The gut microbiota in IBD

Chaysavanh Manichanh, Natalia Borruel, Francesc Casellas and Francisco Guarner

Abstract | IBD—ulcerative colitis and Crohn's disease—is emerging as a worldwide epidemic. An association between the increased incidence of IBD and environmental factors linked to socioeconomic development has been persistently detected in different parts of the world. The lifestyle in developed countries might impair the natural patterns of microbial colonization of the human gut. The interaction of microbes with mucosal immune compartments in the gut seems to have a major role in priming and regulating immunity. In IBD, mucosal lesions are generated by an excessive or dysregulated immune response against commensal microbes in the gut. In individuals with a genetic susceptibility to IBD, abnormal microbial colonization of the gastrointestinal tract might be the origin of such dysregulation. Developments in gene-sequencing technologies, as well as increased availability of powerful bioinformatic tools, have enabled novel insights into the microbial composition of the human gut microbiota and the effect of microbial communities on human physiology and disease. Studies that used these technologies indicate that dysbiosis (that is, abnormal microbiota composition) and decreased complexity of the gut microbial ecosystem are common features in patients with Crohn's disease or ulcerative colitis. Whether such changes are a cause or a consequence of the disease remains to be elucidated.

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Introduction

Ulcerative colitis was first described in Europe during the 19th century,[1] and Dr Burril B. Crohn and his colleagues reported the first cases of a condition designated as 'regional ileitis' in New York, NY, USA, in 1932.[2] In contrast to most gastrointestinal infections (which tend to be acute), ulcerative colitis and Crohn's disease are chronic conditions of unexplained origin, and patients often have persistent ulcerations at the small or large bowel mucosa. Ulcerative colitis and Crohn's disease are clearly distinct clinical and pathophysiological entities, but are commonly defined as chronic IBDs. Both conditions show an undulating course of activity, with a low rate of spontaneous remission of the intestinal lesions and a constant threat of relapsing attacks after periods of spontaneous or medically or surgically induced remission. These are important diseases, disabling for many patients, and generating a considerable burden on health-care systems.

IBD was rare in North America and Europe until the second half of the 20th century, and is still rare in many Asian countries.[3] However, it is extraordinary how disease incidence has changed in a fairly short period of time. Industrialized countries in Northern Europe and North America experienced a steady rise in incidence of both ulcerative colitis and Crohn's disease after World War II. Impressively, during certain periods of time, the incidence rates were doubling every decade.[3] When pooling together data on ulcerative colitis and Crohn's disease, prevalence values of IBD are now almost reaching 1% of the population in North America and some European countries.[3] Rapid increases in the incidences of ulcerative colitis and Crohn's disease were also recorded during the 1970s in Southern European countries such as Spain and Italy, and are now being observed in Japan, South Korea, Australia, New Zealand and some regions of India and China. Such epidemiological patterns are leading to the assumption that IBD will emerge as a worldwide epidemic in the coming years.[3]

Even if a genetic predisposition to develop IBD has been clearly established and documented, the missing genetic contribution to disease susceptibility is presently calculated at 77% in Crohn's disease and 84% in ulcerative colitis.[4] As genetic susceptibility factors within the general population are fairly stable, environmental factors are essential components of the pathogenesis of ulcerative colitis and Crohn's disease, and are primarily responsible for its growing incidence around the globe. Epidemiological, clinical and experimental evidence support an association between IBD and a number of environmental factors, such as diet, antibiotic use,[5] social status and microbial exposure early in life[6] and during life.[7] Some of these factors, including diet, might have an important effect on the composition of the gut microbiota.[8] As pointed out by Bernstein and Shanahan,[7] many of the novel features in a modern lifestyle, that is, features that were not present in previous generations, can be linked to alterations in the microbial colonization of the gut (Table 1).

Microbial colonization has an important effect on the instruction and regulation of the immune system.[9] Abnormal communication between gut microbial communities and the mucosal immune system has been

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Key points

- Environmental factors are necessary contributors to the pathogenesis of IBD —most individuals with genetic susceptibility do not develop the disease—and are primarily responsible for its growing incidence around the globe
- The lifestyle in developed countries can be linked with alterations in the microbial colonization of the human gut
- Microbial colonization has an important effect on the instruction and regulation of the immune system
- The gut microbiota is an essential factor in driving inflammation and the development of mucosal lesions in IBD; certain microbes exacerbate inflammation, but some others mitigate inflammation
- Dysbiosis and decreased complexity of the gut microbial ecosystem are common features in patients with IBD

identified as the core defect that leads to chronic intestinal inflammation.[10] Thus, expanding our knowledge of the human gut microbiota to understand its potential involvement in the pathogenesis of IBD is justified. Development of novel gene sequencing technologies and the increased availability of powerful bioinformatic analysis tools have enabled dramatic advances in this field over the past few years. The purpose of this article is to briefly review our current knowledge of the human gut microbiota, describe changes observed in patients with IBD and discuss whether such changes might explain the pathophysiological characteristics of the disorders and the epidemiological trends.

Bacteria in intestinal inflammation

The gastrointestinal tract has a large mucosal interface (300–400 m^2) that has structures and functions for the immunological recognition of the external environment. A complex network of interactions between epithelial cells and different sorts of immunocompetent cells enables a detailed scrutiny of foreign bodies transiting along the tract. From a functional point of view, gut-associated lymphoid tissues generate either immunoinflammatory responses for rejection of potential pathogens, or an active immune response of tolerance for dietary and microbial antigens that does not induce clinically relevant inflammation.[11] Paradoxically, either diminished or exacerbated immune signalling might trigger the breakdown of intestinal homeostasis, leading to inflammation.[10]

Strong evidence indicates that intestinal inflammation in IBD results from the interaction of the gut microbiota with the mucosal immune compartments (intestinal immune cells can be divided into two compartments—organised inductive and diffusely distributed effector sites). Studies have shown that the gut microbiota is an essential factor in driving inflammation in IBD.[12,13] In Crohn's disease, diversion of the faecal stream induces inflammatory remission and mucosal healing in the excluded intestinal segment, whereas infusion of intestinal contents reactivates the disease.[14] In patients with active ulcerative colitis, short-term treatment with an enteric-coated preparation of broad-spectrum antibiotics rapidly reduced metabolic activity of the microbiota and mucosal inflammation.[15] These observations indicate that luminal bacteria provide the stimulus for immunoinflammatory responses, which lead to mucosal injury. In addition, patients with ulcerative colitis or Crohn's disease show abnormal mucosal secretion of IgG antibodies against commensal bacteria (physiological response is based on IgA antibodies that do not trigger inflammation),[16] and mucosal T cells are hyper-reactive against antigens of the common intestinal microbiota.[17] Thus, in these patients, local tolerance mechanisms towards commensal microbes seem to be abrogated.

Studies of inflamed mucosal samples from patients with Crohn's disease or ulcerative colitis in organ culture have shown that co-culture with nonpathogenic *Escherichia coli* strains strongly stimulate the release of proinflammatory cytokines (tumour necrosis factor, IFN-γ, IL-6, IL-23p19, IL-12p35 and IL-17F) and chemokines (IL-8, CXCL1 and CXCL2), thus stimulating the inflammatory cascade.[18–20] The inflammatory process activates matrix metalloproteinases that provoke matrix degradation, epithelial cell detachment and ulceration; matrix metalloproteinases are the main mediators of tissue damage.[21] Despite the evidence that microbes are necessary to drive the inflammation, certain microbial species of various genera (*Lactobacillus*, *Bifidobacterium* and *Faecalibacterium*) might actually protect the mucosa from inappropriate inflammatory responses that would damage the host.[18–20,22–24] Some bacteria strains (*Lactobacillus casei*, *Lactobacillus plantarum* and

Table 1 | A modern lifestyle might be linked with alterations of gut microbial colonization

Modern lifestyle	Traditional lifestyle
Birth in a hospital; increasing rate of caesarean delivery	Vaginal delivery at home
Small family size	Large family size, crowding
Often live in an urban setting, surrounded by concrete	Tend to live in a rural setting in contact with soil microorganisms
Sanitation of living spaces: environment colonized by resistant microorganisms (including resistant bacteria, fungi and acari)	Ancestral colonization of the living environment
Antibiotic usage early in life	No antibiotics in infant life
Daily body wash with hot water and soap	Limited access to hot water and soap
Low rate of *Helicobacter pylori* colonization	High rate of *Helicobacter pylori* colonization
Decline in endemic parasitism	Parasitic worms common
Food conserved by refrigeration	Food conserved by microbial fermentation
Consumption of processed foods	Consumption of natural foods

Faecalibacterium prausnitzii) downregulate the expression of key proinflammatory cytokines and chemokines and neutralize the proinflammatory effects of *E. coli*.[18–20] Moreover, some strains of the above mentioned genera are able to stimulate production of the anti-inflammatory cytokine IL-10.[22–24] Thus, certain members of the gut microbial community might exacerbate inflammation, but some others can induce immunoregulatory pathways that mitigate inflammatory reactions.

The vast numbers of bacteria that line mucosal surfaces interact directly with the host and have evolved mechanisms for coexistence over millions of years. A dynamic mutualism between the human host and the commensal microbiota probably has important implications for health. As reviewed previously,[9] the decision between induction of productive systemic-type immunity, with the potential for inflammation and damage to host tissue, or a tolerogenic response seems to be largely determined by the microbial effect on antigen-presenting cells and naive T cells of gut-associated lymphoid tissues (follicle-associated epithelia and lymphoid follicles). In the absence of 'danger signals' triggered by microbe-associated molecular patterns, conditioned antigen-presenting cells might induce various subsets of T regulatory (T_{REG}) cells, which via their cytokines IL-10 and transforming growth factor β, or by direct cellular interactions, might suppress effector T-helper (T_H)1, T_H2 and T_H17 responses, as well as innate immuno-inflammatory responses. The T_H cell subset that secretes IL-17 was proposed as the main cause of destruction in many different autoimmune and autoinflammatory diseases.[25] However, the paradoxical exacerbation of Crohn's disease in the clinical trials of a neutralizing antibody to IL-17 has cast into doubt a universal proinflammatory and harmful role for T_H17 cells.[25] In any case, microbial colonization might usually provide a natural barrier against immune pathology by triggering homeostatic proliferation of T_{REG} cell subsets, a phenomenon frequently called 'bystander immune suppression'.[26] This concept emphasizes a putative role of 'natural' microbial communities (regular colonizers of the human gut since ancestral times) in the origin of the imbalance of the immune system in immune-mediated disorders. The absence or deficiency of these microbial communities that are able to stimulate regulatory pathways and T_{REG} cell expansion might compromise immune homeostasis in patients with IBD (Figure 1).

Experimental evidence in animal models shows that a number of commensal bacteria of the dominant gut microbiota have an important role in keeping intestinal mucosal inflammation within 'physiological' levels (that is, without epithelial cell damage) by multiple mechanisms, including direct suppression of inflammatory pathways or expansion of T_{REG} cell subsets.[27] Some bacteria might diminish the production of proinflammatory cytokines by inhibiting activation of NF-κB.[28] Strong evidence indicates that some bacterial lineages (including specific *Bacteroides* and *Clostridium* spp.) that are therapeutically active in mouse models of intestinal inflammation can induce T_{REG} cell expansion.[29,30]

In summary, IBD seems to be characterized by dysregulated immune responses towards the intestinal microbiota.[10] Several factors have been suggested that might contribute to the loss of tolerance towards some of the indigenous microbiota in patients with IBD, including genetic susceptibility, defects in mucosal barrier function and imbalance in the composition of the gut microbiota (for example, excess of aggressive versus 'friendly' commensal bacteria). Experts proposed that either primary dysregulation of the mucosal immune system leads to excessive immunological responses to normal microbiota, or changes in the composition of the gut microbiota elicit pathological responses from a normal mucosal immune system.[31] The latter raised the hypothesis that an altered composition of the gut microbiota has a key role in the pathogenesis of IBD, and it is currently the focus of intensive research.

Novel technologies

The study of isolated microbes by culture techniques has been, and still is, very useful for identifying pathogens and understanding their virulence capabilities. However, it does not help us to describe microbial communities in nature and their ecological characteristics as most of the human microbiota is not easily cultured in a laboratory, mainly owing to the lack of appropriate culture media. Furthermore, laboratory cultivation does not enable characterization of the biology of these organisms in their natural environment. Indeed, the great discrepancies between direct microscopic counts and numbers of cultured bacteria from human microbial samples has motivated microbiologists to turn to culture-independent approaches, such as metagenomics, to investigate human microbes.

Metagenomics is defined as the study of genetic material extracted directly from environmental samples, bypassing the need to isolate and culture individual members of the bacterial community.[32] The first revolution in culture-independent approaches came in the late 1970s from the use of the 16S ribosomal RNA gene (16S rDNA) as a phylogenetic marker to describe microbial diversity and structure.[33] The second revolution started only a few years ago with the appearance of high-throughput technologies with next-generation sequencing, microarrays and the development of bioinformatics tools, enabling a deep characterization of the microbiota functions.[34] Next-generation sequencing techniques parallelize the sequencing process using beads, slides or solid surfaces and produce thousands or millions of sequences at once. These techniques are applied to DNA amplicons or to fragmented community DNA. They consequently led to a drastic reduction of costs and facilitate access to full metagenomic sequencing. As a result of the use of metagenomics in human microbiota studies, microorganisms have been described from different body sites. A range of different approaches can be applied to a human sample (Figure 2).

When the culture of a microbe of interest is still possible (Figure 2, left side), the genomic DNA is directly extracted and sequenced. When the culture of the

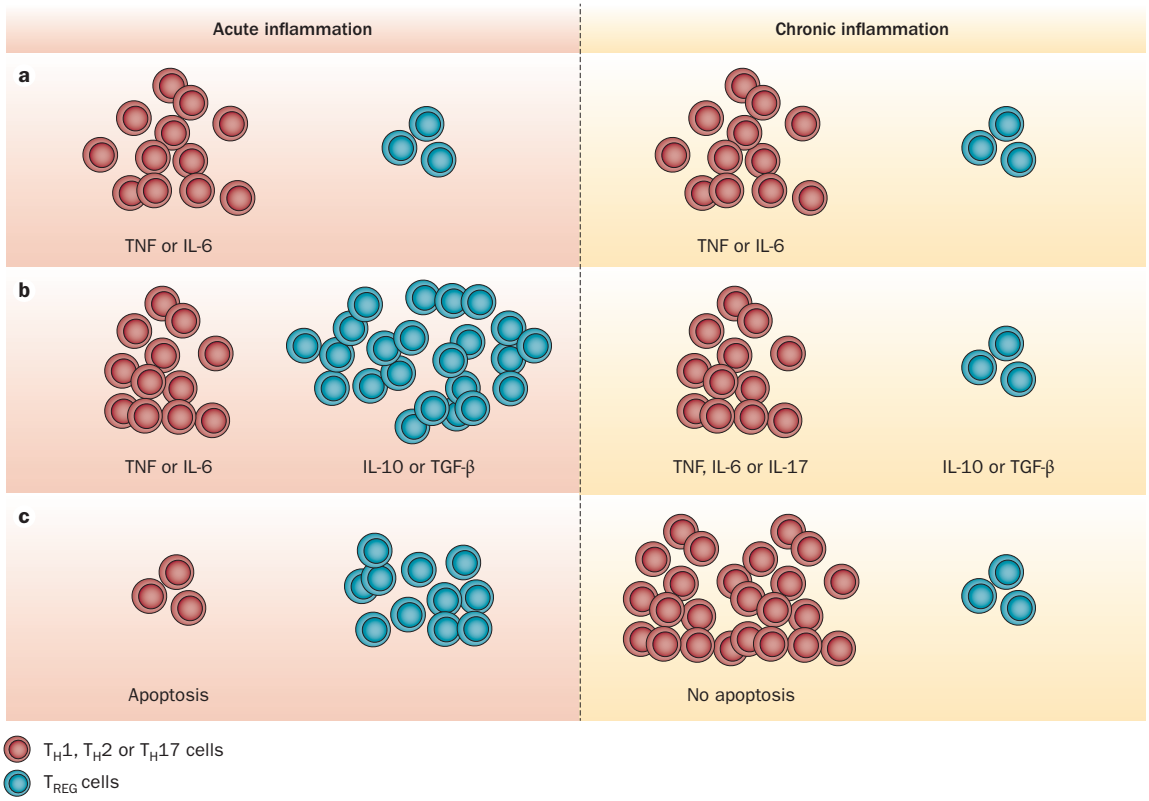

Figure 1 | Suggested model of perpetuation of intestinal inflammation in IBD. **a** | Epithelial insult by enteric pathogens or irritating drugs (such as NSAIDs) would be followed by expansion of T_H1 and T_H2 cell clones against invading antigens; inflammatory cytokines would drive the acute inflammatory response for rejection of the invaders. **b** | Under normal circumstances, after clearance of the offending agents, regular colonizing symbionts would expand the pool of T_{REG} cells producing IL-10 and TGF-β; in IBD, the absence or deficiency of certain crucial symbiont communities might compromise the phase of T_{REG} cell expansion. **c** | IL-10 and TGF-β would drive reduction of IL-6 and TNF and thus apoptosis of T_H1 and T_H2 cell clones and resolution of the mucosal lesions; when the pool of T_{REG} cells is insufficient, the inflammatory response would persist towards chronicity (including T_H17 cell expansion) with consequent mucosal damage. Abbreviations: TGF, transforming growth factor; T_H, T-helper; TNF, tumour necrosis factor; T_{REG} cell, T regulatory cell.

microbe of interest is impossible, a useful alternative is to amplify the genome of a single bacterial cell to then be sequenced and annotated.[35] Both approaches have been reported by the Human Microbiome Project,[36] which is an NIH initiative.[37] This project has released a catalogue of 178 reference genomes representing over 550,000 predicted genes, 30,000 of which were novel. The samples originated from the gastrointestinal tract, oral cavity, urogenital and/or vaginal tract, skin and respiratory tract. According to a report published in 2011, 1,900 microbial strains have been sequenced, or are in progress, for inclusion in the Human Microbiome Project reference strain catalogue.[38]

For organisms that cannot be cultured or amplified as a single cell (Figure 2, right side) the genetic information can be obtained by extracting the nucleic acids from the sample. Different approaches to study the genetic information can be applied to the extracted nucleic acids, such as metagenomics and metatranscriptomics. Metagenomics analysis includes a survey aimed at only sequencing 16S rDNA in the sample, or the shotgun method in which all genetic material in the sample will be sequenced. The 16S rDNA approach includes amplification of extracted samples by PCR using universal primers, followed by sequencing of 16S rDNA. This approach enables the taxonomical identification of microorganisms of the community by matching the sequences to well-annotated databases.[39–42] The shotgun sequencing method, which implies direct sequencing of fragmented genomic DNA without amplification, enables the identification of genes and metabolic pathways. The approach has been used for stool samples by the MetaHIT consortium,[43] a European initiative.[44] Metatranscriptomics analysis, applied to total extracted RNA, can provide information regarding the network of genes that have been expressed by the microbial community. The use of microarray printed with specific gene probes could bypass the need to remove ribosomal RNA (rRNAs) that represent >90% of the total RNA, whereas the use of high-throughput sequencing technology requires a pretreatment to remove them.[45] Either of these approaches can be used, depending on the facilities accessible to the investigators.

In summary, methods that rely on isolation of microbes in pure culture, or on targeting specific bacterial groups (at phylum, genus, species or strain level) using molecular probes, although valuable for specific purposes, cannot provide a reliable picture of

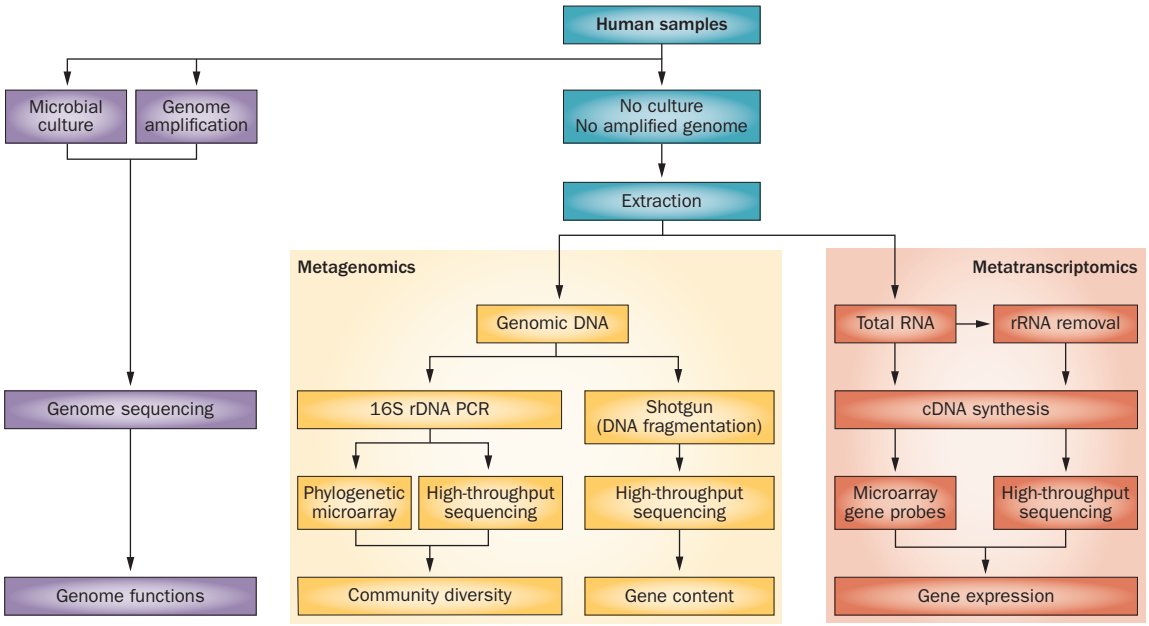

Figure 2 | Technologies to investigate the gut microbiota. On the left side, the approaches used when culture of an individual microorganism or the amplification of its genome is conceivable. On the right side, when most of the bacteria in the sample are not cultivable, approaches including metagenomics and metatranscriptomics are applied to the whole microbial community in the sample to collect information on microbial diversity, gene content and gene expression.

membership within complex microbial communities such as the gut microbial ecosystem. In addition, these methods cannot address ecological questions to reveal interactions and variability inside the community. By contrast, developments in the field of environmental microbiology, as described above, now permit the study of the genomes of entire populations of microorganisms in parallel. These studies provide a complete profile of the organisms that inhabit an environment and delineate the genetic and metabolic capacities of the entire community.[46]

What is a 'normal' gut microbiota?

A 'healthy' gut microbiota must be defined to understand what would be the biological importance of the different patterns of microbial colonization associated with disease states. Admittedly, the composition and functional characteristics of a healthy gut microbiota remain to be elucidated. However, the latest developments in molecular biology have enabled accurate investigations of the microbial communities in human faecal and gut mucosal samples. A number of studies published in the past few years have profiled the 'normal' patterns of the human gut microbiota, and will be briefly summarized in this section.

Studies based on 16S rDNA sequencing have highlighted that only 7–9 of the 55 known divisions or phyla of the Bacteria domain are detected in faecal or mucosal samples from the human gut.[47] Moreover, >90% of all the phylotypes (sequences with 97% identity, assumed to represent a single species) belong to just two divisions: the Bacteroidetes and the Firmicutes.[47] The other divisions that have been consistently found in samples from the human distal gut are Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia. Of the 13 divisions of the domain Archea, only one or two species seem to be represented in the human distal gut microbiota.[47] Thus, at the division level, the human intestinal ecosystem is less diverse than other ecosystems on Earth, such as soils and ocean waters that might contain 20 or more divisions. However, at the lower taxonomic levels (species or strain), considerable variation exists in the composition of the gut microbiota among human individuals.[47]

A study in two healthy people, a male and a female who were sampled daily for 15 months and 6 months, respectively, has shown that there are permanent fluctuations in the composition of the faecal microbiota over time.[48] However, the faecal microbiota tends to return to its typical compositional pattern, in a phenomenon termed resilience. Temporal variation might arise following exposure to different types of foods, medications, or physical environments, and also from changes in transit time, as microbial composition in the lumen varies from caecum to rectum. In addition, the community of mucosa-associated bacteria differs from that in the colonic lumen.[47] Interestingly, mucosa-associated communities are highly stable from the terminal ileum to the large bowel in a given individual.[47,49]

A whole-genome shotgun sequencing study revealed a total of 3.3 million nonredundant microbial genes in faecal samples from a cohort of adult Europeans, mostly formed of healthy individuals but also including a few patients with IBD or metabolic syndrome.[43] For the first time, this study provided a gene catalogue of the human gut microbiome, defined as the collective genome of the microbial symbionts in a host. Up to 98% of the genes in the catalogue are bacterial, and the rest belong to yeasts, viruses (including bacteriophage)

or protist microorganisms. Each human individual carries an average of 600,000 microbial genes in their gastrointestinal tract. It was found that around 300,000 microbial genes are common, in that they were present in 50% of individuals of the cohort. The study identified 1,150 prevalent bacterial species, with at least 160 species per individual.[43]

Bacteroides, *Faecalibacterium* and *Bifidobacterium* are the most abundant genera in human faecal samples, but their relative abundance is highly variable between individuals.[50] Network analysis of genus abundance suggested that the overall structure of the human gut microbiota in each individual conforms to discrete and distinct patterns defined by interactions between members of the microbial community. Multidimensional analysis of metagenomic sequences of faecal samples from adult North American, European and Japanese individuals revealed three robust clusters that have been designated as 'enterotypes', around which all samples would merge on the basis of similarity in composition. Interestingly, distribution into these clusters was not related to apparent phenotypic characteristics such as gender, age, body mass index, race, or country and continent of residence. Each of the three enterotypes is identifiable by the variation in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). The enterotype concept suggests that enteric microbiota variations across individuals are generally stratified, not continuous. This observation further indicates the existence of a limited number of well-balanced host–microbial symbiotic states. This finding might be relevant for future research studies, as different enterotypes generate profile dissimilarities unrelated to host phenotype, and such dissimilarities might not necessarily reveal abnormalities or disease-associated patterns. In a study in healthy adults, the *Prevotella* enterotype was associated with long-term dietary patterns characterized by a high proportion of carbohydrates and simple sugars.[51]

Dysbiosis in ulcerative colitis

Ulcerative colitis is characterized by the presence of diffuse inflammation restricted to the mucosal layer of the colon that causes recurrent symptoms, which seriously affects the patient's quality of life. An infectious origin of ulcerative colitis was thoroughly investigated in the past, but never demonstrated. According to classic notions, infectious diseases are produced by specific microbial agents that possess the capacity of transmitting the disease to susceptible individuals. Ulcerative colitis has never been associated with infection by a single specific pathogen, that is, one present in patients and absent in control individuals, the disease does not consistently improve with antibiotic treatment and contagious transmission has never been documented.

As reviewed in this section, studies of faecal or gut mucosal-associated microbiota have demonstrated quantitative and qualitative changes in composition, suggestive of an imbalance between protective and harmful bacteria, also termed dysbiosis. The main changes observed in the gut microbiota of patients with ulcerative colitis include a reduction in diversity, a decrease in stability and overexpression or underexpression of certain individual species. The potential effect on these changes by the drugs regularly used by patients as maintenance therapy or during previous phases of the disease (such as, mesalazine, azathioprine, steroids and antibiotics) is not known.

Investigation of mucosa-associated microbial communities in biopsy specimens from patients with active ulcerative colitis showed reduced diversity of taxa as demonstrated by single-strand conformation polymorphism,[52] a profiling technique based on 16S rDNA amplification using universal primers and crude fractionation of dominant components by means of gel electrophoresis. To a lesser extent, reductions of diversity have also been observed in other diarrhoeal conditions, such as acute infectious colitis or IBS.[53,54] However, a subsequent study confirmed a reduction of species diversity by sequence analysis of 16S rDNA in extracts from surgical specimens from patients with active ulcerative colitis.[55] The study suggested that several commensal bacteria, notably members of the phyla Firmicutes and Bacteroidetes, are depleted in some, but not all, patients with active ulcerative colitis undergoing surgery.

Analysis of 16S rDNA sequences in faecal samples from twin pairs who where concordant or discordant for Crohn's disease or ulcerative colitis, revealed no substantial differences between healthy individuals and individuals with ulcerative colitis during remission.[56] By contrast, another twin study in which 16S rDNA sequences in mucosal biopsy samples from the sigmoid colon were analysed showed less bacterial diversity with more Actinobacteria and Proteobacteria and less Bacteroidetes in patients with ulcerative colitis than in their healthy twin (Figure 3).[57] The lower proportion of Bacteroidetes in patients with ulcerative colitis is mainly attributable to bacteria from the Prevotellaceae family. Only three of 11 patients with ulcerative colitis were in clinical remission at the time of the study. Interestingly, unaffected siblings from ulcerative colitis discordant pairs also showed a lower bacterial diversity than healthy individuals without an affected sibling, which would support the potential heritability of the changes.

The reduction of species diversity in patients with ulcerative colitis is associated with temporal instability of the dominant taxa.[58] In faecal samples sequentially collected from patients with ulcerative colitis remaining in remission and with stable medication during a year of follow-up, only one-third of the dominant taxa were persistently detected, as revealed by the similarity index between samples of the same individual at different time points during follow-up. By contrast, healthy individuals showed a remarkable stability, with intraindividual similarity indices averaging at 80%.[58]

Specific modifications in microbial composition, specifically an increased presence of aggressive bacteria in patients with ulcerative colitis, have also been described. A study of crypt-associated mucous gel obtained by laser capture microdissection of colonic biopsy samples

from patients with ulcerative colitis has demonstrated an increased load of *Desulfovibrio* subspecies identified by PCR.[59] These are Gram-negative, anaerobic and sulphate-reducing bacteria that are involved in the pathogenesis of ulcerative colitis as a result of their capacity to generate sulphides.[60] *In vitro* studies demonstrated that 5-aminosalicylic acid inhibits faecal sulphide production, and in patients with ulcerative colitis not on this drug, faecal levels of sulphide were considerably higher than in healthy control individuals.[61]

Increased densities of bacteria attached to the colonic epithelium are also a prominent finding in morphological studies in biopsy specimens from patients with ulcerative colitis.[62] Techniques to identify the presence of bacteria—quantitative PCR (qPCR), fluorescence *in situ* hybridization and electron microscopy—reveal high concentrations of mucosal bacteria in patients with active ulcerative colitis, but not in control individuals with a normal colonoscopy.[62] In cultures of biopsy specimens from inflamed colons of patients with ulcerative colitis, bacterial species able to invade the epithelium, such as *Fusobacterium varium* strains, have been identified.[63] *F. varium* isolated from colonic mucosa of patients with ulcerative colitis, as well as supernatants of the cultured isolates, are cytotoxic to Vero cells *in vitro*. In addition, when mice are infused with *F. varium* by rectal enema, the microbial suspension generates colonic mucosal erosions, inflammatory infiltrate and apoptotic bodies.[64] Strains of *Fusobacterium nucleatum* isolated from inflamed biopsy tissue from patients with ulcerative colitis were also shown to be invasive in a Caco-2 cell assay.[65] Similarly, *E. coli* has been detected at increased levels by molecular techniques in faecal samples from patients with ulcerative colitis,[66] and some of the *E. coli* isolates express virulence factors or invading properties.[67]

Finally, *F. prausnitzii*, a major representative of the *Clostridium leptum* group with known anti-inflammatory properties, is under-represented in patients with ulcerative colitis who have active disease[68] and during remission.[69]

Dysbiosis in Crohn's disease

Crohn's disease is a heterogeneous disease that might affect any part of the gastrointestinal tract, causing a wide variety of inflammatory lesions with different phenotypic characteristics (penetrating, inflammatory or stricturing). Despite intensive investigation, searches for organisms that cause Crohn's disease have not led to the identification of a single specific pathogen. In particular, *Mycobacterium avium paratuberculosis* was proposed as a causative agent of the disease following its isolation in 1984 from the diseased intestinal tissue of patients with Crohn's disease by Chiodini and co-workers.[70] However, antimycobacterial therapy does not benefit patients with Crohn's disease,[70] and the involvement of this species in the aetiology of the disease remains controversial. However, wide consensus does exist on the critical role of the intestinal microbiota in the pathogenesis of the lesions.[67]

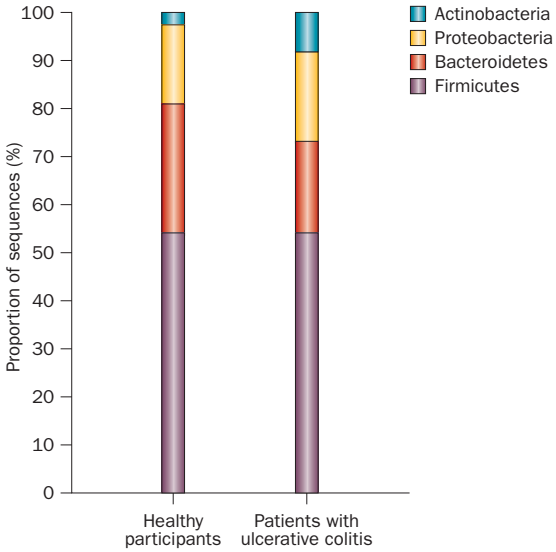

Figure 3 | Dysbiosis in ulcerative colitis based on data by Lepage *et al.*[57] from 62 participants. Analysis of 16S ribosomal DNA gene sequences in mucosal biopsy samples showed a higher proportion of Actinobacteria and Proteobacteria and a lower proportion of Bacteroidetes in patients with ulcerative colitis than in their healthy siblings.

In the study by Ott and co-workers,[52] the diversity of mucosa-associated microbiota in specimens from patients with active Crohn's disease undergoing surgery was markedly reduced compared with mucosal specimens from control individuals without inflammation. The study used a 16S rDNA-based profiling technique in which dominant components of the microbiota can be distinguished and separated by means of gel electrophoresis without sequencing. A full metagenomic approach was used for the first time by Manichanh and co-workers,[71] who investigated faecal samples from patients in remission by construction of 16S rDNA libraries and subsequent analysis of all 16S rDNA sequences. A striking difference was found in microbial diversity between patients with Crohn's disease and healthy participants, which was essentially attributable to a reduced complexity of the phylum Firmicutes in patients with Crohn's disease (Figure 4). Similarly, in a study of monozygotic twin pairs, healthy individuals had a considerably higher bacterial diversity than individuals with Crohn's disease; however, this study used a low-resolution profiling technique (T-RFLP) without 16S rDNA sequencing.[72] In agreement with these findings, a study published in 2010 that used microarray with 500 16S rDNA probes reported decreased abundance of several bacterial species of the phylum Firmicutes in patients with Crohn's disease.[73] As observed in ulcerative colitis, reduced species diversity of the gut microbiota in Crohn's disease seems to be associated with temporal instability of dominant species when compared with healthy individuals.[74]

Accurate characterization of the intestinal microbiota is influenced not only by intraindividual variability but also by interindividual variations, such as enterotype or

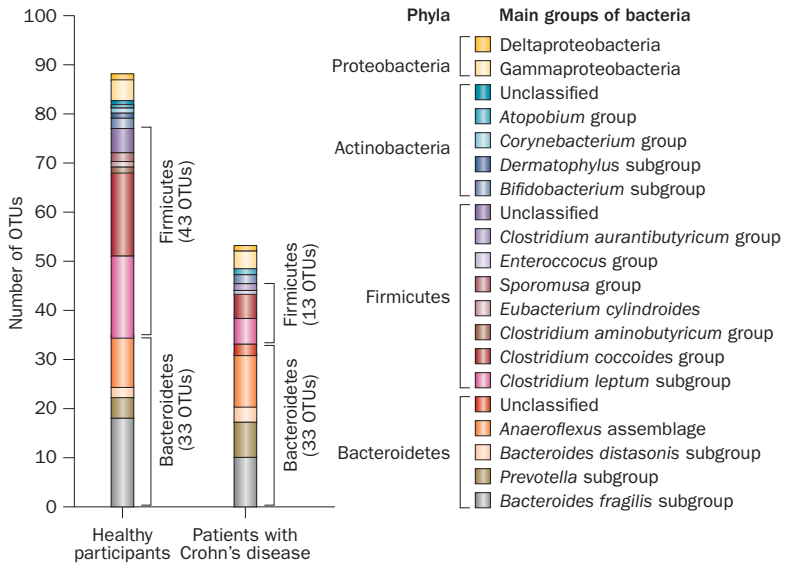

Figure 4 | Reduction of bacterial diversity in patients with Crohn's disease based on data by Manichanh *et al.*[71] from 12 participants. Taxonomy of the dominant OTUs identified in the faecal microbiota of healthy participants and patients with Crohn's disease. Abbreviation: OTUs, operational taxonomic units.

genetic background, that make it difficult to correlate specific microbial signatures with disease. Studies with pairs of twins discordant for disease status might help to overcome such difficulties. As mentioned previously, Willing and co-workers[56] investigated the microbial composition of faecal samples by 16S rDNA sequencing in 40 pairs of twins who were concordant or discordant for Crohn's disease or ulcerative colitis and in mucosal samples from a subset of the cohort. Microbial communities in individuals with Crohn's disease differed from those in healthy individuals and profiles from individuals with Crohn's disease that predominantly involved the ileum differed from those with Crohn's disease that predominantly involved the colon. Changes specific to patients with ileal Crohn's disease included the disappearance of core genera, such as *Faecalibacterium* and *Roseburia*, and increased amounts of *Enterobacteriaceae* and *Ruminococcus gnavus*.[56] These findings seem to be consistent across studies of cohorts of patients with Crohn's disease from different countries.[24,56,68,71,73] Interestingly, a reduction of *F. prausnitzii* abundance in ileal mucosal samples is associated with a higher risk of postoperative recurrence of ileal Crohn's disease.[24] Furthermore, in mouse models of intestinal inflammation, administration of *F. prausnitzii* resulted in anti-inflammatory effects.[24] Counts of *F. prausnitzii* in faecal samples were considerably lower in patients with active Crohn's disease than in healthy control individuals, but samples from patients with infectious colitis also showed reduced counts.[68] This later observation might suggest that decreased abundance of *F. prausnitzii* could be secondary to either diarrhoea or mucosal inflammation.

By contrast, a greater relative abundance in *Enterobacteriaceae*, particularly *E. coli* species, has been consistently observed in patients with Crohn's disease; the change is more notable in mucosal specimens than in faecal samples.[67] Several independent studies have reported increased numbers of mucosa-associated *E. coli* with invasive properties or the presence of intramucosal *E. coli* in inflamed mucosal samples from patients with Crohn's disease, as reviewed by Chassaing and Darfeuille-Michaud in 2011.[67] A new, potentially pathogenic group was designated adherent-invasive *E. coli* (AIEC) and has been isolated from ileal mucosal specimens of patients with Crohn's disease from different countries.[71,76] The AIEC strains are able to adhere to intestinal epithelial cells, invade epithelial cells, survive and replicate within macrophages.[77] AIEC can also be found in samples from healthy individuals, although it is less prevalent in healthy people than in those with Crohn's disease, but does not adhere to ileal enterocytes isolated from individuals without Crohn's disease.[77] These findings suggest that AIEC strains are associated specifically with the ileal phenotype of Crohn's disease. The potential effect of therapeutic agents on dysbiosis in Crohn's disease is not known.

Dysbiosis in pouchitis

Restorative proctocolectomy with ileal pouch–anal anastomosis has become the surgical treatment of choice for patients with severe ulcerative colitis who do not respond to medical therapy or who develop neoplasia, and for patients with familial adenomatous polyposis. Pouchitis, which is a relapsing condition characterized by bouts of inflammation of the ileal pouch mucosa, is the most common complication of this procedure, with a prevalence rate of 23–40%.[78] Interestingly, chronic pouchitis occurs almost exclusively in patients who undergo proctocoloctomy to treat ulcerative colitis, and is rarely seen in patients with familial polyposis.[78] Clinical and basic scientific evidence suggests that dysbiosis has a key role in the initiation and progression of chronic inflammation in the pouch reservoir. Analyses of 16S rRNA gene sequences extracted from ileal pouch biopsy samples showed a notable increase in Proteobacteria (*E. coli* and other enterobacteria belong to this phylum), as well as a marked decrease in Bacteroidetes and one Firmicutes species (*F. prausnitzii*).[79] This compositional pattern is found in patients who previously had ulcerative colitis with or without pouchitis at time of study, compared with control patients with familial polyposis and no pouchitis. Bacterial diversity was considerably greater in pouchitis-free patients than in those with pouchitis. However, no individual species have been specifically associated with pouchitis.[78]

Conclusions

Abnormal host–microbe interactions in individuals with genetic susceptibility generate intestinal inflammation and tissue injury in IBD. No evidence exists for contagious transmission of ulcerative colitis or Crohn's disease. However, several commensal microbes with or without virulence properties have been identified that could be involved in the induction of immunoinflammatory cascades that lead to tissue destruction. Hypothetically, an abnormal microbial composition and/or the absence of

ancestral residents of the human gut could be the origin of the imbalance in mucosal immune homeostasis. This hypothesis is supported by experimental data showing that a number of dominant genera of the gut microbiota induce immunoregulatory pathways and can mitigate immunoinflammatory responses.

Current studies on the gut microbiota in patients with IBD reveal an abnormal microbial composition that is characterized by low diversity of species in the gut microbial communities, but high density of mucosal surface colonization and epithelial invasion in areas with active disease. The potential causative role of previous antibiotic use in the origin of such a defect cannot be dissociated with our current data, as antibiotics can reduce diversity and antibiotic use in childhood is a known risk factor for IBD.[5] The defect in species richness of the microbial ecosystem in IBD is linked with a trend of instability of the profile of dominant species over time that is much more pronounced than in healthy control individuals. At the species level, most findings suggest a reduced abundance of *F. prausnitzii*, which is a dominant species in the healthy human gut microbiota, in Crohn's disease and perhaps in ulcerative colitis, and an increased prevalence of AIEC strains with invasive properties in ileal Crohn's disease. However, it is not clear whether such microbial changes can cause IBD or are a consequence of the disease.

Review criteria

In writing this review, we relied on original articles and reviews that appear in PubMed, as well as on our current readings on the topic. The search terms used were "microbiota IBD", "microbiota ulcerative colitis", "microbiota crohn", "bacteria IBD", "bacteria ulcerative colitis", "bacteria crohn" and "human metagenomics". Due to space limitations, the number of studies quoted has been restricted. Articles were chosen for citation on the basis of the relevance of its contents without any bias toward author or journal.

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Author contributions
All authors contributed equally to all aspects of this article.