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Intestinal microbiota and its host: harmony or discord?

The Microbiota in Inflammatory Bowel Diseases

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INTRODUCTION

The microbial community of the human gastrointestinal tract, referred to as the "gut microbiota", is fundamental to health. The gut microbiota has a major role in digestive physiology and the maintenance of intestinal homeostasis. Dysfunction in the regulation of the intestinal immune response to the microbiota can upset the fragile balance of this complex ecosystem by modifying its composition, thus creating dysbiosis.[1-3] Because it promotes the implantation of bacteria that induce local inflammatory reactions, dysbiosis predisposes the individual to pathological or uncontrolled inflammation. This process is involved in many diseases including chronic Inflammatory Bowel Diseases (IBD) such as Crohn's disease and ulcerative colitis, two complex diseases that evolve by inflammatory flares alternating with remission phases.

THE INTESTINAL MICROBIOTA IN THE PATHOGENESIS OF CHRONIC INFLAMMATORY BOWEL DISEASES

Involvement of genetic and environmental factors

Crohn's disease and ulcerative colitis are diseases that relate to an activation of the intestinal immune system towards the microbiota in hosts who are genetically susceptible and under the influence of environmental factors. This pattern is commonly accepted, although the pathophysiology of IBD is more complex.[4] The intestinal microbiota is not the sole factor. Other genetic and environmental factors also impact these diseases, regardless of the microbiota. In fact, recent large genetic analyses have made it possible to identify more than 150 genes that predispose individuals to these diseases.[5]

In addition, since the prevalence of these diseases is increasing considerably in industrializing countries, environment is suspected to be involved in their occurrence, especially pollution (microparticles, heavy metals).[6,7] Food and diet may also be involved.[8,9] Nevertheless, some genetic factors and some environmental factors that are involved in Crohn's disease and ulcerative colitis play a role via their effects on the gut microbiota; these factors have been recently studied.

Involvement of the microbiota in the development of IBD: the arguments

The involvement of the microbiota in the development of IBD has long been suspected. Different arguments have been put forward.

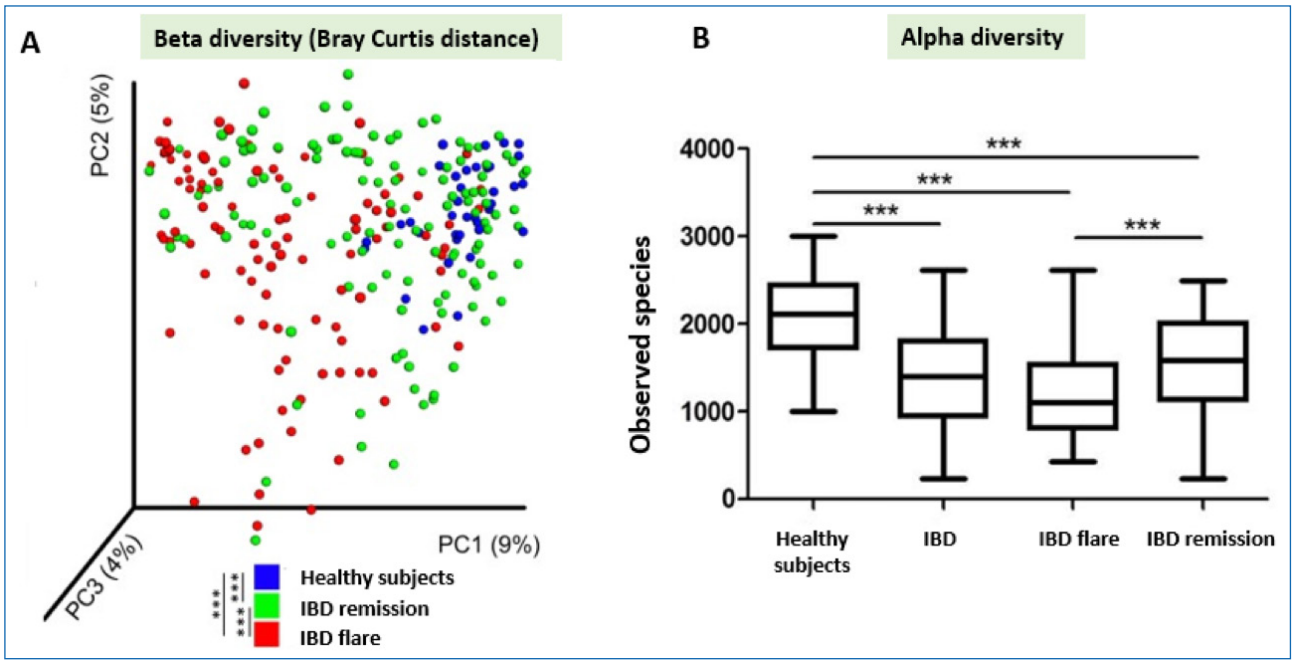
Genetic analyses have shed some light. The vast majority of genes associated with these diseases are either genes involved in the recognition of microorganisms, or genes involved in the response to microorganisms. In Crohn's disease, the diversion of the fecal flow leads to the healing of downstream lesions. Once the fecal stream is restored, inflammatory lesions reappear within days. Similarly, in research on murine models of colitis, it is generally impossible to induce inflammation in axenic mice.

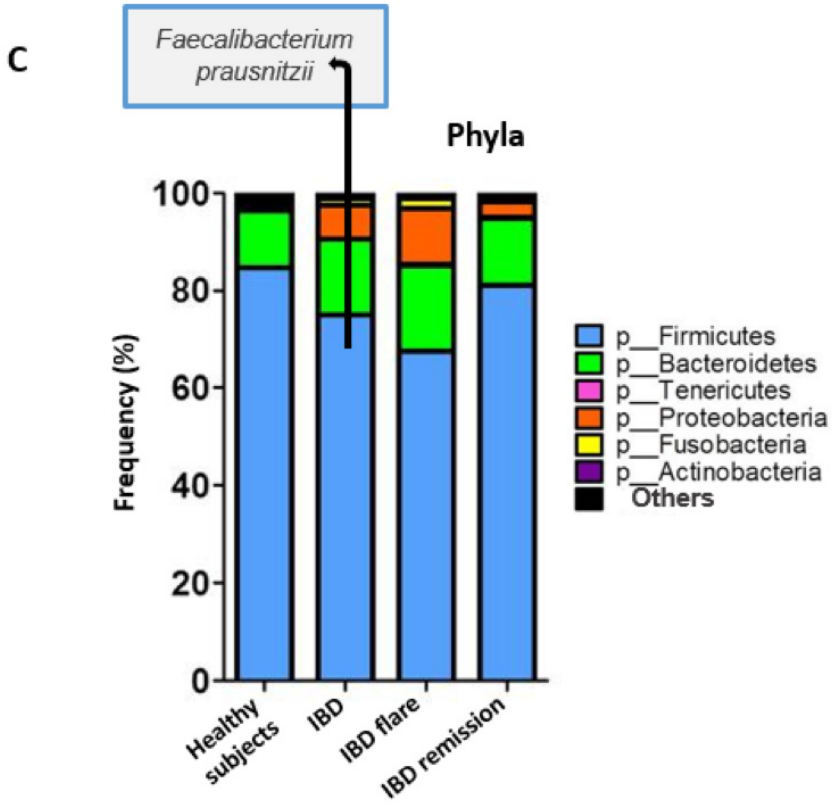
Another argument lies in the difference that may be observed between the microbiota of a patient with IBD and that of a healthy subject. Dysbiosis, or abnormalities of the intestinal microbiota, occur in IBD. Among our recent observations, as also reported in many previous studies, are:

1) a difference in composition between the microbiota of healthy volunteers, that of patients experiencing IBD flare, and that of IBD patients in remission (Figure 1A),

2) a decreased bacterial diversity in patients, all the more important while they are experiencing a flare episode (Figure 1B).[1]

Figure 1. Anomalies of the microbiota in inflammatory bowel disease (IBD). The scatter of points (Bray Curtis distance) reveals the significant difference ($p<0.001$) between the microbiotas of healthy subjects and those of patients in IBD flare or IBD remission (A). Compared with healthy subjects, the diversity was significantly ($p<0.001$) decreased in IBD patients, either at an inflammatory stage or in remission (B). In all phenotypes, the bacterial microbiota is dominated by phyla of firmicutes, bacteroidetes and proteobacteria. During IBD, firmicutes (especially *Faecalibacterium prausnitzii*) are decreased in favor of proteobacteria (C).[1]



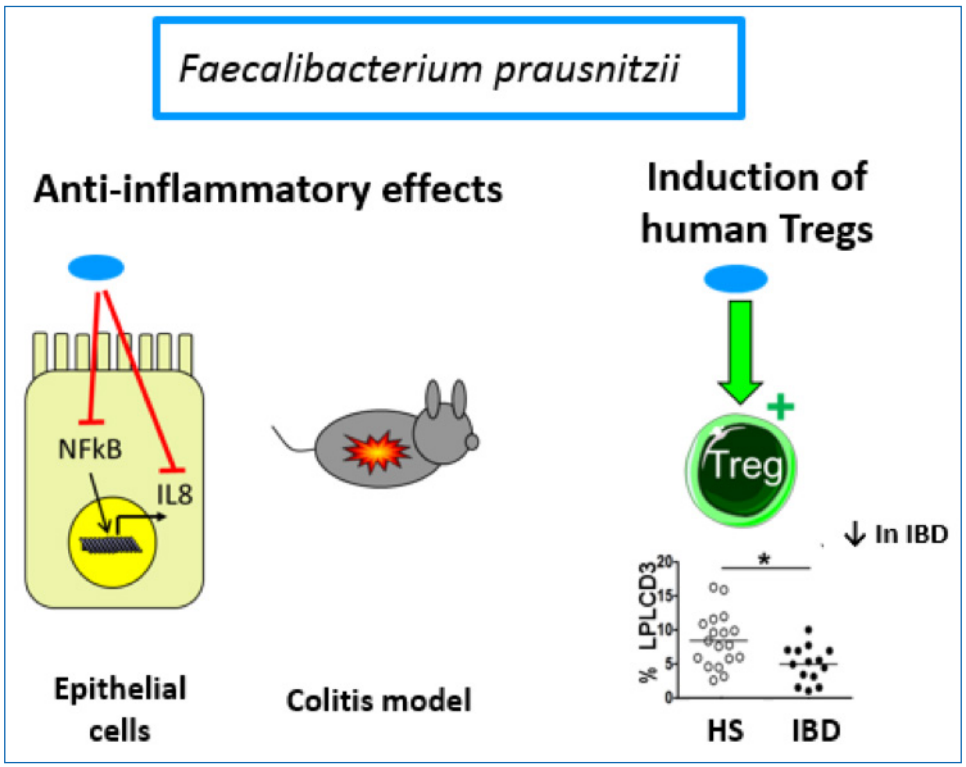


With regards to composition, we observed, in particular, a decrease in the count of bacteria belonging to the firmicutes phylum with an increased count of those belonging to the proteobacteria phylum (Figure 1C).[1] *Faecalibacterium prausnitzii* is one of the dominant bacteria in the firmicutes phylum, and in the human intestinal microbiota overall. The amount of *Faecalibacterium prausnitzii* appears particularly reduced in patients with IBD, especially those with Crohn's disease, and it is observed that its levels may predict the risk of relapse.[2,3]

It has also been shown that *Faecalibacterium prausnitzii* has anti-inflammatory effects via various mechanisms, including the blockade of inflammatory pathways in intestinal epithelial cells and the stimulation of a specific subpopulation of regulatory T lymphocyte expressing both CD4 and CD8 (Figure 2).[2,3, 10-13]

IBD pathogenesis, therefore, appears to be marked by complex interactions between microbiota, host genetics and the environment.

Figure 2. Mechanisms involved in the anti-inflammatory action of *Faecalibacterium prausnitzii*.



COMPLEX HOST-MICROBIOTE INTERACTIONS IN IBD

Role of host genetics in the modulation of gut microbiota and IBD

In order to determine in humans whether the genetics of the host has an effect on the microbiota when intestinal inflammation occurs, we recently studied the intestinal microbiota of patients with three types of rare primitive immune deficiency inducing a phenotype that resembles IBD: chronic septic granulomatous, XIAP deficiency and TTC7A deficiency.[14] These three genetic defects have extremely different consequences in terms of immunity and epithelial cells, resulting from different mechanisms, but all are characterized by IBD-like intestinal manifestations. The analysis showed a clustering of patients dependent on the causal gene of their disease. Compared with the microbiota of healthy subjects, patients with TCC7A deficiency had an increase in some proteobacteria and a decrease in firmicutes; patients with chronic septic granulomatosis had a different signal, as did patients with a defect in XIAP.[14] In the microbiota of the latter, we observed among the increased bacteria a majority of bacteria usually found in the oral microbiota.[14]

Another characteristic that should be highlighted in patients with XIAP deficiency is the sharp increase in *Lactococcus garviae*, a bacterium that is found only in this type of patient and which is a pathogen of seawater fish.

These human data suggest that alterations of the microbiota in an inflammatory context are not related to the sole intestinal inflammation and that dysbiosis is not only a non-specific process related to inflammation. Host genes could also actively modulate dysbiosis, with, more specifically, an effect on the type of microbiota alteration. These correlations must be tested in experimental murine models seeking causal relationships.

Causal relationship in the genetic interactions between host and microbiota in intestinal inflammation

CARD9 (Caspase Recruitment Domain 9) is one of the most important IBD-associated genes. It is an important gene of innate immunity against many types of microorganisms. Expressed primarily in antigen-presenting cells, CARD9 integrates signals downstream of innate immunity receptors.

To understand the role of this gene in intestinal inflammation, knockout mice for *Card9* (*Card9* KO) and non-genetically modified control WT mice were submitted to a DSS (Dextran Sodium Sulphate) colitis model.[15] *Card9* KO mice are more sensitive to DSS-induced colitis than unmodified mice, with a defect in the recovery phase following the active inflammatory phase. The susceptibility to inflammation seen in *Card9* KO mice is transferable to WT mice via intestinal microbiota transfer. The mechanism involves a defect in tryptophan metabolism by the microbiota of *Card9* KO mice.[2]

In fact, some microbiota bacteria are known to be able to use tryptophan in the production of indole derivatives, AhR receptor agonists (aryl hydrocarbon receptor) that stimulate this receptor on a number of epithelial or immune cells. They have very important effects on intestinal homeostasis, especially in the production of interleukin-22 and antimicrobial peptides. Activation of this pathway also promotes the integrity of epithelial cells.
A defect in the tryptophan metabolism of *Card9* KO mice microbiota induces decreased production of AhR agonists, AhR pathway activation, production of interleukin-22 and antimicrobial peptides, disruption of epithelial integrity, ultimately inducing greater susceptibility to inflammation.[15]

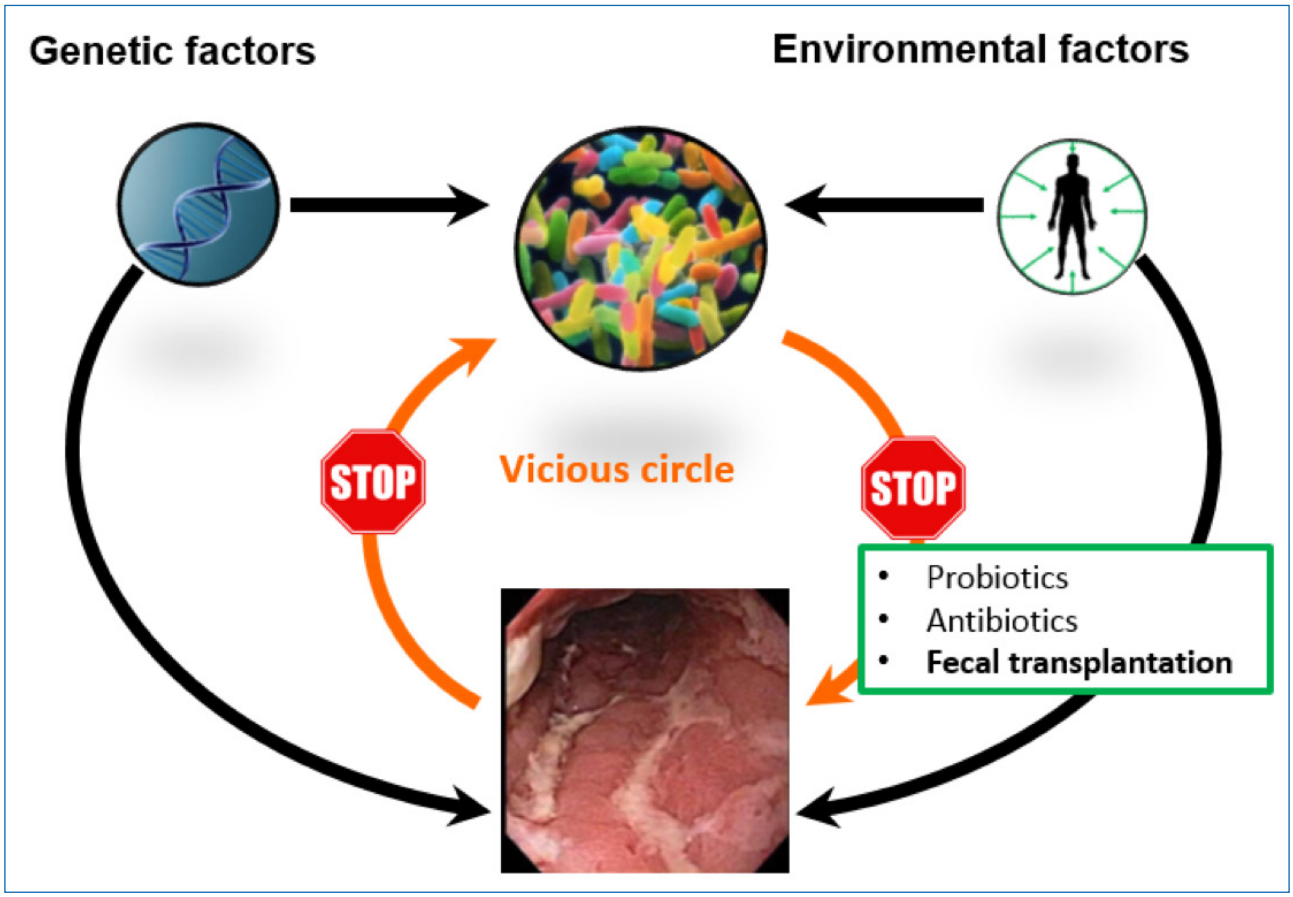
In order to evaluate whether the observations made in mice are relevant in humans, the stools of patients with IBD and healthy subjects were analyzed, revealing that overall AhR agonist production capacity is decreased in patients with MICI, including a decrease in indole-acetic acid.[15] These results show that host genes have an effect on the composition and function of the gut microbiota, altering the production of microbial metabolites and intestinal inflammation.

Altered microbiota: cause or consequence?

There is a long-standing question about the role of microbiota in IBD, but also in other diseases: are microbiota abnormalities the cause or the consequence of the disease? The answer seems to be "both!". The microbiota does not change spontaneously, it changes due to certain factors. On the other hand, if some important protective functions are lost, or if deleterious functions appear in a modified microbiota, both disease and inflammation are promoted. In fact, it is now established that:
1) genetics and environmental factors have an impact on intestinal inflammation and on the microbiota, and
2) the inflammation impacts the microbiota, which itself has an effect on the disease. This induces a vicious circle (Figure 3) that the treatment will have to target.

In terms of treatment, it appears necessary to block both pathogenic immune response and microbiota at the same time (Figure 3). Current drugs act primarily on immunity but the microbiota is not targeted.

Figure 3. Genetics, environment and microbiota in inflammatory bowel disease: the vicious cycle between inflammation and dysbiosis of the microbiota. For real therapeutic efficacy in IBD, both targets of pathogenesis - inflammation and microbiota - must be addressed.



A TREATMENT TARGETING THE GUT MICROBIOTA IN INFLAMMATORY BOWEL DISEASES

The treatments proposed for targeting the gut microbiota are either conventional or next-generation probiotics, antibiotics or drugs that target bacteria to block them, and fecal microbiota transplantation (FMT). FMT is the replacement of the dysbiotic microbiota by a healthy microbiota. Currently the only indication for this procedure is recurrent *Clostridium difficile* infection. With the exception of this indication, FMT takes place only in a research setting.

Four randomized controlled trials conducted in IBD have been published.[16-19] In these studies, the objective was to induce remission using FMT in patients experiencing an active inflammatory phase of their ulcerative colitis. The published results are positive, with significant differences in three of the four trials. However, there are some limitations: a great heterogeneity in the methods applied, small samples of patients, and lack of maintenance data.

No randomized controlled trials within this arena have been published in Crohn's disease. Nevertheless, a randomized controlled pilot study was conducted at Saint-Antoine Hospital (Paris) but it has not yet been published. This trial, which included a small number of patients experiencing a flare of Crohn's disease, aimed to target both the immune system and the microbiota. After corticosteroid induction of clinical remission, patients were randomized to either FMT or dummy FMT. Follow-up was carried out over 6 months, with gradual reduction of corticosteroid therapy, in order to obtain maintenance data. The results of this study should be published shortly.

COMMENTS, CONCLUSIONS

Given its proven role in IBD pathogenesis, the gut microbiota is now considered a potential therapeutic target. New generation probiotics and FMT are actively being studied.

FMT appears to be an interesting therapeutic option, but it is not yet fully controlled. The main interest of FMT studies carried out in IBD may be the identification of microorganisms, metabolites and microbial functions conveying therapeutic effects. These may be simpler and more controlled therapeutic tools than FMT, such as with the new generation probiotics including *Faecalibacterium prausnitzii*.

REFERENCES (Underlined references link to PubMed abstracts)

1. Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, et al. Fungal microbiota dysbiosis in IBD. Gut 2017; 66(6):1039-1048.
2. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. PNAS 2008; 105 (43):16731-16736.
3. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm Bowel Dis 2009; 15(8):1183-9.
4. Ni J, Wu GD, Albenberg L, Tomov VT. Gut microbiota and IBD: causation or correlation? Nat Rev Gastroenterol Hepatol 2017; 14(10):573-584.
5. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012; 491(7422):119-124.
6. Dutta AK, Chacko A. Influence of environmental factors on the onset and course of inflammatory bowel disease. World J Gastroenterol 2016; 22:1088-1100.
7. Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. Gastroenterol Hepatol 2010; 6:339-346.
8. Forbes A, Escher J, Hébuterne X, Kłęk S, Krznaric Z, Schneider S, et al. ESPEN guideline: clinical nutrition in inflammatory bowel disease. Clin Nutr 2017; 36(2):321-347.
9. Rapozo DC, Bernardazzi C, de Souza HS. Diet and microbiota in inflammatory bowel disease: the gut in disharmony. World J Gastroenterol 2017; 23(12):2124-2140.
10. Miquel S, Leclerc M, Martin R, Chain F, Lenoir M, Raguideau S, et al. Identification of metabolic signatures linked to anti-inflammatory effects of Faecalibacterium prausnitzii. MBio 2015; 6(2).
11. Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn's disease. Gut 2016; 65(3):415-425.
12. Sarrabayrouse G, Bossard C, Chauvin JM, Jarry A, Meurette G, Quévrain E, et al. CD4CD8αα lymphocytes, a novel human regulatory T cell subset induced by colonic bacteria and deficient in patients with inflammatory bowel disease. PLoS Biol 2014; 12(4):e1001833.
13. Godefroy E, Alameddine J, Montassier E, Mathé J, Desfrançois-Noël J, Marec N, et al. Expression of CCR6 and CXCR6 by gut-derived CD4+/CD8α+ T-Regulatory Cells, which are decreased in blood samples from patients with inflammatory bowel diseases. Gastroenterology 2018; 155(4):1205-1217.
14. Sokol H, Mahlaoui N, Aguilar C, Bach P, Join-Lambert O, Garraffo A, Seksik P, et al. Intestinal dysbiosis in inflammatory bowel disease associated with primary immunodeficiency. J Allergy Clin Immunol 2019; 143(2):775-778.e6
15. Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nat Med 2016; 22(6):598-605.
16. Rossen NG, Fuentes S, van der Spek MJ, Tijssen JG, Hartman JH, Duflou A, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. Gastroenterology 2015; 149(1):110-118.
17. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, Armstrong D, Marshall JK, Kassam Z, Reinisch W, Lee CH. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterology 2015; 149(1):102-109.e6.
18. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet 2017; 389(10075):1218-1228.

19. Costello SP, Hughes PA, Waters O, Bryant RV, Vincent AD, Blatchford P, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. JAMA. 2019; 321(2):156-164