The Microbiome in Visceral Medicine: Inflammatory Bowel Disease, Obesity and Beyond

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Summary

It has become increasingly evident over the past two decades that the microbiota plays a nurturing role in the development of the immune system. This appears to be important since the amplitude of immune responses has a crucial regulatory function in homeostasis and the prevention of unwanted inflammation. Hence, a malfunctioning gut flora has been shown to play a key role in visceral medicine. Strong evidence demonstrates for example that intestinal inflammation can develop as a result of a dysregulated microbiota, deficient antimicrobial responses, and aberrant bacterial translocation into the bowel wall. In healthy individuals, the bacterial translocation is blocked by a single layer of highly specialized intestinal epithelial cells which forms a strong barrier that lines the gut wall. This structure is responsible for an efficient absorption of nutrients while keeping the luminal flora at bay. In susceptible individuals, for yet incompletely understood reasons, either defective epithelial barrier function or dysregulated microbial composition or microbial pathogens drive intestinal inflammation. Many therapeutic strategies focusing on the modulation of the microbiota have been proposed recently but future research including prospective human studies and gnotobiotic mouse models are still needed to evaluate the contribution and potential therapeutic value of individual bacteria to human health.

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Introduction

The human gut flora which is contained within the lumen of the gastrointestinal tract is notoriously known to represent one of the most complex ecosystems on earth, harboring a plethora of viral, bacterial, fungal, protozoan and archaeal species which altogether represent the intestinal microbiome [1–3]. Studying the ‘human microbial organ’ in homeostasis and disease states revealed the existence of a strong interaction between the intestinal mucosa and the microbiota. For example, microbiota components degrade non-digestible products and synthesize vitamins and short-chain fatty acids that provide up to 10% of the total energetic expenses of the body [4]. Equally important has been the realization that many of these byproducts are also being used to nurture the developing immune system [5, 6]. In this context, the interaction between microbes and mucosal immune compartments in the gut plays a major role in priming and regulating immunity [7]. Unprecedented technical advances of the last decade (e.g. next-generation sequencing and metabolomics studies) uncovered for the first time the diverse composition and dynamics of the intestinal microbiome. The upcoming decade holds great promise in uncovering why specific strains are localized to different segments of the gut and how microbiota supports visceral homeostasis [8].

Different layers of passive and active defense including the physical integrity of the intestinal epithelial cell layer, the mucus, antimicrobial peptides, glycoproteins, and secretory immunoglobulin A protect viscera from the potentially aggressive microbial content of the intestinal lumen. In situations in which different levels of primary defense are compromised, microbial components may reach the underlying immune cell layer, thus initiating local inflammatory responses [9]. Under various circumstances (e.g. genetic predisposition, low general health status, and predisposing environmental factors) the self-restricted immune control mechanisms including T cell exhaustion fail [10]. As a consequence, chronic activation of the immune system takes central stage, thus laying the foundations for the development of chronic visceral inflammation such as inflammatory bowel disease (IBD).

It is currently believed that IBD comprises of a group of idiopathic, chronic and relapsing disorders of the gastrointestinal system in which homeostasis has been compromised by exaggerated immune responses to microbiota-derived components in a genetically predisposed individual [11]. Crohn's disease (CD), one of the two main forms of IBD, can affect every segment along the gastrointestinal tract from the mouth to the anus and is characterized by segmental inflammation affecting the entirety of the gastrointestinal wall. In contrast, ulcerative colitis (UC), the other major form of IBD, affects the large intestine only and is described as a continuous bloody mucosal inflammation. Given its highly debilitating character, there has been great interest to elucidate the etiopathogenesis of IBD and to find better treatments for the disease. Genetic predisposition, environmental factors, intestinal microbiota, and overreacting immune responses to gut microbiota are currently considered the four pillars of IBD pathogenesis. It is now largely accepted that uncontrolled immune responses against commensals or their products are critically involved in IBD pathophysiology [12, 13]. In contrast, alterations in the composition of microbiota have been discussed as causative agents in IBD [14, 15]. The major implication of the microbiota in this context is highlighted by the observation that inflammation is ameliorated or even absent in the absence of bacteria in different models of IBD [12, 13].

In this review we discuss the role of intestinal microbiota in visceral medicine in the context of gut health and intestinal inflammation and highlight some of the strategies aiming to functionally modulate microbiota as a new treatment frontier for visceral health.

Essential Role of the Intestinal Microbiome in Visceral Homeostasis

Although the structural and functional complexity of different ecosystems established within the gastrointestinal tract is daunting, it has become evident over recent years that deciphering interactions between the microbiome and the intestinal immune system holds great promise for understanding homeostasis of the entire body. There is great confidence that these interactions control pathophysiological mechanisms in obesity, diabetes, cardiovascular disease, metabolic syndrome, and chronic liver disease as well as IBD and colorectal cancer [16–19]. In this context, the impact of the microbiota on the host seems to be best reflected by thinking of ourselves as being 'married to our microbiota' [5]. Fueled by unprecedented technical advances mainly in the field of next-generation sequencing and computational analysis of functional genomic data, the interest in unraveling the 'microbiota code' has taken central stage in the last decade. Hence, we are witnessing an exponential increase in the number of large-scale multi-centric research initiatives focusing on this topic. One prominent example is the human microbiome project (www.hmpdacc.org) which aims to establish basic knowledge about the composition and interactions of the 'healthy gut' microbiota in health and disease states [20].

Colonization of the sterile fetal gut begins at birth from maternal vaginal and fecal flora as well as first contacts with environment [21] or even in utero by contamination with microbes from the mother [22]. Skin-to-skin contacts and breastfeeding subsequently provide first encounters with important building blocks of the healthy microbiota (e.g. *Bifidobacterium* and *Lactobacillus*) [5, 23]. By the age of 2–3 years, the by now diversified and complex microbiota stabilizes [22, 24–26]. It has been estimated that the intestinal microbiota in healthy adults harbors ten times more bacteria than the number of human cells in the entire body whereas the microbial genome encodes 100 times more genes than the human genome [2].

Besides bacteria, however, the luminal content of our gut is home to many viruses, bacteriophages, fungi, protozoa, and archaea whose presence and relations with bacteria on the one hand and the immune system on the other hand are largely unknown, owing mainly to the fact that standard techniques of the past decades (e.g. 16S-based sequencing) could not characterize the vast majority of these intruders.

Although previously thought to consist of a common core of 'good' bacteria shared among healthy individuals, recent next-generation sequencing approaches revealed a high level of microbial diversity between individuals [1]. In contrast, the stability of the intraindividual microbiome over time argues for the fact that either genetic or lifestyle habits dictate the composition of the microbiota [27]. Noteworthy, the microbiome is most similar in monozygotic twins compared to siblings [28], suggesting a primary role for genetic determinism of the microbiota buildup. Very importantly, however, recent studies revealed the fact that although generally stable, the microbiota can be altered by infections and antibiotic treatment, environmental factors, surgery, and nonetheless changes in individual lifestyle including long-term change in diet [3, 29]. It has been recently suggested that changing diet composition could promote a 'healthy microbiota', thus allowing for therapeutic interventions [30].

Bacteria have been shown to modulate visceral health by the products of their fermentation. For example, fermentation of carbohydrates in the proximal colon provides not only a source for cross-feeding of bacterial communities but also accounts for 10% of the daily energy expenses of the host [4]. The availability of carbohydrates becomes limited in distal parts of the colon where fermentation of other substrates including proteins and amino acids becomes predominant [18]. Interestingly, it has been demonstrated that whereas products of carbohydrate fermentation (e.g. butyrate, propionate) induce immunomodulatory effects, oral tolerance, and restitution of inflammation [31], products of protein fermentation in the distal colon (e.g. ammonia, phenols) have the potential to initiate inflammation, DNA damage, and colorectal cancer progression [32]. Recognition of the carbohydrate fermentation as a main homeostatic resort of gut microbiota provides the scientific basis for improving gut health by diet [18, 30]. These observations underline the importance of prioritizing the dietary intake of plant-based foods as compared to protein-rich animal products in order to prevent disease [18, 19].

Dysbiosis and Its Relation with the Development of Intestinal Bowel Disease and Obesity

Most of the gut microbiota resides in the large intestine, and the vast majority (98–99%) of the 'healthy' gut flora is accounted for by four phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Altogether, more than 150 species make up the core of the bacterial contents of the large intestine [2, 33, 34]. Although not all these species are shared between unrelated individuals, the functional outcome of different microbial make-ups is similar across the healthy population. This indicates that various microbiotas have evolved to ensure a common core of functions in men [18]. Recently, the concept of 'gut health' which is based on the effective digestion and absorption of food, the presence of normal and stable intestinal microbiota, the absence of gastrointestinal illness, and the subjective status of well-being has been defined as a new objective in preventive medical care [35].

Dysbiosis, defined as an alteration in the composition as well as a decreased complexity of the microbiota, is commonly observed in IBD patients. Whether such changes are a cause or a consequence of the disease remains hotly debated [7, 36]. In this context, bacterial diversity is significantly reduced in CD patients with active inflammation but also during remission phases as compared with healthy controls; such data suggested that dysbiosis may be a causative factor of the inflammation in CD [37]. This view is also sustained by animal studies showing that germ-free animals are protected from experimentally induced intestinal inflammation [13, 15]. In IBD, *Actinobacteria* and *Proteobacteria* are increased whereas *Bacteroidetes* and *Firmicutes* are decreased which results in major shifts in oxidative stress pathways as well as decreased carbohydrate metabolism and amino acid biosynthesis [38–40]. Very recently, it has been described that a consortium of bacteria that can be triggered by low-fiber diet promotes expansion and activity of colonic mucus-degrading bacteria and that fiber-deprived gut microbiota promotes aggressive colitis by an enteric pathogen [30]. Collectively, these studies indicate that the microbial composition can have a major impact on immune homeostasis in the gut. However, whether these changes are the cause or consequence of IBD has yet to be convincingly shown.

Based on its important implications in the maintenance of host energy homeostasis, the intestinal microbiota can be regarded as a separate endocrine organ [41]; in particular, it is thought that dysbiosis can, by promoting low-grade inflammation, contribute to the metabolic syndrome (i.e. hyperglycemia, hypertriglyceridemia, dyslipidemia, hypertension) and obesity-related pathologies such as type 2 diabetes [42, 43]. Like for IBD, the pathogenesis of obesity has been linked with phylum-level changes in the intestinal microbiota as well as a reduced diversity in gut bacterial composition [28]. Initial results indicated that an increase in the abundance of *Firmicutes* was associated with diet-induced obesity [44]; moreover, the *Firmicutes*:*Bacteroidetes* ratio was found to positively correlate with the obese phenotype in mice independently of the diet [16]. One of the major advances in our understanding of the interactions between microbiota and obesity stems from ingenuous fecal microbiota transfer experiments between humans and mice

[45]. The authors of this study recruited four pairs of human adult female twin pairs discordant for obesity (i.e. one of the siblings was lean and the other one was obese) and introduced the fecal microbiota of each one into germ-free mice. Mice receiving bacteria from the obese sibling became obese, whereas those receiving bacteria from the slim sibling stayed slim. As shown by co-housing experiments, microbiota from lean donor mice can invade the guts of obese-recipient mice, reducing total body and fat mass, thus preventing the metabolic syndrome in mice that are fed with appropriate low-fat, high-fiber diet [45]. Remarkably, this protection disappeared when mice were fed a diet that was high in saturated fat but low in fiber. This observation correlated with the invasion of *Bacteroidetes* components, particularly *Bacteroides* spp., from the lean microbiota-transferred mice into the obese microbiota-transferred cage-mates, in a diet-dependent setting. The fact that this invasion process only occurred in one direction could reside in the observation that the microbiota from obese individuals is less diverse compared to lean people, thus leaving niches that can be filled by lean bacterial components [28]. Taken together, these results highlighted the existence of transmissible, rapid, and modifiable effects of diet-by-microbiota interactions [45].

An Unresolved Matter: Can Inflammation Be Triggered by Specific Microbiota Components?

Several studies have provided evidence for a causative role of specific bacterial strains in mouse models of intestinal inflammation (e.g. *Bacteroides fragilis* or *Helicobacter hepaticus*) [46, 47], whereas others could not confirm these data [48]. Currently, there is no clear evidence that infection due to specific components of the microbiota is causing IBD according to Koch's postulates. In the strict sense, this would require: i) the culprit microorganism is present in all patients, ii) disease can be transferred upon inoculation, and iii) the infectious agent can be re-isolated after experimental infection [20]. Hence, the present general consensus is that whereas a number of microbes strongly support IBD development, the unequivocal evidence for a causative role of the microbiota in IBD is still missing [49].

Studies in genetically altered mice provided evidence for the implication of specific microbial components in inflammation. For example, in immunocompromised *Rag2*^{-/-} mice, the presence of *H. hepaticus* causes chronic intestinal inflammation resembling features of human IBD [50]. In another study, *H. hepaticus* triggered colitis in specific-pathogen-free *Il10*^{-/-} mice through an IL(interleukin)-12- and interferon- γ -dependent mechanism [51]. The same authors later demonstrated that *H. hepaticus* induces wild-type regulatory T cells to produce IL-10 which suppress interferon- γ production, thus preventing bacteria-induced colitis [47]. Nevertheless, others found that specific-pathogen-free *Il10*^{-/-} mice had similar colitis activity as compared to wild-type animals in the *H. hepaticus* model, therefore questioning the predominant role of this bacterium in experimental IBD [48].

Escherichia coli is the predominant non-pathogenic facultative flora in the human intestine [52]. The fact that commensals can

cause intestinal inflammation is strengthened by the observation that some species may acquire traits of pathogenicity including the potential to adhere to the intestinal epithelium or to express toxins as in the case of adherent/invasive *E. coli* [52]. It has been demonstrated that adherent/invasive *E. coli*, which is absent in healthy individuals, can be abundantly found in the inflamed ileal mucosa of CD patients where it causes intestinal inflammation by disrupting the intestinal barrier [53]. Such bacteria might infect macrophages without killing them, a mechanism by which subsequent chronic stimulation of immune cells might be triggered [49]. Nevertheless, as with most of the facultative pathogen strains, there is a requirement for genetic or immune-related predispositions of the host in order for colitis to develop.

In another study, *Atg16L1* mutant mice were highly susceptible to norovirus infection which was dependent on commensals. This observation strengthens the hypothesis of the multistep pathogenesis model of IBD wherein the first infection primes the immune system for the commensal-driven chronic inflammation [54]. In this respect, individuals with an episode of *Salmonella/Campylobacter* gastroenteritis were shown to have increased short- and long-term susceptibility to develop IBD later in life [55]. The underlying mechanism is unknown but might rely on the prolonged toxic effect of these infections on the homeostatic barrier function of the gut epithelium. Since a disrupted barrier cannot ward off commensals, these could penetrate the lamina propria to elicit innate and adaptive immune reactions. Subsequent encounter of primed effector T cells with bacteria antigens could then trigger the pathogenic cascade observed in IBD [15].

Other commensal species in turn have the ability to shape intestinal immunity by primarily interfering with adaptive immune responses. In elegant experiments using gnotobiotic mice, it was found that colonization of the small intestine with a single symbiont, i.e. segmented filamentous bacterium, a bacterial species related to *Clostridia*, was sufficient to induce the appearance of Th17 cells in the lamina propria of the small intestine [56]. One particular feature of segmented filamentous bacteria that distinguishes them from other commensals is their ability to tightly adhere to the surface of epithelial cells in the terminal ileum. Importantly, the increased expression of proinflammatory and antimicrobial genes resulted in enhanced resistance to infection by *Citrobacter rodentium*, an intestinal pathogen related to enterohemorrhagic and enteropathogenic *E. coli* in humans [56]. Very recently, researchers were able for the first time to grow mouse segmented filamentous bacteria outside their host in a co-culturing system. Like in vivo, intracellular offspring attached to mouse (and human) host cells and potently induced innate defense genes, proinflammatory cytokines, and chemokines. By mimicking the in vivo niche, this technique provides unprecedented insights into growth requirements and their immunostimulatory potential, opening new avenues for investigating the microbial-host interactions at the molecular levels [57]. In contrast, organotypic culture of intestinal crypts that has been established and refined over the past decade, the so-called organoids [58], currently provides a great tool for addressing molecular mechanisms governing intestinal epithelial cell physiology. Combining organoid culture with immune

cell and/or microbiota culture, by eliminating the need of using live animals, would dramatically accelerate the pace at which the cellular and molecular understanding of the interactions between microbiota and the gut will advance.

The Eternal Question: Microbiota – Cause or Consequence of Inflammation?

This predilection for IBD lesions in locations with greatest bacterial concentration (i.e. colon and terminal ileum) offered circumstantial evidence to link bacterial colonization to IBD pathogenesis [59]. The presence of dysbiosis in IBD patients has immediately raised the question of whether they represent the cause of disease or the consequence of ongoing inflammation. The major limitation in drawing sound conclusions about the causative or secondary nature of microbiota in IBD pathogenesis has been the fact that dysbiosis could be observed only retrospectively. Although some of these issues could be overcome by e.g. sequencing microbiota samples from patients and their relatives without clinical inflammation, no definitive response to this question was granted. Nevertheless, since alterations in the microbiota certainly entertain and possibly aggravate ongoing inflammation, the analysis of dysbiosis is critical in understanding visceral disease and establishing novel therapeutic approaches [15, 33, 40]. The complexity of the problem is highlighted by different observations; e.g., *E. coli* has been linked to the development of IBD and colorectal cancer, whereas another *E. coli* strain is used as a probiotic to induce resolution of inflammation [60, 61]. Whether such contradicting observations represent a reflection of our currently incomplete understanding of microbiota or is rather due to e.g. host-dependent genetic backgrounds is yet to be defined.

In mice, two main strategies were used to ascertain the existence of a link between bacteria and intestinal inflammation. The first approach used broad-spectrum antibiotics to clear the content of the intestine of microbiota. Although results differed slightly depending on the experimental conditions, there is a general agreement that the elimination of bacteria blocks intestinal inflammation [15]. A more precise and elegant strategy is the use of germ-free mice which are born under aseptic conditions, usually by CPS-sarean section, and immediately transferred and maintained in a sterile environment. These mice can then be colonized with one or several well-characterized strains of bacteria giving rise to gnotobiotic mice with precisely defined flora. Experiments in animals with spontaneous intestinal inflammation (e.g. *Il10^{-/-}*, or transgenic *HLA-B27* or *Tg(CD3E26)*) have demonstrated the critical role of microbiota in promoting this process. Time and again, it was shown that the spontaneous phenotype present in mice with genetic predisposition can be abrogated by housing the animals in germ-free conditions. Moreover, a germ-free environment was shown to protect animals in various induced models of intestinal inflammation. Importantly, when mice were transferred to conventional conditions inflammation ensued, providing a direct causative implication of the microbiota in disease under these experimental settings [12, 15, 62–64].

This idea for a causative role of the microbiota in intestinal inflammation was further extended and consolidated by microbiota transfer experiments [65]. In that study, microbiota from colitis-bearing animals was shown to induce intestinal inflammation in healthy *Tbet*^{-/-} × *Rag2*^{-/-} mice. Strikingly, the flora of these mice in which colitogenic bacteria became dominant due to the lack of T-bet and adaptive immune responses was able to induce colitis even in healthy controls. The culprit pathogens were identified as *Klebsiella pneumoniae* and *Proteus mirabilis*. Confirming their prominent role, these individual strains were able to induce colitis in *Rag2*^{-/-} and wild-type mice upon adoptive transfer, establishing the concept of transferable susceptibility [66].

Expanding the idea of transferable susceptibility even further, it has been recently proposed that microbiota does not only transfer disease but importantly that a healthy microbiota can limit intestinal inflammation in colitis models [67]. The practical implications of the latter results could be reflected in strategies focused on establishing a 'healthy' microbiota in inflamed patients by changing their diet and/or lifestyle or more drastically by stool transplantation approaches.

Genetic Predisposition Modulates the Microbiota in Intestinal Bowel Disease

Currently, the number of susceptibility genes for IBD has surpassed 200 [68–70]. The vast majority of them (e.g. *CARD9*, *IL10*, *IL23R*, *JAK2*, *TNFSF15*) are implicated in mucosal barrier function, microbial recognition, antimicrobial defense, mucus production, immune regulation, or restitution after injury. As such, dysregulated antimicrobial responses to commensals or the appearance of dysbiosis as a direct consequence of genetic alterations might cause IBD [15, 68–73].

In 2001, work from two independent groups described *NOD2/CARD15* as the first genetic risk locus for CD [72, 73]. NOD represents one of a series of intracellular innate immune receptors implicated in microbial recognition. There is an increased risk (up to 20 times in homozygous NOD2 carriers) to develop IBD, and mice with *Nod2* deletion had increased susceptibility to intestinal inflammation. Importantly, the inflammatory phenotype in these mice could be rescued by housing them in a germ-free environment underlying the intimate relation between genetic predisposition and microbiota (reviewed in [12, 15]). It was later found that *Nod2* is required for the regulation of commensal microbiota in the intestine and *Nod2*-deficient mice harbored increased loads of commensals. Moreover, these mice had a significantly delayed clearance of the opportunistic pathogen *H. hepaticus* as compared to wild-type mice which resulted in the break-down of intestinal homeostasis [74]. Mechanistically, NOD2 might negatively regulate excessive toll-like receptor activation by inducing adequate low-intensity innate immune responses upon bacterial dipeptide peptidoglycan recognition. In contrast, mutated NOD2 variants would result in hyperactivation of e.g. NF-κB pathway and dysregulated immune responses [75, 76]. An alternative explanation sug-

gested that bacterial phagocytosis might be defective in mutated NOD2 variants [77]. Yet another hypothesis is that defective NOD2 impairs α-defensins production by Paneth cells which would in turn compromise antimicrobial responses in the gut. In any case, flawed bacterial recognition and handling might result in intestinal barrier defects and the chronic exposure of commensals to the underlying immune system. In this context, CD patients with ileal lesions show compromised Paneth cell-related antimicrobial defense [78], and Paneth cell death has also been related to persistent inflammation in CD patients and experimental colitis models [79]. A recent study from our group found that mice lacking caspase-8 specifically in their intestinal epithelial cells developed spontaneous ileitis. The most striking phenotype of these mice was the lack of Paneth cells and the reduced number of goblet cells, suggesting once again that a defect in the innate immune response is driving intestinal inflammation [79]. Whereas Paneth cell dysfunction clearly modulates microbiota composition and function, the underlying mechanisms remain obscure. It has been suggested that an altered production of antimicrobial peptides might induce dysbiosis in the gut by enabling for example the direct contact of luminal components with the epithelium. Barrier integrity could also be affected by Paneth cell dysfunction since these cells contribute to the maintenance of its integrity by providing nutrients/signals to the stem cell niche. Moreover, impaired production of trefoil factor 3, which contributes to the structural integrity to the mucus [80], or deficient mucus production as observed in *Muc2*^{-/-} mice favor epithelial barrier dysfunction. This in turn could be followed by the uncontrolled activation of lamina propria immune cells which amplifies local immune responses and induces even more epithelial cell death, perpetuating a vicious cycle.

As another example, mice lacking NF-κB essential modulator specifically in epithelial cells presented spontaneous, TNF-α-driven apoptosis of intestinal epithelial cells which resulted in altered barrier function and microbial-induced severe pancolitis [81]. Extending these studies, it has been recently shown that the NF-κB essential modulator gene prevents intestinal inflammation by inhibiting receptor-interacting protein kinase 1 kinase activity-mediated epithelial cell death, suggesting that inhibition of this kinase could be used in treating patients with NF-κB essential modulator gene mutations [82].

Another single nucleotide polymorphism conferring increased susceptibility to CD is the autophagy gene *ATG16L1* [71]. Defective autophagy has been associated with impaired Paneth cell function in both mice and humans [83]. The authors of this study made the unexpected observation of a gain of function specific to *ATG16L1*-deficient Paneth cells which led to various modifications in the secretory functions that resulted in increased peroxisome signaling and lipid metabolism which promoted inflammation. It has been proposed that by interfering with autophagy, intracellular bacteria cannot be efficiently eliminated, thus perpetuating the immune activation.

In summary, genetic alterations surely contribute to IBD pathogenesis, but further research is needed for definitive answers on how genetic defects result in microbiota alterations in intestinal inflammation.

Therapeutic Targeting of the Microbiota

Antibiotics

Antibiotics have been used to treat the complications of chronic inflammation. Their use is dramatically restricted by several major concerns: i) scarcity of clinical trials that document the long-term benefit of antibiotic use; ii) interference with the overall energetic metabolism and the capacity to transport and metabolize bile acid, cholesterol, hormones, and vitamins [84]; iii) observations indicating that antibiotics alter the microbiome and possibly epigenome with unpredictable long-time effects [85]. Importantly, alteration of the microbiome results in the reduction of the species' diversity which itself has been identified as a possible contributing factor of obesity, diabetes, cardiovascular, metabolic and liver disease, or IBD and colorectal cancer [86]. Antibiotic therapy is therefore currently reserved to cases in which the aim is to reduce the bacterial burden of harmful and invasive families such as *Enterobacteriaceae*, to treat acute flares of disease, or to offer short-time postoperative prophylaxis.

Probiotics

Probiotics are live microorganisms that are thought to provide health benefits when consumed in adequate amounts. The most well-known include strains of the genera *Bifidobacterium* and *Lactobacillus*. Studies in mice indicated that oral administration of *Bifidobacteria* or *Lactobacilli* significantly reduces spontaneous inflammation in the *Il10*^{-/-} colitis model [87, 88]. Similarly, *Lactobacillus* GG prevented recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment, suggesting that antibiotics and probiotics might act synergistically to induce immunomodulation [89]. Although the mechanisms by which probiotics block inflammation remain obscure, it has been proposed that they control the balance between anti-inflammatory and proinflammatory responses [90], e.g. by competing with pathogens or pathobionts for ecological niches or nutrients. Alternatively, some may protect themselves by producing toxins that affect the latter. Another possibility is the induction of regulatory T cell responses by probiotics. Despite good conscious acceptance by the public, there is only one probiotic, i.e. *E. coli* Nissle, that has been recommended in the guidelines of the European Crohn's and Colitis Organisation (ECCO) as effective for maintaining remission in UC patients [91].

Prebiotics

Prebiotics represent dietary supplements that selectively support the growth of protective intestinal organisms, particularly of the genera *Bifidobacterium* and *Lactobacillus*. The key hallmark of prebiotics is their non-digestible nature; i.e., they pass the small intestine untouched and are only being processed by the microbiota in the colon in order to yield short-chain fatty acids (e.g. acetate, propionate, butyrate) and lactate. Well-known examples include carbohydrates, oligosaccharides or short polysaccharides including lactulose, inulin, oligofructose, galactofructose, galacto- and xylo-oligosaccharides [18, 92]. For example, lactulose, inulin, and/or goat's milk oligosaccharides significantly ameliorated mu-

cosal inflammation in chemically induced colitis models triggered by e.g. trinitrobenzenesulfonic acid in mice [93] or dextran sodium sulfate in rats [94] as well as in the spontaneous *Il10*^{-/-} colitis mouse model [87], in part by increasing fecal *Bifidobacterium* and *Lactobacillus* levels. Nonetheless, despite convincing and reproducible animal data showing a beneficial role of prebiotics in models of IBD, colon cancer, obesity, diabetes, and other metabolic or cardiovascular disease, their efficacy in humans remains controversial. Whereas fructo-oligosaccharide treatment increased *Bifidobacterium* levels and reduced disease activity in CD in one study [95], it showed no clinical benefit over placebo in a more recent study [96]. Hence, it remains disputed whether prebiotics can really become part of the therapeutic options to treat visceral disorders. Further studies aiming to clarify their mode of action in animal models and their validation in larger multi-centric clinical trials are granted to provide definitive evidence for their usefulness in IBD.

Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) is the procedure by which stool obtained from healthy donors is introduced as a suspension into the gastrointestinal tract of the recipient by the oral (nasogastric/nasoduodenal feeding tubes) or rectal route (enemas or during colonoscopy). Stemming from encouraging results in animal models of intestinal inflammation and the successful use of the method in patients with severe relapsing *Clostridium difficile* infection, the method has been recently proposed as a potential future treatment for various visceral metabolic disorders and IBD [97–99].

One major drawback for the implementation of FMT in visceral medicine is represented by the lack of large randomized controlled trials. The vast majority of the available studies included one to several patients. Although the mechanism by which transplanted microbiota induces remission of inflammation is unknown, it was observed that in UC responders there was an increase in the α -diversity and the microbiota was shifted towards a protective one which resembled the transplanted material, as revealed by post-transplant 16S-based metagenomics analysis [100]. In another randomized controlled trial, FMT induced significantly more remission in patients with active UC compared to water enemas (placebo group) [101]. Interestingly, although no statistically significant difference in clinical and endoscopic remission could be observed between UC patients receiving FMT from healthy donors and those who received their own fecal microbiota, the microbiota of responders was distinct from that in non-responders, suggesting that differences in microbiota correlate to clinical response [102]. Encouraging is the fact that most of the studies indicate FMT to be a safe and efficient method for reestablishing normality [99, 103], although the risk of transferring susceptibility to cancer cannot be excluded [104].

Since the characterization of the intestinal microbiota is currently in its infancy, additional studies are needed before well-characterized transplants are going to become available for large-scale FMT. Undoubtedly, the long-term effects of this procedure will play a dominant role in the implementation of the legal concerns and regulations related to FMT.

Fig. 1. Schematic representation of the interactions between the intestinal microbiota and the gut immune system during **a** homeostasis, **b** pathology, and **c** restitution of inflammation.

Future Perspectives

The last decade has experienced an explosive interest in analyzing the composition of the intestinal microbiome and its interactions with the host. Human studies addressing the involvement of the microbiota in disease provided a solid basis for understanding how microbiota can affect human health. However, most conclusions regarding the direct implication of the microbiota in the development of the disease are limited by their retrospective nature. Moreover, the complexity of established human disease hinders ascertaining the particular role of individual species. Another limitation is the fact that actual knowledge mainly comes from European and North American patients, whereas Asian, African and South American populations are underrepresented [18]. Taking into consideration the complex microbiome-host intertwining that together shape the metabolic profile of the host, the question of whether future therapies should target human cells or also include the microbial component has been raised [5]. Future well-conducted prospective clinical studies and the use of gnotobiotic mice hold great promise for elucidating how microbiota controls vis-

ceral health. Nevertheless, the challenge remains of how basic knowledge can be translated into clinical practice. In any case, as medical care moves towards personalized approaches, a better understanding of the individual gut microbiota and its possible manipulation, e.g. by the rational design of functional foods that increase carbohydrate fermentation or by FMT, might open new therapeutic avenues for microbiota-linked diseases (fig. 1).

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