
INFLAMMATORY
BOWEL DISEASES
BIOCODEX
Microbiota Institute

The prevalence of inflammatory bowel diseases (IBD) is particularly high in Western countries: it affects about 3 out of 1,000 people in North America, Oceania and many European countries[1]. In emerging countries, numbers are growing together with urbanization. Although bacterial, fungal and viral dysbiosis have been described in patients with IBD, it is not yet known whether they are the cause or the consequence of the disease. Or do they play a role in the onset, maintenance or severity of inflammation, thus giving rise to a vicious circle?

[1] Ng SC, Shi HY, Hamidi N et al. *Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies.* Lancet. 2018 Dec 23;390(10114):2769-2778. doi: 10.1016/S0140-6736(17)32448-0. Epub 2017 Oct 16

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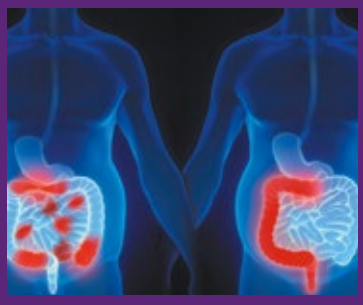

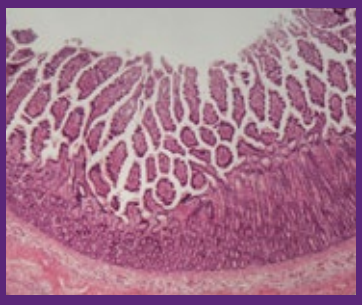

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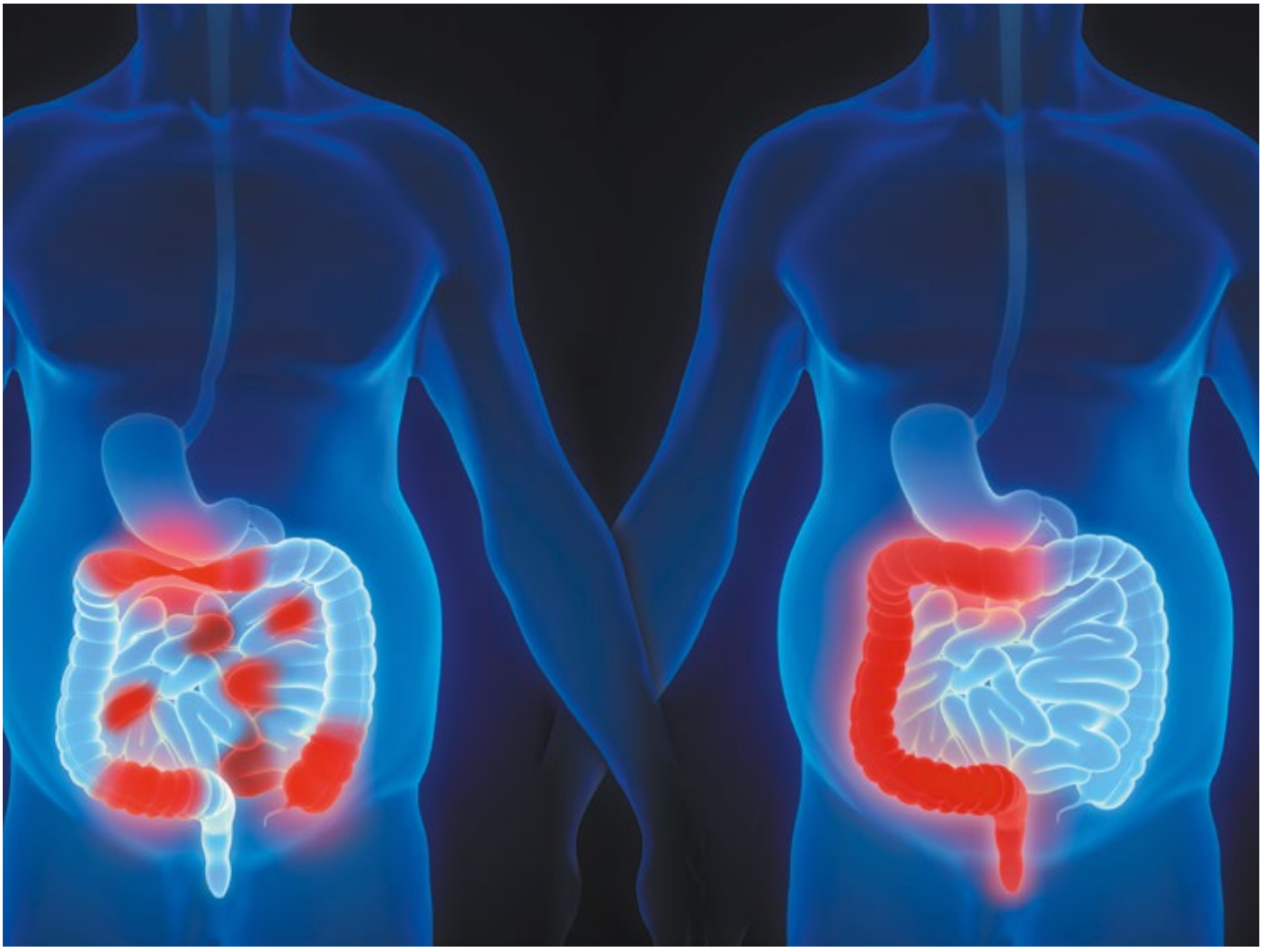
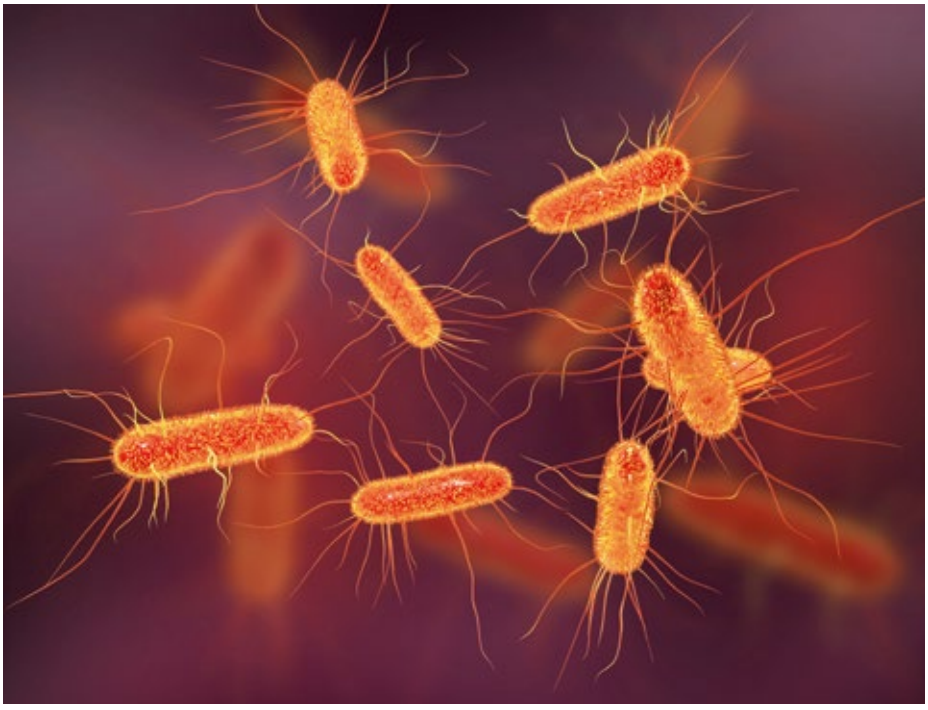
DYSBIOSIS IN IBD

Inflammatory bowel diseases (IBD) are characterized by an inflammation of the gastrointestinal wall that may affect either the entire intestinal tract (Crohn's disease, CD) or be confined to the rectum and colon (ulcerative colitis, UC). They are associated to an impairment of the biodiversity and composition of bacterial, fungal and viral microbiotas, which are believed to play a role in the disease pathogenesis and/or progression.



A bacterial dysbiosis characteristic of IBD

A double bacterial gut dysbiosis, characterized by a decrease in some beneficial strains and an increase in pathogenic strains, is associated to IBD. These composition abnormalities could be both the cause and the consequence of these disorders, thus inducing a vicious circle.



Escherichia coli

In patients with IBD, structural and functional alterations of the gut microbiota have been observed. The composition is also different in patient undergoing an acute episode compared to those in remission[2].

DECREASE IN BENEFICIAL BACTERIA AND INCREASE IN PATHOGENS

First feature: decreased Firmicutes/Bacteroidetes ratio. A decrease in some beneficial bacteria from the Firmicutes phylum is observed, for instance, lower abundance of *Faecalibacterium prausnitzii*, a commensal bacterium with anti-inflammatory properties and whose decrease seems to be a marker of CD[3]; reduced rate of Firmicutes, commonly observed in patients with IBD[4]; significant decrease in *Bacteroides fragilis* (Bacteroidetes), a bacterium with proven protective effects in murine models of induced colitis[5].

In patients undergoing an acute phase of IBD, there is also a lower abundance of *Clostridium coccoides, Clostridium leptum, Faecalibacterium prausnitzii* and *Bifidobacterium*[2].

Second feature: excess of potentially harmful microorganisms, especially Gammaproteobacteria and Actinobacteria. In one out of three patients with CD, the mucosa is invaded by a strain of *Escherichia coli* called AIEC (*Adherent-invasive Escherichia coli*)[3]. Contrary to other infectious agents, these strains are able to cross the intestinal mucus barrier, adhere to it, then invade the gut epithelial cells, and survive and replicate in macrophages. This leads to the secretion of large quantities of TNFα, which in turn causes inflammation.

DYSBIOSIS: CAUSE OR CONSEQUENCE OF IBD?

This bacterial gut dysbiosis, which seems to be a marker of IBD, is suspected to play a role in its pathogenesis. A study conducted in mice genetically predisposed to UC revealed a two-way relation between this disease and gut dysbiosis[6]. Bacterial dysbiosis could thus not only contribute to the onset of IBD, but also be a secondary consequence of gut inflammation.

Different hypotheses were suggested to explain this dual phenomenon: some species from the Firmicutes phylum have anti-inflammatory properties and are major producers of short-chain fatty acids (SCFA)–especially butyrate–, which represent the main energy-producing substrate for colonocytes. Moreover, a decrease in the number of Firmicutes could trigger or intensify a local inflammation by decreasing the levels of anti-inflammatory cytokines (key regulators of mucosal immunity) and/or by altering the colon barrier function through a deficit of SCFA[4].

[2] Aleksandrova K, Romero-Mosquera B, Hernandez V. *Diet, Gut Microbiome and Epigenetics: Emerging Links with Inflammatory Bowel Diseases and Prospects for Management and Prevention.* Nutrients. 2017 Aug 30;9(9). pii: E962. doi: 10.3390/nu9090962

[3] Torres J, Mehandru S, Colombel JF et al. *Crohn's disease*. Lancet. 2017 Apr 29;389(10080):1741-1755

[4] Kho ZY, Lal SK. *The Human Gut Microbiome - A Potential Controller of Wellness and Disease.* Front Microbiol. 2018 Aug 14;9:1835

[5] Coretti L, Natale A, Cuomo M et al. *The Interplay between Defensins and Microbiota in Crohn's Disease.* Mediators Inflamm. 2017 ;2017:8392523

[6] Nagao-Kitamoto H, Shreiner AB, Gillilland MG 3rd et al. *Functional characterization of Inflammatory Bowel Disease-Associated gut dysbiosis in gnotobiotic mice.* Cell Mol Gastroenterol Hepatol. 2016 Mar 3;2(4):468-481. eCollection 2016 Jul.

Each IBD has its own virome

In addition to bacteria, the gut microbiota is also composed of viruses. Although studies focusing on viruses are still rare, the presence or absence of some families seems to be specific markers of CD and UC.

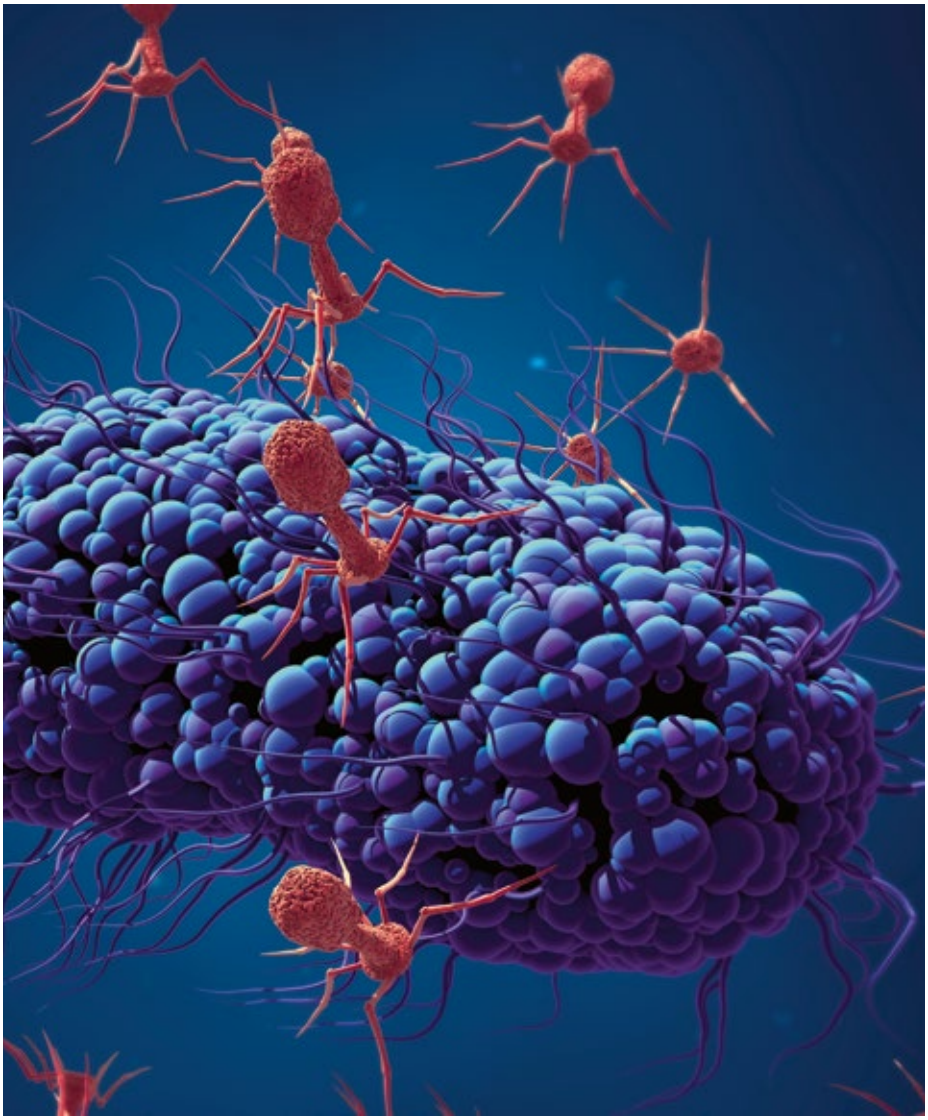
The second microbiota component that could be involved in IBD is the virome (viral component of the microbiota), made up of both eukaryote-infecting viruses and bacteriophages infecting bacterial cells, which are the most studied. In patients with IBD, a dysbiosis of this virome has been observed: loss of diversity in addition to a greater variability of gut viruses in patients with CD. A study conducted in the United States and United Kingdom in 2015 also revealed an increased abundance and diversity of the enteric virome in patients with CD or UC[7].

IMPACT OF BACTERIOPHAGES ON THE BACTERIAL MICROBIOTA

Bacteriophages are ten times more numerous than bacteria and are involved in the microbiota mechanism through the control of bacterial abundance and diversity, which leads to an either protective or harmful effect: in patients with CD, the expansion of Caudovirales bacteriophages is associated to a loss in bacterial diversity and could be involved in the bacterial dysbiosis and gut inflammation[8].

VIROME SIGNATURE

While studies on the virome are rare, those focusing specifically on eukaryotic viruses are even more so. One of them compared the gut mucosa of healthy controls to that of treatment-naive young patients whose IBD had been diagnosed early[8], and suggested that some eukaryote-infecting viruses could be involved in the onset of gut inflammation and contribute to IBD pathogenesis, with a specific signature depending on the disease: more viruses from the *Hepadnaviridae* family compared to controls and patients with CD, and less *Polydnaviridae* and *Tymoviridae* in patients with UC; increased abundance of *Hepeviridae* (a family of viruses including HEV for instance) and less *Virgaviridae* in patients with CD compared to controls. These virome signatures could be acquired early in life (for instance through diet) and later increase host susceptibility to IBD[8].



[7] Zuo T, Kamm MA, Colombel JF et al. *Urbanization and the gut microbiota in health and inflammatory bowel disease.* Nat Rev Gastroenterol Hepatol. 2018 Jul;15(7):440-452
[8] Ungaro F, Massimino L, Furfaro F et al. *Metagenomic analysis of intestinal mucosa revealed a specific eukaryotic gut virome signature in early-diagnosed inflammatory bowel disease.* Gut Microbes. 2019;10(2):149-158

Association between fungal dysbiosis and environment

The fungal portion of the gut microbiota (or mycobiota) has been much less studied than the bacterial portion but could also be involved, since a fungal dysbiosis was also observed in patients with IBD. Possible interactions between bacteria and fungi have been mentioned.

It appears that the bacterial and viral components of the microbiota are not the only ones to be affected in patients with IBD. The mycobiota, i.e. all fungi present in the gut ecosystem, also seems to be disrupted.

DISRUPTED MICROBIOTA

A study conducted in 235 patients with IBD and 38 healthy controls brought to light the presence of a fungal dysbiosis in affected patients: increase of the *Basidiomycetes/Ascomycetes* ratio, decrease in the proportion of *Saccharomyces cerevisiae*, and increase in that of *Candida albicans*[9]. In patients with CD, it is believed that the development of fungi occurs to the detriment of bacteria, triggering a loss of diversity. Moreover, interactions between these two kingdoms (bacteria and fungi) also seem to be degraded compared to those observed in healthy subjects, thus revealing inter-kingdom alterations specific to IBD. Although data on the fungal portion of the microbiota are still very fragmented, these initial results suggest that the mycobiota plays a role in the pathogenesis of IBD. This dysbiosis, characterized by alterations in biodiversity and composition, adds itself to the bacterial dysbiosis.

URBANIZATION AS A CAUSE OF THIS DYSBIOSIS?

Meanwhile, some research teams focused on the link observed between rapid urbanization and increased incidence of autoimmune diseases, including IBD[7]. Several hypotheses were suggested, including one involving the mycobiota: the Western diet, rich in carbohydrates, which promotes the development of *Candida* in the intestines; the impact of atmospheric pollution in urban areas which could reduce fungal biodiversity; and quality of urban air, less rich in some spores (*Actinomyces, Botrytis*...) than rural air. It seems that urbanization does not only impact the mycobiota but could also be related to the dysbiosis of other microorganism communities (bacteria, viruses, parasites...).

ROLE OF ENTERIC HELMINTHS[7,10]

Helminths are parasitic worms. They include nematodes (roundworms) and platyhelminths (flatworms).

The absence of helminths has been associated to the development of IBD, while their presence seems to prevent the development of IBD.

They probably play an immunoregulatory role within the gut microbiota (development of anti-inflammatory mechanisms, increase of mucus and fluid secretion in the intestinal lumen...)

Ingesting *Trichuris suis* eggs could have a protective effect against IBD.

[9] Sokol H, Leducq V, Aschard H et al. Fungal microbiota dysbiosis in IBD. Gut. 2017 Jun

[10] Helmby H. *Human helminth therapy to treat inflammatory disorders - where do we stand?* BMC Immunol. 2015 Mar 26;16:12. doi: 10.1186/s12865-015-0074-3

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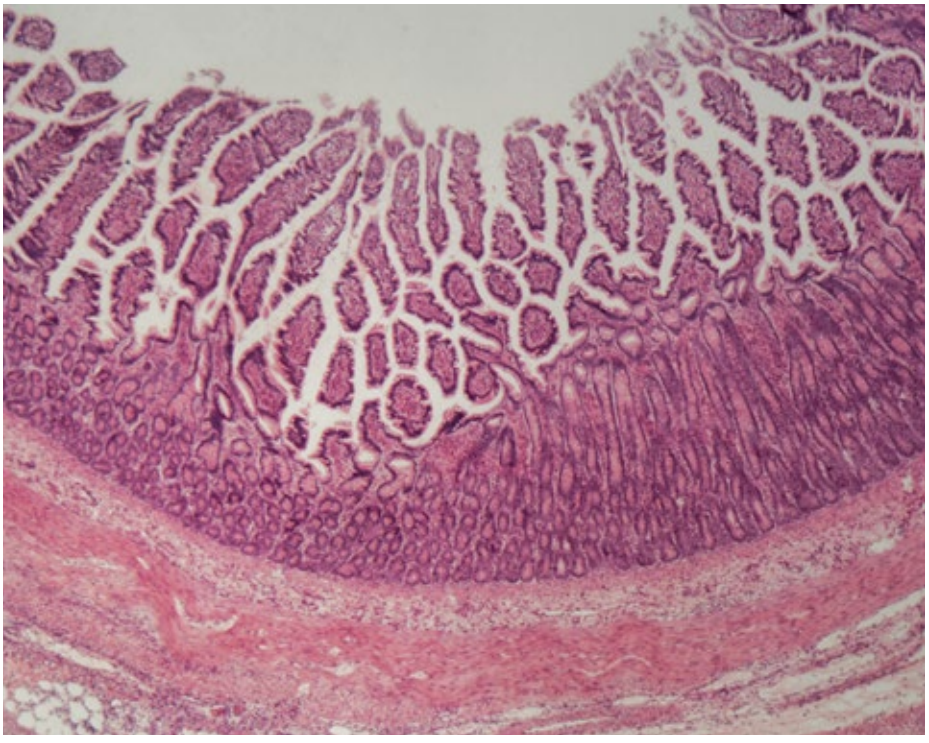
PATHOPHYSIOLOGY OF DYSBIOSIS

The dysbiosis observed in IBD could be related to an alteration of the gut epithelium, which is no longer able to play its barrier role, as well as to a dysregulation of the local innate immune response that promotes inflammation. Explanations and focus on antimicrobial peptides.

Role of the intestinal epithelium and the innate immune response

The alteration of the intestinal barrier observed in patients with IBD could explain the pathophysiology of dysbiosis: not only would this mechanical frontier be altered, but also its first line of immune defense.



Besides its role in the absorption of ions, water and other nutrients, the intestinal barrier serves as a wall and prevents the entry of bacteria into the gut lumen. But its permeability increases in acute phases of Crohn's disease (CD), which promotes translocation of bacteria through the mucus as well as local inflammation[5].

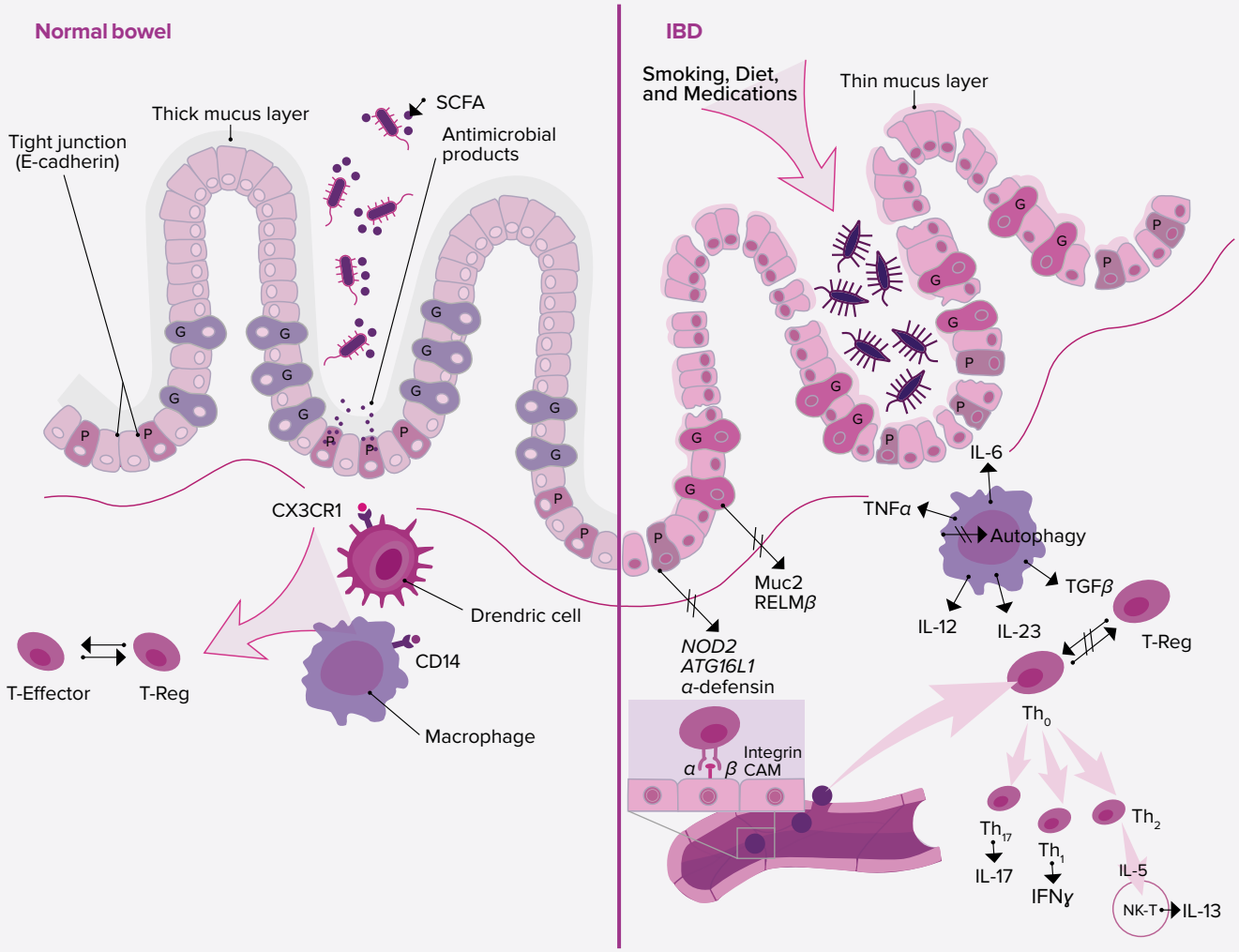
THE ROLE OF THE INTESTINAL BARRIER

Potential causes: alteration of the tight junctions of intestinal epithelial cadherins (glycoproteins playing a key role in intracellular adherence); involvement of some transcription factors[11] related to epithelial regeneration. Other mechanisms under discussion involve the gut mucus, whose thickness keeps pathogenic bacteria at bay, but which is considerably decreased in patients with IBD. This phenomenon could be explained by the alteration of mucus-producing goblet cells whose disruption induces the development of colitis in murine models. It could also be explained in patients with CD by the impairment of Paneth cells located at the bottom of small intestinal crypts, known to be involved in homeostasis and with a defensive role of the gut mucus through antimicrobial secretion[5,11].

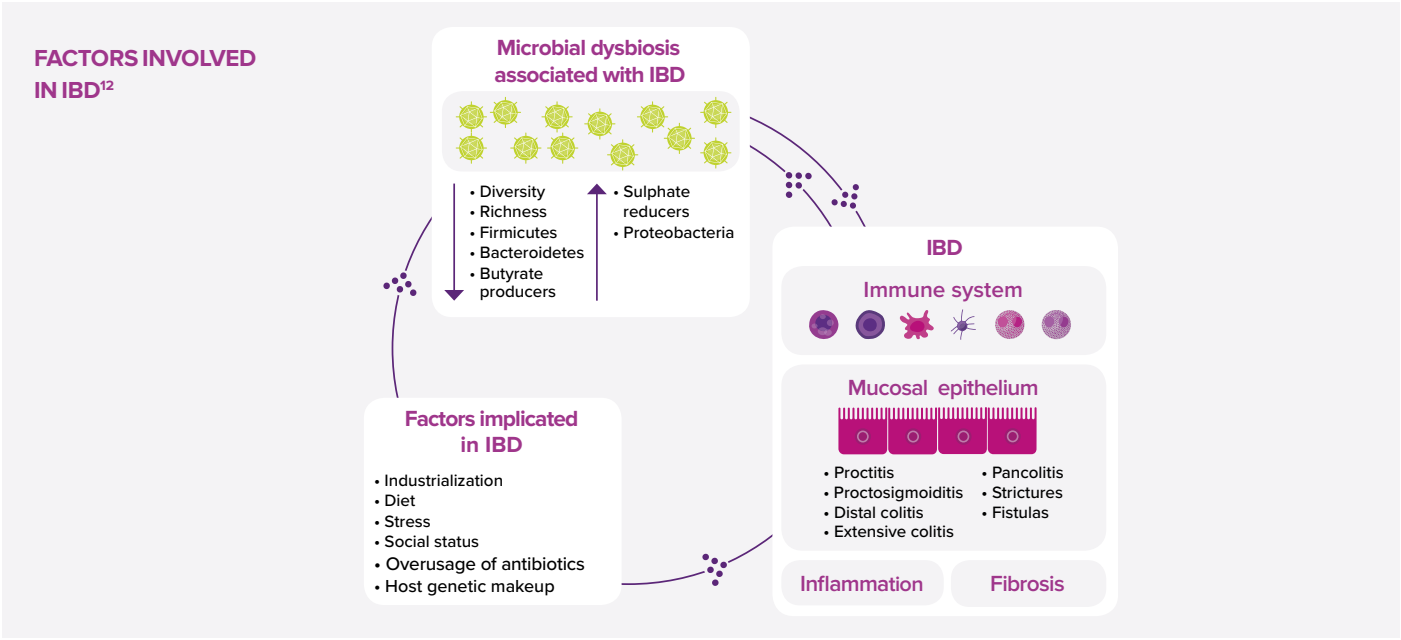
FIRST LINE OF DEFENSE OF THE IMMUNE SYSTEM

The scientific literature also mentions several mechanisms involving innate immunity, and more precisely dendritic cells, macrophages, innate lymphoid cells and neutrophils. These cells, which complete the previously described system, act as the first line of defense of the immune system. In the intestines of healthy subjects, macrophages are hyporeactive (reduced proliferation and activity) and can produce anti-inflammatory cytokines[11]. On the contrary, in patients with IBD, the imbalance in innate immunity cell populations could be associated to several phenomena[11]: bacteria crossing the mucus which became permeable due to macrophage activity decrease and defective neutrophil recruitment; inflammation induced by the production of large quantities of pro-inflammatory cytokines (TNF-α and IL-6) by specific macrophages; and inflammatory T-cell recruitment through the accumulation of dendritic cells which induce an adaptive immune response.

ALTERATION OF GUT MUCOSA IN PATIENTS WITH IBD[11]

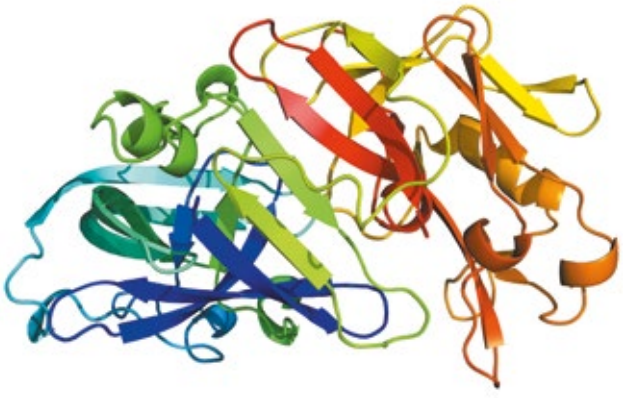


[11] Ramos GP, Papadakis KA. *Mechanisms of Disease: Inflammatory Bowel Diseases.* Mayo Clin Proc. 2019;94(1):155-165



Focus on the role of antimicrobial peptides

Antimicrobial peptides (AMP) are small multifunctional peptides present in the animal and plant kingdoms that protect the host against attacks from pathogenic microorganisms. Among them are defensins, thus called because they act as host defense peptides.



Defensins play a major role in innate immunity. Ten defensins have been identified in humans: six α-defensins that are mainly secreted by Paneth cells, neutrophils and some populations of small intestine macrophages; and four β-defensins that are secreted by epithelial cells[5].

MECHANISMS OF ACTION

Mechanisms of action of antimicrobial peptides are variable[8]: some puncture bacterial membranes and cause ion and nutrient efflux, loss of structure, or even bacterial lysis; other "bind" to bacteria and limit they passage through the gut epithelium without killing them; in other words, they reduce pathogen colonization and change the composition and density of bacterial communities in the intestinal lumen.

CROHN'S DISEASE AND DECREASE IN DEFENSINS

When CD affects the ileum, the production of some α-defensins decreases, which reduces antibacterial activity and promotes pathogenic bacteria penetration[5]. Among possible explanations there is NOD2 gene mutation, involved in the recognition of bacterial surface and onset of defensin production; and impaired signaling pathways, whose dysregulation is involved in the development of many human cancers. Some researchers believe that the decrease in α defensin content is not the cause but the consequence of inflammation[4]. In colonic CD, only β-defensin rates are disrupted5: increase in secretion of β-defensin type 2 and decrease in type 1. Some scientists believe that the smaller number of copies of the gene located on chromosome 8 in affected patients (3 instead of 4) is at fault.

[12] Somineni HK, Kugathasan S. *The microbiome in Inflammatory Bowel Disease: from prevailing clinical evidence to future possibilities.* Clin Gastroenterol Hepatol. 2019

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WHAT ROLE COULD MICROBIOTA MODULATION PLAY?

Since gut dysbiosis seems to be associated to IBD, microbiota modulation could be a new avenue for relevant therapeutic care. Two possible options: fecal microbiota transplant or use of probiotics.

Fecal microbiota transplant: mixed results

Fecal microbiota transplant allows patients to receive approximatively 10^{11} bacteria per gram of stool, but also fungi, viruses and archaea. Its objective is to correct gut dysbiosis. The number of necessary attempts and results vary according to the individuals and their pathology, and the protocol requires seamless controls[13] .

Gut microbiota modulation is a potential treatment for IBD. In practice, this strategy could be carried out through fecal microbiota transplant (FMT). The fecal matter comes from one or several healthy donors and is then transplanted into the distal gastrointestinal tract of the patient thanks to a nasogastric tube, during a colonoscopic enema, or more recently through oral capsules. This protocol is already being successfully used in case of recurrent Clostridium difficile infections. But what about IBD?

ULCERATIVE COLITIS

Although safety conditions of the first tests were satisfying and the therapy led to partial or total remission in some patients with ulcerative colitis (UC), other attempts were more controversial[14] . The donor's fecal composition quality, the number of transplants and early therapeutic care, could modulate the chances of success and appear as a post-hoc explanation of heterogeneity of results.

CROHN'S DISEASE (CD)

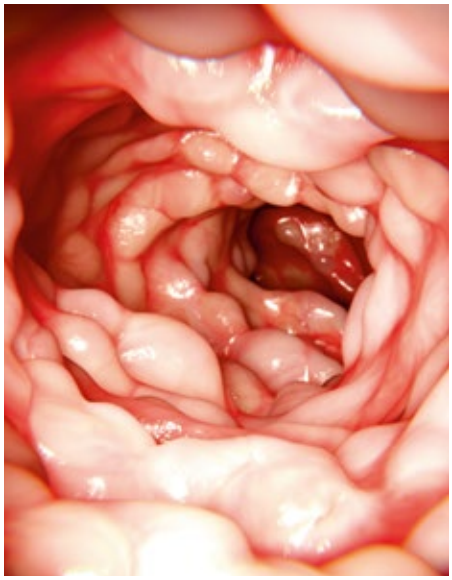
Studies conducted on Crohn's disease (CD), although fewer in number, produced either positive (clinical and endoscopic remission) or neutral results.

[13] https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse

In the only study that did not show a significant disease reduction, patients still reported an improvement in their quality of life[14]. To confirm the clinical efficacy of fecal transplant in Crohn's disease, additional studies are still necessary.

MANY PENDING ISSUES

Whatever the disease, several questions remain unanswered regarding: FMT engraftment, which could require several fecal transplants; the quality of the transplanted microbiota often obtained from donors living in countries where IBD prevalence rate is high; the restoration of microbiota altered functions after the transplant (for instance, production of protective short-chain fatty acids such as butyrate); and of course, the role of inflammation, either as a cause or consequence of dysbiosis[14].



Use of probiotics: the VSL#3 strain stands out

Treatment with strains of living beneficial bacteria was long considered the safest and most sustainable approach against IBD. Although some of them seem effective to treat UC, studies regarding their use against CD are still inconclusive.

Probiotics are living microorganisms which, when ingested in adequate quantities, provide health benefits. Several mechanisms of action might underlie the beneficial effects of probiotics in IBD: inducing changes in the gut microbiota composition thus reducing gut dysbiosis; regulating metabolic activity of the gut microbiota; eliminating pro-inflammatory processes; and immunomodulation[12].

CONTRASTED RESULTS DEPENDING ON THE DISEASE

In UC, the efficacy of probiotics containing one and only strain (from the *E. coli* Nissle 1917 species), as well as milk fermented with *Bifidobacterium*, in inducing disease remission was comparable to that of standard anti-inflammatory treatments (mesalazine)[12]. Different combinations of bacterial strains were also tested, but with no effect on disease remission, with one exception: a cocktail of 8 different strains[15], called "VSL#3" induced a significant reduction in UC symptoms[13,16],

[14] Weingarden AR, Vaughn BP. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. Gut Microbes. 2017 May 4;8(3):238-252

(rectal bleeding and stool frequency) and a study proved its efficacy in maintaining remission. This same cocktail also seems to be efficient in patients with pouchitis: it prevents inflammatory flares arising from coloproctectomy and maintains remission[17,18], However, probiotics have not shown to be useful in CD so far. Results from the rare studies performed are weak and inconclusive, including with strains that have proven to be efficient against UC and pouchitis[12,15].

FURTHER INVESTIGATIONS

Heterogeneity of non-clinical and clinical results could be at least partially attributed to factors related to the host (age, gender, diet, disease location, severity, family history of IBD) and the probiotic preparations that were used (type of strain, concentration, mode of administration, potential colonization, and strain survival rate). Other factors, such as dose and duration of probiotics administration, are also supposed to play a primary role in the success of this therapeutic approach, whose adverse effects are minimal, or even nonexistent[12].

SANITARY AND DIETARY RULES[2]

Excess in energy and/or macronutrients intake (saturated fat? refined sugar?) seem to increase gut inflammation, while several micronutrients could modulate it: vitamins A, C, E and D, folic acid, beta-carotene, trace elements (zinc, selenium, manganese and iron).

Since there are no existing dietary recommendations specific to IBD, patients are advised to avoid food which might worsen symptoms (high-fiber foods during flares, caffeine, alcohol, excess fatty foods, etc.).

The specific carbohydrate diet (SCD) which limits complex carbs and eliminates simple sugars, the low FODMAP diet (no fermentable sugars) and the Mediterranean diet have shown anti-inflammatory properties and may induce improvement under certain conditions. However, elimination diets are controversial because of a nutrition imbalance risk.

No smoking.

[15] *Lactobacillus paracasei, L. plantarum, L. acidophilus, L. delbrueckii, Bifidobacterium longum, B. breve, B. infantis, Streptococcus thermophilus*
[16] Derwa Y, Gracie DJ, Hamlin PJ et al. *Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease.* Aliment Pharmacol Ther. 2017;46:389–400
[17] Shen J, Zuo ZX, Mao AP. *Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials* (ERRATUM). Inflamm Bowel Dis. 2014;20:21-35
[18] Dong J, Teng G, Wei T et al. *Methodological Quality Assessment of Meta-Analyses and Systematic Reviews of Probiotics in Inflammatory Bowel Disease and Pouchitis.* PLoS One. 2016 Dec 22;11(12):e0168785

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TOWARDS AN INNOVATIVE MANAGEMENT OF IBD

How is IBD diagnosed?

Because there is no specific test available, IBD diagnosis is based on an array of tests that the physician has to combine to confirm the inflammation and its chronic nature. In Crohn's disease (CD) the inflammation may affect the entire gastrointestinal tract, while in ulcerative colitis (UC) it is confined to the rectum and colon. Usually, the diagnosis is made by a specialist. It is relatively simple and is supported by endoscopy and biopsies, and sometimes an MRI of the intestines. It should be reminded that 1 European out of 100 will develop IBD at one point in life, with Northern people being even more affected. IBDs are often detected in young patients: 28 years old on average for CD, and around thirty for UC–for which a second peak is also observed at around fifty years old, a few months after quitting smoking. (While smoking worsens CD, it paradoxically limits UC symptoms).

"The gut microbiota is undoubtedly a promising research avenue"

Which type of solutions can be offered to patients?

The difficulty of managing CD and UC lies on the adjustment of the background treatment that prevents anatomical damages caused by successive flares, and the need to delay as much as possible any surgical procedure. As

for the treatment of exacerbations, it is important to avoid the use of corticosteroids, which are responsible for too many adverse effects, morbidity and mortality. In the absence of a reliable biomarker of clinical severity, finding a balance is quite a subtle exercise... Moreover, patients with colonic IBD must be closely monitored to confirm the absence of dysplasia (thus the absence of cancer over time) and to avoid any infection (tuberculosis, herpes...) when prescribing one or several immunosuppressants (vaccination schedule and serological surveillance for instance). And finally, patients must be accompanied on their everyday life: studies, travels, sexuality, marriage, children, diet... because IBD can be very disabling in some patients (30 to 50%).

The microbiota, a major therapeutic avenue for today and tomorrow?

Research surrounding IBD is very active and includes the search for new molecules, flare management and implementation of treatment strategies. Among them, the gut microbiota is undoubtedly a promising research avenue: we are starting to understand that the gut microbiota is responsible for triggering and maintaining the inflammation of the gastrointestinal system. The microbiota and its host communicate through many symbiotic actions related to species coevolution. But, for unknown reasons, these symbiotic relationships sometimes malfunction, which is why research is greatly needed to find mechanisms of action to modulate the microbiota and restore potentially impaired functions through the administration of probiotics, metabiotics (microbiota metabolites) or fecal microbiota transplant (a pragmatic method to replace the unbalanced microbiota by another one deemed healthy). ●

Chronical inflammatory bowel diseases

There are two main inflammatory bowel diseases (IBD): Crohn's disease (CD) and ulcerative colitis (UC). Both are characterized by the inflammation of all, or part, of the gastrointestinal tract and their prevalence keeps increasing, particularly in Western countries.

IBD are associated to imbalances in gut microorganism populations. These dysbiosis can be of bacterial (microbiota), but also viral (virome) and fungal (mycobiota) origin. They are suspected to be both a cause and a consequence of the disease and may sometimes be its specific signature.

The alteration of the intestinal barrier in patients is a major factor promoting bacterial translocation as well as local inflammation and disrupting the innate immune response (decrease in defensins in the case of Crohn's disease), thus exposing the organism to pathogens.

Beyond sanitary and dietary rules aiming at limiting gut inflammation, microbiota modulation could be a key avenue for future therapy. Fecal transplant or probiotics could restore host/microbiota symbiotic relations which are impaired in patients with IBD.

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