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Jérémy Denizot, Nicolas Dreux, Arlette Darfeuille-Michaud, Nicolas Barnich

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Importance of Bacteria as Trigger in Inflammatory Bowel Disease

Jérémy Denizot[1,2], Nicolas Dreux[1,2], Arlette Darfeuille-Michaud[1,2,3] and Nicolas Barnich[1,2,3]*

[1]Clermont Université, M2iSH, Microbe intestin inflammation et Susceptibilité de l'Hôte, UMR 1071 Inserm/Université d'Auvergne, France
[2]Unité Sous Contrat Institut National de la Recherche Agronomique 2018, Clermont-Ferrand F-63001, France
[3]Institut Universitaire de Technologie, Génie Biologique, Aubière F-63172, France

Abstract

Inflammatory bowel diseases (IBD) provide a complex model of host-microbe interactions underpinning disease pathogenesis. Although there is no widespread agreement on the aetiology of IBD, there is evidence that micro-organisms lead to the often severe inflammatory response characteristic of the disease. IBD is thought to result from an inappropriate and continuing inflammatory response to pathobionts microbes in a genetically susceptible host. In this review, we discuss the complex microbial ecosystem of the mammalian gut, the underlying genetic factors that predispose to IBD, and how these gene variants may alter host-microbe interactions and propagate inflammation. Incentive should be given to research that will promote a better understanding of host-microbial interactions in the intestine and lay the foundations for new therapeutic approaches to both treat and prevent onset and relapse of intestinal inflammation in genetically susceptible hosts.

Keywords: Inflammatory bowel disease; Dysbiosis; Pathobiont; Microbiota; Mice models of inflammation

Abbreviations: IBD: Inflammatory Bowel Disease; CD: Crohn's Disease; UC: Ulcerative Colitis; AIEC: Adherent-Invasive *E. coli*; SFB: Segmented Filamentous Bacteria; InPEC: Intestinal Pathogenic *E. coli*; MAP: *Mycobacterium avium paratuberculosis*; ETBF: Enterotoxigenic *Bacteroides fragilis*; NTBF: Non Toxigenic *Bacteroides fragilis*; CEACAM: Carcinoembryonic Antigen-Related Cell-Adhesion Molecule; NOD: Nucleotide Oligomerization Domain; TLR: Toll-Like Receptors; NLR: Nucleotide Oligomerization Domain (NOD)-Like Receptors; MAMP: Microbe-Associated Molecular Pattern; GWAS: Genome-Wide Association Studies; T6SS: Type VI Secretion System; TNF: Tumour Necrosis Factor; IL: Interleukin; ER: Endoplasmic Reticulum; IFN: Interferon; LPF: Long Polar Fimbriae; DSS: Dextran Sulphate Sodium; IEC: Intestinal Epithelial Cells; PPs: Peyer's Patches; AJC: Apical Junctional Complex; HD: Human Defensin; HM: Hypomorphe

Introduction

Inflammatory bowel disease (IBD) arises from complex interactions of genetic, environmental, and microbial factors [1]. The two major subtypes of IBD, ulcerative colitis (UC) and Crohn's disease (CD), exhibit distinct and overlapping clinical and pathologic features. Recent progress in IBD host genetics has provided a critical framework to evaluate microbial contributions to pathogenesis [1-3]. Mucosal inflammation has a profound effect on the host's mucosa, on nutrient availability in the gut and on microbiota composition. Chronic gut inflammation could be due to the effects of commensal microbiota and/or to acute inflammation caused by enteric pathogens. However, some functional principles might be common to both types of disease. One important challenge will be to understand the role of commensal and/or pathogenic bacteria when specific host genotypes are concerned.

Microbial/Host Interactions in Physiologic Conditions

The intestine is an open ecological system that is colonized immediately after birth by a microbial population that reaches 10^{12} bacteria per gram of luminal content in the distal gut [4]. Apart from contributing substantial beneficial functions to the host, the microbial population has enormous potential for physiological and pathological interactions with the host. For example, the microbiota drives the development of the mucosal and systemic immune system and controls the regeneration of the intestinal epithelium [5,6]. The detection of pathogens by the host is achieved through the families of Pattern Recognition Receptors (PRR) that recognize conserved molecular structures known as pathogen-associated molecular patterns (PAMP) and induce the production of innate effector molecules. Because these structures are also found on non-pathogenic micro-organisms, the term microbe-associated molecular pattern (MAMP) is increasingly used, particularly when speaking of host-commensal interactions. These signaling receptors are divided into three families: toll-like receptors (TLR), retinoic acid inducible gene I (RIG-I)-like receptors (RLR), and nucleotide oligomerization domain (NOD)-like receptors (NLR) [7]. Homeostatic mechanisms depend on down-regulating bacterial receptors, which induce intracellular and secreted molecules that activate innate and adaptive immune responses, and stimulate protective molecules that mediate mucosal barrier function. In addition, bacterial exposure activates protective pathways that prevent subsequent injurious responses to the same stimuli. TLR ligation down-regulates NF-κB activation upon re-exposure to the same or different TLR ligands through the induction of multiple inhibitors, including IRAK-M, toll-interacting protein (TOLLIP), single immunoglobulin IL-1 receptor (SIGIRR), A20, *NOD2*, and peroxisomal proliferator-activated receptor-γ (PPAR-γ) [8]. The immune system continuously adapts to the microbiota in a cyclic, dynamic cross-talk in which intestinal epithelial cells play an important role in instructing non-inflammatory responses for a steady-state control of bacterial growth, or in triggering inflammatory mechanisms that can clear the gut of harmful invaders [9]. The system is complex and robust in the sense that many players with partially overlapping roles act to keep the integrity of the intestinal mucosal barrier. Failure of these mechanisms involves genetic and environmental factors that trigger inflammatory bowel disease.

***Corresponding author:** Nicolas Barnich, M2iSH, Inserm/Université d'Auvergne UMR 1071, CBRV, 28 place Henri Dunant, 63001 Clermont-Ferrand, France, Tel: (33)4-73-17-83-76; Fax: (33)4-73-17-83-71; E-mail: nicolas.barnich@u-clermont1.fr

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Micro-organisms Involved in Intestinal Inflammation: Lessons from Animal Models

Mouse models of intestinal inflammation, including genetically engineered mice, congenic mouse strains, chemically induced models, and a cell-transfer model, have played a key role in understanding the mechanisms that govern the inflammatory response in the intestine, and in designing new therapeutic strategies in the treatment of patients with IBD [10]. In the event of infection, the epithelium switches from inertia to pronounced pro-inflammatory response, initiating and orchestrating immune defense against incoming pathogens [9,11].

Common models of infectious murine colitis are infection with the murine epithelial-adherent pathogen, *Citrobacter rodentium*, and infection of streptomycin-pretreated mice with *Salmonella* typhimurium. Studies in these models have helped to define the interactions between bacterial pathogens and host immune defenses, thus broadening the understanding of host-microbial interactions in the intestinal tract. Studies on acute *Salmonella* and Citrobacter infection models have revealed that enteropathogenic bacteria can subvert the inflammation [12]. For example, *Salmonella* may use different strategies to exploit intestinal inflammation and out-compete the microbiota: (i) *Salmonella* uses mucin-derived carbohydrates, which are released in the gut in the course of inflammation, (ii) *Salmonella* can use tetrathionate as an electron acceptor which is more abundant in the inflamed gut, (iii) *Salmonella* is resistant to a variety of host-derived defensins and antimicrobial peptides and avoids inhibition of the host-derived antimicrobial peptide lipocalin-2 that sequesters the siderophore enterochelin by the production of salmochelin [13]. This competitive edge against the commensals explains why *Salmonella* spp. and other gut pathogens benefit from mucosal inflammation and subvert an immune response designed to fight infections.

Specific members of the commensal microbiota known as segmented filamentous bacteria (SFB), with the candidate name *Arthromitus*, are potent inducers of CD4+ T helper cells that produce IL-17 and IL-22 (Th17 cells) in the small intestine *lamina propria* of mice. SFB are spore-forming Gram-positive bacteria most closely related to the genus *Clostridium* and have been reported to colonize the intestine of numerous species, including humans [14,15]. Colonization of the small intestine of mice with SFB is sufficient to induce the appearance of Th17 cells in the lamina propria. SFB adhere tightly to the surface of epithelial cells in the terminal ileum of mice with Th17 cells but are absent from mice that have few Th17 cells [16]. Colonization with SFB is correlated with the increased expression of genes associated with inflammation and antimicrobial defenses and resulted in enhanced resistance to the intestinal pathogen *Citrobacter rodentium*. Thus, manipulation of this commensal-regulated pathway may provide new opportunities for enhancing mucosal immunity and treating diseases [17].

Colonization of lower bowel and biliary tract in mice with *Helicobacter hepaticus* causes a chronic inflammatory response similar to human IBD. In contrast to infections with *Citrobacter rodentium* or *Salmonella enterica* serotype Typhimurium, which induce acute and self-limiting infections, *H. hepaticus* only causes disease in immunocompromised Rag2-deficient mice that lack immune regulation and mount inflammatory responses toward intestinal bacteria [18]. The analysis of *H. hepaticus* genome led to the identification of bacterial type VI secretion systems (T6SS) that have a role in chronic intestinal inflammation. During prolonged intestinal colonization of animals, *H. hepaticus* intimately contacts the epithelium and uses its T6SS to create a tolerogenic immune environment. Indeed, T6SS of *H. hepaticus* limits colonization of animals and actively suppresses innate and adaptive immune responses. In addition, infection with T6SS deficient *H. hepaticus* mutant induces higher expression of pro-inflammatory mediators by IECs and CD4+ T cells in the colon, including IL-17, IL-23, IL-1β, TNFα, and IFNγ than infection with WT *H. hepaticus* [19]. Although T6SS have been largely studied in the context of bacterial virulence, growing evidence supports the notion that T6SS may have also evolved for non-pathogenic purposes in symbiotic bacteria and that bacterial T6SS are one mechanism for forging host-microbial interactions.

Rodent models of spontaneous or induced intestinal inflammation provide compelling evidence that enteric bacterial antigens continuously drive chronic, immune-mediated colitis and ileitis [20]. Genetically engineered mice and rats with systemic immunoregulatory defects may display inflammation usually limited to the colon, with occasional ileal, duodenal, and gastric antral involvement. The predominant colonic phenotype suggests involvement of the complex anaerobic commensal enteric microbiota. In addition, the observation that most germ-free susceptible rodents, such as interleukin-10-deficient mice, HLA-B27/human β2 microglobulin transgenic rats or Tg(ε26) mice, have no intestinal inflammation or immune activation but rapidly develop disease and pathogenic immune responses after colonization with specific pathogen-free enteric bacteria provides convincing evidence that bacteria are key players in inflammatory process [21-24]. Thus, the intestinal microbiota profoundly affects host immune composition under physiological conditions [25].

Involvement of Micro-organisms in IBD Etiology

IBD, such as CD and UC, preferentially occur in the colon and distal ileum, which contain the highest intestinal bacterial concentrations [26,27]. An important role for microbial agents in the pathogenesis of CD is suggested by clinical, experimental, and therapeutic studies, but less convincing evidence is available for UC. For example, histomorphological features of CD, such as aphthous ulcers of the mucosa, mural abscesses, suppurative fistulas, macrophage and epithelioid cell granulomas, have been suggested to be of microbial origin. The evidence that enteric bacterial antigens continuously drive chronic, immune-mediated colitis and ileitis is provided by rodent models of spontaneous or induced intestinal inflammation [1]. Moreover, the composition and function of the microbiota in CD and UC are abnormal. A general dysbiosis of the intestinal microbiota has been well established in IBD patients with both culture-dependent and cuture-independent techniques, but a systematic characterization of this dysbiosis is lacking [27,28]. Patients with IBD, compared with healthy controls, have fewer bacteria with anti-inflammatory properties and/or more bacteria with pro-inflammatory properties. Several metagenomic-based studies reported that members of the phyla *Bacteroidetes* and *Firmicutes* were reduced in patients with CD or UC [27,29-31]. A recent study have provided a faecal microbiota dysbiosis signature associated with CD, characterised by a decreased presence of *F prausnitzii, B adolescentis, D invisus* and an unknown species of *Clostridium* cluster XIVa, and an increased presence of *R gnavus* [32]. In general, the composition of the predominant faecal microbiota of patients with IBD was studied in comparison with the predominant composition in unaffected controls, but the characterization of microbiota composition associated with intestinal mucosa is often missing.

To support the infectious disease hypothesis, Koch's postulates need to be fulfilled. They are defined as (i) the presence of the micro-organism in all diseased individuals; (ii) its isolation and culturing; (iii) the occurrence of infection upon inoculation; and (iv) the re-

isolation from the experimental infection, and hence, on this basis, Koch's postulates fail in IBD. Neverthless, since IBD are multifactorial diseases, a modified Koch's postulate taking into account the host genetic susceptibility should be considered. IBD could result from a dysfunctional innate immune response to persistent intracellular bacteria, possibly opportunistic pathogens, in patients having defects in innate immune response, mucosal barrier or bacterial killing of intracellular bacteria. There are at least three possible mechanisms that can drive pathogenic immunologic responses in CD to luminal microbes: (1) changes in microbial composition leading to dysbiosis, (2) functional alteration of commensal bacteria as described for Adherent and Invasive Escherichia coli (AIEC), toxigenic *Bacteroides fragilis, Campylobacter* and *Helicobacter* species or (3) involvement of traditional microbial pathogens such as *Mycobacterium avium* subspecies *paratuberculosis*.

Consequences of Dysbiosis in IBD Patients

Dysbiosis, an altered composition of the commensal bacterial populations, is a major factor in disease pathogenesis that occurs with genetic susceptibility and leads to the dysregulation of the immune response to bacterial antigens observed in IBD [33,34]. Dysbiosis can result from an overgrowth of bacteria having pro-inflammatory properties or a loss of beneficial commensal bacteria, leading to elevated host intestinal inflammation. The most reliable features of dysbiosis involve an overall decrease in biodiversity, a low stability, a decrease in bacteria from the *Firmicutes* phylum (notably from the *C. leptum* and *C. coccoides* groups), an increased number of Proteobacteria and the presence of unusual bacteria [27,31,35]. Some characteristics of dysbiosis seem to be specific for each IBD type, whereas others are shared by all forms of IBD and probably also by other gastrointestinal inflammatory conditions such as acute self-limited colitis.

Comparison of the intestinal microbiota of CD patients to that of healthy controls has revealed compositional changes. In most studies these changes are characterized by an increase in the abundance of *Bacteroidetes* and Proteobacteria and a decrease in that of Firmicutes. Reduced numbers of *Bacteroides fragilis* might contribute to inflammation because this prominent human symbiont has protective effects in mice following experimental induction of colitis by *Helicobacter hepaticus*, a murine commensal bacterium with pathogenic properties [36,37]. This beneficial activity requires a single microbial molecule (polysaccharide A, PSA) and in animals harbouring *B. fragilis* not expressing PSA, *H. hepaticus* colonization leads to disease and pro-inflammatory cytokine production in colonic tissues. Thus, molecules of the intestinal microbiota can mediate the critical balance between health and disease.

A lower proportion of *Faecalibacterium prausnitzii* on resected ileal Crohn's mucosa was associated with endoscopic post-operative recurrence. *F. prausnitzii* induced a tolerogenic cytokine profile on peripheral blood mononuclear cells with very low levels of IL-12 and high levels of IL-10 [30]. On Caco-2 intestinal epithelial cells, *F. prausnitzii* supernatant abolished IL-1β-induced IL-8 production and NF-κB activation and its supernatant improved colitis score and survival rate in mice with DSS-induced colitis. In contrast, several studies document selective expansion of *Enterobacteriaceae* [12], with in particular increased proportion of *E. coli* of the fecal and mucosally associated microbiota in CD patients, which invade the mucosa and are present within granulomas and adjacent to fistulae and ulcers [4]. The reduced abundance of *F. prausnitzii* and increased abundance of *E. coli* are indicative of an ileal CD phenotype, distinct from colonic CD. Based on these observations, the relative abundances of these specific bacterial populations are promising biomarker candidates for differential diagnosis of CD and eventually customized treatment.

The presence of bacteria that penetrate the mucus layers was observed in 30% of mucosal biopsy specimens from patients with IBD, compared with 3% of mucosal biopsies from healthy controls, which indicates that the microbiota could have closer contact with the mucosa of IBD patients [38]. This might result from the increased numbers of some mucolytic bacteria, such as *Ruminococcus gnavus* and *Ruminococcus torques*, observed in macroscopically and histologically normal colonic epithelium from patients with colonic CD and UC [39,40].

The composition of colonic microbiota influence transcriptional profile of the mucosa. Bacterial products, such as butyrate production, might affect mucosal gene expression. Patients with UC had different gene expression profiles and lower levels of biodiversity than their healthy twins, as well as unusual aerobic bacteria. Patients with UC had lower percentages of potentially protective bacterial species than their healthy twins [41]. Loss of natural intestinal diversity and a shift of bacterial composition toward a more deleterious profile might reflect the net effect of environmental factors over the past decades leading to the dramatic increase in the incidence of IBD in the industrialized world [42].

Functional Alteration of Commensal Bacteria

A particular subset of bacteria further highlights how the behaviour of the microbiota is dependent on the immune status of the host. Although these bacteria, which are known as pathobionts, colonize the gastrointestinal tract of many individuals asymptomatically, they also have the potential to cause disease [16]. Pathobionts are microbial symbionts that can cause disease in predisposed hosts following changes in the gastrointestinal environment, such as Crohn's disease associated *E. coli*, enterotoxigenic *Bacteroides fragilis* or *Helicobacter hepaticus*.

Adherent-invasive *E. coli* (AIEC)

In CD patients, *E. coli* abnormally colonizes acute and chronic ileal lesions and accounts for up to 100% of total aero-anaerobic flora [43]. Bacteria colonizing the gut mucosa have the ability to strongly adhere to intestinal epithelial cells (IEC) [43-45]. There is increasing interest in AIEC since they have been reported to be more prevalent in CD patients than in controls in several countries: France [44], United Kingdom [45], Spain [46] and USA [47-49]. The greater richness of AIEC in CD patients suggests that this pathovar might be more permanent in CD intestinal mucosa owing to better host-environmental conditions. Although no single *E coli* isolate was found in Crohn's ileal mucosa, some genotypes of *E. coli* were more likely than others to be associated with chronic or early recurrent ileal lesions. However, the presence of AIEC in healthy subjects suggests that AIEC are facultative pathogens that cause disease in susceptible hosts. AIEC phenotype is very frequent among animal intestinal pathogenic *E. coli* (InPEC) strains, particularly of feline and canine origin. These data strengthen the particular identity and disease specificity of the AIEC pathotype and the putative role that animals might play in the transmission of AIEC-like strains to humans [50]. Of interest, diseases with granulomatous response to *E. coli* have been reported in animals, and AIEC were isolated from Boxer dogs with granulomatous colitis [51].

Adherent invasive *Escherichia coli* appear specific to ileal CD and have been shown to induce the release of IL-8 by infected IEC. Differential secretion of IL-8 and CCL20 by IEC was dependent on the CD-associated *E. coli* strain and the contribution of the *E. coli* O83:H1

flagella are crucial in inducing inflammatory response in IEC [48,52]. CD-associated AIEC bacteria, by expressing long polar fimbriae (LPF), can use Peyer's Patches (PP) as an open gate to induce early stages of the disease [53], which support clinical observations suggesting that the sites of initial inflammation in ileal CD are the lymphoid follicles [54]. Using AIEC strain LF82, several studies have demonstrated the ability of these intestinal microbes to disrupt the integrity of epithelial cells in an *in vitro* cell model and an *in vivo* model. IBD-associated *E. coli* strains significantly decreased trans-epithelial resistance, induced disorganization of F-actin and displacement of ZO-1, and E-cadherin from the apical junctional complex (AJC) [49,55]. In addition, infection of mice with AIEC bacteria led to a significant increase in intestinal permeability and to disruption of mucosal integrity in a type 1 pili-dependent mechanism. This is consistent with the abnormal expression of the tight junction-associated pore-forming claudin-2 protein at the plasma membrane of IEC observed in AIEC-infected mice [56]. This disruption allows the bacteria to penetrate into and beyond the epithelial monolayer triggering a chronic immune response. These findings provide a link between microbes related to IBD, disruption of the intestinal epithelial cell barrier, and disease pathogenesis [3,57].

AIEC LF82 induces aggregation of infected macrophages, some of which fuse to form multinucleated giant cells and subsequent recruitment of lymphocytes [58]. Although the cell aggregates do not completely mimic natural CD-associated granulomas, they are very similar to early stages of epithelioid granulomas. AIEC bacteria are also able to survive and to replicate extensively within macrophages [59]. The bacteria replicate in large vacuoles that have phagolysosome-like properties within macrophages, where they are exposed to low pH [60]. AIEC-infected macrophages release large amounts of tumour necrosis factor-alpha (TNF-α), a key cytokine in IBD inflammation. Although most bacteria are successfully internalized and eliminated by macrophages, this pathobiont bacterium has developed survival strategies that interfere with the internalization and/or phagosomal maturation processes, specifically by interfering with the autophagy machinery [61]. In addition, AIEC bacteria, via expression of flagella, potentiate an inflammatory mucosal immune response involving increased expression of TLR5 and IPAF flagellin receptors in DSS-treated mice [62].

Enterotoxigenic *Bacteroides fragilis*

The *Bacteroides fragilis* species includes both key commensals and important opportunistic human pathogens [63]. A subgroup of *B. fragilis*, termed enterotoxigenic *B. fragilis* (ETBF), can be distinguished from non-toxigenic *B. fragilis* (NTBF) strains because it expresses a 20 kDa zinc-dependent metalloprotease known as *B. fragilis* toxin (BFT). ETBF, which can persistently colonize the human intestine, has been associated with active IBD and colorectal cancer in humans [64,65]. In some hosts, ETBF acts via secretion of BFT to induce colitis. However, the full spectrum of clinical disease related to ETBF and the impact of chronic ETBF colonization on the host remain to be defined.

Traditional Pathogens

Yersinia

Several studies have demonstrated that *Yersinia* enterocolitica is associated with CD and might function as a trigger for IBD [66,67]. Indeed, the presence of pathogenic *Yersinia* DNA was observed in 31% of CD patient samples. In addition, enteropathogenic *Yersinia* strains display a tropism to lymphoid tissue [68]. The bacteria bind to and invade M cells within the follicle-associated epithelium overlying the lymphoid follicles of the PPs [69,70]. Following their entry into PPs, the bacteria induce the host immune response, which is characterized by inflammation with infiltration of neutrophils and macrophages [71]. Further functional studies are needed to establish a relation between *Yersinia* infection and IBD aetiology.

Bacteria Responsible for Gastroenteritidis

Nontyphoid *Salmonella* and thermophilic *Campylobacter* have been suggested as having a role in the aetiology of IBD [72-74]. An increased risk of IBD in *Salmonella* or *Campylobacter* gastroenteritis patients as compared with an age- and gender-matched background population was observed. This risk was higher during the first year but remained high up to 15 years after the Salmonella/*Campylobacter* gastroenteritis. The differences related to UC vs CD, *Salmonella* vs *Campylobacter*, age groups, and gender were minor [75]. Moreover, acute inflammation triggered by enteric pathogens, such as Salmonella typhimurium is accompanied by changes in the bacterial community structure marked by an outgrowth of the pathogen. Recent studies show that S. typhimurium can harness benefit from the host response to edge out the beneficial bacterial species that dominate in the healthy gut [76].

Listeria

The development of the cold-chain paralleled the outbreak of CD during the 20th century, which suggests that psychrotrophic bacteria such as *Listeria* spp. could contribute to the disease [77]. *Listeria monocytogenes* has been occasionally found in the gastrointestinal tract of healthy persons and has been implicated in causing granulomatous hepatitis in animals and humans [78]. The oral administration of *L. monocytogenes* initiated infection in the PPs of the small intestine of mice [79]. The PPs appeared to be the only site where *L. monocytogenes* made an initial invasion and survived in the intestinal tissues. It is tempting to suggest that on interacting with the host mucosa, *Listeria* may induce the aphthous ulcer, regarded as the earliest lesion of CD [80] and the first to recur after surgery [81].

Mycobacterium avium paratuberculosis (MAP)

Mycobacterium avium paratuberculosis (MAP) is an obligate intracellular organism that has frequently been associated with CD. MAP is a recurrent candidate for several reasons: it causes epidemic chronic colitis in cattle and other species, including primates; it has similarities with Johne's disease; higher levels of MAP have been found in the tissues and blood of CD patients than in controls; antibodies directed against MAP are often associated with CD; and in some cases, anti-mycobacterial drugs improve disease status. Molecular techniques, such as polymerase chain reaction, and culture methods, have enabled to demonstrate that there is an association between MAP and CD. Recently, genome-wide association studies (GWAS) identified novel susceptibility genes for CD, which are critical for generation of an adaptive immune response that is protective against intracellular pathogens, including *M. tuberculosis* infection.

Host Factors Favoring Bacteria as Inducer of Inflammation: Lessons from Mice Models

Many genetic predisposition factors have been recently discovered. GWAS have identified 71 interesting candidate genes as being associated with CD including *NOD2, ATG16L1* and *IRGM* [82]. In innate immunity, the association of CD with polymorphisms in the two autophagy-related genes, *ATG16L1* and *IRGM*, and in *NOD2 (CARD15),* leads to defects in the recognition and handling of intracellular bacteria, defects in the synthesis of anti-microbial peptides

by Paneth cells, which argues in favour of bacterial involvement in CD aetiology.

Autophagy-related genes

It has recently been recognized that *NOD2* exerts an important role in the induction of autophagy associated with the intracellular presence of bacteria [83,84]. Because *NOD2* polymorphisms account for the vast majority of the currently explained cases of genetic heritability of CD, and as *ATG16L1* is among the genes most strongly associated with CD, autophagy and the intracellular sensing of bacteria may be part of a major shared mechanism in the development of CD [85].

NOD2-mediated sensing of bacterial products facilitates control of bacterial infection. In vitro, *NOD2* has been found to be involved in the bacterial clearance of *Salmonella* and *Streptococcus* species [86,87], and *Nod2–/–* knock-out mice display increased susceptibility to *per-os Listeria monocytogenes* infection, a finding paralleled by a decreased Paneth cell secretion of some anti-bacterial cryptidins [11]. By contrast, *NOD2* does not appear to play a role in the clearance of *Yersinia pseudotuberculosis* in a murine model [88]. Patients harbouring *NOD2* mutations have decreased levels of mRNA for alpha-defensins HD-5 and HD-6 in involved ileal mucosa compared with un-involved ileal mucosa [89]. However, reduction in HD-5 and HD-6 transcription may not have been associated with *NOD2* status but could have been the consequence of inflammation [90]. Moreover, a defect in *NOD2* gene revealed an abnormal presence of *Bacteroidetes* species in the ileum of *NOD2* deficient mice suggesting the involvement of *NOD2* in the control of flora composition and diversity [91]. This mutation also could favour the persistence and virulence of *Helicobacter hepaticus* in *NOD2* deficient mice. Together, all these studies underscore the importance of *NOD2* and suggest that the CD-specific mutated *NOD2-L1007fs* can participate in a functionally impaired epithelial barrier and in sensitivity to bacterial infection and to a particular microbiota due to defects in defensin secretion.

IRGM and ATG16L1 deficient epithelial cells and macrophages failed to control intracellular replication bacteria, as shown with *Salmonella, Mycobacterium* or Crohn's disease-associated adherent-invasive *E. coli* [61]. As autophagy is an innate defense mechanism acting as a cell-autonomous system for elimination of intracellular pathogens, this observation lends weight to the notion that intracellular bacteria including AIEC might play a role in CD pathogenesis and that deficiency in the autophagy process due to mutations might allow pathogenic bacteria to persist within patients' mucosa. A recent study showed that the association of IRGM with CD arises from a miRNA-based alteration in IRGM regulation that affects the efficacy of autophagy, thereby involving a synonymous polymorphism as a likely causal variant [92]. Studies in *Atg16l1* hypomorphic (*Atg16l1^{HM}*) mice gave insights into the potential mechanism of *ATG16L1* in CD. *Atg16l1^{HM}* mice were shown to develop normally without any evidence of intestinal inflammation but they exhibited substantial structural abnormalities in Paneth cells, suggesting defects in cryptidin secretion [93]. This is a consequence of the presence of a murin norovirus persistent infection in the context of the *Atg16l1* hypomorphic protein [94]. These data strongly support the involvement of infectious agent in modulation of host gene expression in a genetically predisposed host.

Endoplasmic reticulum stress related genes *XBP1* and GP96

Endoplasmic reticulum (ER) stress is induced by the accumulation of misfolded proteins in the ER arising from either primary (genetic) or secondary (environmental) factors that affect the folding of proteins within the ER. A genetic locus in the vicinity of *XBP1* was initially involved as a risk factor for IBD based upon GWAS analysis [95,96].

Total loss of *XBP1* function, as observed in homozygotically deleted mice, resulted in a nearly complete absence of Paneth cells, leading to defects in anti-microbial synthesis, hyper-responsiveness to the microbial antigens and reduction of goblet cells due to apoptosis. All together, these events lead to spontaneous small intestinal inflammation and increased susceptibility to challenge with *L. monocytogenes* [97]. These studies with *XBP1* reveal the possibility that IBD may primarily emanate from genetically determined abnormalities within the intestinal epithelium.

The ER-stress response chaperone Gp96 is strongly expressed on the apical surface of ileal epithelial cells in CD patients and acts as a host cell receptor for outer membrane vesicles from AIEC bacteria, promoting AIEC invasion [98]. Hence, it is speculated that AIEC could take advantage of the abnormal expression of Gp96 in patients with CD to invade the ileal mucosa, promoting gut inflammation.

Carcinoembryonic antigen-related cell-adhesion molecule 6

Carcinoembryonic antigen-related cell-adhesion molecule 6 (*CEACAM6*) is overexpressed in the ileal epithelial cells of patients with CD, as compared with those of controls, and allows CD-associated AIEC to colonize the gut and to induce strong inflammation in transgenic mice expressing human *CEACAM6* [99,100]. The presence of AIEC causes an increase in the expression of *CEACAM6* on the surface of cultured intestinal cells, as does incubation with IFN-γ or TNF-α, pro-inflammatory mediators of which increased levels are typically found in the intestine of CD patients. Of note, AIEC can induce the secretion of TNF-α from cultured macrophages [59]. Thus, AIEC may directly and indirectly induce epithelial cells to up-regulate *CEACAM6*, thereby allowing their own adhesion to these cells. By increasing the adhesion of AIEC, *CEACAM6* may contribute to an amplification loop of increased colonization and inflammation [101]. To date, no polymorphism in *CEACAM6* gene have been associated with CD in GWAS analysis. A recent study reported the presence of polymorphism in *CEACAM6* gene but no relationship between *CEACAM6* variants and IBD susceptibility, in particular with the ileal CD involvement, was observed [102]. This suggests a particular gene regulation in CD leading to *CEACAM6* abnormal expression and allowing AIEC to trigger ileal inflammation.

Role of Probiotics in IBD Therapy

Immunosuppressive therapies, for example with TNF antagonists, are currently being used as remedies against severe human inflammatory diseases. The use of antibiotics as primary or adjuvant treatments for active luminal IBD is controversial despite promising data from clinical trials [103]. With the identification of dysbiosis in IBD patients, there is growing evidence that probiotic micro-organisms might influence disease outcome of IBD in both animal models and humans [104,105].

Probiotics are viable microorganisms that proliferate in the gut and exert positive health effects. Examples of species of probiotics that are currently in use or under evaluation are: *Lactobacillus rhamnosus, L. reuteri, L. acidophilus, L. bulgaricus, Bifidobacterium infantis, Saccaromyces boulardii, Enterococcus faecium, the Nissle strain of Escherichia coli and Clostridium butyricum* (For review, [106]). These probiotics are able to (1) compete for binding sites decreasing pathogen attachment and subsequent invasion of the mucosa, (2) stimulate epithelial barrier function and (3) modulate host defense genes expression such as antimicrobial peptides in the gut. A recent study described the ability of probiotic *Escherichia coli* Nissle 1917

to reduce pathogen invasion and to modulates cytokine expression in Caco-2 cells infected with CD-associated *E. coli* LF82 [107]. *E. coli* Nissle, *Lactobacillus* and the VSL#3 bacterial mixture are able to strengthen intestinal barrier functions through the up-regulation of human beta defensin-2 via induction of proinflammatory pathways [108,109]. Moreover, probiotics enhance barrier function and intestinal immunity. Many recent studies report the benefic effect of probiotics on epithelial barrier function *in vitro* and *in vivo*. For example, *Lactobacillus rhamnosus* GG accelerates intestinal barrier maturation, induces claudin-3 expression [110] and attenuates interferon-γ and tumour necrosis factor-α-induced barrier dysfunction *in vitro* [111]. Gram-positive probiotic *Lactobacillus* is able to modulate barrier function *in vitro* by increasing adherent junction protein expression and complex formation [112].

Lactobacillus casei has been shown to antagonize the pro-inflammatory effects induced by commensal *E. coli* in human CD by down regulating pro-inflammatory mediators [113]. The protective effects of commensal bacteria or probiotics have been analysed in different mouse models of experimental colitis. For example, (i) yeast *Saccharomyces boulardii*, which is increasingly prescribed as a probiotic agent against human IBD [114], alleviates *Citrobacter rodentium*-induced colitis in mice by modulating the expression of type 3 secretion system [115], (ii) polysaccharide A from *B. fragilis* (a commensal non toxigenic *B. fragilis* NTBF strain) exerts protective effect against *H. hepaticus*-induced intestinal inflammation in mice [37], (iii) the presence of segmented filamentous bacteria improves colonization resistance to *C. rodentium* and enhances intestinal barrier functions and T cell maturation [17] and (iv) lactic acid bacteria, such as *Lactobacillus plantarum* and *Lactobacillus brevis*, prevents the production of pro-inflammatory cytokines in a DSS-induced colitis model [116].

To conclude, the beneficial effects of probiotic intervention on intestinal inflammation could be the result of many different mechanisms, including improvement of colonization resistance, barrier function, metabolic effects, modulation of signal transduction and immune responses [117].

Conclusion

In summary, genetic studies and murine models have emphasized the role of genetic predispositions and how they affect interactions with microbial and environmental factors, leading to pro-colitogenic perturbations of the host-commensal relationship. This review provides important insights into the role of intestinal microbiota in induction of IBD. In contrast to acute inflammation which can be triggered by a single micro-organism, there is clear evidence that a single microbial species is not sufficient to trigger chronic IBD and that specific bacterial subsets can induce IBD, whereas other subsets cannot. This does not exclude, however, the possibility that additional pathobionts species may also be capable to induce disease. As additional animal models of IBD based on more human susceptibility mutations become available, the experimental criteria and conceptual framework developed will allow us to better understand the role of bacteria in IBD aetiology.

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