Eubiosis and dysbiosis: the two sides of the microbiota

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SUMMARY

The microbial ecosystem of the gastrointestinal tract is characterized by a great number of microbial species living in balance by adopting mutualistic strategies. The eubiosis/dysbiosis condition of the gut microbiota strongly influences our healthy and disease status. This review briefly describes microbiota composition and functions, to then focus on eubiosis and dysbiosis status: the two sides of the microbiota.

KEY WORDS: Intestinal microbiota, Eubiosis, Dysbiosis.

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INTRODUCTION

All multicellular organisms live in close association with the surrounding microbes, and humans are no an exception. Every part of our body surface, in communication with the environment, its colonized. The number of these microorganisms, collectively known as "microbiota", is tenfold higher than that of our cells (Sekirov *et al*., 2006), the human beings are now considered as "hybrid organisms" (Sekirov *et al*., 2006), consisting of both human and microbial cells. The coding capacity of microbiota, called "microbiome", is a hundred times higher than that of our cells (Ley *et al*., 2006). Microbes colonize our body from birth, and persist until death, interfering with our anatomical, physiological and immunological development. After a brief description of its composition and functions, we will look at the strategies activated by human and microbes to maintain an eubiosis status in the gut microbiota ecosystem.

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GUT MICROBIOTA COMPOSITION AND FUNCTIONS

The gastrointestinal tract (GIT) is unquestionably the most populated organ. The colon contains more than 70% of the microorganisms colonizing GIT (Sartor, 2008). Taxonomically speaking, compared to the 100 and more bacterial phyla existing on the planet Earth, only a few divisions have been identified in the human gut: Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Actinobacteria and Fusobacteria (Zoetendal *et al*., 2008). Furthermore, 99% of the identified species belong mainly to the two phyla Firmicutes and Bacteroidetes, representing together 70% of the total microbiota (Mariat *et al*., 2009).

The anaerobic bacteria exceed by two or three orders of magnitude the facultative anaerobic and aerobic bacteria (Harris *et al*., 1976). The similarity among individuals, observed at phylum taxonomic level, is lost considering the taxonomic level of species. Considering the gut microbiota at the species taxonomic level, we can observe a significant variation among individuals (Frank *et al.,* 2007), so that the microbiota composition could be compared to a fingerprint (Eckburg *et al.,* 2005). The diversity among individuals is easily understood if

we consider the myriad of factors influencing the composition of the intestinal microbial ecosystem. Host genetic background that through bacteria attachment sites exert an important role for the first colonizing bacteria (pioneer flora) arrive.

Pioneer flora in turn modulates host genes expression, influencing the successive microbial flora (Hooper *et al.*, 2001). Moreover environmental factors, such as age, diet, stress, drugs, will strongly influence the composition of the human microbiota (Rawls *et al.,* 2006). Both endogenous and exogenous factors will contribute to the microbiota composition. In order to verify the changes in microbiota composition in different geographical areas, the faecal microbiota of subjects belonging to different ethnic groups, states and continents were examined (Arumugam *et al*., 2011). The study (Arumugam *et al.*, 2011) highlighted that human gut flora can be classified into three different groups, named Enterotypes, based on variations of specific genera. Specifically: Enterotype 1, with a prevalence of the Bacteroides; Enterotype 2 with a prevalence of Prevotella; Enterotype 3 with a prevalence of Ruminococcus.

There would seem to be no relationship between Enterotype geographic area, sex, age or body mass index (Jeffery *et al*., 2012). Each Enterotype has unique properties, but still has a number of essential common functions, probably representing those necessary to survival in the intestinal habitat. Even if this finding needs to be confirmed, it implies that each Enterotype has different capabilities, and different metabolic responses to diet or medication, giving a reason why different persons exhibit different responses to medical treatments.

Complicated symbiotic relations were evolved between men and microbes. In general, we speak of co-evolution, co-adaptation, and co-dependency. The correct term to define the kind of relation between men and their microbiota is mutualistic (both, human and microbes, have their benefit). About 50 years ago, the ecologist *Theodor Rosebury* coined the term *Amphibiosis* (Blaser *et al*., 2006) to define the relationship between humans and microbes that could be beneficial or pathological, depending on the context in which it occurs. A community highly efficient in recovering energy from food may constitute a risk factor for obesity in a person with easy access to food, while it may be healthy in an individual with limited access to food.

The intestinal microbiota must be considered a real organ, with well-defined functions, composed of different cell lines (represented by the different microbes), that communicate with each other and with the host, consuming, preserving and redistributing the energy, operating physiologically important chemical transformations and able to maintain and repair itself by self-replication (Possemiers *et al.*, 2011). The functions carried out by the gut microbiota organ include: barrier *versus* hexogen microbes, structural and metabolic functions (Hooper *et al.,* 2001; Gill *et al.,* 2006), together with an important role in immune system development and activation (Kamada *et al.,* 2013; Belkaid *et al.,* 2014). Gut microbiota exerts a barrier function versus hexogen microbes by means of competition phenomena for nutrients and ecological niches (Buffie *et al.,* 2013), and production of antimicrobial substances (Alakomi *et al.,* 2000).

The development of the intestinal tract involves the formation of a surface area large enough to allow a blood supply appropriate for nutrient acquisition, containing a suitable number of attachment sites for microbes, and able to support the resident bacterial community. It must also be resistant to systemic translocation of foreign antigens and microbiota-derived catabolites. In addition, subsequent to an injury, it must be able to maintain its homeostasis and to restore itself. Several studies showed the strong influence of the microbiota in the development of the gastrointestinal tract.

Members of the microbiota are able to induce the transcription of the angiogenc-3 protein, with angiogenic activity (Hooper *et al.,* 2001); *B. thetaiotaomicron* strongly influences the transcription of host factors involved in the functionality of the enteric nervous system, suggesting that it may have a rule in the postnatal development of peristalsis (Hooper *et al.,* 2001). Gut microbiota contributes to the maintenance of the integrity of the intestinal epithelial barrier through the maintenance of cell-

cell junctions, and the promotion of epithelial repair after damage (Sekirov *et al.,* 2010). Gut microbiota intervenes in the structural development of the gastrointestinal tract and immune system (Kamada *et al.,* 2013). It is therefore conceivable that the composition of colonizing flora influences immune individual variations (Lee *et al.,* 2014).

Furthermore, several studies indicated the influence of microbiota on structural development and functioning, of organs outside the intestinal tract (Sommer *et al.,* 2013). Experiments conducted with germ-free mouse indicate that the microbiota influences the regulation of mood and behaviour, contributes to the pathophysiology of the humour disorders (Diaz *et al.,* 2011) and influences the development of the nervous system. Germ-free mouse showed deregulation in the hypothalamic-pituitary-adrenal axis (HPA) influencing the host response to stress, (Nobuyuki *et al.,* 2004), and a decreased perception of inflammatory pain.

Our distal intestine is comparable to an anaerobic bioreactor, housing most of our intestinal microorganisms and in which otherwise indigestible polysaccharides, including those derived from plants such as pectin, cellulose, hemicellulose, resistant starch are degraded (Sonnenburg *et al.,* 2006). Humans contain a very limited arsenal of enzymes necessary to digest common polysaccharides, which are provided by the microbiome (Sonnenburg *et al.,* 2006). Intestinal microbiota maximizes caloric availability of nutrients ingested by:

1) the extraction of additional calories from otherwise indigestible oligosaccharides;
2) the modulation of intestinal epithelium absorption capacity and nutrient metabolism (Hooper *et al.,* 2001), thereby promoting the absorption of nutrients and their use.

Moreover, the important role of microbiota in the metabolism of xenobiotic compounds, such as drugs administered for therapeutic purposes, should not be underestimated. Today pharmaco-genetic examination, essential for the production and administration of therapies, is a well accepted concept. This notion should be extended, and include drug metabolomics, which takes into account the contribution to drug metabolism of both the host and its microbiota (Nicholson *et al*., 2005).

MONITORING THE MICROBIOTA

A healthy intestinal microbial ecosystem is balanced but flexible enough to tolerate the intrusion of potential pathogens from hexogen flora (food, water, and various environmental components), normally contained in a regular flow condition. A healthy intestinal flora is essential to promote the health of the host, but the excessive growth of the bacterial population leads to a variety of harmful conditions. To avoid this outcome humans have implemented various strategies. The mucosal immune system must satisfy two functions:

1) be able to control the intestinal microbiota preventing overgrowth and translocation to systemic sites (Sekirov *et al*., 2010);
2) tolerate microbes, and prevent the induction of an excessive and injurious systemic immune response.

The excessive growth of the bacterial population and the penetration/translocation of the microbiota outside its luminal compartment, it is hindered in different ways as the production of secretory IgA (Macpherson *et al*., 2004), and antimicrobial peptides (AMP_S) (Cash *et al*., 2006; Ostaff *et al.*, 2013). Numerous antimicrobial peptides (AMP), (defensins, cathelicidins, lectins, etc.) and diverse groups of compounds, acting by breaking the surface structures of both commensal and pathogenic bacteria, are produced by mammalian gastrointestinal cells, and by various microbial species. Furthermore, several products of microbial metabolism have been shown to stimulate the host to produce several types of AMPs. The AMPs have a different spatial distribution throughout the gastrointestinal tract (GIT): the maximum antimicrobial activity level was found in the intestinal crypts and mucus layer, while at lumen level a reduced activity was found (Meyer-Hoffert *et al.,* 2008). The microbiota stimulates the host to produce AMPs, and produces AMPs itself. Lactobacillus and Bacillus, produce antimicrobial substances active against a wide range of entero-pathogenic bacteria, both Gram positive, and Gram negative bacteria (Liévin-Le Moal *et al.,* 2006). Lactobacillus and Bifidobacterium prevent Listeria infection of cultured epithelial cells (Sanz *et al*., 2007). Nevertheless, these antimi-

crobial substances often tend to show activity against bacterial groups similar to the bacteria producer, a strategy to keep potential competitors out of the niches occupied. A lower level of AMP transcripts might be induced by single bacterial species such as *B. thetaiotaomicron* (Cash *et al.*, 2006), but the presence of the entire microbial community is required to promote high and complete levels of expression. Moreover, the close contact of commensal bacteria with the intestinal epithelium seems to be a necessary condition for induction (Cash *et al.*, 2006). Also several microbial metabolites have been shown *in vitro* to induce the expression of AMPs. The short chain fatty acids and lithocholic acid have been shown to induce the expression of a cathelicidin LL-37 (Schauber *et al.*, 2003; Kida *et al.*, 2006; Termén *et al.*, 2008). So, the commensal bacteria and/or their structural components and metabolic products have the ability to induce the expression of AMP and to promote their activation. AMP induction can be mediated through different signalling pathways, reflecting the different nature of inductive stimuli. Finally, the microbiota monitors itself modulating the mucosae glycosylation (Hooper *et al.*, 2001), an important factor in the colonization of the GIT.

Antimicrobial activity peaks occurs in the intestinal crypts and in the mucus layer along the mucosa. Given their close proximity to the underlying mucosal immune system, these intestinal areas play a very important role in maintaining homeostasis. In healthy subjects, the intestinal epithelium is not strongly colonized and a lot of energy is spent both by the host and its microbiota in preventing colonization of this district. In a recent study, we demonstrated the presence of a predator bacterium, *Bdellovibrio bacteriovorus*, in human gut microbial ecosystem (Iebba *et al.*, 2014). Predation is an important mechanism in nature to keep bacterial populations under control.

B. bacteriovorus seem to be closely associated with the mucosa area of healthy subjects, in which it probably exerts control over the colonization of this site. *B. bacteriovorus* was not found in the highly colonized mucosa of inflammatory bowel diseases (IBD) and coeliac disease patients, in which an extensive microbial colonization occurs.

ECOLOGICAL CONSIDERATIONS

In order to focus on and understand the guidelines governing the stability of the microbial ecosystem we must go into a discipline called microbial ecology, studying the microorganisms, the interrelationships that exist among them and the specific environment where they live. Definitely we should consider ecological parameters in order to understand, and to interfere with the human microbial ecosystems. Martin and collaborators (Martin *et al.*, 2009) tried to explain the rules governing the stability of ecosystems with the "*Nash Equilibrium*". *Nash Equilibrium* defines an ecosystem in which none of the components of the ecosystem is advantaged by changing its strategy. The existing balance is a function of the cooperation among all the members of the ecosystem and individual success, based on the strategic choices of a species, depends on the choices of the others. In this context, there are rules and limits that strongly disadvantage the transgressors. Any form of life that is outside the rules, or deviates from equilibrium, inevitably is disadvantaged compared to others. Ecological theories indicate that "head to head" competition inevitably leads to the loss of some species, and the community will tend to be a monoculture composed of the winner (with a loss of biodiversity). The model defined by "Nash Equilibrium" allows the co-evolution of organisms in competition, that otherwise would destroy each other. This also supports the idea that it is the overall balance of the gut microbial community that must be considered in the healthy *status*. The structure of the microbial community is an important factor that can strongly influence an individual's susceptibility to specific diseases. The habitat where the microbial community resides must also be taken into account. In a "healthy" habitat, we find a high spatial and temporal heterogeneity. In order to loosen the competition among different strains and obtain a fully colonized healthy habitat, a high number of genomic variants will be needed. Each genomic variant will colonize a different niche. In a balanced ecosystem, all niches will be occupied, and the colonization of potential pathogens coming with the allochthonous flora becomes difficult unless they adopt mutu-

alistic strategies respecting the nature of the *Nash equilibrium*. Our microbial populations are subjected to two strong selective pressures. Pressure exerted by the microbiota itself, which tends to diversify microbial genomics in order to decrease the competition among them, and that exerted by the host that, on the contrary, tends to homogenize the genomes, promoting functional redundancy. In this way, the host ensures that functions exerted by the microbiota, important for his health, are not codified by a single species, but by more, evolutionarily distant, species. In this way the loss of one bacterial species does not correspond to the loss of the function achieved by the species lost. These two selective pressures coexist in a healthy ecosystem in perfect forces equilibrium: the overrunning of one of the two would inevitably lead to an imbalance in the ecosystem, favouring individual genomic diversity or functional redundancy. Ecological parameters computed by mathematical equations should be considered in the study of human microbial ecosystems. These parameters include Simpson biodiversity (Hsi), reflecting the number and relative abundances of the bacterial species within a sample; Simpson evenness (Esi), reflecting the distribution of species density within a sample; Carrying capacity (Rr), reflecting the carrying capacity of the habitat and richness of microbial community; the Gini coefficient of concentration (C), reflecting the variation of the bacterial population structure, and should be used to evaluate the healthy status of the microbial ecosystem (Marzorati *et al.,* 2008).

MICROBIAL TOLERANCE AND PHYSIOLOGICAL INFLAMMATION

In preventing an excessive response to myriad microbes, an important role is played by the intestinal epithelium, which reads and interprets signals from the luminal environment through receptor systems such as the "Toll-like receptors "(TLR), and "Nucleotide-Binding Oligomerization" (NODs) (Bertin *et al*., 1999; Inohara *et al*., 1999; Inohara *et al*., 2003; Kawai *et al*., 2010), and allows the underlying mucosal immune system to make a continuous sampling through its dendritic cells (Lavelle *et al*., 2010). Through the modulation of immune responses, we prevent an excessive immune response against bacteria of the intestinal microbiota. The recognition/binding of the host receptor proteins to specific microbial molecules or structures (PAMPs) stimulates a signalling cascade that ultimately involves the nuclear factor NF-kb (nuclear transcription factor) stimulating the expression of genes codifying pro-inflammatory molecules (Hayden *et al*., 2006). In a healthy host the continued activation of inflammation, named "physiological inflammation", is strictly controlled by specific mechanisms that allow a constricted regulation of the pro-inflammatory signal, and the maintenance of homeostasis (Cario *et al.,* 2002). The failure of this control mechanism could lead to a persistent inflammatory state (Haller, 2006).

The microbiota contributes to the control of the immune response (homeostasis). Under the continuous stimulation of the various components of the commensal bacteria, a reduced expression of TLRs in the intestinal epithelium and a low production of inflammatory cytokines occurs (Cebra, 1999). Furthermore, a strategic distribution of the receptor systems is else important for the discrimination between pathogenic and commensal (Neish, 2009). Important for homeostasis is the site at which the bacterial ligands interact with the receptor systems. When the interaction takes place in areas where the host/bacteria coexistence is not expected, microbes are sensed as pathogens and consequently an adequate inflammatory response is induced.

EUBIOSIS AND DYSBIOSIS

The composition of our microbiota is influenced by host genotype, environment and diet. Signalling molecules and metabolic products of the microbiota influence several intestinal functions: visceral-sensing, motility, digestion, permeability secretion, energy harvest, mucosal immunity, and barrier effect (Montalto *et al*., 2009). Furthermore, the components of the microbiota and/or the microbiome may enter the circulation and be transported to various organs affecting their functionality: brain (cognitive functions), liver (lipid and drug metabo-

lism), and pancreas (glucose metabolism), (Korecka *et al*., 2012). The awareness that bacterial structural components and/or metabolites is sufficient to induce the development and maturations of organs, and physiological processes in the host, makes us realize the importance of the microbial ecosystem in maintaining a healthy status. The intestinal microbial ecosystem balance, called eubiosis, is a fundamental concept. As early as 400 B.C. Hippocrates said "death is in the bowels" and "poor digestion is the origin of all evil". Ali Metchnikoff, who lived from 1845 to 1916, suggested that most disease begins in the digestive tract when the "good" bacteria are no longer able to control the "bad" ones. He called this condition dysbiosis, meaning an ecosystem where bacteria no longer live together in mutual harmony. A gut microbiota in a eubiotic *status* is characterized by a preponderance of potentially beneficial species, belonging mainly to the two bacterial phylum Firmicutes and Bacteroides, while potentially pathogenic species, such as that belonging to the phyla Proteobacteria (Enterobacteriaceae) are present, but in a very low percentage. In case of dysbiosis "good bacteria" no longer control the "bad bacteria" which take over (Zhang *et al*., 2015).

THE PHYSIOLOGICAL IMPORTANCE OF THE "EUBIOTIC STATUS"

The factors that can disturb the balance of intestinal microbiota include: lifestyle, antibiotic treatments and pathogens. The importance of maintaining a eubiotic condition in the intestinal microbial ecosystem is quickly highlighted when we look at some of the deleterious *sequelae* after antibiotic treatment (Sekirov *et al*., 2010). The main consequence of antibiotic treatment is the disruption of the ecosystem balance, leading to antibiotic-associated diarrhoea. The aetiopathology of diarrhoea may be due to the pathological proliferation of opportunistic pathogens of the endogenous microbiota, such as *Clostridium difficile* (McFarland, 2008) and vancomycin-resistant enterococci (Crouzet *et al.,* 2015). Moreover, after antibiotic treatment patients are more receptive to infection sustained by hexogen pathogens, due to the loss of microbiota integrity and barrier function. We can emphasize that both opportunistic and exogenous pathogens benefit from the dysbiosis *status*. Additionally, it should be highlighted that the host response to exogenous infectious agents amplifies/ promotes a dysbiosis *status*. The host responses include inflammation induction, leading to an alteration of the intestinal nutritional environment, and often to a secretory diarrhoea, having strong effects on the microbiota ecosystem. Under an inflammatory condition, we can observe an unexpected decrease in the vitality of the intestinal microbiota, enhancing the availability of ecological niches for pathogen colonization. Furthermore, substances such as nitrate, *S*-oxides, and *N*-oxides are generated as by-products of inflammation. Such compounds can represent a growth advantage for potentially pathogenic species, such as the member of the Enterobacteriaceae family, in particularly *Escherichia coli*, as demonstrated in the experiments carried out in mouse (Winter *et al*., 2013). Facultative aerobe-anaerobe Gram-negative bacteria are usually present in low loads in the microbiota of healthy subjects, where strictly anaerobe bacteria are predominant. The presence of by-products generated by the inflammatory response promotes the growth of aerobic-anaerobic facultative bacteria able to use by-products as terminal electron acceptors for anaerobic respiration (Winter *et al*., 2013). Finally, diarrhoea leads to instability of the indigenous microbial population. Destabilization of the resident microbiota, resulting from an intestinal infection, has a negative impact both on its protective and immune-modulatory functions, predisposing the host to more unpleasant infectious *sequelae*. Moreover, malfunction of the microbiota "organ" could have a negative impact even in distant organs.

The incidence, morbidity, mortality and costs related to diseases such as *Clostridium difficile* (CD) infection have been increasing in the last decade, and CD infection is currently one of the most common nosocomial infections in the West (1-2-3). CD infection has a high incidence of relapse ranging from 15 to 26% of patients (Pépin *et al*., 2006; Musher *et al*., 2005). Generally, disease recurrences are treated with repeated cycles of vancomycin, with an esti-

mated therapy efficacy at the first administration of about 60%, which is radically reduced in patients with multiple recurrences (Walters *et al*., 1983; Kelly *et al*., 2008). At present, there are no standardized treatments, and there is a gap in the management of patients with multiple recurrences (Cohen *et al*., 2010). Considering these therapeutic limitations, the technique of faecal microbiota transplantation has been proposed in recent years in the treatment not only of CD recurrence (Gough *et al*., 2011; Kassam *et al*., 2013; Van Nood *et al*., 2013), but also of severe recurrent infections (Brandt *et al*., 2012; Gallegos-Orozco *et al*., 2012; Neemann *et al*., 2012). There are many cases of patients with recurrent CD infections that have been treated with transplantation of faecal microbiota, and the percentage of efficacy has been greater than 90% (Guo *et al*., 2012). The faecal samples to be transplanted were obtained from healthy donors, preferring subjects closely related to the patient. The astonishing success achieved with this therapeutic strategy, an alternative to antibiotics, clearly shows the importance of a healthy/eubiotic microbial ecosystem for defence against infections. Furthermore, studies designed to evaluate the possible use of the faecal transplant are being undertaken in patients suffering from obesity, inflammatory bowel diseases, or liver diseases. In all these pathologies a dysbiotic status of the intestinal microbiota was proved (De Palma *et al.,* 2010; Schippa S. *et al.* 2010; Parekh *et al.,* 2015). Today, the condition of dysbiosis has been associated with important diseases. The list of disorders related to the intestinal microbiota is growing daily: pathologies are usually complex and multifactorial in terms of both pathogenesis and complications.

ESCHERICHIA COLI AND *FAECALIBACTERIUM PRAUSNITZII*: THE DYSBIOTIC INDEX

Facultative anaerobic bacterial species, such as *Escherichia coli,* prevail over an inflamed intestine because unlike anaerobic bacteria, they can use the by-products of inflammation as terminal electron acceptors (Winter *et al*., 2013). Studies carried out with adult and paediatric IBD patients have reported that mucosa-associated microbiota is significantly increased particularly in the Enterobacteriaceae family, such as *Escherichia coli* (Conte *et al*., 2006; Martinez-Medina *et al.,* 2014.). An *E. coli* increase has also been observed in other pathologies such as coeliac disease and cystic fibrosis (Schippa *et al*., 2010). Genomic characterization of mucosa-associated *E. coli* strains isolated from bioptic samples collected from ulcerative colitis, Crohn's disease (CD) patients, and healthy subjects, showed the association among particular of *E. coli* genomic variants to different type of IBD, and healthy controls (Schippa *et al*., 2009). Characterization of *E. coli* strains isolated from the mucosa of adult (CD) patients, led to the identification of a new pathotype within the species, named: "adherent-invasive *Escherichia coli* "(AIEC) (Darfeuille-Michaud, 2002). These strains act like entero-invasive pathogens, but with specific characteristics that differ from other subtypes of entero-invasive *E. coli*. The prototype strain, the first to be isolated and the most studied is AIEC LF82, isolated from ileum chronic lesions of adult CD patients (Barnich, *et al*., 2007). The AIEC strains were isolated in 60% of adult patients with CD and in 16.7% of control subjects (Darfeuille-Michaud *et al*., 2004). This clearly indicates that the *E. coli* genomic variant, resembling AIEC strains, can be normally preset in a healthy subject, in a lower relative abundance. Such variant can represent pathobiont strains (Iebba *et al*., 2012; Schippa *et al*., 2012), whose growth is favoured in an inflamed habitat. A recent study on the characterization of *E. coli* strains isolated from CD paediatric patients indicated the presence of AIEC strains in both CD and non-IBD controls, confirming the "pathobiont" nature of AIEC strains. The AIEC-like isolates were more abundant in CD patients indicating the positive selection of this variant in genetic predisposed subjects (Conte *et al.,* 2014).

When dysbiosis occurs in addition to a significant increase in *E.* coli, a decrease of beneficial species is reported too. Among beneficial species, the relative abundance of the obligate anaerobe *Faecalibacterium prausnitzii*, a butyrate producer defined as an anti-inflammatory bacterium, is reported to be significantly reduced (Sokol *et al*., 2008). The decrease in relative abundance of *F. prausnitzii*, has been observed

not only in IBD patients, but also in patients with colorectal cancer, cystic fibrosis, and elderly or obese subjects (Miquel *et al*., 2013). The ratio of the relative abundances of *F. prausnitzii*/*E. coli* is currently used to evaluate the dysbiosis status (Lopez-Siles *et al*., 2014). However, it should be noted that the decrease of the relative abundance of *F. prausnitzii* in adult CD patients is not observed in paediatric CD patients where a significant increase has been reported (Cao *et al*., 2014). This apparent contradiction could be explained as a response of the microbiota to contrast the inflammation,-increasing/favouring the growth of anti-inflammatory species. The disease progression and persistence of inflammation drastically changes intestinal habitat conditions, shaping an environment no longer favouring *F. prausnitzii* growth (Duboc *et al.*, 2013).

However, we should not forget that it is the overall composition of the intestinal microbiota rather than the presence of single species that is relevant to the health or disease status of the host. Particular genomic variants could represent an additional risk factor increasing the mucosa damage and the severity of diseases such as IBD. The importance of the overall composition of the intestinal microbiota can be appreciated observing the total recovery that has occurred in patients undergoing faecal transplantation performed to restore a healthy community in subjects with *C. difficile* diarrhoea, where antibiotic therapies had failed (Cammarota *et al.*, 2014; Kronman *et al*., 2014).

HUMAN VIROME

Finally, we should always remember that the microbial ecosystems contain not only bacteria, but also viruses, bacteriophages and mycetes that strongly contribute to the maintenance of the balance. Metagenomic analysis demonstrated that a large community of viruses, most of which are unique to each individual, regularly colonize our body (Minot, *et al.*, 2013). The human viruses group includes both eukaryotic and prokaryotic viruses. The viruses infecting eukaryotes have important effects on human health, whereas prokaryotes infecting viruses can also affect human health by impacting bacterial community structure and function. Therefore, the definition of the virome is an important step to understand how microbe networks influence human health or disease (Wylie *et al.*, 2012). Further work is necessary to appreciate the virome effects on human health, immunity, and response to co-infections. It was recently reported that: "Influenza virus infection of intestinal cells raises the exposure of galactose and mannose residues on the cell surface, significantly increasing bacterial adhesiveness, independently of their own adhesive ability, indicating that influenza virus infection, could constitute an additional risk factor" (Aleandri *et al.*, 2015).

CONCLUSION

From this brief discussion, it is clear that it is important to have a holistic vision of our microbial ecosystem that takes into account all the components of the community. The mammal's intestine is a complex and rich ecosystem that provides multiple levels of intercellular signalling among:

1) the microbiota components,
2) the microbiota and the host,
3) the microbiota and hexogen pathogens,
4) the host and hexogen pathogens.

Understanding the communication and the pathways involved in these interactions is essential to improve our knowledge of human physiology, and our ability to treat or prevent pathophysiological processes. The intestinal microbiota influences the host at every level, with an incredible adaptation capability to our lifestyles. We should not underestimate the effects/consequences of our behaviour on our microbial ecosystem, inevitably having an impact on our health.

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All the authors of the manuscript declare that they have no conflict of interest in connection with this paper.

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