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Perspective

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Delving the depths of 'terra incognita' in the human intestine — the small intestinal microbiota

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Abstract

The small intestinal microbiota has a crucial role in gastrointestinal health, affecting digestion, immune function, bile acid homeostasis and nutrient metabolism. The challenges of accessibility at this site mean that our knowledge of the small intestinal microbiota is less developed than of the colonic or faecal microbiota. Here, we summarize the features and fluctuations of the microbiota along the small intestinal tract, focusing on humans, and discuss physicochemical factors and assessment methods, including the technical challenges of investigating the low microbial biomass of the proximal small bowel. We highlight the essential protective mechanisms of the small intestine, including motility, the paracellular barrier and mucus, and secretory immunity, to show their roles in limiting excessive exposure of host tissues to microbial metabolites. We address current knowledge gaps, particularly the variability among individuals, the effects of dysbiosis of the small intestinal microbiota on health and how different taxa in small intestinal microbiota could compensate for each other functionally.

Sections

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Introduction

The history of microbiota research has been driven by a series of technical innovations. In 1683, Leeuwenhoek first glimpsed the previously unseen world of microorganisms, including bacteria, hidden in his dental plaque. The pioneering work of Louis Pasteur in the latter half of the nineteenth century on microbial selective culture led to the later identification and characterization of different microbial taxa by many different researchers. In the mid-twentieth century, the development of microbial genetics initially through conjugation[1] and phage transduction techniques facilitated the identification of biochemical pathways[2]. Despite these advances, research activity was still hampered until the 1990s by the laborious nature of determining the detailed composition of a highly complex microbiome. This aspect all changed after a revolution in engineering and computational solutions for highly parallel nucleic acid sequencing microbiology. The rich automated data-stream caused a revolution in microbiology by the use of omics technologies and bioinformatics, making it practical to address the composition and transcriptomic activity of microbiotas at an individualized level. We now have a better appreciation of the intriguing role of gut microbiota in our health and disease, although the groundbreaking studies have primarily been focused on the colonic microbiota, mainly using faecal samples to probe the largest and densest population of these microscopic health influencers in the human body.

In contrast to the extensively studied large intestinal microbiota, small intestinal microbiota (SIM) has faced challenges in investigation, mainly due to technical challenges in the analysis of the low microbial biomass of the proximal small intestine and difficulties in ethical access to the distal small intestine without invasive methods[3]. Various measurement methods for SIM in humans, including surgical procedures such as stomas, offer distal sampling access but are limited to specific medical conditions[4]. Endoscopy provides a less invasive option for visualization and targeted sample collection, but requires preparative fasting. Smart capsules enable non-invasive data collection from the entire gastrointestinal tract, but are only recovered in the faeces following extended colonic transit times[5]. Post-mortem samples from brain-dead organ donors offer another avenue[6–8], although their limited availability and the delays in collection can ultimately affect the cultivation of microbiota. Due to all these constraints, much of our understanding of SIM is derived from studies in patients with gastrointestinal conditions, potentially biasing our knowledge. This reliance on patient-derived data, coupled with the under-representation of healthy individuals in SIM studies, poses a major limitation, impeding our ability to draw comprehensive conclusions about the composition and role of 'typical', disease-free SIMs.

In this Perspective, our purpose is to demonstrate some current ideas of the way in which the human SIM functions in health — and dysfunctions in conditions such as small intestinal bacterial overgrowth (SIBO) — given the underlying problems of competition between the host and its microbiota for nutrients set against the benefits of mutualism. We highlight non-invasive sampling techniques and their potential caveats, heralding a new era of comprehensive digestive tract profiling by addressing the distinct composition and metabolic functions of SIM, including the bioavailability of bile salts and pharmaceuticals. We explore the dynamic shifts in SIM across time and the feed–fast cycle that limits SIBO. Our purpose is to highlight the importance of the SIM in both disease prevention and the maintenance of health, as a somewhat different perspective on the human colonic microbiome.

Biogeographical and fluctuating dynamics

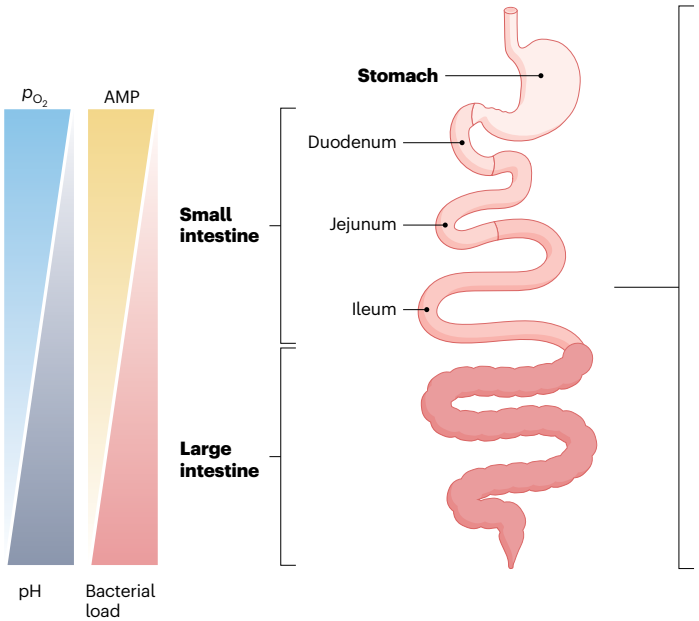
The small intestine, comprising the duodenum, jejunum and ileum, has a vital role in the digestive system, primarily focusing on nutrient absorption and digestion. This role is distinct from that of the colon, whose physiological role is to contain the bulk of the intestinal microbiota and reabsorb approximately 97% of the 9 l of fluid entering daily from the small intestine[9]. The human small intestine accomplishes its absorptive and secretory functions with a specialized anatomical and physiological setup. The large surface of the human small intestine, with only a thin mucus layer, allows efficient absorption of macronutrients and micronutrients as well as immune modulation[10]; it mediates the absorption of 90% of all host-digestible calories. Thus, metabolic changes within the small intestine have a notable effect on host metabolism[10,11]. This unique environment is bounded by a single layer of polarized intestinal epithelial cells comprising absorptive, goblet, Paneth, tuft and enteroendocrine lineages. The subepithelial microvasculature selectively oxygenates mucosal tissues setting up an oxygen gradient between the mucosa and the lumen[12].

The junction of the small and large intestines is at the ileocecal valve. Human studies of ileocecal physiology are also limited by accessibility, but both human and large-animal studies indicate the presence of a functional high-pressure zone[13] that prevents free reflux of large intestinal contents[14], with neuromuscular control allowing drainage of small intestinal contents both in digestive and interdigestive phases[15].

Characteristics of the small intestine

The small intestinal luminal environment has distinct histological and physiological characteristics[16,17] (Fig. 1): a longitudinal pH gradient (in humans: pH ~6.0 in the duodenum, 6.8 in the jejunum, and ~7.45 in the terminal ileum)[18–21]; higher partial pressures of oxygen than in the lower intestine (10–50 mmHg)[19,22]; high concentrations of antimicrobial peptides in the lower small intestine (2 μg/ml)[23]; continuous exposure to digestive enzymes and bile[24,25]; an shorter transit of the contents of a meal than the colon, typically 3–6 h[5,26,27], with motility modulated according to the episodic intake of food by the host[28]. The small intestine is less acidic than the stomach and small intestinal conditions allow microbial populations with lower biomass and diversity than the colon. The labile environment of the proximal small intestine requires exceptional microbial adaptability, as rapid adjustments are needed to cope with dynamically changing conditions. Bacterial populations are observed to range from approximately $\leq 10^4$–10^5 colony-forming units per millilitre (cfu/ml) in the healthy duodenum, increasing to 10^7–10^8 cfu/ml in the distal ileum in humans[29]. This gradient reflects both the decreasing influence of gastric acid and the increasing bioavailability of nutrients as food transits through the intestine.

The biogeographical features along the transverse axis of the gut exhibit notable variations, influencing the distribution of microbial populations. The predominant microbial community in the lower small intestine is characterized by the prevalence of rapidly proliferating facultative anaerobes. These microorganisms are resilient to the combined effects of bile acids[30], as demonstrated in *Escherichia coli* via efflux pumps[31], and to antimicrobials[32], such as the resistance of *Enterococcus faecalis* through the production of altered cell wall precursors that prevent drug binding and overexpression of efflux pumps[33]. Therefore, they can compete with the host and other bacteria for the available simple carbohydrates within the intestinal lumen[4]. Bile acids, released through the ampulla of Vater in the duodenum, are bactericidal due to their surfactant nature. These bile acids markedly influence the composition of the SIM[34,35] as they are toxic to



	Human	Mouse
Length	4.5–5.5 m	30–40 cm
pH	6.6–7.49	5.9–7.45
O_2	10–70 mmHg	56–60 mmHg
AMP	<2 μg ml^{-1}	<2 μg ml^{-1}
Transit time	3–6 h	2–4 h
Plicae circulares	Yes	No
Mucus thickness	?	~100–300 μm
SI/colon	400	18
Microbiota	Lactobacillaceae Enterobacteriaceae Streptococcaceae Actinomycetaceae Prevotellaceae Veillonellaceae	Lactobacillaceae Enterobacteriaceae Muribaculaceae Actinomycetaceae

Fig. 1 | Biogeographical characteristics of the gastrointestinal tract in humans and mice. The longitudinal axis of the intestines shows variations in both environmental conditions and bacterial populations, including changes in oxygen partial pressure (p_{O_2}), pH, antimicrobial peptides (AMP) and bacterial biomass. More distally there is a decrease in p_{O_2} and AMP levels, whereas pH and bacterial load show a general increase although this aspect is not necessarily continuous and depends on the interdigestive or postprandial state. The small intestine exhibits distinct biogeographical characteristics, including length, pH, transit times, plicae circulares, mucus thickness, surface area ratio and microbiota profiles, compared with the large intestine. Mice are shown for comparison as they are frequently used as in vivo models, although they naturally re-inoculate their intestinal microbiota through coprophagia. SI/colon, small intestine to large intestine surface area ratio.

many bacterial taxa[36]. For instance, an excess of bile acids might promote the growth of Bacillota (formerly Firmicutes) whilst inhibiting Bacteroidota (formerly Bacteroidetes)[36].

Given the shorter transit time in the small intestine (3–6 h)[37] than in the colon (48–72 h)[38,39], bacterial adherence to tissue or mucus becomes advantageous for sustained small intestinal colonization. The transition from proximal to distal segments of the small intestine and into the colon is marked by an equilibrium shift favouring a higher prevalence of strict anaerobes. This shift is attributed, in part, to the oxygen consumption patterns of aerobic and facultative anaerobic microorganisms in the upper intestinal sections. Additionally, the gradual increase in pH from the duodenum to the ileum, reaching approximately ~pH 7.5, coupled with a diminishing oxygen gradient distally, creates a more favourable environment for bacterial growth[18,40].

Technical caveats of analysis and animal models

The most robust analysis of the SIM is made from samples with the highest biomass from the lower small intestine where culture techniques can be used to verify taxonomy by molecular determination of species or genus-specific microbial DNA sequences. Unfortunately, whilst DNA sequencing techniques are highly sensitive and can detect non-culturable organisms, they are very susceptible to contamination, especially when processing samples with a low microbial biomass such as those from the duodenum and proximal jejunum[41]. As DNA is a stable molecule, detection does not necessarily prove that live organisms were present in the sample. The arguments against a low biomass placental microbiome in the past few years illustrate the caveats that must be applied to interpreting DNA analyses of samples with a low microbial biomass[42].

Comparative analyses between different animal species show fundamental differences in intestinal physiology and microbiota mutualism (for example, in ruminants), but these are outside our current scope[43]. Mice are nevertheless valuable animal models (Fig. 1) as they can be maintained germ-free and both their microbiome and germ-line genetics can be experimentally manipulated to determine causal mechanisms of mutualism or disease. An important caveat is that they are naturally coprophagic and re-inoculate their intestinal microbiome unless special experimental measures are taken.

Small intestinal microbial composition and variability

The initial studies of the lower human SIM were with ileal samples taken at surgery or from ileostoma effluents. These showed a biomass of 10^5–10^7 microorganisms per millilitre of contents, which is many orders of magnitude lower than the biomass in the colon, with compositions including *Streptococcus*, *Bacteroides*, *Clostridium*, *Lactobacillus* and Enterobacteriaceae as relatively common taxa. Examinations of mucosal samples obtained from autopsies[44], endoscopies[45], colonoscopies[46] and ostomies[47] verified these data. *Streptococcus*, *Veillonella*, *Prevotella*, *Fusobacterium* and *Haemophilus* have also been identified as constituents of the small intestinal microbiota, and are consistently detected throughout the small intestine[48–50]. Studies using nasoileal catheters and analysing ileostoma effluent, which enable continuous collection, have supported these observations[4,47,51–53]. By contrast, tube aspirates have shown very a low microbial biomass of 10^1–10^4/ml in the jejunum[54–56].

The shifting microbial landscape along the length of the small intestine involves differences in the relative contributions of different

taxa as well as increased distal microbial density. The duodenum exhibits a predominance of Bacillota, with an increase in Pseudomonadota (formerly Proteobacteria) from the proximal to the distal sections. The ileum has a higher abundance of Bacteroidota than the duodenum, resembling conditions found in the colon[48,49,57,58]. By contrast, the human colonic microbiota exhibits a predominance of Bacteroidota and Bacillota with substantial representation from the Clostridiaceae, Bacteroidaceae, Lachnospiraceae, Enterococcaceae and Ruminococcaceae families, with their relative contributions varying considerably between individuals[53,59,60].

The reasons for the low microbial density in the proximal small intestine can be understood in terms of relative sterility of the gastric input, postprandial and interdigestive intestinal motility, and susceptibility of different taxa to bile acids and secreted antimicrobial peptides. As the host digests and absorbs the diet, available nutritional carbon sources change along the length of the small intestine. Ileostomy fluids contain dietary fibre and complex carbohydrates resistant to human digestion, particularly from plant cell walls that have escaped absorption in the small bowel, and are mainly hydrolysed once they reach the microbial biomass of the lower intestine[61–63]. In other words, bacteria in the small intestine could potentially metabolize simple sugars and amino acids[4] whereas ileal and colonic bacteria mainly ferment large polysaccharides remaining from mucus or nutritional fibre.

Comparative stability and dynamic responses of SIM

In work where we were able to analyse samples from human patients directly from the ileal contents during surgery, before the creation of an ileostoma, and those collected from the ileostoma later, we found that there were no statistically significant differences in the microbiota composition of the stoma samples from individual patients compared with their unmanipulated lower SIM[53]. Further studies of the ileostoma samples from patients during the feed–fast cycle showed purging of the small intestinal biomass during overnight fasting, followed by rapid blooms in small intestinal microbial taxa after the patient had eaten[53] (Fig. 2). Through deep sequencing and computational reconstruction, we were able to infer the substrain compositions in the distal

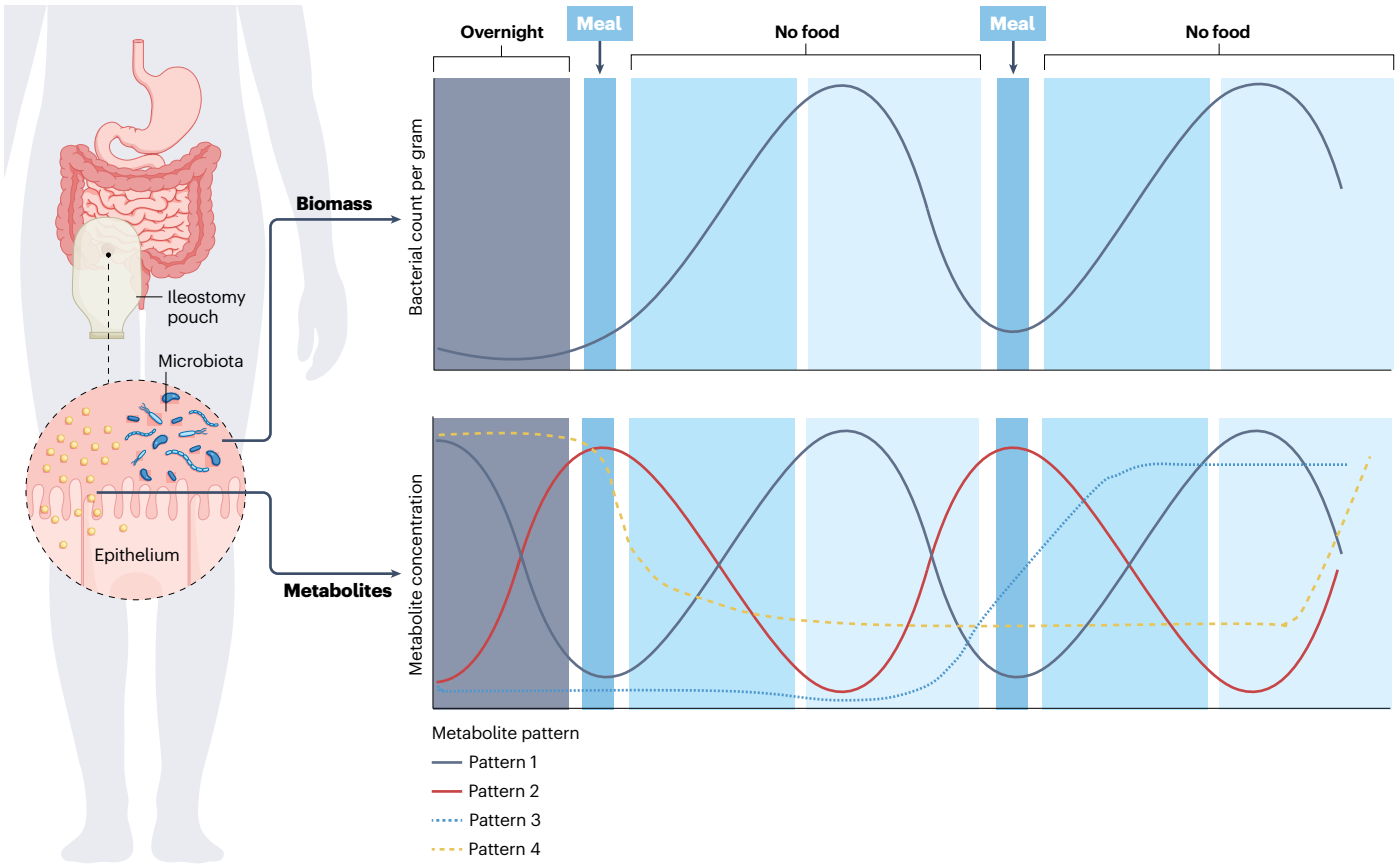


Fig. 2 | Feeding affects the abundance of gut microbial members and metabolites in the small intestine during the day. Overnight fasting induces a reduction in bacterial biomass within the small intestine. Subsequent reintroduction of nutrients through feeding triggers an exponential increase in bacterial mass, predominantly characterized by a proliferation of facultative, rapidly proliferating bacterial substrains. This dynamic fluctuation underscores the major effect of feeding patterns on the microbial landscape and different metabolite patterns of the small intestine. In contrast to biomass, the pattern of metabolite concentrations is more intricate. Pattern 1 demonstrates that overnight fasting leads to a reduction in specific metabolites within the small intestine. The subsequent reintroduction of nutrients triggers a substantial increase in these metabolites. Conversely, Pattern 2 shows an opposite trend, with an increase in metabolite levels during fasting and a decrease after meals. Pattern 3 shows a reduction in metabolite concentrations following a meal, which remain lower until the end of the day. Lastly, Pattern 4 shows an increase in metabolite concentrations after the second meal of the day, which are sustained at higher levels. Each pattern is specific for different metabolites depending on whether feeding induces or represses the uptake or release of small intestinal molecules. See ref. 53 for further information.

section of small intestine; some of these substrains rapidly increased or decreased in frequency within hours after the patient had eaten. These changes suggest very fast adjustments in substrains even within a single microbial species during digestion, that probably represent adaptation to the changing conditions as the wave of dietary metabolites pass through the gut[53]. The purging of the small intestinal biomass during an overnight fast is probably dependent on the interdigestive motor programme of migrating motor complexes — a cyclical pattern of motility that occurs every 90–120 min[64] — and explains why problems of SIBO occur in patients with diseases of intestinal dysmotility and those with surgically created blind loops.

The SIM, therefore, shows features of functional elasticity. This dynamic response concept has so far mainly been investigated in the colonic microbiota. The microbiota needs to resume its original composition and functionality after regular perturbations. Thus, sequential environmental challenges can be accommodated in several ways: without changes in composition (for example, by upregulation of metabolic activities) ('resistance'); by limiting alterations in composition over time ('resilience'); and by maintaining sufficient microorganism diversity so that loss of a metabolic function in one strain can be compensated by its presence in another ('functional redundancy')[65].

Microbial adaption and transmission dynamics

It is still unclear if bacterial strains consistently maintain their identity during passage throughout the entire gastrointestinal tract, or if they adapt to the specific intestinal niches they inhabit. Extensive microbial movement can be observed from the oral cavity to the lower gastrointestinal tract in both healthy individuals and individuals with disease[66]. In a study of 125 species common to both the mouth and the gut, 77% exhibited evidence of orofaecal transmission, with 59% of these showing substantially higher strain similarity within individuals than across a broader cohort, indicating frequent and coherent strain transfers along the intestine[66]. Specifically, core oral taxa such as *Streptococcus*, *Veillonella*, *Actinomyces* and *Haemophilus* were identified among those frequently passaged, whereas 22 species showed occasional passage, including all 21 members of the *Prevotella* genus, a key component of the oral and gut microbiome. However, 23% of the species prevalent in both sites did not meet the criteria for transmission, highlighting a complex and selective transmission landscape within the oral–gut–microbiome axis[66]. The genetic makeup of bacterial strains in particular locations within the intestine is distinctly unique, yet these strains are interconnected and seem to originate from a common founder strain that has colonized multiple areas. Strains such as *E. coli*, *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*) and *Ruminococcus gnavus* show remarkable genetic similarity when compared across different locations within the same intestine, despite differing in their growth rates depending on their location[67]. Notably, there is a gap of 300–400 genes between microbial strains that are distantly related, and a difference of 10–30 genes among strains closely related but isolated from distinct individuals[67]. These data indicate that the intestinal microbiota is formed by microbial transmission between individuals and along the intestine as well as the evolution of strains over time within an individual.

Shielding from microbial invasion

The essential problem of small intestinal host–microbial mutualism and physiology is that, whereas microorganisms can metabolize, and therefore harvest energy from, complex carbohydrates and other macromolecules that are resistant to host digestive enzymes, they also compete with the host for simple saccharides, amino acids and lipids, and compete with each other for carbon sources[68]. This aspect means that compartmentalization between host tissues and the different cellular components of the microbiota in the human small intestine needs to work along two axes: a transverse barrier between the intestinal lumen and host tissues, formed by junctional complexes between epithelial cells, and motility along its length to maintain a gradient of microbial biomass. Even in the absence of the full sterilizing effect of stomach acid, as seen in patients treated with proton pump inhibitors, the proximal small intestine prioritizes digestion and absorption of bioavailable dietary components. This section of the gastrointestinal tract is not sterile and maintains a small microbial population adapted to its unique environmental pressures and resource availability, as discussed below (Fig. 3).

Epithelial cells and immune mediators

The single cell layer of intestinal epithelial cells connected by junctional complexes is the key physical and permeability barrier, selectively absorbing nutrients whilst excluding luminal pathogens and pro-inflammatory molecules from the host tissues. The intestinal epithelium also consists of specialized cell lineages that are relevant to the microbiota barrier[69]. Much of the evidence discussed in this section was derived from studies in animal models, which provide insights into these mechanisms.

Paneth cells are located at the base of small intestinal crypts and secrete antibacterial peptides[70] such as defensins, lysozyme and DMBT1, which mix into the mucus and are secreted at the crypt opening[71]. Mucus, enriched with antibacterial peptides, then spreads to cover spaces between the villi. Epithelial cells produce additional bactericidal substances, such as REG3A which targets Gram-positive bacteria[72–74]. The production of reactive oxygen species by DUOX2 in the upper villi generates hydrogen peroxide that has potential biocidal activity for luminal microbes[75].

Goblet cells generate mucins that form an uninterrupted barrier. The mucus layer, a gel-like barrier composed of proteins and glycans, has a crucial role in the cross-sectional structure of the host–microbiota interface. This layer is generally thin and easily detachable in the small intestine, compared with two layers and a thicker coating in the colon, of which an inner compact layer is tightly bound to the epithelium and excludes most bacteria[76,77] (Fig. 3). Mucus also contains a variety of goblet-cell-derived antimicrobial molecules, including IgGFc-binding protein, calcium-activated chloride channel regulator 1, zymogen granule membrane protein 16, anterior gradient protein 2 homologue, trefoil factor 3 and kallikrein 1, alongside MUC2 (refs. 78,79), which contribute to intestinal epithelial shielding from the luminal microbial contents[80]. Although the loose small intestinal mucus layers allow infiltration of some bacteria[76], the epithelial cells are effectively protected by the presence of a high concentration of antimicrobial proteins (for example, defensins produced by Paneth cells[71] and IgA from lamina propria plasma cells) and oxygen in the zone surrounding the villi[12]. The intestinal microbiota is also restrained by bacterial competition for metabolites and the action of mucus-adhering bacteriophages, with bactericidal activity particularly intense in the small intestinal mucus[81]. Despite these defences, certain mucosa-adherent microorganisms in the small intestine manage to establish themselves due to specialized adaptations. These adaptations include the expression of surface structures such as pili and fimbriae[82], which facilitate attachment to the epithelial cells, and the induction of a common set of genes specifically to enable growth in host mucin glycans[83].

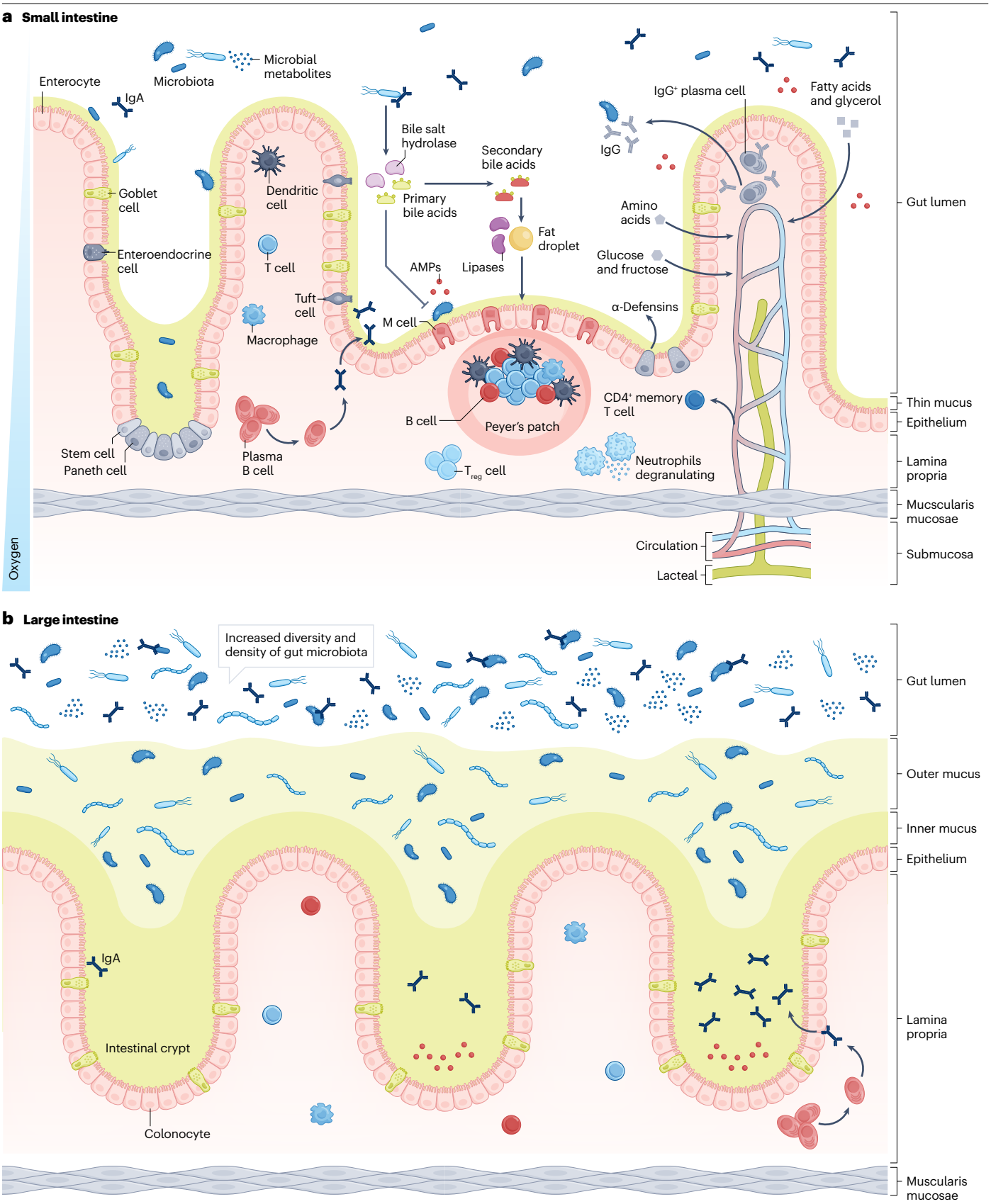

a Small intestine
Enterocyte
Microbiota
Microbial metabolites
IgA
Goblet cell
Enteroendocrine cell
Dendritic cell
T cell
Tuft cell
Macrophage
Stem cell
Paneth cell
Plasma B cell
Bile salt hydrolase
Primary bile acids
Secondary bile acids
Fat droplet
Lipases
AMPs
M cell
B cell
Peyer's patch
T$_{reg}$ cell
IgG$^+$ plasma cell
IgG
Fatty acids and glycerol
Amino acids
Glucose and fructose
α-Defensins
CD4$^+$ memory T cell
Neutrophils degranulating
Circulation
Lacteal
Oxygen
Gut lumen
Thin mucus
Epithelium
Lamina propria
Mucscularis mucosae
Submucosa
b Large intestine
Increased diversity and density of gut microbiota
IgA
Intestinal crypt
Colonocyte
Gut lumen
Outer mucus
Inner mucus
Epithelium
Lamina propria
Muscularis mucosae

Fig. 3 | Structure and immune functions of the small and large intestine. **a**, Interplay between the small intestinal microbiota and the host immune system. Multiple barriers restrict gut bacteria from reaching host cells, including the mucus layers, antimicrobial peptides (AMPs) produced by Paneth cells at crypt bases, secreted IgA and translocated IgG and a sharp oxygen gradient affecting the survival of anaerobic bacteria near the epithelial surface. These interactions show how the small intestinal microbiota shapes mucosal immunity through mechanisms such as antigen presentation, induction of secretory IgA and modulation of inflammatory responses. Primary bile acids are converted by bacterial enzymes, promoting lipid digestion and offering protection against infections. **b**, Distribution of the mucus and microbiota density in the large intestine. A schematic longitudinal section of the large intestine highlights the relative thickness of the inner (firm) mucus layer, inhabited by few mucin-digesting bacteria and the outer (loose) mucus layer. This distribution underscores the dynamic nature of the interface between host mucosal surfaces and resident microbial communities, and illustrates the different host–microbiota interactions in maintaining intestinal homeostasis. M cell, microfold cell; T_{reg} cell, regulatory T cell.

Finally, tuft cells, that act as chemosensory receptors, generate a unique blend of effector molecules such as IL-25, allergy-associated eicosanoids and acetylcholine[84]. The secretion of IL-25 can promote the recruitment and activation of immune cells that affect bacterial communities[85]. Moreover, tuft-cell-derived acetylcholine has been shown to regulate epithelial fluid secretion, which might create niches that favour the growth of specific bacterial taxa[86]. Microbial-derived succinate also stimulates tuft cells to trigger a type 2 immune response, increasing Paneth cell numbers and antimicrobial peptide expression[87].

Mucosal immunity and non-pathogenic taxa

Microfold (M) epithelial cells with a thin glycocalyx promiscuously allow dendritic cells in the dome of Peyer's patches to sample the luminal contents and induce mucosal B and T cell reactions. The B cell arm of these responses triggers the induction of plasma cells producing high amounts of IgA, which is transported through the epithelial layer and can act on microorganisms in the intestinal lumen[88]. Reconstitution experiments in mice with different specificities of induced IgA show that it exerts a series of inhibitory functions by binding to the intestinal bacterial cell wall[89]. Although clearance of small intestinal microorganisms fundamentally depends on intestinal motility, there is evidence from animal models that intestinal secretory IgA contributes to promoting microbial passage into the colon and limiting premature microbial death with the release of microbial metabolites[10]. IgA generally coats the outer surface of intestinal microorganisms[90], including the flagella, restricting bacterial motility in the small intestine and promoting clearance into the colon. Surface coating with IgA also protects against bile acid-induced damage and bacterial cell death, therefore preventing the release of lipopolysaccharide and other inflammatory bacterial molecules[89].

Enteric nervous system

The enteric nervous system functions autonomously through networks of enteric neurons that belong to molecularly and functionally distinct subtypes organized into discreet circuits with reflex activity[91]. The bidirectional interactions between the microbiota and the enteric nervous system have been reviewed in detail elsewhere[92]. In brief, the enteric nervous system controls the transit time through the small intestine of humans with different characteristics in both fed and fasting states. Luminal receptors for small intestinal microbial metabolites and bile acids (following bacterial metabolism) can directly stimulate motility through enteroendocrine signalling through basolateral secretion of serotonin. Alarmins, such as IL-33, can potentially exert similar effects, and enteric nervous system afferents can be stimulated through a range of microbial-derived molecules[93]. Nuclear receptor systems, such as the aryl hydrocarbon receptor, also stimulate intestinal motility of both dietary and microbial molecules[94]. The small intestinal microbial biomass is, therefore, generally limited by intestinal motility with coupling between microbial molecular sensing and motile function.

Bile acids

Bile acids exert bactericidal effects due to their surfactant nature which can disrupt bacterial membranes, and destroy bacterial cells[36]. They are deconjugated and dehydroxylated by small intestinal microbial enzymes. Some dehydroxylases are expressed by a very limited range of microbial taxa, making the personalized composition of the small intestinal microbiota critical for individualized bile acid homeostasis[95]. Apart from their direct bactericidal effects bile acids also limit small intestinal microbial biomass indirectly through activation of the farnesoid X receptor, and stimulation of inducible nitric oxide synthase and IL-18 transcription, which engage the immune system and providing protection against the overgrowth[96,97].

Microbial metabolites and systemic influences

Bacterial metabolites pervasively penetrate all host tissues in the body. Evidence from mass spectrometry and NMR spectroscopy analyses shows that about 10% of all metabolites in the bloodstream are from the microbiome[98,99]. Despite the lower bacterial biomass than the large intestine, small intestinal microbial metabolites enter the body very readily[10]. The relative contribution that the SIM makes to this pervasive microbial metabolite penetration probably depends on the level of the biomass at any given time. Most bacterial metabolites are rapidly cleared in the urine. Clearance of microorganisms into the colon (where mucus is thicker and its inner layer is almost free of bacteria) also restricts the accumulation of bacterial metabolites[10].

As bacteria are replicating, growing and dying in the intestine, and exchanging molecular compounds between different taxa, many of the bacterial metabolites that penetrate the body are endogenous compounds or other end products of metabolism synthesized from dietary carbon sources or shed host molecules. Drug xenobiotics are another starting point for microbiota metabolism in humans. Well-known examples are the microbiota-dependent cleavage of the azido bond of sulfasalazine to release anti-inflammatory 5-aminosalicylate from bound sulfapyridine for therapy of ulcerative colitis and reductive inactivation of digoxin by *Eggerthella lenta*[100]. The microbiota uses a wide range of different reductive, oxidative and conjugative (such as acetylation or proprionation) reactions in metabolizing drug xenobiotics. The combination of mass spectrometry and reverse genetic techniques has shown that although some drugs (for example, dexamethasone) are metabolized by single bacterial species or a limited range of taxa, microbial drug metabolism is generally promiscuous with homologous enzymes present in parallel in many different taxa[101]. This diversity is analogous to the redundancy in metabolic functions for general metabolism between microbial taxa, so that despite wide

differences in microbiota composition between individuals, the overall encoded metabolic capability remains remarkably similar between individuals[102].

Microbiota metabolism of drug xenobiotics has been largely studied taking the microbiota as a unit rather than discriminating reactions by those taxa in the lower small intestine (for example, by studies of ileostoma samples). Nevertheless, non-encapsulated oral drug treatment is usually effective in patients with ileostomas given the large absorptive surface of the small intestine[103]. Despite the redundancy between drug metabolic pathways of different taxa described here, the reduced biomass and diversity of the SIM means that drug metabolism by different small intestinal consortia in different individuals is likely to be underestimated in understanding personalized differences in pharmacokinetics.

Dysfunctional microbial colonization

The microbiota, both in the intestine and at other body sites, is acknowledged to play an important part in both intestinal and non-intestinal diseases. Comparisons of germ-free and colonized mice have shown that every organ system is potentially affected. In the small intestine, there is extensive reprogramming of epithelial, mucosal immune and mesenchymal cells, and enteric neural function. Although a few live microorganisms translocate to reach the mesenteric lymph nodes or the systemic circulation, the microbiota is well contained in the intestinal lumen and most of these local effects (which adapt the small intestine to the presence of luminal microorganisms) are generated in the small intestine itself. There is an extensive human and animal model literature — outside the scope of this Perspective — on how such adaptive mutualism can break down in Crohn's disease[104], how small intestinal microbial residents can potentially generate systemic autoimmunity[105], and how colonization resistance with a non-pathogenic microbiota can prevent the ingress of pathogens[32]. As described above, the composition of the SIM community is highly dynamic with highly variable compositions. As different microbial taxa are countered by distinct immune responses, the states of healthy mutualism are probably highly personalized, although better insights into the specifics between different individuals are still emerging.

In this section, we provide an overview of situations in which the SIM biomass is abnormally distributed or increased or in which small intestinal microbial metabolism rather than host immunity is primarily responsible for causing symptoms.

Small intestinal bacterial overgrowth

SIBO is a heterogeneous condition characterized by excessive bacterial biomass within the small intestine. Typically, SIBO results from gut dysmotility or the loss of small intestinal continuity of flow (diverticula, surgically induced blind loops or strictures). The interdigestive motor programme of migrating motor complexes normally clears much of the small intestinal biomass[53]; when this programme is ineffective, inefficient removal of bacteria and their dietary carbon sources allows microbial blooms[106]. The consequences are that the bacterial breakdown of carbohydrates in the proximal small intestine results in the formation of organic acids, aldehydes, alcohols and gases[106]. Hydrogen and methane as respiratory acceptors detected in breath tests are used as surrogates for diagnosis[107]. Excessive fermentation in the small intestine can lead to the production of metabolic by-products, potentially causing symptoms such as bloating, nausea, abdominal pain, distension and acidic stools. Only a proportion of microbial taxa can synthesize vitamin B_{12}; in instances in which the overgrowing microbial mass is made up of consumers rather than producers, low serum B_{12} levels can result[108].

For SIBO, as with other conditions potentially arising from small intestinal microbial dysbiosis, a critical challenge lies in accurately defining both the proximal extent and the size of the microbial biomass. This task becomes particularly complex given that the biomass often varies between the fed and fasting states. These fluctuations are important not only for understanding SIBO but also because they can complicate the differential diagnosis of conditions with overlapping symptoms such as bloating, discomfort and altered bowel habits, as is the case for functional dyspepsia. Although levels of bacteria in fasting proximal intestinal aspirates of $>10^3$ cfu/ml on MacConkey agar[109] are potentially diagnostic of SIBO[110,111], non-invasive diagnostic methods such as glucose breath testing (GBT) or lactulose breath testing (LBT) are also more commonly used. However, their pooled sensitivity and specificity are disappointing (LBT 42.0% and 70.6% and GBT 54.5% and 83.2%, respectively, over 14 different studies)[112–114].

The vast majority of bacteria detected in patients with SIBO belong to the phyla Bacillota and Pseudomonadota. These bacteria can be divided into two subgroups: Gram-positive bacteria such as *Streptococcus*, *Staphylococcus* and *Lactobacillus* originating from the oropharynx, referred to as small intestinal oral bacterial overgrowth (aerodigestive tract SIBO, defined as $>10^5$ cfu/ml of oropharyngeal bacteria)[58,115], and coliform Gram-negative bacteria, or coliform SIBO, predominantly characterized by an increased presence of Enterobacteriaceae pathogens such as *Escherichia*, *Klebsiella* and *Proteus*[116]. Overgrowth can also include *Bacteroides*, *Clostridium*, *Veillonella*, *Fusobacterium* and *Peptostreptococcus* in patients experiencing diarrhoea and malabsorption[116] as assessed by culture-based methods[111,117–119] and/or 16S ribosomal RNA gene and/or shotgun metagenomic sequencing[58,110,111,118,120]. This diversity supports the concept that SIBO is a microbial community problem in the face of altered motility or intestinal anatomy, rather than a single type of bacteria.

Within this heterogeneity, there is a major caveat. Even with the strict criterion of $>10^5$ cfu/ml in duodenal aspirates, SIBO can also be found in healthy individuals consuming high-fibre diets[121]. Conversely, in patients selected with non-specific intestinal symptoms, the symptoms were not significantly associated with SIBO but with a potentially dysbiotic upper intestinal microbiota[121], as detailed below.

Functional gastrointestinal disease

Despite the potential challenges of sampling or analytical contaminants in samples from the upper small intestine with a low microbial biomass, there is evidence that the taxa composition of such low abundance communities can be associated with functional intestinal symptoms. Patients selected with symptoms such as diarrhoea, abdominal pain and bloating have reduced upper intestinal diversity on the basis of 16S microbial DNA sequences, loss of anaerobes and the selective presence of some facultative aerobes such as *E. coli*[121]. Animal model experiments show that *E. coli* blooms are associated with increased luminal oxygen when the intestinal epithelial layer is damaged and oxygen-sensitive anaerobes cease to produce short-chain fatty acids that normally drive epithelial oxygen consumption through β-oxidation[122]. There is preliminary evidence from dietary shift experiments in 16 healthy individuals with a high duodenal microbial biomass[121] that dietary fibre (which provides the complex carbohydrate carbon source for the generation of short-chain fatty acids by anaerobes) can protect individuals from dysbiosis and symptoms such as diarrhoea, bloating and abdominal pain[123].

The antithesis of a high-fibre diet aiming to protect the proximal SIM is the fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet, which has reduced fibre and includes other fermentable saccharides and polyols, and is used to treat irritable bowel syndrome (IBS). IBS lies at the interface of lower intestinal function and central pain appreciation, defined by the relationship between abdominal pain and defaecation with changes in the frequency of defaecation or faecal consistency. Amongst the multifaceted potential causes of IBS, including immune dysfunction, biogenic amine imbalance, altered barrier function, bile acid homeostasis and the enteric nervous system, it is recognized that dietary modifications, such as reducing intake of FODMAPs, can alleviate symptoms in some patients, probably due to reduced fermentation by colonic microbiota[124]. This finding leaves us with the uncertainty of which microbiota taxa are metabolizing which nutritional sources and where in the intestine this event occurs. The inaccessibility of the lower small intestine and proximal colonic microbiotas and their variability between individuals make this a challenging problem to answer on a personalized level. Nevertheless, the importance of staged metabolism along the small intestine is shown in short bowel syndrome in which simple sugars can reach the lower gastrointestinal tract to be fermented by D-lactate-producing *Lactobacillus* and pro-inflammatory Pseudomonadota[125,126].

Coeliac disease

Coeliac disease presents a compelling case in which, despite a clearly defined nutritional aetiology involving dietary gluten, emerging evidence suggests that low-abundance gut microorganisms could contribute to disease pathogenesis (reviewed elsewhere[127]). The condition is precipitated by gluten ingestion, and strict adherence to a gluten-free diet remains the cornerstone of effective management. Coeliac disease is strongly linked to haplotypes encoding HLA-DQ2/DQ8 (ref. 127), and gliadin protein epitopes of gluten-triggering pathogenic T cell clones have been identified[128]. The intestinal damage is normally confined to the proximal duodenum where the small intestinal microbial load is very low[129], yet analysis of 16S microbial DNA sequences from duodenal aspirate and biopsy samples indicates that patients with coeliac disease and enteropathy have different upper intestinal microbial patterns from those in healthy individuals as controls. Microbiota composition was analysed in different sections (proximal-D1, D2 and distal-D3) and luminal compartments (small intestinal and faecal) from 24 patients with coeliac disease and 41 healthy individuals[130]. Gut location emerged as a primary determinant of microbiota composition, explaining 41% of the variation between samples, whereas disease status (coeliac disease versus controls) accounted for less than 1%. Bacillota dominated across all sites, with Bacteroidota enriched in duodenal aspirates and Pseudomonadota in biopsy samples. Different bacterial genera were enriched at each sampling location[130]. However, in patients with coeliac disease, microbial differences across different sampling sites revealed increased levels of *Escherichia*, Prevotellaceae (unclassified), *Neisseria* and *Peptostreptococcus*, whereas *Dolosigranulum*, *Phenylobacterium*, *Acidovorax*, *Moraxella*, *Methylobacterium*, *Staphylococcus*, *Bacillus*, *Sellimonas*, *Bradyrhizobium*, *Delftia*, *Acinetobacter* and *Leuconostoc* were decreased compared with levels in controls[130,131]. Evidence for a mechanistic role of the microbiota differences has been obtained from transferring these microbial consortia to germ-free mice and showing that carboxypeptidase gene content and gluten degradation are lower in samples from patients with coeliac disease than in samples from healthy individuaks[130]. It will be interesting to know whether persistent abnormalities of the duodenal microbiota can explain differences in sensitivity to gluten challenge in patients with primed mucosal T cells, yet whose intestinal histology has reverted to normal on a gluten-free diet.

Conclusions

In this Perspective, we highlight the intricacies of gut microbiota biogeography, emphasizing the stratification of microbial communities across various gut regions, specifically focusing on the small intestine. The reason that microbiotas can have such different compositions in different healthy individuals is largely explained by most metabolic functions being contributed by different taxa; yet altering the diet in each individual changes the microbiota composition through preferred consumer-resource effects. Understanding both the ecology and its effects on the host remains a challenge, given the oscillatory nature of small intestinal physiology.

There is evidence that even the low microbial biomass in the proximal small intestine could be relevant to intestinal function and disease susceptibility. Investigating these problems presents substantial technical challenges due to low microbial biomass and high risks of host DNA and environmental contamination, complicating the distinction between microbial signals and background noise. Longitudinal studies are required to understand the function of different consortia in individual patients.

Innovative methodologies such as stoma sampling, capsules and endoscopy, have propelled forward our understanding of the protective mechanisms of the small intestine, microbial metabolism, and their implications for drug bioavailability and health[3–5,53]. Yet, the complexity of the SIM and its dynamic relationship with the host remain under-investigated. Future work will leverage non-invasive techniques to ethically and effectively study the SIM, aiming to uncover the nuanced interrelations between diet, host genetics and microbial communities.

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