Debugging the intestinal microbiota in IBD
Quel rôle pour la flore intestinale dans les maladies inflammatoires chroniques intestinales ?

R. Dessein\textsuperscript{a}, P. Rosenstiel\textsuperscript{b}, M. Chamaillard\textsuperscript{c,*}

\textsuperscript{a} INSERM U801, Lille, F-59019 France; University of Lille 2, Lille, F-59000, France
\textsuperscript{b} Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Germany
\textsuperscript{c} INSERM U795, Lille, F-59037, France

Summary
Besides its role in repelling enteropathogenic infections, the gastrointestinal tract is in intimate contact with commensal microbiota. Tremendous advances have been made in determining the pivotal role of the microbiota in both tissue homeostasis and metabolism, as well as in the initiation and maintenance of inflammatory lesions in inflammatory bowel diseases. A better understanding of human gut microbiota could provide innovative targets for treating and/or curing such common immunopathologies of the gastrointestinal tract.

Résumé
Une des fonctions du système inflammatoire et immunitaire digestif est d’assurer la défense de l’organisme vis-à-vis des agents microbiens pathogènes. Il est aussi en contact permanent avec la flore intestinale commensale. Au cours des dernières années, des progrès conséquents ont permis de mieux comprendre le rôle clé du microbiote intestinal, non seulement comme élément de l’homéostasie de la muqueuse digestive et acteur métabolique à part entière, mais aussi comme facteur participant à l’initiation et à l’entretien de l’inflammation muqueuse au cours des maladies inflammatoires chroniques intestinales. Mieux caractériser et mieux comprendre les nombreuses propriétés de la flore intestinale laisse ainsi espérer l’identification de nouvelles pistes pour le traitement des maladies inflammatoires chroniques intestinales.

* Corresponding author:
E-mail address: mathias.chamaillard@ibl.fr (M. Chamaillard).
Inflammatory Bowel Disease (IBD) is an intestinal disease affecting millions of mainly young adults worldwide. The two major clinical subentities of IBD, namely Ulcerative Colitis (UC) and Crohn’s disease (CD), are characterized by an unpredictable succession of relapses and remissions. While UC lesions are confined to the intestinal mucosa and limited to the colon and the rectum, CD-associated lesions are transmural and may occur anywhere in the gastrointestinal tract. Since the 1940’s, the incidence of these gut immunopathologies has markedly increased in “westernized” countries in Europe, North America and more recently in East Asia, resulting in millions of IBD patients worldwide [1]. Epidemiological data highlight the physiopathological role of certain environmental factors related to hygiene and industrialization, such as refrigeration [2]. Over the last decades, our understanding of the pathogenesis of IBD has markedly increased thanks to genetic and immunological studies in mice and humans. It is believed to be the result of a breakdown in the intestinal barrier against microbes in genetically predisposed individuals [3]. Evidence suggests that there are at least four mutually non-exclusive scenarios of gut defects, which may lead to chronic intestinal inflammation through sustained exposure of the mucosal immune system to lumen-derived microbial products: i) impaired mucosal tolerance (i.e. CD-associated mutations in NOD2, a cytosolic receptor for bacterial muramyl dipeptide [4-6]; ii) defective antimicrobial immunity (i.e. impaired α- and β-defensins expression in ileitis and colitis respectively and abnormal autophagy [7-11]); iii) increased intestinal permeability (i.e. CD-associated mutations in NOD2 [12] and UC-associated mutations in MDR1, an efflux transporter controlling epithelial homeostasis [13]) and/or iv) abnormal responsiveness of T cells to microbial signals (i.e. bacterial flagellin is an immunodominant antigen in CD [14] and mutations in the interleukin-23 receptor [15-16]). For further details on each of these physiopathological hypotheses see the following excellent reviews [17-19]. All these results suggest that the microbiota is the master regulator of the initiation and perpetuation of chronic mucosal inflammation in IBD. In this opinion article, we highlight the microbial point-of-view of the physiopathology of IBD, which could help to develop alternative treatments to restore immunological integrity in IBD.

The evolving role of the microbiota in intestinal immunopathologies

The gastrointestinal tract coexists in intimate contact with the commensal microbiota, which plays an essential role in both tissue homeostasis and metabolism. Notably, the microbiota protect against numerous enteropathogens by priming the immune system, competing for attachment and/or secreting antimicrobial molecules such as bacteriocins [20, 21]. The composition of the microbiota is influenced by multiple factors including age, environmental and genetic factors. Iron stimulates growth and virulence of intracellular bacteria, whereas aluminum is an adjuvant for bacterial stimulation of the immune response [22]. Dietary iron and aluminum can potentiate experimental colitis [23, 24]. Using germ-free and conventional mice as model systems, it has been suggested that the pathogenesis of IBD is driven by the indigenous intestinal microbiota (and certain enteric pathogens in particular). Similarly, predisposition to sequelae of morbid obesity (e.g. steatosis of the liver and insulin resistance) are linked to certain prokaryotes constituting the flora, which control metabolic function by extracting energy from the diet [25, 26]. Conversely, obesity alone has been shown to alter the diversity of gut microbiota [27]. The physiological role of innate immunity in this process remains to be elucidated. Last but not least, progression towards colorectal malignancy is also directly favoured by innate immune signalling and/or by certain commensal strains through conversion of dietary compounds into carcinogens [28].

Decoding fecal-associated microbiota in IBD

Tremendous advances have been made in determining the pivotal role of microbiota in the initiation and maintenance of inflammatory lesions in IBD. Notably, several reports showed imbalances in the diversity and magnitude of microbiota associated with CD, a phenomenon which has been referred as dysbiosis [29]. Both microbiologists and gastroenterologists are now faced with a difficult challenge. What is the impact of each microbial species in the pathogenesis of IBD? Culture methods are ineffective in fully determining the diversity of microbiota. Recent technological progress has renewed the interest of dissecting this microbial ecosystem by providing the unique opportunity of assessing the diversity, function and resistance to antibiotics of individual strains and the role of microbiota in health and disease [30]. Even if the use of the unique 16S rDNA has certain limitations for discriminating between micro-organisms [31], what was mainly observed is the limited diversity of microbiota in CD patients compared to healthy controls and UC patients [32]. In particular, certain members of the Firmicutes phylum are nearly absent in CD but present in controls and UC. Conversely, CD-associated microbiota are characterized by the omnipresence of certain Proteobacteria, such as Escherichia and Bacteroides genus [33-36]. Interestingly, the physiological presence of non-bacterial microbiota (e.g. intestinal viruses, parasites or fungi) is far less characterized. To assess the inter-individual variability, comprehensive high-throughput analyses of microbial-derived sequences are now awaited in large cohorts of controls, patients with IBD and their healthy relatives.

Decoding the mucosal-associated microbiota in IBD

One argument in favour of an initiating and/or perpetuating role for gut microbiota is the increased prevalence of mucosal-associated microbes in IBD [37], including sulfate-reducing bacteria and adherent-invasive Escherichia coli (AIEC) [38, 39]. The differences between fecal- and mucosal-associated microbiota question the use of stools as the gold standard for research [40]. Mucosal-associated bacteria correlate with an increased risk of postoperative recurrence of CD [41,42]. AIEC are found in about 36.4% of ileal biopsy specimens in CD compared to 6.2% in controls [43]. These bacteria adhere and invade intestinal epithelial cells by synthesizing α-haemolysin [39] and replicate intracellularly in macrophages without inducing cell death [44] and by escaping endocytic compartments. Despite major
progress in the field, several important issues must still be addressed. Is there one type of AIEC or several? How have AIEC evolved and/or were they selected from commensals to pathogenic bacteria? What is the reservoir of these AIEC? What are the immunological strategies that humans have developed to repel AIEC infection? Can these AIEC drive CD pathogenesis under natural conditions? Alternatively are these AIEC a reflection of the consequences of an impaired mucosal barrier? Martin et al. have begun to answer these questions by showing equivalent amounts of mucosa associated E. coli in both CD and colorectal cancer [38].

**Chronic susceptibility or resistance to infectious agents in IBD**

For almost a century, the similarity between the granulomatous lesions in the mucosa of CD patients and those in digestive tuberculosis suggest the existence of an etiologically relevant infectious agent, such as *Mycobacterium avium paratuberculosis* (MAP) [45, 46]. The idea of a chronic persistent infection was supported by the description of familial clustering and phenotypic concordance of the disease [47], incomplete concordance between monozygotic twins [48], the presence of relapses interspersed with clinical remission and the erosions of follicle-associated epithelium, which might precede the development of apoptotic lesions in CD [49]. In ileal CD, the onset of the disease is correlated with the involution of Peyer’s Patches, the main lymphoid structure of the gut [50]. These clinical observations suggest a protective role of Peyer’s Patches in the pathogenesis of CD by causing a long-lasting immune response against enteropathogens targeting the Peyer’s Patches, such as *Yersinia* spp [51] and MAP [52].

**MAP** causes a chronic intestinal disease called Johne’s disease in several animals, which presents clinical and pathological similarities to CD [53]. Found in human breast milk by both culture and PCR [54], intracellular MAP can also resist a large spectrum of antibiotics, as well as commonly used pasteurization techniques in the food industry and for water supplies [55, 56]. This might explain the elevated incidence of MAP in the human population and its potential implications in the pathogenesis of CD. Notably, MAP infection has been shown to precede the development of distal ileitis [57]. In CD, culture- and molecular-based techniques have shown an increased incidence of MAP in intestinal biopsies and/or blood samples from CD patients compared to healthy control [58-61]. However, the Koch’s postulates for MAP in CD are under debate [62], because epidemiological data on the transmissibility of MAP is lacking and the detected MAP strains are extremely variable in the different reports [63]. Furthermore the MAP genotypes isolated from CD patients differ from the diseases-causing strains in other mammalian species [64]. Other evidence also challenges this hypothesis, because the mycobacterial cell wall remains undetectable by immunochemical staining [65] and opportunistic infection by MAP is not associated with the use of immunosuppressive agents. Recently, a prospective, parallel, placebo-controlled, double-blind, randomized trial in active CD evaluated a two-year combination of the anti-tuberculin molecule rifabutin associated with a macrolid (namely clarithromycin) and a bacteriostatic molecule active against both *M. leprae* and atypical mycobacteria (namely clarithromycin). As in a previous study using rifampicin, isoniazid, and ethambutol [66], this study failed to show any significant benefit after one-year of follow-up [67]. However, the interpretation of these results are still being debated [68], because anti-inflammatory properties have also been linked to clofazimine and clarithromycin [69]. Furthermore, the antibiotic property of the molecule is not restricted to MAP and pathogens have strategies to resist antibiotics by persisting in a specific niche, such as the mesenteric adipose tissue [70]. Further studies are now needed to follow-up methotrexate and 6-mercaptopurine in CD, as these immunomodulatory molecules also have antimycobacterial properties [71]. Alternatively, although the decreased seroprevalence of certain pathogens such as *Helicobacter pylori*, might support the hygiene hypothesis [72], their clinical relevance in CD needs to be investigated.

**Harnessing microbiota to restore gut barrier function in IBD**

The gut barrier function is increased by signals derived from commensals, such as bacterial polysaccharide [73] and Toll-like receptor agonist [74-76]. Upon detection of such signals, the composition of the microbiota is influenced by a set of effectors, such as defensins [77], innate immune exclusion molecules (such as DMBT1 [78, 79]), the c-type lectin regllg [80] or angiogenin-4 [81]. Fortifying the intestinal barrier could therefore be achieved by therapeutic manipulation of the microbiota with either antibiotics, intrabiotics, probiotics and/or prebiotics. The latter include dietary components that foster the growth of “beneficial” bacteria [82], such as inulin and fructose oligosaccharides that enhance the growth of *Bifidobacterium* and *Lactobacillus* species and provide a substrate for the production of short-chain fatty acids [83]. To assess the potential therapeutic value of prophylactic molecules, double blind, randomized, placebo-controlled trials have evaluated the efficiency of budesonide [84], ornidazole [85], metronidazole [86, 87], and rifaximin [88]. While rifaximin and a combination of ciprofloxacin/metronidazole/budesonide were ineffective, the recurrence of Crohn’s disease after ileocolonic resection was prevented by ornidazole and metronidazole. However, the efficacy of these antibiotics against the biofilm remains questionable [89]. Swidinski and collaborators are now providing preliminary answers by evaluating the impact of acute and chronic metronidazole-ciprofloxacin therapy on mucosal-adherent bacteria in CD [90].

**Concluding remarks**

Despite the tremendous progress in this field, several questions remain unanswered. How does the composition of the flora of genetically predisposed patients and their healthy relatives change throughout their life? Besides an increased understanding of the pathogenesis of IBD, recent technological advances have also provided a better understanding of the microbiome of each patient, which might clarify the pathogenesis of common gut immunopathologies and suggest innovative therapeutical targets. Notably overall 25-30% of
patients may be refractory to therapy regardless of the drug. This usually results in a significant delay before appropriate treatment is found and also an accumulation of adverse events. To help physicians in predicting clinical relapses of IBD, valuable non-invasive biomarkers are needed. Testing certain species within the microbiota might be useful to predict the risk of clinical relapse of disease activity, thus helping physicians to choose the optimal therapeutic strategy for each patient.

Acknowledgments:
We apologize to our colleagues whose work was not cited here owing to space limitations.

Funding
Mathias Chamaillard is supported by grants from INSERM, the Région Nord-Pas-de-Calais, the Association François AuPetit, and the IRMAD. Philip Rosenstiel is supported by grants from BMBF and the Deutsche Forschungsgemeinschaft (SFB 617, ExC Future Ocean and Inflammation at Interfaces)

Conflict of interest:
Rodrigue Dessein, Philip Rosenthal and Mathias Chamaillard have no conflict of interest.

References


