Cryptosporidium parvum lectin p30	Gal/GalNAc

Lectin type	Lectin	Ligand
	Giardia lamblia	Mannose
	Toxoplasma gondii TgMIC1	NeuAcα2,3Galβ1,4GlcNAc