

# Interactions of commensal and pathogenic microorganisms with the intestinal mucosal barrier

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**Abstract** | The intestinal mucosal barrier is composed of epithelial cells that are protected by an overlying host-secreted mucous layer and functions as the first line of defence against pathogenic and non-pathogenic microorganisms. Some microorganisms have evolved strategies to either survive in the mucosal barrier or circumvent it to establish infection. In this Review, we discuss the current state of knowledge of the complex interactions of commensal microorganisms with the intestinal mucosal barrier, and we discuss strategies used by pathogenic microorganisms to establish infection by either exploiting different epithelial cell lineages or disrupting the mucous layer, as well as the role of defects in mucus production in chronic disease.

## Commensal

Refers to symbiotic microorganisms that do not seem to harm (pathogens) or benefit (mutualists) their host. Commensal is arguably an imprecise term to apply to a microorganism, especially considering that any microorganism may display behaviours that are both beneficial and detrimental.

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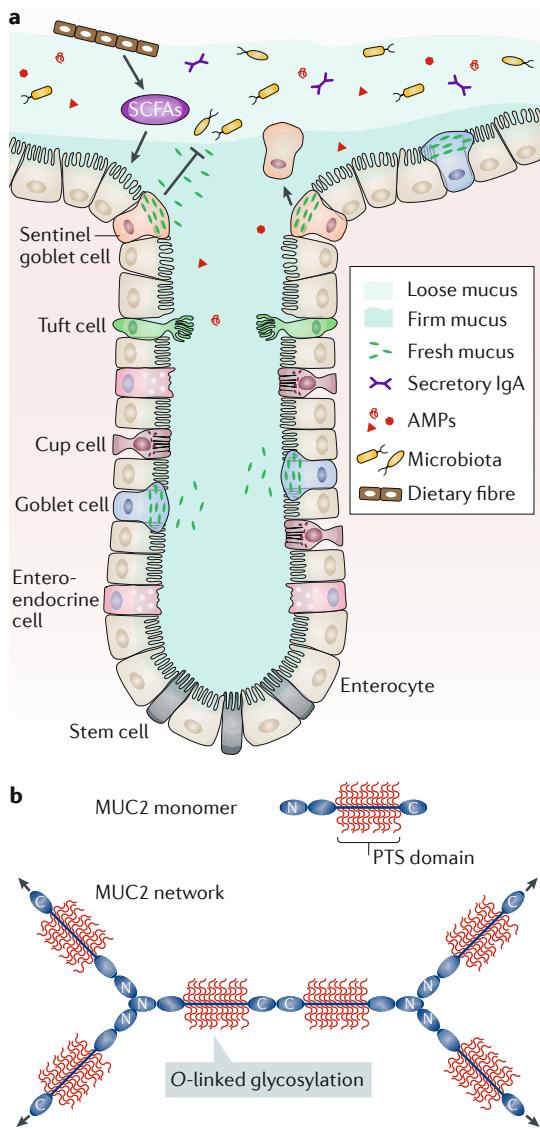
The human small and large intestines harbour a diverse community of commensal bacteria commonly referred to as the microbiota, which, at  $10^{11}$ – $10^{12}$  microorganisms per  $\text{cm}^3$  in the colon, is unrivalled in its population density compared with bacterial abundance at other body sites<sup>1</sup>. The composition of the human colonic microbiota is mainly defined by Bacteroidetes and Firmicutes, along with members of a few other phyla, but it strongly varies between individuals<sup>2</sup>. The microbiota is shaped by environmental exposure, including to maternal microorganisms, starting at birth<sup>3–5</sup>. Once established in early childhood, the hundreds of coexisting species inhabiting the gut respond to environmental and lifestyle stimuli in timescales as short as hours to days<sup>6</sup> and as long as reproductive generations<sup>7</sup>. An important function of the gut microbiota is digestion of dietary fibre, which is a broad category of nutrients encompassing dozens of chemically diverse polysaccharides present in plant cell walls and other sources and functions as a prominent energy source for the microbiota. These dietary polysaccharides are largely indigestible by human digestive enzymes, which mostly target starch and a few simple sugars<sup>8</sup>. Bacterial fermentation of fibre-derived sugars produces short-chain fatty acids (SCFAs) that influence gut epithelial cell and lymphocyte homeostasis<sup>9–11</sup> as well as nutrient storage<sup>12</sup>. In recent years, the role of the gut microbiota in the causation and progression of intestinal diseases, such as inflammatory bowel disease (IBD) and colorectal cancer, has become more relevant owing to changing dietary lifestyles, including reduced consumption of fibre polysaccharides in industrialized societies<sup>13</sup>.

The first line of host defence against both encroaching commensal bacteria and invading enteric pathogens

is the intestinal mucosal barrier<sup>14</sup>, which is a physical barrier that includes both biochemical and immunological components; the physical barrier is composed of epithelial cells that are connected by tight junctions and is protected by an overlying host-secreted mucous layer that contains two layers in the colon (FIG. 1a). The immunological aspects of both the physical and biochemical barriers are reviewed elsewhere in detail<sup>15,16</sup>, and we only briefly highlight the immunological components of the mucosal barrier here (BOX 1).

The intestinal epithelium consists of six differentiated cell types with defined functions: enterocytes, enteroendocrine cells, Paneth cells, tuft cells, goblet cells and microfold (M) cells, which have roles in absorption, hormone secretion, antimicrobial peptide (AMP) secretion, taste-chemosensory responses, mucus production and antigen sampling, respectively<sup>17</sup>. Not all cell types run the length of the intestine, and at least one additional type, cup cells, exists but has no known function<sup>17</sup>. Intestinal mucus is secreted by goblet cells to form a physical barrier in places such as the stomach and colon. Mucus also presents biochemical decoy receptors for potential pathogens, harbours components of the host immune response<sup>16</sup> and may even retain memory of recent antimicrobial activity by binding and retaining bacteriophage populations<sup>18</sup>.

In addition to being composed of mostly mucin glycoproteins (FIG. 1b), mucus is a reservoir for other proteins, including secretory IgA and AMPs<sup>19,20</sup>. Mucin 2 (MUC2) is the predominant gel-forming mucin expressed in the lower intestine, although other mucins are involved<sup>16</sup>. Mucin glycoproteins contain a bewildering repertoire of mostly O-linked and some N-linked glycan



**Fig. 1 | Structure, function and production of the gut mucosal barrier.** **a** | Model of large intestinal crypt architecture and metabolic connections with the microbiota in the gut lumen. The large intestine harbours commensal bacteria that ferment dietary fibre into short-chain fatty acids (SCFAs), which are an important energy source for colonic enterocytes and goblet cells. Mucus secreted by goblet cells continually replenishes the mucous layer that overlies the gut epithelium as a first barrier against commensal bacteria and invading pathogens. Secretory immunoglobulin A (IgA) and antimicrobial peptides (AMPs) are secreted into the mucus as a defence against pathogens and potentially harmful commensal bacteria. The colonic epithelium consists of enteroendocrine cells, tuft cells, cup cells, goblet cells and sentinel goblet cells, which are located at the crypt entrance to reduce bacterial encroachment into the crypt. When challenged with bacterial Toll-like receptor ligands, sentinel goblet cells expel mucus and are ejected into the intestinal lumen to be replaced. **b** | Host-secreted mucin 2 (MUC2) glycoproteins are a major constituent of small intestinal and colonic mucus. The monomer is organized into multiple domains, with the PTS core domain, which contains mainly proline (Pro), serine (Ser) and threonine (Thr), being the largest; the amino-terminal and carboxy-terminal domains mediate disulfide crosslinking to build a very large mucin network comprising thousands of monomers (black arrows denote additional network connections). A series of glycosyl transferase enzymes (BOX 2) add thousands of individual O-linked glycans to the side chains of Ser and Thr residues that ultimately comprise up to 80% of the final MUC2 mass. These glycans are remarkably varied in their chemical structure, with up to 100 different variants in secreted MUC2 in humans and mice.

#### Microbiota

A community of microorganisms, such as the taxonomically diverse and densely populated assemblage of species that inhabits the human gut.

**Inflammatory bowel disease (IBD).** A group of chronic diseases characterized by episodes of relapsing and remitting inflammation. Crohn's disease and ulcerative colitis are the most common types of IBDs and are thought to develop from a combination of host genetic predisposition, including defects in the gut mucosal and immunological barriers, and environmental factors, such as diet and the microbiota.

#### Tight junctions

Multi-protein complexes that form near the apical ends of intestinal epithelial cells, providing tight, water-impermeable and ion-impermeable seals between cells. Proteins that are included in tight junctions include claudins and occludins, which span the membrane and are anchored together outside the cell and are connected to the intracellular cytoskeleton.

#### Mucous layer

Mucus, composed mostly of secreted mucin glycoproteins and other substances, is secreted by goblet cells and overlies the intestinal epithelium, forming an adherent and insoluble inner layer and a looser outer layer in the colon.

#### Tuft cells

Taste-chemosensory cells that are present in the intestinal epithelium and have an important role in initiating a type 2 immune response to clear parasitic infection. They have a unique appearance that includes a tubulovesicular system and an apical bundle of microfilaments that are attached to a tuft of long, lumen-facing microvilli.

structures, which despite their complex structural variation can be targeted by several common species of gut bacteria as nutrient sources<sup>21</sup>. Moreover, several intestinal pathogens secrete enzymes or possess physical capabilities that disrupt the mucous barrier or promote its invasion, facilitating pathogen colonization and successful infection<sup>22</sup>. Other intestinal pathogens exploit signals and/or metabolic cues that are derived from mucosal nutrients but are liberated by the commensal microbiota<sup>23</sup>.

In this Review, we discuss the interaction of commensal microorganisms with the intestinal mucosal barrier and strategies used by pathogenic microorganisms to establish infection by either exploiting different epithelial cell lineages or disrupting the mucous layer. The latter may be caused by either the commensal microbiota or defects and variations in host mucus production. Although the mucosal immune system is integral to the intestinal mucosal barrier, the focus of this Review is on the main physical aspects of this system: mucus and epithelial cells. Recent reviews provide complementary summaries with deeper focus on aspects of the mucosal immune system<sup>16,24,25</sup>.

#### The gut mucosal barrier

To promote the continual health and resilience of the mucosal barrier, epithelial cells are constantly shed into the lumen and replaced by proliferation and differentiation of stem cells, which are located near the crypt bottom, with complete renewal every few days<sup>26</sup>. Likewise, the secretory activity of goblet cells (FIG. 1a) continuously replenishes the mucous layer that overlies the epithelium and lubricates the intestine. In the colon, gel-forming mucins assemble into a firm inner layer that is adherent to the epithelium and resistant to dense microbial colonization<sup>27</sup>; however, a loose, outer mucous layer also forms, probably through the action of host and microbial enzymes. This layer is home to a dense population of commensal bacteria that can vary in both its identity and physiology relative to luminal populations<sup>28</sup>. For example, two well-studied commensals, *Bacteroides thetaiotaomicron* and *Escherichia coli*, exhibit different global transcriptional patterns during existence in the colonic mucous layer compared with matched, nearby luminal contents<sup>28</sup>. In addition, bacterial colonization also influences mucus production and shapes the mucous barrier. *B. thetaiotaomicron* and *Faecalibacterium prausnitzii* are two known examples of gut bacteria that modulate mucus production by augmenting goblet cell differentiation and inducing expression of genes involved in mucin glycosylation<sup>29</sup>. The activity of mucin-secreting goblet cells has been linked to innate immune signalling through the discovery of specialized

**Box 1 | The immune system as a key part of the mucosal barrier**

In addition to the physical and biochemical aspects of the mucosal barrier, the immune system has a crucial role in intestinal homeostasis, and many of its components are physically integrated into the epithelium and mucous layer. Pathogens and commensal microorganisms that reach close to or invade host tissue are sensed by a variety of pattern recognition receptors and antigen-presenting cells. Colonic mucus is a reservoir for immunoglobulin A (IgA), which is secreted into the lumen of the intestinal tract<sup>126</sup>. Pathogen detection leads to T cell-dependent production of IgA, which is released into the intestinal lumen, followed by coating and neutralization of the invading microorganism<sup>119</sup> as recently shown for *Salmonella enterica* subsp. serovar *enterica* Typhimurium<sup>127</sup>. Another protein found in the mucous layer, the recently discovered protein Ly6/PLAUR domain containing 8 (LYPD8)<sup>128</sup>, binds to flagellated members of the microbiota, such as *Proteus mirabilis*, and inhibits their motility, preventing the invasion into the mucous layer<sup>128</sup>. Resistin-like-β (RELMβ), another bactericidal protein present in the mucus, limits the access of Proteobacteria to the mucous layer<sup>129</sup>.

Microbial-activated Toll-like receptors (TLRs) trigger the myeloid differentiation primary response protein MYD88, which leads to the activation of the transcription factor nuclear factor-κB (NF-κB)<sup>130</sup>. TLR deficiency can have severe effects on hosts. For example, TLR4-deficient mice exhibit a reduced number of inflammatory cells and are more susceptible to dextran sodium sulfate (DSS)-induced colitis<sup>131</sup>. The mouse commensal bacterium *Helicobacter hepaticus* interacts with TLR2 to induce IL-10 production in macrophages via an immunomodulatory polysaccharide, which triggers a nuclear mitogen- and stress-activated protein kinase (MSK)-cAMP response element-binding (CREB)-dependent anti-inflammatory response<sup>132</sup>. A toxin secreted by *Bacteroides fragilis* activates a pro-carcinogenic signalling cascade in colonic epithelial cells involving the cytokine IL-17 and the transcription factors NF-κB and signal transducer and activator of transcription 3 (STAT3)<sup>133</sup>. MYD88-deficient mice show a higher susceptibility to experimentally induced colitis<sup>134</sup>, and inhibition of MYD88 leads to increased colitis-associated cancer<sup>135</sup>. Likewise, mice lacking TLR5 fail to control epithelial-associated Proteobacteria populations and subsequently develop colitis<sup>136</sup>. Germ-free MYD88-deficient and TIR-domain-containing adaptor inducing IFN-β (TRIF)-deficient mice show a decreased susceptibility to azoxymethane-associated and DSS-associated cancer<sup>137</sup>.

Short-chain fatty acids (SCFAs) regulate several aspects of barrier function, including immune components, through G protein coupled receptors (GPCRs) on epithelial and T cells. GPR43-dependent regulatory T cell ( $T_{reg}$  cell) induction by SCFAs protects mice against colitis and requires expression of this receptor in T cells themselves<sup>138</sup>. Activation of GPR109a by the SCFA butyrate induces  $T_{reg}$  cells as well as IL-10-producing T cells<sup>139</sup>. Additionally, butyrate inhibits IL-17 by increasing the plasma levels of IL-10 and IL-12 (REF.<sup>140</sup>). Segmented filamentous bacteria induce T helper 17 ( $T_{H}17$ ) cell development in the intestine of mice<sup>141</sup>, and acetate produced by Bifidobacteria protects mice from death by enteropathogenic infections by suppressing inflammatory responses<sup>142</sup>.

The involvement of tuft cells in helminth elimination has become evident only recently<sup>143</sup>. A newly discovered role of tuft cells is the development of a type 2 immune response against the helminth *Nippostrongylus brasiliensis*<sup>144</sup>, which triggers the secretion of the cytokine IL-25 and leads to generation of innate lymphoid cells (ILCs)<sup>145</sup>. These ILCs generate IL-13, which leads to expansion of tuft cells and goblet cells, creating extra mucus to expel the parasite.

**Glycoproteins**  
Class of proteins comprising oligosaccharide chains connected to a backbone polypeptide, sometimes thousands of amino acids long, which is modified with glycans in the endoplasmic reticulum.  
**Mucins and other**  
glycoproteins contain O-linked and N-linked glycans attached to serine or threonine and asparagine, respectively.

**Secretory IgA**  
Immunoglobulin A (IgA) is the most abundant antibody isotype in the human body and occurs in two subtypes: IgA1 and IgA2, which are distinguished by their heavy chains. IgA1 is predominant in the serum, and IgA2 is most abundant in the intestinal tract to bind pathogens by reducing mobility and decreasing proliferation.

**Carbohydrate-active enzymes**  
(CAZymes). This term encompasses several different groups of enzymes: glycoside hydrolases, polysaccharide lyases, carbohydrate esterases and glycosyltransferases. These groups, and hundreds of more specific enzyme families that they collectively contain, are involved in building and degrading polysaccharides and glycans.

sentinel goblet cells, which are located at the entrance of colonic crypts and presumably impede luminal bacteria from entering the crypt<sup>30</sup> (FIG. 1a). Bacterial SCFAs and metabolites are able to induce mucus biosynthesis in germ-free mice<sup>29,31</sup>. Of the 17 currently known human or mouse mucins<sup>16</sup>, the importance of MUC2 is underscored by the fact that mice genetically deficient in the *Muc2* gene develop inflammation and colon cancer owing to closer contact of bacteria with the epithelium<sup>32,33</sup>.

Human MUC2 is a notably large (5,289 amino acid) polypeptide that is organized into multiple domains, the largest being a PTS core containing mainly proline (Pro), serine (Ser) and threonine (Thr)<sup>34</sup>. The mostly O-linked glycosylation of MUC2 occurs on Ser and Thr side chains and contributes essential biophysical properties to mucus, such as hydrophilicity, which holds secreted mucin glycoproteins in an extended form. Cysteine-containing regions in MUC2 promote crosslinking into much larger lattices via disulfide bonds at the amino and carboxyl termini (BOX 2; FIG. 1b).

### Commensal microorganisms

Although a major function of secreted mucus is to protect the host epithelium from both commensal microorganisms and pathogens, the glycoproteins in this barrier also create a beneficial nutrient environment for some colonic microorganisms. Dietary fibre

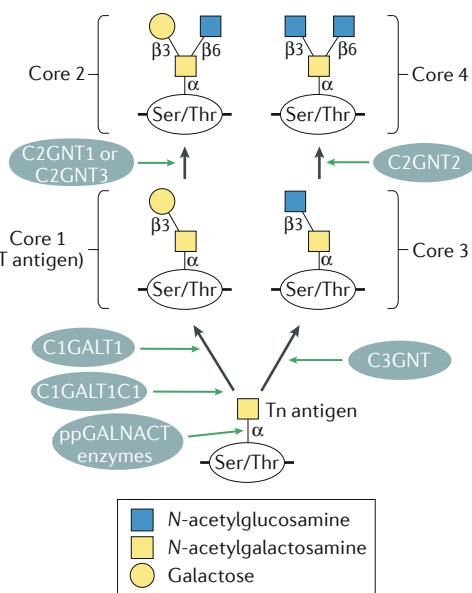
provides a major energy source for gut microorganisms. Although the endogenous niche created by host glycans is always available and is therefore likely to be occupied by bacteria, the importance of this niche increases in the absence of dietary fibre polysaccharides because it pushes the microbiota to rely more heavily on endogenous nutrients, including mucins, to sustain their metabolism<sup>35,36</sup>.

Dietary fibre encompasses an array of distinct polysaccharides, which are mostly from plant cell walls and plant storage polymers and are also present in other sources, such as animal tissue, fermented foods, fungi and food additives<sup>37</sup>. To depolymerize fibre polysaccharides, host-associated bacteria collectively produce thousands of carbohydrate-active enzymes (CAZymes) that display different catalytic specificities for the linkages in these molecules before microorganisms can ferment the liberated sugars into SCFAs<sup>38</sup>. Similarly, members of the microbiota that have evolved to use host glycans, such as the abundant O-linked glycans present in mucus, must produce substrate-specific CAZymes and proteases (see below) to cleave the unique linkages that are present in these molecules and share little overlap with dietary fibre (FIG. 2a).

The plant cell wall harbours multiple polysaccharides in a 3D matrix of varying solubility, which presents these nutrients to the microbiota in a complicated biophysical scaffold that transcends just the chemistry

## Box 2 | Synthesis of mucin glycans

Synthesis of ~100 different structures that simultaneously occur on human mucin 2 (MUC2) or its mouse homologue<sup>146</sup> and other mucins is initiated in the Golgi apparatus by a series of glycosyltransferase enzymes<sup>147</sup>. An  $\alpha$ -linked N-acetylgalactosamine (GalNAc) is first added by 1 of 20 different polypeptide N-acetylgalactosaminyltransferase (ppGALNACT) enzymes to the hydroxyl group of Ser or Thr residues to build a basal structure called Tn antigen. Tn antigen, in turn, functions as the substrate for additional glycosyltransferases that further elongate and diversify different core structures that are defined by one or two sugars that are directly attached to peptide-linked GalNAc. In contrast to the many ppGALNACT enzymes in mice and humans, synthesis of these cores is initiated by a single glycosyltransferase. For example, to build core 1, the enzyme core 1  $\beta$ 1,3-galactosyltransferase (T synthase or C1GALT1) adds galactose to the Tn antigen, generating Gal $\beta$ 1-3GalNAc1-Ser or Gal $\beta$ 1-3GalNAc1-Thr (T antigen)<sup>148</sup>. Biosynthesis of core 3-derived O-linked glycans is likewise initiated by a single core 3  $\beta$ 1,3 N-acetylglucosaminyltransferase (C3GNT) transferring GlcNAc to GalNAc1-Ser or GalNAc1-Thr (Tn antigen)<sup>148</sup>. These structures may then be further elongated with additional sugars and linkages, for example, by  $\beta$ 1,6-linked N-acetylglycosamine attached either to core 1 (by C2GNT1 or C2GNT3, to produce core 2 from core 1; or, C2GNT2, to produce core 4 from core 3). These core structures can be further elongated into monoantennary or biantennary structures to make extended glycan chains. C1GALT1C1, glycoprotein-N-acetylgalactosamine 3- $\beta$ -galactosyltransferase 1 chaperone 1.



## Prebiotics

Dietary supplements (most often oligosaccharide or polysaccharide fibres) that selectively increase certain taxonomic groups in the gut microbiota and also exert a positive impact on host health.

## Hidden Markov models

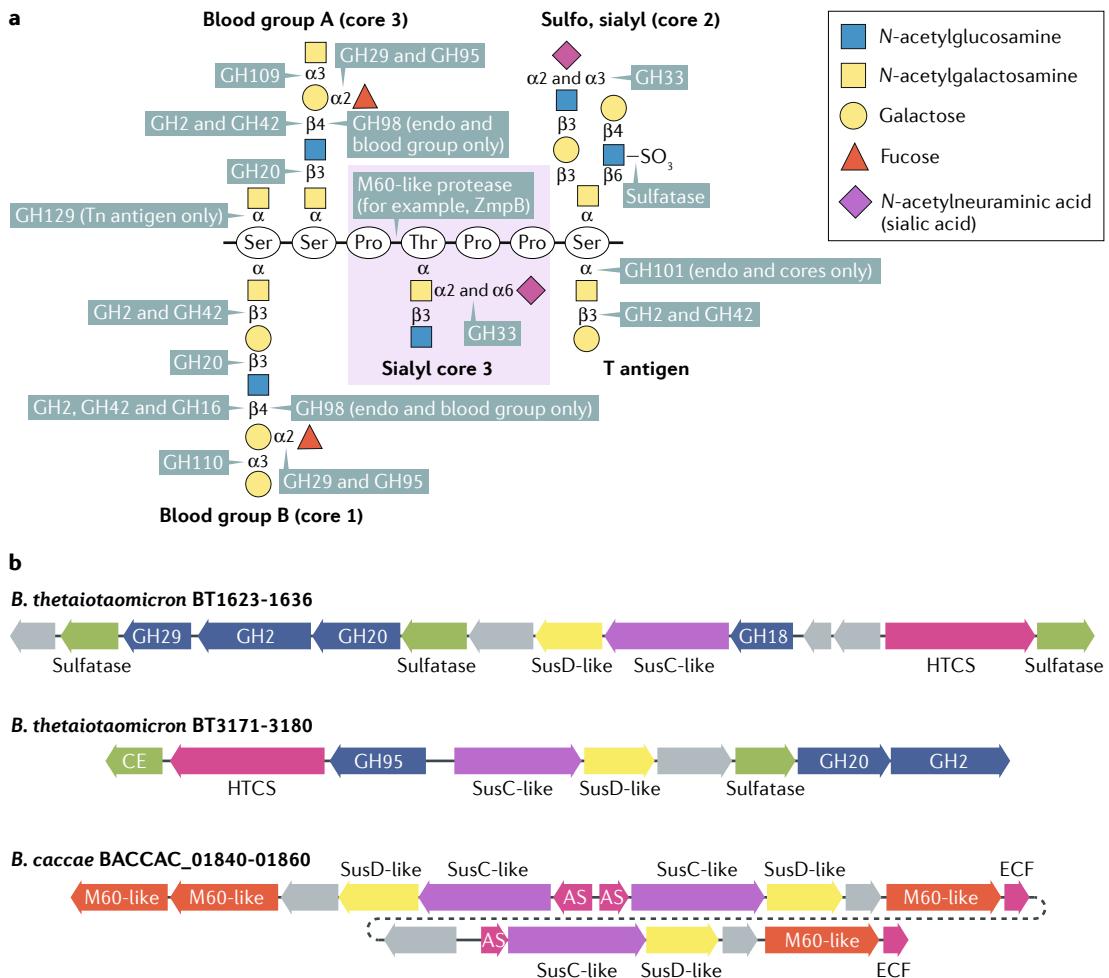
A statistical approach that is often used in bioinformatics to identify hidden, but meaningful, amino acid sequence signals in proteins and can be used to group them into families with similar function.

be important for disease because they equip bacteria with the ability to reach closer to host tissue than most microorganisms.

The gut microbial enzymes required for fibre degradation have been intensively studied in recent years, especially among members of Bacteroidetes<sup>10–14</sup>. However, those required for mucus degradation have been investigated in far less detail, partly owing to the complexity of this substrate and difficulty in analytical methods. The known enzyme families required for removing specific glycosidic linkages in mucin O-linked glycans have been recently reviewed<sup>21</sup>, and emerging work has begun to identify gut bacterial enzymes that degrade high mannose N-linked glycans<sup>45</sup>. CAZymes are grouped into families, with the nomenclature including the enzyme type (for example, glycoside hydrolase (GH) or polysaccharide lyase) and a family number that represents shared homology via hidden Markov models<sup>8</sup> and provides some indication of sugar and linkage specificity, which may be broad or narrow depending on the particular family. Although members of several CAZyme families have been associated with the degradation of host mucosal glycans, for example, GH2, which contains  $\beta$ -galactosidase activity among others (FIG. 2a), there is still sufficient ambiguity in most families to impede predicting the ability of their constituent enzyme members to target mucin glycans based on annotation alone<sup>46</sup>. For example, other members of GH2 are also known to degrade  $\beta$ -linked galactosides present in non-mucin fibre polysaccharides.

Future work is needed to understand how various taxa of human gut bacteria have evolved to recognize and degrade mucosal glycans, as well as cope with the biophysical complexity of secreted mucin glycoproteins. A useful approach has been to first identify strains and/or species that grow on purified O-linked or N-linked glycans as sole carbon sources, identifying genes that are activated during those growth conditions and then perform detailed enzymatic characterizations with recombinant proteins<sup>36,41,47</sup>. This approach is facilitated in genera such as *Bacteroides*, which groups multiple genes together in individual polysaccharide utilization loci (PULs), which are gene clusters that often contain multiple CAZyme-coding genes (FIG. 2b).

New activities are being actively discovered for gut microorganisms that use mucus as a nutrient or degrade it during pathogen invasion. A recently described protease family (M60-like proteases; also known as Pfam13402)<sup>48</sup> has provided evidence for enzymes that cleave the mucin glycoprotein backbone in a manner that is dependent on the presence of specific glycan sidechain structures<sup>49</sup>. Zinc metalloprotease ZmpB from *Clostridium perfringens* cuts adjacent to glycosylated Ser and/or Thr residues, with optimal cleavage mediated by recognition of  $\alpha$ 2,6-sialylated core 1 or core 3 structures (FIG. 2a). By contrast, another protease family member from *B. thetaiotaomicron* (BT4244) prefers a simpler structure with only Tn antigen<sup>49</sup>. Some common commensals encode large repertoires of M60-like proteases. For example, the type strain of *Bacteroides caccae*, an organism recently implicated in low dietary fibre-induced destruction of the colonic mucous layer<sup>36</sup>, possesses 16



**Fig. 2 | Microbial pathways involved in the metabolism of colonic mucus.** **a** A variety of different O-linked glycan structures, with a mixture of glycosidic linkages, are attached to mucus and encountered by gut bacteria as potential nutrient sources. These glycans vary widely in their structure and also in ‘capping’ attachments, which include the host’s blood group (A, B, H or non-secretor) and attached sulfate, fucose or sialic acids, some of which can be present on the same molecule (for example, the sulfo, sialyl (core 2) example illustrated). Some carbohydrate-active enzymes (CAZymes) produced by the microbiota are able to degrade O-linked glycans by cleaving specific linkages in these molecules. The target linkages of several different GH families, M60-like proteases and sulfatases are indicated, showing how bacteria degrade some of these structures. Note that some activities only work in an endo-fashion (that is, cut internally within glycan chains) and in particular contexts, such as blood-group-removing GH98 enzymes that liberate the terminal trisaccharide from either blood group A or B structures or GH101 enzymes that cleave peptide-attached N-acetylgalactosamine (GalNAc) but only when it has an attached  $\beta$ 1,3-linked galactose (T antigen, a historical term for the unsubstituted core 1 structure derived from its increased abundance on tumour cells). **b** | Schematics of polysaccharide utilization loci (PULs) for three gene clusters from *Bacteroides* that have been implicated in the degradation of O-linked glycans (host mucin). Genes encoding degrading enzymes are shown in green, red or blue and distinguish sulfatases and carbohydrate esterase (CE) activities, M60-like proteases or GHs, respectively (with family numbers indicated to match to potential target linkages as shown in part **a**). A number of other binding, transport and sensory proteins are encoded in these loci, including hybrid two-component sensor regulator (HTCS), extra-cytoplasmic function (ECF) sigma factor/anti-sigma (AS) factor regulators and homologues of the prototypic *Bacteroides theta iotaomicron* starch utilization system (Sus), TonB-dependent receptor SusC and starch-binding protein SusD. *B. caccae*, *Bacteroides caccae*.

of these enzymes, many of which are also grouped in co-regulated PULs (FIG. 2b). Furthermore, a recently discovered intramolecular *trans*-sialidase (RgNanH) from the commensal gut bacterium *Ruminococcus gnavus* enables the bacterium to bind to mouse and human mucus using an adhesive carbohydrate-binding module and to cleave terminal sialic acid sugars from this substrate in a form (2,7-anhydro-Neu5Ac) that

can be metabolized only by *R. gnavus*<sup>50,51</sup>. Given that mucin-degrading bacteria can cause damage that at least predisposes to disease, possibly in the absence of overt pathogens in genetically susceptible hosts, these enzymes may provide targets for inhibitory drugs to block intestinal diseases. Furthermore, increased mucin degradation by the gut microbiota may function as a marker for IBD.

Finally, a few commensal bacterial lineages have evolved specialized strategies that enable them to interact with or even live inside various niches within the mucosal barrier. Examples include segmented filamentous bacteria, which make direct, intimate contacts with small intestinal epithelial cells<sup>52</sup>, and some *Bacteroides* species (*Bacteroides fragilis* and *B. thetaiotaomicron*)<sup>53,54</sup>, which colonize the lumen of some colonic crypts. Accordingly, *B. fragilis* has been shown to require special crypt colonization factor proteins for crypt colonization, of which *B. thetaiotaomicron* has several homologues. These are encoded by a PUL with homology to others known to be involved in mucin utilization, and *B. fragilis* mutants missing these functions fail to occupy crypts<sup>53</sup>. These results imply that specific host-derived glycan structures of crypt mucus are an important nutrient source or adherence site for these *Bacteroides* spp. A separate study using laser capture microdissection identified *Acinetobacter* species as abundant bacteria in the colonic crypt of mice<sup>55</sup>, leading to the conclusion that members of this genus possess an additional, unknown crypt colonization strategy. Even deeper into intestinal tissue, *Alcaligenes* species have been identified as lymphoid-resident commensal bacteria, occupying an even more specialized niche inside of small intestinal lymphoid tissue<sup>56</sup>. *Alcaligenes* species proliferate in the lymphoid tissue of Peyer's patches when interleukin-22 (IL-22)-producing innate lymphoid cells (ILCs) are depleted and may cause systemic inflammation in mice<sup>56</sup>. Such examples of apparently non-harmful commensals invading the mucosal barrier further blur the lines between commensal and pathogenic host–microorganism interactions. Mechanisms used by pathogens to subvert the gut mucosal barrier are discussed in the following sections.

### Pathogens in the small intestine

The architecture of the mucosal barrier in the small intestine is fundamentally different to that in the large intestine. The small intestine is characterized by thinner mucus to promote nutrient absorption and antigen sampling, which is, in part, offset by secretion of AMPs to exclude bacteria from the epithelial surface<sup>57</sup>. Enteric pathogens have evolved several different strategies to gain entry into the small intestinal mucosal barrier by exploiting distinct cell lineages.

**Enterocytes and microfold cells.** Together with specialized secretory cells (goblet cells, enteroendocrine cell, Paneth cells and tuft cells), the small intestinal mucosal barrier contains specialized M cells, which cover underlying lymphoid aggregates called Peyer's patches (FIG. 3a). Thus, the major role of M cells is the sampling and transcytosis of antigens through the epithelial barrier, but these cells and Peyer's patches are also exploited by certain pathogens to gain entry through the mucosal barrier<sup>58</sup>. For example, although *Salmonella enterica* subsp. *enterica* serovar Typhimurium can directly invade epithelial or dendritic cells, it primarily uses an M cell-dependent mechanism to traverse the mucosal barrier<sup>59</sup>. The pathogen can even gain access to the underlying tissue through ingestion by dendritic cells located in

the Peyer's patch, which may be followed by systemic spread<sup>60</sup> (FIG. 3a).

Host cell surface proteins such as integrins are expressed on M cells and several other cell types and are frequent receptors for pathogen invasion. Compared with other intestinal enterocytes, M cells express a higher number of  $\beta 1$ -integrin proteins that are receptors for *Yersinia* spp<sup>61</sup>. *Yersinia pseudotuberculosis* uses its outer membrane protein invasin to transit through M cells into the underlying Peyer's patch by interacting with  $\beta 1$  integrins expressed on the surface of the targeted cells<sup>62,63</sup> (FIG. 3b). A similar mechanism, which includes formation of a fibronectin bridge between  $\beta 1$  integrins and the invading bacterium<sup>64</sup> (FIG. 3b), is used by *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

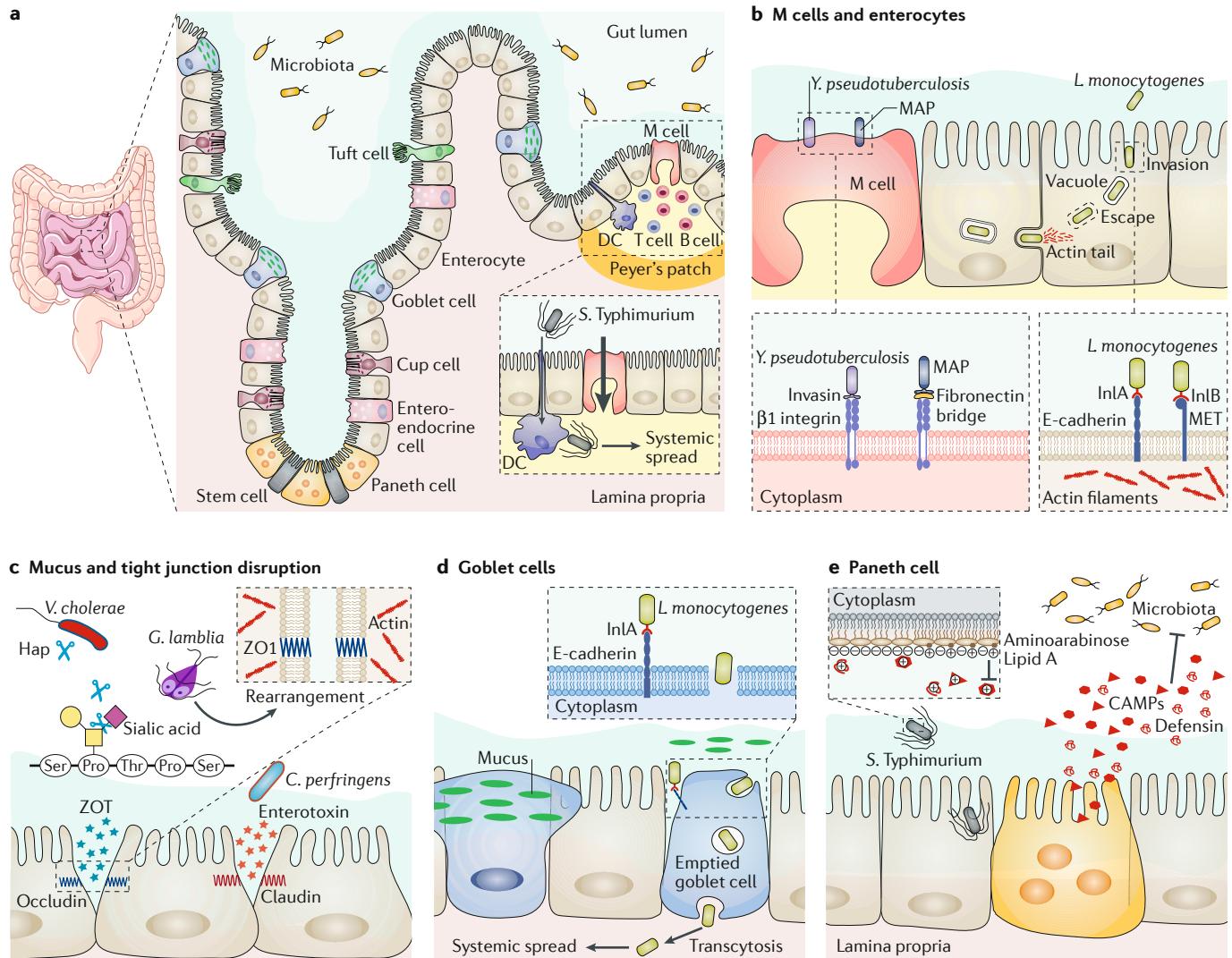
E-cadherin (a transmembrane glycoprotein) and the hepatocyte growth factor receptor (MET) are two other proteins presented on M cells. *Listeria monocytogenes* uses two of its main virulence factors, the surface proteins internalin A (InlA) and/or internalin B (InlB), to bind to E-cadherin and MET and invade epithelial cells, including M cells<sup>65,66</sup> (FIG. 3b). The docking of InlA to E-cadherin induces local, intracellular actin recruitment, activates the Arp2/3 complex<sup>67</sup> and enables *L. monocytogenes* to invade cells, break out of its vacuole using listeriolysin O and then move within the cytoplasm and spread from cell to cell via actin assembly-inducing protein (ActA)-dependent actin polymerization<sup>68</sup> (FIG. 3b).

**Mucus degradation and tight junction disruption.** Some pathogens secrete enzymes that cleave mucus or disrupt tight junctions in the underlying epithelial cells to destabilize host tissue (FIG. 3c). *Vibrio cholerae* secretes a soluble Zn<sup>2+</sup>-dependent metalloproteinase (haemagglutinin protease (Hap)) that possesses mucinolytic activity<sup>69</sup>. By contrast, another toxin produced by this bacterium, zonula occludens toxin (ZOT), interacts with the extracellular domains of the tight junction proteins occludin and zonula occludens 1 protein (ZO1) to disrupt epithelial integrity<sup>70,71</sup>. Similar disruption of a different tight junction protein, claudin, is performed by the bacterial pathogen *Clostridium perfringens* through the activity of its enterotoxin<sup>72</sup>. Such barrier disruption strategies are not limited to bacteria, as the protozoan *Giardia lamblia* (synonymous with *Giardia duodenalis*) possesses abilities to degrade or disrupt mucus<sup>73</sup>. Furthermore, the protozoan disrupts ZO1 to increase permeability of the epithelium<sup>74</sup> and reorganizes cytoskeletal F-actin filaments<sup>75</sup> (FIG. 3c).

**Pathogen invasion or evasion of secretory cell activity.** Despite the mucus-secreting and AMP-secreting activities of goblet and Paneth cells, some pathogens have also evolved mechanisms to invade through these cells. Besides the routes described above for *L. monocytogenes* infection (FIG. 3b), goblet cells can be an alternative target site for entry<sup>76</sup>. The host receptor E-cadherin is also expressed on goblet cells but is situated below the tight junctions and only accessible from the lumen if the cell has expelled mucus. *L. monocytogenes* recognizes E-cadherin at the surface of villi located at the tip of mucus-expelling goblet cells, is internalized and quickly transcytosed through the mucosal barrier and

**Interleukin-22**  
(IL-22). A cytokine that is part of the IL-10 cytokine family that is produced by activated natural killer and T cells and, in the gut, is responsible for activating epithelial cell regeneration, mucus production and antimicrobial peptide secretion.

**Innate lymphoid cells**  
(ILCs). A group of innate immune cells that are characterized by the absence of antigen-specific B or T cell receptors.



**Fig. 3 | Interactions of pathogens with the mucosal barrier in the small intestine.** **a** Four secretory epithelial cell lineages are present in the small intestine, which include goblet cells, enteroendocrine cells, Paneth cells, and tuft cells. Microfold (M) cells are absorptive cells, which are part of the Peyer's patch. *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* predominantly invades M cells (thick arrow, inset) before being engulfed by dendritic cells (DCs) located in the Peyer's patch, although *S. Typhimurium* can directly invade trans-epithelial DCs as well (thin arrow, inset) before systemic spread. **b** Receptor-mediated invasion strategies for *Yersinia pseudotuberculosis*, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and *Listeria monocytogenes*. *Y. pseudotuberculosis* uses its outer membrane protein invasin to transit through M cells into the Peyer's patch by interacting with  $\beta 1$  integrins expressed on the cell surface, whereas MAP forms a fibronectin bridge to  $\beta 1$  integrins. *L. monocytogenes* uses its surface proteins internalin A (InLA) and/or internalin B (InLB) to bind to E-cadherin and hepatocyte growth factor receptor (MET) and invade epithelial cells, including M cells. Docking of InLA to E-cadherin induces local, intracellular actin recruitment, activates the Arp2/3 complex and enables the pathogen

to invade cells, break out of its vacuole using listeriolysin O, then move within the cytoplasm and spread from cell to cell via actin assembly-inducing protein (ActA)-dependent actin polymerization. **c** Mechanisms through which *Vibrio cholerae*, *Clostridium perfringens* and the protozoan *Giardia lamblia* disrupt mucus and/or tight junctions between epithelial cells. *V. cholerae* secretes a haemagglutinin/protease (Hap), which possesses mucinolytic activity, and its zonula occludens toxin (ZOT) interacts with the extracellular domains of the tight junction proteins occludin and zonula occludens 1 protein (ZO1) to disrupt epithelial integrity. The protozoan *G. lamblia* reorganizes cytoskeletal F-actin filaments and disrupts ZO1 to promote tight junction disruption and increase permeability of the epithelium. **d** *L. monocytogenes* uses goblet cells that have expelled their mucus to traverse via transcytosis into the lamina propria as their E-cadherin receptors are easier to access by the pathogen. **e** Paneth cells secrete cationic antimicrobial peptides (CAMPs), such as defensin, to inhibit pathogenic and non-pathogenic microorganisms. *S. Typhimurium* can change the anionic charge of its surface molecule lipid A to reduce negative charge by adding positively charged amino groups via aminoarabinose.

is then transferred in the lamina propria via exocytosis<sup>76</sup> (FIG. 3d). Moreover, *S. Typhimurium* has evolved to circumvent host defences during invasion of the epithelial barrier (FIG. 3e). This pathogen can evade the activity of Paneth cell-secreted cationic antimicrobial peptides (CAMPs), including defensins, by altering the anionic

character of the cell surface<sup>77,78</sup>. The pathogen uses phase variation to activate functions that incorporate positively charged aminoarabinose into lipid A, which decreases the net negative charge of the outer membrane of the pathogen<sup>77,78</sup>. The ordinarily anionic lipid A is thought to attract cationic CAMPs, and reducing

the negative charge inhibits this process<sup>79,80</sup> (FIG. 3e). Along the same lines, some bacteria display anionic polysaccharide capsules above their outer membrane to neutralize AMPs before they reach lipopolysaccharide (LPS) at the cell surface<sup>81</sup>, although a potential role for this strategy in small intestinal pathogens is not clear. While *S. Typhimurium* is an invasive pathogen, different LPS modifications that also block killing by AMPs occur in some commensals<sup>82,83</sup>, underscoring the pressure to circumvent this arm of host defence by both pathogens and mutualists.

### Pathogens in the large intestine

The dense commensal microbiota in the large intestine protects the host from invasion by several pathogens (a process termed colonization resistance). For example, colonization with Clostridiales was recently shown to be protective against lethal pathogens in mice<sup>84</sup>, and depletion of butyrate-producing bacteria by antibiotic treatment promotes expansion of dysbiotic Enterobacteriaceae via microbiota-activated peroxisome proliferator-activated receptor-γ (PPAR $\gamma$ ) signalling<sup>85</sup>. Nevertheless, alterations in the colonic microbiota composition or physiology can also promote defects in the intestinal mucosal barrier. Enteric pathogens that colonize the large intestine use numerous mechanisms, including some dependent on the activities of co-resident commensal microorganisms, to promote their colonization or persistence through invasion of the mucosal barrier.

**Defects in colonic mucous barrier promote invasion of enteric pathogens.** Studies in genetically modified mice have revealed the importance of mucus in the host defence against colonic pathogens. One study used MUC2-deficient (*Muc2*<sup>-/-</sup>) mice to understand the role of the colonic mucous barrier in slowing disease by the attaching and effacing (A/E) pathogen *Citrobacter rodentium*, revealing that mice lacking a normal mucous layer experience accelerated disease progression and even lethal colitis in response to this ordinarily self-limiting pathogen<sup>86</sup> (FIG. 4a). Another study investigated the amount of time required for expulsion of a parasitic nematode, *Trichuris muris*, in the caecum of *Muc2*<sup>-/-</sup> mice<sup>87</sup>, which was significantly delayed compared with wild-type mice that instead exhibit goblet cell hyperplasia to probably increase mucus production and aid expulsion. A recent study using gnotobiotic mice provides a novel perspective on invasion of *C. rodentium* based on diet-microbiota interactions that influence mucus thickness and, in turn, pathogen susceptibility<sup>86</sup>. When mice are deprived of fibre, the main exogenous nutrient source for colonic microorganisms, the composition of the microbiota changes in favour of increased mucus-degrading bacteria (FIG. 4a). Consequently, mucus is increasingly used as an alternative nutrient and the thickness of the mucous layer, measured in Carnoy's solution-fixed histological sections, overlying the epithelium is eroded. *C. rodentium* can more rapidly traverse this eroded mucous layer and elicit similar disease kinetics and lethal colitis as in *Muc2*<sup>-/-</sup> mice (FIG. 4a).

The host mucosal barrier requires the cytokine IL-22, a cytokine involved in epithelial regeneration, AMP

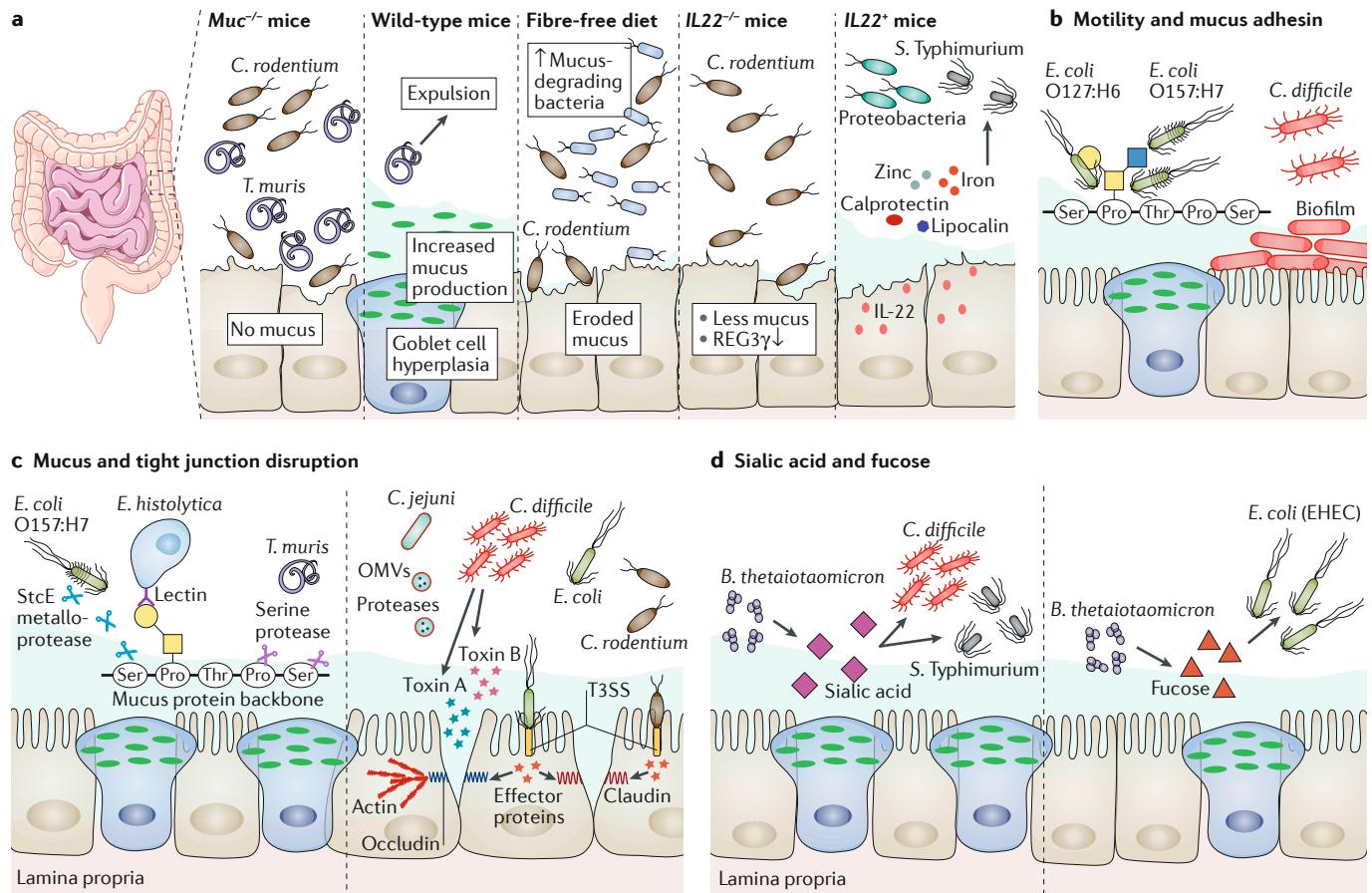
production and mucin synthesis, for an efficient defence against pathogens. Mice deficient in IL-22 clear *T. muris* infection more slowly, presumably owing in part to the lack of goblet cells and mucus<sup>88</sup>. IL-22-dependent induction of the AMPs regenerating islet-derived protein 3β (REG3 $\beta$ ) and REG3 $\gamma$  in colonic mouse epithelial cells provides increased survival after *C. rodentium* infection<sup>89</sup>. IL-22-deficient mice experience accelerated and lethal colitis similar to that observed in fibre-deprived and *Muc2*<sup>-/-</sup> mice, further underscoring the importance of proper mucosal barrier and immunological responses in slowing disease progression caused by this pathogen<sup>89</sup> (FIG. 4a). *S. Typhimurium* has evolved strategies to use host-dependent IL-22 production, which triggers metal sequestration by increased host lipocalin and calprotectin production, to its advantage. This pathogen can use siderophores that are resistant to lipocalin neutralization and high-affinity zinc transporters, promoting its own colonization while competing commensals are suppressed by this response<sup>90</sup> (FIG. 4a).

### Motility and adherence facilitate pathogen colonization.

Expression of phase-variable flagella and fimbriae helps adapt pathogen subpopulations to suitable infection sites by providing some pathogens with adherence properties and others with motility. Beyond promoting motility through the mucous layer, flagella confer another advantage to some pathogens during infection at mucosal surfaces. Enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) strains possess flagella termed H6 and H7, which possess adhesive properties to bind to bovine mucins<sup>91</sup> (FIG. 4b), suggesting that this additional property contributes to colonization by these pathogens on mucosal surfaces<sup>91</sup>. Similar flagellum-related mucus adherence by *Clostridium difficile*, a nosocomial pathogen associated with antibiotic treatment<sup>92</sup>, is thought to promote colonization by this organism<sup>93</sup>, which can also form a biofilm near the epithelial surface<sup>94</sup> (FIG. 4b). Emerging data further support a role for multi-species bacterial biofilms in the pathogenesis of colorectal cancer<sup>95</sup>. Tumour-associated biofilms, mainly composed of *E. coli* and *B. fragilis*, were recently discovered in humans, and tumour-prone mice colonized with these species exhibit increased tumour formation<sup>96</sup>. As adherence to and movement through the mucous layer are requisite steps before epithelial colonization and biofilm formation for many commensals and pathogens, understanding the factors that mediate these events will uncover strategies to prevent some enteric infections and chronic diseases.

**Disruption of colonic mucus and tight junctions by pathogens.** Some colonic pathogens are adept at breaking peptide bonds and glycosidic linkages in colonic mucin structures and/or epithelial tight junctions, conferring competitive advantages during infection (FIG. 4c). StcE, a well-studied *E. coli* metalloprotease, cleaves mucins<sup>97</sup>, which could help the pathogen to reach the epithelium and initiate disease. A recent study confirmed the proteolytic activity of StcE in human colonic mucosal biopsy samples and mucin-producing LS174T colon carcinoma cells<sup>98</sup>. An in vitro study demonstrated that pathogenic *E. coli* secretes a zinc-metalloproteinase SslE, another member

**Colonization resistance**  
A phenomenon by which the presence of a healthy microbiota inhibits invasion by external pathogens. In the gut, this state has been ascribed to the inhibition of many enteric pathogens, which are more adept at gaining an infection foothold after a major perturbation, such as antibiotic treatment.



**Fig. 4 | Pathogen adaptations that promote colonization and invasion of the mucosal barrier in the large intestine.** **a** Mice genetically deficient in mucin 2 (*Muc2<sup>-/-</sup>* mice) lack a normal mucous layer, which results in accelerated disease progression following infection with *Citrobacter rodentium*. Wild-type mice exhibit goblet cell hyperplasia, likely increasing mucus production and expelling the nematode *Trichuris muris*. However, *Muc2<sup>-/-</sup>* mice cannot clear *T. muris* owing to the lack of mucus production. Mice fed a fibre-free diet exhibit an altered microbiota composition that reflects a higher abundance of mucus-degrading bacteria. Increased foraging on the mucous layer leads to accelerated disease progression following infection with *C. rodentium*. Mice infected with *C. rodentium* and deficient in IL-22 show reduced mucus production and experience lethal colitis as the cytokine fails to induce the antimicrobial peptides (AMPs) regenerating islet-derived protein 3β (REG3β) and REG3γ in colonic mouse epithelial cells. *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* uses host-dependent IL-22 production, which triggers metal sequestration (zinc and iron) by increased host lipocalin and calprotectin production, to its advantage by promoting its own colonization while suppressing competing commensals. **b** In addition to promoting motility through the mucous layer, flagella confer another advantage to some pathogens during infection at mucosal surfaces. Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) strains express flagella (H6 and H7) with mucin adhesion

properties, which might contribute to colonization on mucosal surfaces. Similar flagellum-mediated adherence to mucus is thought to promote colonization by *Clostridium difficile* and biofilm formation near the epithelial surface. **c** *E. coli*, *Entamoeba histolytica* and *T. muris* produce enzymes and lectins that either cleave or bind to mucins, whereas other pathogens, such as *C. difficile* and *Clostridium perfringens*, secrete toxins that disrupt colonic tight junctions. StcE, an *E. coli* metalloprotease, cleaves mucins, which helps the pathogen to reach epithelial cells. *E. histolytica* can adhere to mucins using a galactosamine-N-acetylgalactosamine (Gal/GalNAc)-binding lectin. *T. muris* secretes serine proteases that act on the amino-terminal disulfide crosslinking domain of MUC2, leading to depolymerization of the MUC2 network. Outer membrane vesicles (OMVs) filled with proteases are secreted by *Campylobacter jejuni* and cleave the tight junction proteins E-cadherin and occludin. *C. difficile* toxin A and toxin B disrupt the cellular actin cytoskeleton, which affects its interactions with tight junction components claudin and occludin. *E. coli* and *C. rodentium* use their type III secretion systems (T3SS) to attach to epithelial cells, reorganize the cytoskeleton and alter tight junction protein integrity. **d** Commensal organisms such as *Bacteroides thetaiotaomicron* can use mucin-degrading enzymes to liberate sugars, such as sialic acid and fucose, which are either directly used by pathogens as nutrients (*S. Typhimurium* and *C. difficile* use free sialic acid) or modulate virulence activities of pathogens (fucose sensing by EHEC).

of the M60-like protease family that cleaves mucin glycoproteins<sup>99</sup>. Similarly, the protozoan *Entamoeba histolytica* secretes both cysteine proteases and an M60-like<sup>48</sup> protease that degrades mucus<sup>100,101</sup> (FIG. 4c). *E. histolytica* also promotes colonization of the colonic mucous layer by adhering to mucins using a 170-kDa lectin<sup>102</sup>. The nematode *T. muris* secretes serine proteases that act on the amino-terminal disulfide crosslinking domain of MUC2, leading to depolymerization of the MUC2 network<sup>103</sup>

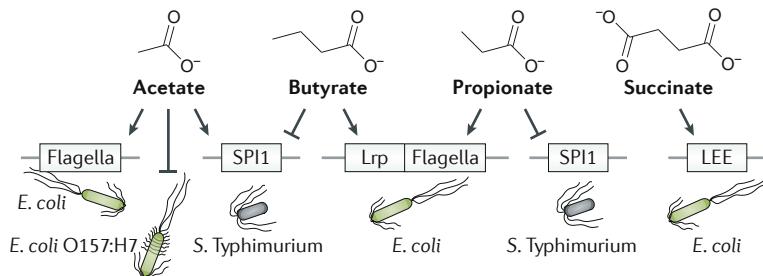
(FIG. 4c). As judged by techniques such as *trans*-epithelial electrical resistance, staining for tight junction proteins and increased passage rates of labelled molecules of various sizes in vivo and in vitro, some pathogens disrupt aspects of epithelial cell fitness, including the integrity of their tight junction proteins. For example, *C. difficile* toxin A and toxin B disrupt the cellular actin cytoskeleton, which affects its interactions with tight junction components, such as claudin and occludin<sup>104</sup>. *E. coli* and

#### Lectin

A broad family of plant, animal and bacterial proteins defined by their ability to bind to certain carbohydrates with moderate to high affinity.

## Box 3 | SCFAs and an organic acid can influence pathogens

Short-chain fatty acids (SCFAs) and organic acids produced by fermenting gut bacteria are ubiquitous and abundant metabolites in the gut, so it is not surprising that pathogens have evolved many ways to sense and respond to these molecules. Changes in the concentrations of SCFAs (acetate, butyrate and propionate) and the organic acid succinate potentiate susceptibility to several pathogens. *Bifidobacterium* produces acetate, which promotes host defence mechanisms in epithelial cells and therefore protects mice from fatal *Escherichia coli* O157:H7 infection<sup>142</sup>. By contrast, acetate enhances *Salmonella enterica* subsp. *enterica* serovar Typhimurium colonization by promoting expression of pathogenicity island 1 (SPI1)<sup>149</sup>, which encodes one of its type 3 secretion systems (T3SSs) and enables it to inject effector proteins into host cell cytoplasm. Other studies have shown that butyrate and propionate reduce the expression of SPI1 in *S. Typhimurium*<sup>150,151</sup>, which indicates that the composition of the SCFAs in the ileum signals the pathogen that it is in a suitable infection site<sup>23</sup>. A study demonstrated that *Clostridium difficile* gains a fitness advantage by metabolizing the organic acid succinate, which is produced by some commensals (for example, *Bacteroides*) and consumed by others (for example, Firmicutes), including *C. difficile*<sup>152</sup>. This metabolite is not abundantly available to pathogens before antibiotic treatment owing to consumption by other bacteria but is transiently enhanced in the presence of some antibiotics, presumably owing to either lysis of bacterial cells or selective killing of succinate consumers<sup>152</sup>. Furthermore, in the presence of *Bacteroides thetaiotaomicron*, succinate enhances the expression of the locus of enterocyte effacement (LEE) in *E. coli*<sup>153</sup>, and the global virulence regulator leucine-responsive regulatory protein (Lrp) and flagellar genes are induced by butyrate<sup>154</sup>. Production of *E. coli* flagella is also promoted by acetate and propionate<sup>154</sup>, underscoring the idea for this and other pathogens that motility and virulence functions are closely tied to SCFA levels.



*C. rodentium* also use the type III secretion systems (T3SSs) to attach to the epithelial cells, to reorganize the cytoskeleton and consequently to alter the architecture and integrity of tight junction proteins<sup>105,106</sup>. Outer membrane vesicles, filled with proteases, are secreted by the pathogen *Campylobacter jejuni* to cleave the tight junction proteins E-cadherin and occludin<sup>107</sup> (FIG. 4c).

**Exploitation of commensal-liberated mucosal nutrients by pathogens.** In addition to roles for commensal-derived SCFAs for pathogens (BOX 3), degradation of mucus by commensal bacteria and changes in the environment following antibiotic treatment create new niches in the gut that facilitate colonization and persistence of pathogens, such as *C. difficile* and *S. Typhimurium*. Some of the direct connections between these events have been elucidated recently. Two studies<sup>108,109</sup> revealed separate sugar-liberation pathways in which the activity of commensal bacteria towards mucosal nutrients augments colonization by these pathogens (FIG. 4d). A first study showed that free sialic acid is released from colonic mucus glycans by some commensal bacteria, such as *B. thetaiotaomicron*, and that the availability of this sugar, which can be directly utilized but not cleaved by *C. difficile* and *S. Typhimurium*, is significantly increased

**Type III secretion systems (T3SSs).** Needle-like complexes related to the bacterial flagellum that are an important virulence factor of Gram-negative bacteria. They enable pathogenic bacteria to inject effector proteins into the cytoplasm of the host target cell and modify a variety of cellular responses.

after antibiotic treatment. The latter effect may be due to elimination of sialic acid-liberating commensals, which could also compete for this sugar, but not the destruction of the sialic acid-cleaving enzymes they produced before antibiotic treatment<sup>108</sup>. A second study revealed that a different host glycan-derived sugar, fucose, which is also cleaved by *B. thetaiotaomicron* and other commensals, positively regulates the virulence of EHEC, presumably constituting a signal to the pathogen that it is within the mucous layer and close to its host epithelial niche<sup>109</sup>. This finding is interesting in light of seminal research into *B. thetaiotaomicron*-host interactions, which showed that introduction of this fucose-consuming bacterium into germ-free mice elicits fucosylation of host glycans<sup>110</sup>, a phenomenon that has been recreated in intestinal organoids<sup>111</sup>. Thus, there is a complex interplay between the presence and activity of commensal bacteria, changes in the host mucosal barrier and the subsequent balance between commensal-pathogen relationships.

**Mucosal barrier and chronic disease**

A major role of the mucosal barrier is to keep bacterial pathogens and commensal bacteria at a physical distance from the epithelium. Disruption of this barrier contributes to inflammatory diseases such as IBD. Additionally, disruption of the small intestinal barrier is emerging as a factor in the development of coeliac disease<sup>112</sup>. If the large intestinal mucous layer is compromised owing to defects in mucus production or glycosylation<sup>113–115</sup>, which might potentially lead to it being broken down more quickly by bacteria<sup>35,36</sup>, commensals that normally occupy the lumen and outer mucous layer may move closer to host tissue and provoke increased immune responses. Although naturally occurring mucus defects in humans are not as severe as the effects observed in engineered mice (such as *Muc2*<sup>-/-</sup> mice) mentioned above, even seemingly small changes in the interaction between the host and the gut microbiota could tip the balance between health and diseases such as IBD. This idea is supported by a study that showed infiltration of commensal bacteria into the inner mucous layer in multiple mouse IBD models and patients with ulcerative colitis, resulting in closer contact of the microbiota with the epithelium and in inflammation<sup>116</sup>. Along the same lines, it was shown that patients with ulcerative colitis lack a mucous layer on inflamed tissue in the gut mucosa, which is not the case in patients with Crohn's disease<sup>117</sup>, which is consistent with the idea that inflammation in patients with ulcerative colitis is associated with altered mucus production or glycosylation. However, it is not clear from these studies whether the inflammation-associated changes in mucus presence or penetrability are the primary cause of the inflammation or a result. In light of the increased mucus foraging activity that occurs in the context of a low-fibre diet discussed above<sup>36</sup>, a recent study found that mice fed a Western style (high-fat and low-fibre) diet also experience increased permeability of the inner mucous layer, as evaluated by ex vivo culture and bead-penetration assay<sup>118</sup>. This study, which also found that certain prebiotics and probiotics reverse specific aspects of this barrier defect, underscores the complexity of microbial interactions with the mucosal barrier and the multiple sites and mechanisms

**Molecular chaperone**

Type of protein that assists in the folding and unfolding of other proteins. They prevent aggregation by binding to non-native structures and therefore support the folding process.

involved<sup>118</sup>. Finally, it has been shown that potentially inflammatory commensal bacteria in patients with IBD are highly coated with immunoglobulin A (IgA) and penetrate the inner mucous layer in a mouse model to drive disease, suggesting that closer proximity of some of these deleterious organisms causes them to be increasingly detected by the immune system of the host<sup>119</sup>.

As the biosynthesis of O-linked glycans is such a complex process, it is not surprising that many components are required to maintain and support robust mucin production and that defects exist in this process<sup>120</sup> (BOX 2). Mutations that reduce function of glycoprotein-*N*-acetylgalactosamine 3- $\beta$ -galactosyltransferase 1 (C1GALT1), the enzyme that catalyses the synthesis of core 1-derived and core 2-derived O-linked glycans, have been shown in patients with colorectal cancer and ulcerative colitis<sup>115,121</sup>. C1GALT1-deficient mice fail to produce core 1-derived O-linked glycans and shift to higher production of core 3-derived O-linked glycans<sup>115</sup>. Interestingly, spontaneous colitis occurs in *C1galt1*<sup>-/-</sup> mice in the distal colon, and the importance of these findings is corroborated by a known somatic missense mutation in the gene encoding the molecular chaperone C1GALT1-specific chaperone 1 (C1GALT1C1) in patients with ulcerative colitis. C1GALT1C1-deficient mice are characterized by abnormal mucus expression, altered mucin glycosylation and reduced thickness of the inner mucus<sup>115</sup>. Similar to core 1-deficient mice, mice with an engineered defect in synthesis of core 3-derived O-linked glycans exhibit a reduction in MUC2 protein levels and increased permeability of the mucous layer<sup>122</sup>, which enhances the contact of intestinal bacteria with epithelial cells. Finally, results from a recent study demonstrated that mice lacking core 1 develop colitis only in the distal colon, whereas the absence of core 1 and core 3 together leads to the development of colitis in the distal and proximal colon<sup>114</sup>. As in many mouse models of IBD, reducing or eliminating the microbiota by treating with antibiotics or re-deriving mice as germ-free reduces colitis in *C1galt1*<sup>-/-</sup> and/or core 1 and core 3 double-knockout mice, highlighting a role for the microbiota in disease progression. Precise mechanisms connecting defects in mucin glycosylation with altered activities of gut microorganisms or changes in microbiota community structure and the onset of disease still remain to be determined. However, it is likely that reduced glycosylation increases mucus susceptibility to bacterial degradative enzymes, which decreases its barrier function and brings bacteria into closer proximity to the host and its immune system. Consistent with this, the mucus of core 1-deficient and core 3-deficient mice is more susceptible to degradation by proteases<sup>114</sup>. An alternative explanation, which is not exclusive of the previous mechanism, is that altered or simplified glycosylation selects for a different microbiota structure that in turn promotes or

exacerbates inflammation. Consistent with this, core 1-deficient mice show altered gut microbiota profiles that are shifted in favour of Bacteroidetes, a phylum with several known mucin degraders<sup>123</sup>. Finally, a third possibility is that altered mucin glycosylation increases the penetrability of the mucous layer to gut microorganisms or their antigens, leading to increased host exposure and activation of the immune system. Given the complex interactions described above, which interconnect diet, gut microorganisms and the immune system with the integrity of the mucosal barrier during health and disease, deciphering these mechanisms will lead not only to better understanding of why these spontaneous inflammatory diseases occur but also how to prevent and treat them.

**Summary and outlook**

The intestinal mucosal barrier is the first line of defence against encroachment by both commensal and pathogenic microorganisms. The status and integrity of this barrier are contingent upon a number of linked factors, including host diet, the community of commensal microorganisms that compose the microbiota, host genetics and exposure to invading pathogens. Diet influences the extent to which commensal bacteria forage on mucus for nutrients, indirectly altering mucous layer status. Host defects in mucin glycosylation, cellular tight junctions and immune responses can damage the overall resilience of the mucosal barrier and are known to contribute to diseases such as IBD. A variety of bacterial pathogens have evolved strategies to overcome the intact mucosal barrier and establish infection in the human host. It is clear that some of the mechanisms (for example, M60-like proteases, motility and adherence) deployed by classically defined pathogens are also used by organisms that are present as commensals in many individuals. Given the complexity of these interactions and the ability to easily control variables, such as the amount and form of dietary nutrients in animal studies, a future goal is to understand the complex interaction between diet, the gut microbiota and the mucosal barrier during health and disease. Further research on diet–mucosal interactions that govern the effects of overt pathogens in healthy hosts or the pathogenic roles of commensal microorganisms in hosts with defects that predispose to diseases such as IBD should reveal new therapies or preventive strategies for these enteric diseases. Finally, future strategies may also involve precision editing of the gut microbiota with specific dietary components or drugs, as shown in a recent study in which Enterobacteriaceae-dependent microbial dysbiosis during gut inflammation was ameliorated by tungstate treatment<sup>124</sup>, as well as prebiotic modulation of the gut microbiome to specifically target enterocyte proliferation and cytokine production<sup>125</sup> to improve health.

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E.C.M., M.N. and M.S.D. researched data for the article, discussed the content, wrote the article, and reviewed and edited the manuscript before submission.

**Competing interests**

The authors declare no competing interests.

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