

Review Article

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The Microbiome: Evolutionary Perspective and Symbiogenesis

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Abstract: The main function of the Immune System (IS) in metazoans is to protect them against invading microorganisms, which are considered from this perspective as competitors that reduce the availability of resources, cause tissue damage and essentially threaten their adaptability. This relationship is seen as a low intensity perpetual armed war, related to the hypothesis of the “Red Queen”. This view suggests that the metazoans’ IS has evolved under a selective pressure imposed by microorganisms of the microbiota, that whether infectious or not, have co-evolved with the host’s IS in an evolutionary symbiogenesis, with reciprocal interactions that have developed local immunity. The microbiota changes with age and communicates with the brain. The above mentioned implies that we are witnessing the birth of a new scientific discipline that could be termed microbial anthropology, and several perspectives that includes the change of our perception of health and established the fundamentals for the use of microorganisms as therapeutic agents. In order for these therapies to succeed considerable information must be available about our microbiota, such as changes during pregnancy, the effects of antibiotic use, new methods for in vitro cultivation and the host-commensal dynamics.

Keywords: innate immune response; microbiome; symbiogenesis; evolutionary perspective

1 Introduction

From a neo-Darwinian point of view, the main function of the Immune System (IS) in metazoans is to protect them against invading microorganisms, which are considered from this perspective as competitors that reduce the availability of resources, cause tissue damage and essentially threaten their adaptability. Hence, this relationship is seen as a low intensity perpetual armed war, related to the hypothesis of the unforgiving “Red Queen”. This view suggests that the metazoans’ IS has evolved under a selective pressure imposed by microorganisms. Numerous observations of this paradigm suggest that whether infectious or not, microorganisms have co-evolved with the host’s IS in an evolutionary symbiogenesis [1] (Figure 1).

This interaction with the IS starts with Innate Immunity (II), which recognizes the associated molecular patterns (AMPs) on bacteria and identifies them using pattern recognition receptors (PRRs) in the cells of their hosts. Some representative examples of AMPs are the lipopolysaccharides (LPS) in Gram-negative bacteria, the glycolipids in mycobacteria, lipoteichoic acid in Gram-positive bacteria, the mannans in yeast, and the RNA in viruses.

When referring to these same molecular arrangements in pathogen bacteria, a “P” is added at the beginning in the acronym: PAMPs. Once the PAMPs are recognized, the mammal host produces a large array of molecules to stop the infection, such as collectins and antimicrobial peptides as part of the II. These two act together with the molecules of effector cells in charge of the Adaptive Immune Response (AIR) [2].

The above mentioned is discussed more extensively in “The Danger Model: a renewed sense of self”, which proposes that immunity is based, rather than in the recognition of self from non-self, in the idea of a system that responds to harmful entities, whether own or strange, and is activated by alarm signals triggered by danger. Matzinger [3] describes the previous models. Burnet’s model was the first one and it was based in the principle that the

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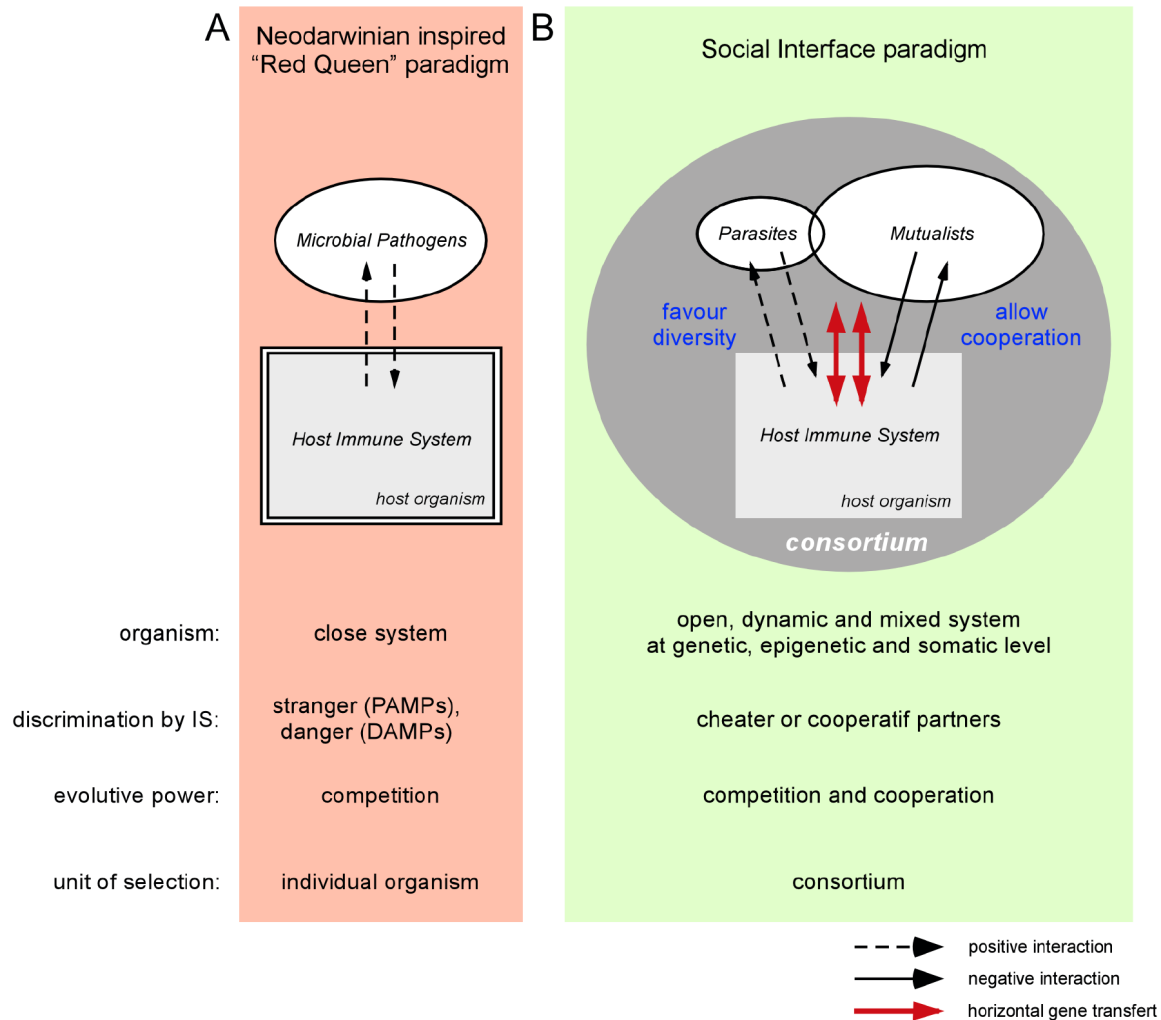


Figure 1 The relationship between the metazoan immune system and the microbial world. **A)** In the neo-Darwinian-inspired Red Queen paradigm, the immune system (IS) fights microbial pathogens and protects the integrity of the host organism. Pathogens are identified by the presence of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). **B)** In the social interface paradigm, the IS allows for the establishment of a symbiotic relationship between the host, parasites, and the microbiota. Discrimination is based on a cheater or cooperative profile. In this new paradigm, the organism loses its unicity and strict boundaries, becoming an open, dynamic, and mixed consortium [1].

Immune System differentiated self from non-self. However, this explanation left many issues unanswered and was considered a fairly simple model with a reductionist and easy solution. Then came the non-self- infectious model, proposing that the IS attacked anything foreign that produced infection. This model did not take into account cases of autoimmune diseases or tumors. Next was the Danger Model, stating that the immune system attacks everything own or foreign whether infectious or not, but capable of causing some kind of damage. This model is the most complete so far as it answers a higher number of previously unanswered questions. For example, it can explain immune response in such cases as transplants, pregnancies, tumors, and autoimmune diseases [3].

Today, the Immune Response is considered to be a part of the inflammatory process. Though inflammation has been conceived for a long time as a beneficial physiological process, it also has a dark side. It fosters the protection of infections and malignant tissue regeneration. Furthermore, under conditions associated with chronic inflammatory processes, it is known to be a driving force behind diseases such as Alzheimer's, diabetes, obesity, cancer, and atherothrombosis among others. In cancers, inflammation serves to feed tumors thus allowing their growth. In these cases, if inflammation could be prevented, blood flow would be reduced and the nutrient supply to the tumor would be eliminated [4].

2 Reciprocal interactions and local immunity

The IS interacts not only with microbial pathogens, but also with mutualist non-pathogenic microbes from the microbiota (Figure 1). This relationship (particularly in mammals, but in reality within all living beings) can be illustrated by the reciprocal interactions between the intestinal microbiota and the immune system of the human body. There are about 100 trillion bacteria living in our intestines without causing disease, and which on the contrary, facilitate its functions providing aid in the decomposition of fiber, amino acids and drugs. There is a metabolic interdependence between the gastrointestinal system and the bacteria it hosts. Their genes and ours together make up a meta-genome that keeps the body functioning at optimal condition. This microbiota and the gastrointestinal microbiome, integrate the genomic assembly of all bacteria living in our bodies. Permanent interaction with them helps us keep our IS active and ready for pathogen invasions. However, this mutualistic relationship that keeps us healthy also makes us dependent on them, and their absence can cause dysfunctions and illness [5]. Moreover, the richer the bacterial microbiome, the less prone the host will be to chronic disease [6,7].

For other tissues, such as skin, the control of immunity compartmentalization performed by resident commensals (similar to those in the intestine) is necessary for a proper innate immune response to take place. The skin microbiota is also key in preventing inflammation and promoting the fine adjustment of T resident lymphocytes. Studies on pathogen-free mice have demonstrated that the microbiota of the skin is essential to prevent infection. Similarly, the intestinal microbiota has been linked to the proper functioning of the immune system and the intestinal tract, however it has also been associated with the promotion of systemic inflammation that can translate into disease. The local nature of the microbiota is fundamental to understand that its relationship with the IS is compartmentalized. Both in skin and intestine, the balance between cytokines and T lymphocytes is closely linked to the signaling of commensals. In the skin, the absence of commensals alters local cytokine production such as IL-1, and this in turn promotes local effector responses of inflammation. The lack of these microbes is also the cause of diseases such as psoriasis, arthritis, and atopic dermatitis. It is important to point out that the skin innate response is completely independent of what takes place inside the intestine, no matter how similar the mechanisms may be [8].

Back to the intestine, the microbiota and the immune system act together in a symbiogenic fashion to form an antipathogenic barrier. Here, microbes compete for space and the displacement of the beneficial ones may end up causing an illness in the host. It is believed that the IS actually favors the adoption of certain microbiota which gives vertebrates the advantages of an enhanced metabolic (and immune) performance. Studies of the intestinal epithelium reveal the presence of beneficial bacteria in the outer and inner linings. Many of them, such as *Bifidobacterium* and *Bacteroides*, produce substances that are toxic to some pathogenic bacteria, but they also produce nutrients for colonocytes. In this context, *Bacteroides* and *Lactobacillus* spp. are important in reducing inflammation by inhibiting the activation of the classical NF- κ B pathway. The colonic mucous membrane favors colonization by *Bacteroides fragilis* and this facilitates the anti-inflammatory processes.

Nevertheless, the complex interactions between the II and AIR of the organism and the microbiota also include examples that are not beneficial at all. Some of the intestinal innate lymphoid cells (ILC) have an aryl hydrocarbon receptor (AhR) which detects microbiota metabolites and xenobiotics. This receptor is required both for maintaining the ILC and for the production of Interleukin-22 (IL-22). Furthermore, macrophages and dendritic cells in the intestine produce Interleukin-23 (IL-23) and Interleukin-17 (IL-17), in response to those metabolites of the microbiota. These two interleukins stimulate the release of antimicrobial peptides that inhibit or kill pathogenic and opportunistic bacteria in the vicinity of intestinal surface epithelial cells. Another important interleukin is the IL-10, which allows the immune system to tolerate the microbiota. Its absence causes inflammation, and there are also a variety of polymorphisms of the IL-10 that may cause different susceptibilities in humans. Minor constituents of the microbiota can amplify intestinal TH17 cell numbers. That is the case of *Clostridia* sp., *Candidatus arthromitus* or segmented filamentous bacteria (SFB). This bacterium populates the ileum and caecum and has long been known to be a potent activator of intestinal immune responses. Induction of TH17 cells by SFB provides protection against gut pathogens. However, TH17 induction by SFB is not entirely benign, because mono-association of intestinal mice with SFB induces TH17-mediated inflammatory arthritis and multiple-sclerosis-like symptoms in the experimental autoimmune encephalomyelitis (EAE) model. Remarkably, however, sensing of a single constituent of the intestinal microbiota can promote autoimmunity in extraintestinal tissues. At present, it is unclear whether SFB, or related organisms, exert similar effects in humans [9].

As illustrated by Figure 2, the adaptive-innate-epithelial barrier protects from microbial antigens, from the microbiota. The intestinal epithelial cells (IECs) secrete mucins and antimicrobial peptides that limit the interaction of microbes with epithelial cells. In eubiotic healthy conditions, PAMPs stimulate secretion of cytokines including thymic stromal lymphopoietin (TSLP), IL-33, IL-25 and transforming growth factor- β (TGF- β) by intestinal epithelial cells (IECs) that promote the development of macrophages and dendritic cells. Dendritic cells, in turn, foster the development of induced

Treg cells (iTreg) via a TGF β and retinoic acid (RA). The anti-inflammatory bowel balance is maintained by inhibiting or dampening potential effector responses, through multiple mechanisms, including TGF- β and IL-10 secretion by the iTreg cells and IL-10 secretion by macrophages. In addition, Treg cells derived from TGF- β , epithelial derived B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) promote development of IgA⁺ plasma cells ensuring a plentiful supply of IgAs in the lumen further limiting microbial interaction with the epithelium.

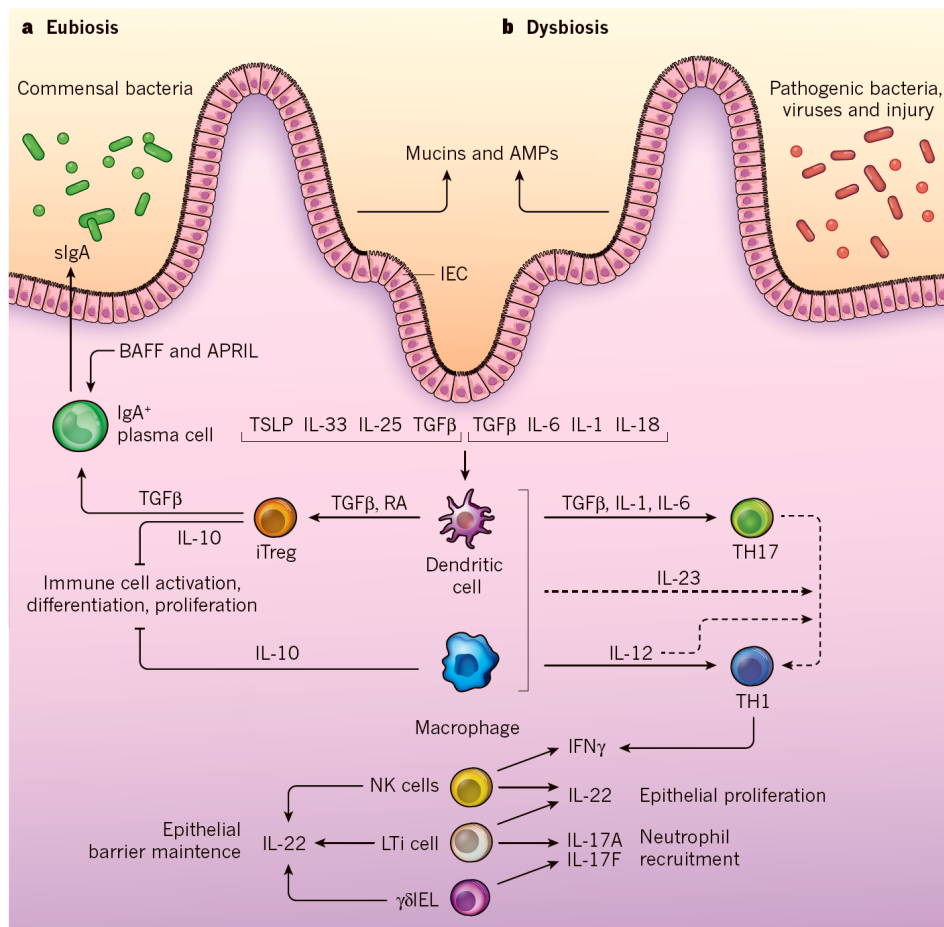


Figure 2 Adaptive-innate-epithelial barrier in response to microbial antigens. **A)** In response to the microbiota, the intestinal epithelial cells (IECs) secrete mucins and antimicrobial peptides that limit the interaction of microbes with epithelial cells. In eubiotic conditions, PAMPs stimulate secretion of cytokines including thymic stromal lymphopoietin (TSLP), IL-33, IL-25 and transforming growth factor- β (TGF- β) by IECs that promote development of macrophages and dendritic cells. Dendritic cells, in turn, foster the development of induced T regulatory cells (iTreg) via a TGF β and retinoic acid (RA). The anti-inflammatory bowel balance is maintained by inhibiting or dampening potential effector responses, through multiple mechanisms, including TGF- β and IL-10 secretion by the iTreg cells and IL-10 secretion by macrophages. In addition, Treg cells derived from TGF- β and epithelial derived B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) promote development of IgA⁺ plasma cells ensuring a plentiful supply of IgAs in the lumen further limiting microbial interaction with the epithelium. **B)** In a pathogenic invasion or mucous membrane injury or dysbiosis, PAMPs stimulate the secretion of pro-inflammatory cytokines by the IECs (including, TGF β , IL-6, IL-1 and IL-18) and intestinal dendritic cells and by the macrophages (including TGF β , IL-6, IL-23 and IL-12) to induce development of TH1 and TH17 cells, the latter of which can transition into the former as a result of IL-23 or IL-12 signaling. Intestinal innate lymphoid cells, including NK cells, lymphoid tissue inducer (LTi) cells, and $\gamma\delta$ IELs, respond to pro inflammatory cytokines to upregulate IL-22, which helps protect the epithelial barrier, and IL-17A and IL-17F, which are involved in neutrophil recruitment [12].

In a pathogenic invasion with mucous membrane injury or dysbiosis, PAMPs stimulate the secretion of pro-inflammatory cytokines by the IECs (including, IL-6, IL-1 and IL-18). Intestinal dendritic cells and macrophages secrete IL-6, IL-23 and IL-12 to induce the development of TH1 and TH17 cells, the latter of which can transition into the former as a result of IL-23 or IL-12 signaling. Intestinal innate lymphoid cells, including NK cells [10], lymphoid tissue inducer (LTi) cells, and $\gamma\delta$ T cells, respond to pro-inflammatory cytokines to upregulate IL-22, which helps protect the epithelial barrier, and IL-17A and IL-17F, which are involved in neutrophil recruitment [9].

3 The microbiota change with age

The microbiota of human beings changes throughout life depending on the lifestyle, antibiotic use and diet. Different types of bacteria can be determined for every age group. Furthermore, if an individual is born by vaginal delivery, *Lactobacillus*, *Prerotella* and *Atopobium* become established in his bowels and if born by cesarean section, then *Staphylococcus* will settle in instead [11]. The microbiota is very important for the baby during breastfeeding, as it is still finishing the integration of its own immune system and its lymphoid tissues require signals from the microbiota for full development. Until that happens, the mother transmits IgA passively for baby's protection [12]. As the individual grows, aerobic bacteria are replaced by anaerobic. Adolescence is associated with *Bifidobacterium* and *Clostridium* sp. and in adulthood *Bifidobacteria* decrease while facultative anaerobes and *Firmicutes* increase. Recent studies have revealed that the absence of some groups of bacteria that perform important functions in the body can increase the risk of disease. For example, the absence of *Lactobacillus* and *Bacteroides* can cause inflammatory bowel disease; the lack of *Clostridia*, *Eubacteria*, and *Roseburia* has inflammatory consequences; and the absence of *Bacteroides*, *Eubacterium*, *Clostridium*, *Lactobacillus* and *Escherichia* generates problems for the absorption of nutrients [11]. During pregnancy, the interactions between the host and his microbiota undergo considerable metabolic changes beneficial to the mother and her product especially between the first and third trimester. The intestinal microbiota is profoundly altered during this period regardless of the culture or ethnicity of the mother. During the first quarter the microbiota is similar to that of healthy non-pregnant women, but in the third quarter the levels of *Proteobacteria* and *Actinobacteria* increase while there is an overall reduction in bacterial diversity.

In this quarter the bacterial population causes reduction of insulin sensitivity, inflammation and weight gain; these changes help at the time of delivery, supporting the growth of the fetus and preparing the body for breastfeeding [12].

Family members normally share the same type of intestinal microbiota. In the cases of families of obese individuals, *Bacteroides* are found in reduced numbers while *Firmicutes* are more abundant. This causes an inhibition of the lipoprotein lipase enzyme and increases lipogenesis. In contrast, families of slim individuals, have more *Bacteroides* and less *Firmicutes*, which enhances fatty acid oxidation (See Figure 3).

A high-fat diet increases the density of Gram-negative microorganisms which will raise the concentration of lipopolysaccharides (LPS), triggering inflammation (one of the factors of obesity). This can be reversed with probiotics, foods that increase the numbers of Gram-positive bacteria. It has been observed that the intestinal microbiota regulates a multitude of metabolic reactions of the host. Some of the most important metabolites produced by these microbes are the short-chain fatty acids (SCFAs), essential for the host. They function as substrates for oxidation and lipid synthesis, as a source of energy, stimulate the nervous system, modulate intestinal motility during transit, and foster the proliferation of epithelial cells [11].

Diet can affect the richness of microbial genes and the metabolic genetic profiles of the individual. These genetic phenotypes may predict the effectiveness of a certain diet in inflammatory variables of overweight or obese individuals. The richness of microbial genes is associated with low levels of adiposity, cholesterol and inflammation [12]. A diet rich in prebiotics that generate bacteria, such as *Clostridium*, increases the number of Treg lymphocytes which produce anti-inflammatory cytokines such as TGF- β and interleukin 10. A close relationship has been recently discovered between bacteria such as *Clostridium* and an important anti-inflammatory mechanism in the intestine [13].

4 The microbiota communicates with the brain

The immune system forms a loop with the nervous system. It starts the signal for action potentials traveling to the sensory arc and receives in return orders coming from the motor arc [14].

The intestine communicates with the brain through the gut-brain axis, a network involving the CNS and the IS. Signals run along this axis in a bidirectional way carrying homeostatic messages originated by hormonal, neural

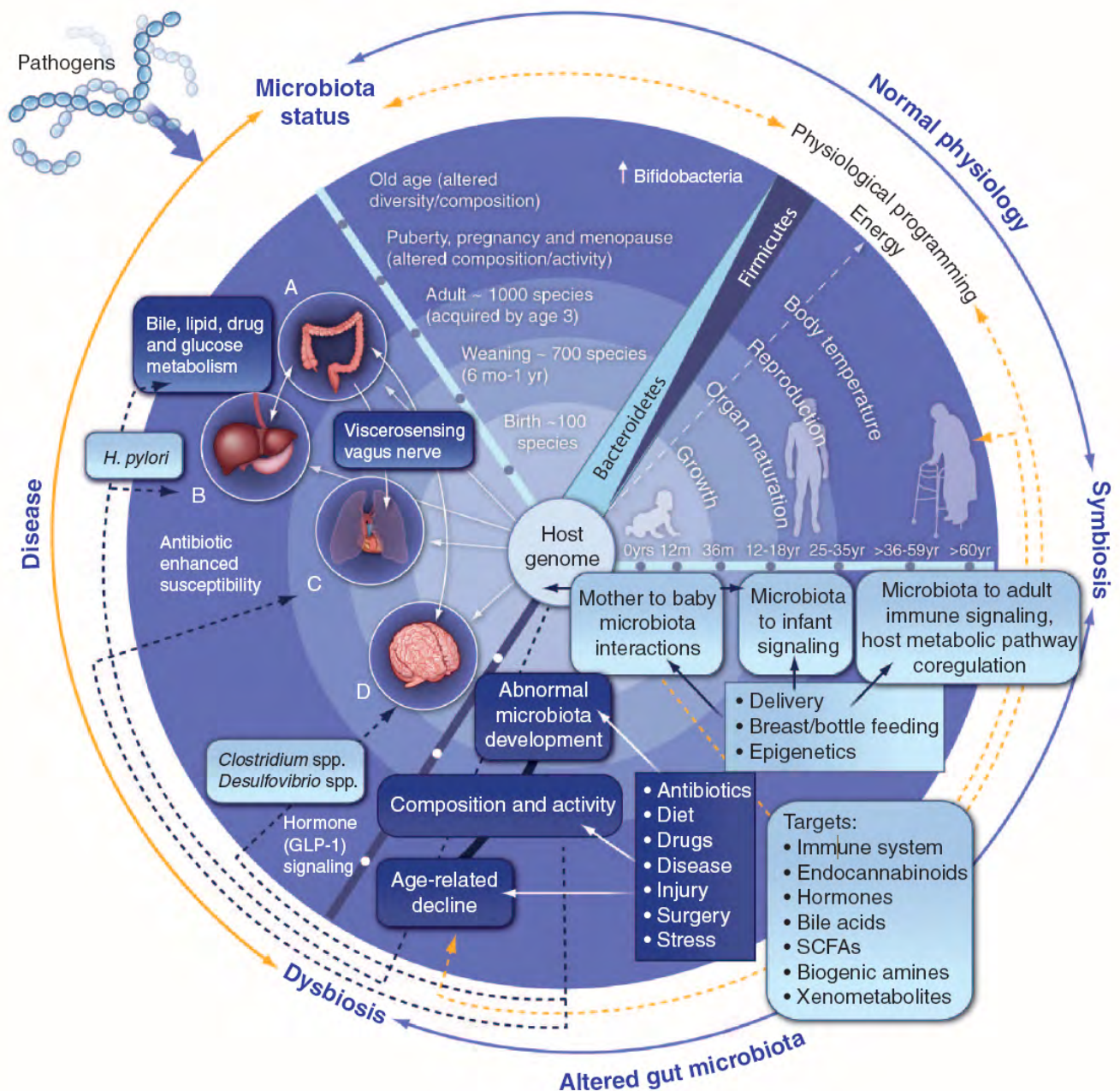


Figure 3 The intestinal microbiota in development and disease. The influence of the gut microbiota on human health is continuous from birth to old age. The maternal microbiota may influence both the intrauterine environment, and the postnatal health of the fetus. At birth, about 100 microbial species populate the colon. Early environmental factors (e.g., method of delivery), nutritional factors (e.g., breast or bottlefeeding), and epigenetic factors have been implicated in the development of a healthy gut and its microbial symbionts. Changes in gut microbial composition in early life can influence the risk of developing diseases later in life. During suckling, the microbial community develops rapidly; shifts in microbial diversity occur throughout childhood and adult life; and in old age, there is a decrease in the Bacteroidetes and an increase in Firmicutes species. The gut microbiota is important for maintaining normal physiology and energy production throughout life. Body temperature regulation, reproduction, and tissue growth are energy-dependent processes that may rely in part on gut microbial energy production. Extrinsic environmental factors (such as antibiotic use, diet, stress, disease, and injury) and the mammalian host genome continually influence the diversity and function of the gut microbiota with implications for human health. Disruption of the gut microbiota (dysbiosis) can lead to a variety of different diseases, including **A**) inflammatory bowel disease, colon cancer, and irritable bowel syndrome; **B**) gastric ulcers, nonalcoholic fatty liver disease, and obesity and metabolic syndromes; **C**) asthma, atopy, and hypertension; and **D**) mood and behavior through hormone signaling (e.g., GLP-1). The gut microbiota is also important for drug metabolism and preventing the establishment of pathogenic microbes [11].

and immunological stimuli. The microbiota modulates this mechanism (Figure 4) producing metabolites capable of altering the functions of the CNS and the IS. It has been observed that the microbiota in the human intestine has an important role in the regulation of anxiety, depression, pain, mood and cognition. Examples of some bacteria that produce neurotransmitters and neuromodulators are *Escherichia* sp., *Bacillus* sp., and *Saccharomyces*

sp. (noradrenaline), *Candida* sp., *Streptococcus* sp., *Escherichia* sp. and *Enterococcus* sp. (serotonin), *Bacillus* sp. (dopamine), and *Lactobacillus* sp. (acetylcholine).

Stress can increase inflammation and bacterial infections are associated with increases in anxiety. All this could be solved with probiotics, which have an important role in reducing stress and anxiety, modulating brain function and behavior, and even in improving mood [15].

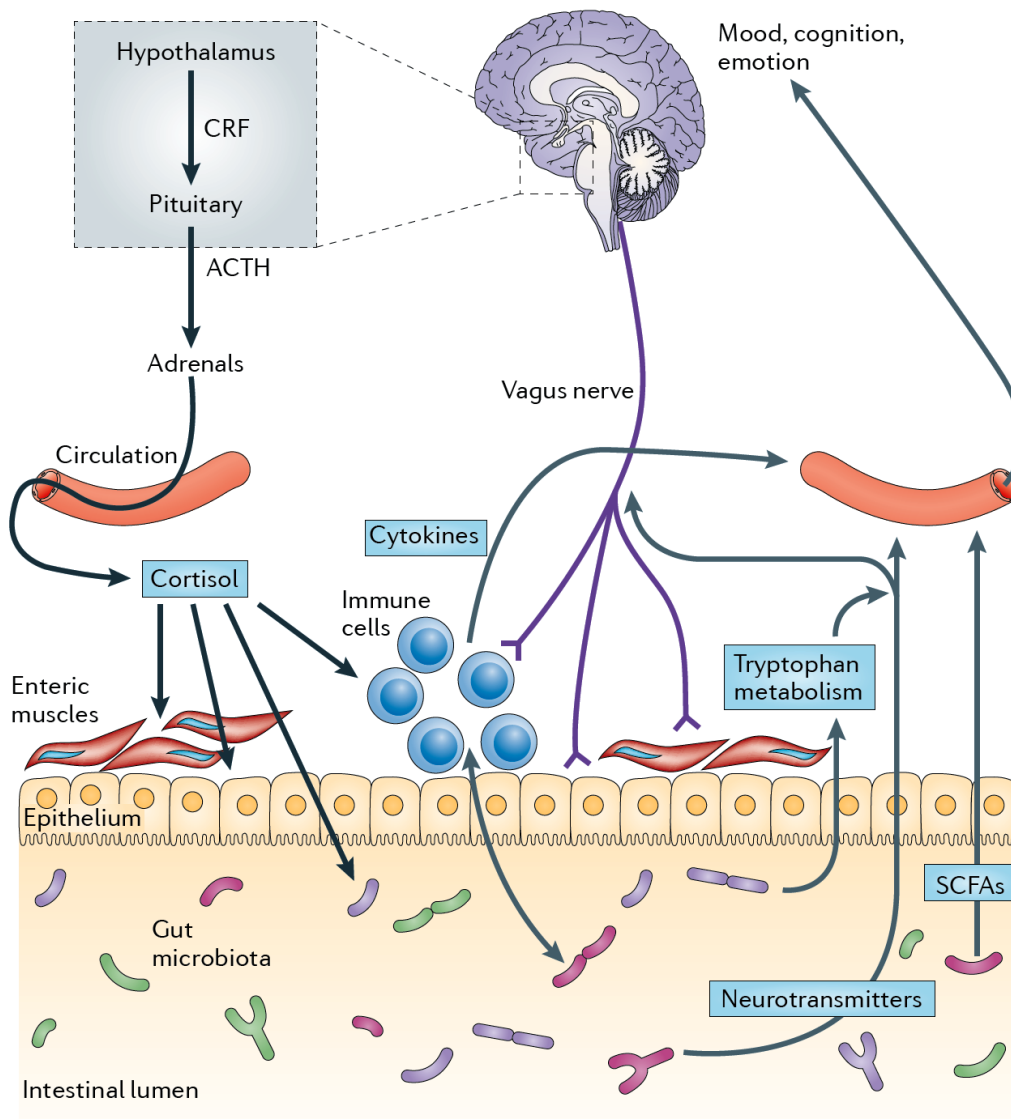


Figure 4 Bidirectional communication pathways between the intestinal microbiota and the brain. There are multiple potential direct and indirect pathways for the intestinal microbiota to send information through the gut-brain axis. These can be the endocrine (cortisol), immune (cytokines) and neural (vagal and enteric nervous system). The brain uses these same mechanisms to influence the composition of the intestinal microbiota, for example, under conditions of stress. The hypothalamus-pituitary-adrenal axis regulates the secretion of cortisol which can affect immune cells (including cytokine secretion), both locally in the gut and systemically. Cortisol can also alter the permeability of the gut, the barrier function and the composition of the intestinal microbiota. Conversely, the intestinal microbiota and probiotic agents can alter the levels of circulating cytokines and this can have a marked effect on brain function. Both the vagus nerve and the systemic levels of tryptophan are strongly implicated in the communication between the intestinal microbiota and the brain. Additionally, the short chain fatty acids (SCFA) are neuroactive bacterial metabolites from dietary fibers that can also modulate the mood, cognition and behavior [15].

5 Perspectives

The influence of stress on microbes is constant and can be anticipated [16]. Studies on the gut microbial communities have shed new light about our evolutionary relationships with these microorganisms and their genes. While some species can cause diseases, others are necessary for our health. This has changed our perception of health and established the fundamentals for the use of microorganisms as therapeutic agents. We are witnessing the birth of a new scientific discipline that could be termed microbial anthropology which in the near future is likely to join forces with computer engineering, nutrition, biochemistry, genetics, microbiology and medicine. The gene databases of the microorganisms we possess in our bodies are ever increasing thanks to powerful computing resources as well as to the increasing range of open-source software, which eventually will allow us to determine all microbial species in the human body, and the differences of species by age groups [17].

One present use of this new knowledge is fecal-microbiota transplants, where the transplant recipients adopt the characteristics of the donor's microbiota and the functions encoded by the donor's microbiome. These results have served as the basis for the emerging field of probiotic therapy. They have also laid the foundations for prebiotic therapies, with foods that encourage the growth of a certain type of microbes. This implies that body weight control will no longer focus on the amount of calories being ingested, but rather on the type of microorganisms encouraged by a specific diet [18]. In order for these therapies to succeed, considerable information must be available about our microbiota, such as changes during pregnancy, the effects of antibiotic use, new methods for in vitro cultivation and the host-commensal dynamics [11].

This technology offers new possibilities for the therapeutic use of prebiotics and probiotics, as they could be designed to manipulate the microbiome and the internal environment of the host in order to improve its health. Another use could be the improvement of foods in the near future. This research field will captivate audiences, allowing for a renewed perception of the self as a super-organism that lives in symbiosis with other species, and will inspire students to expand their knowledge about the human body [17]. Even if what is mentioned above does not pan out, we could still see what happens in a natural life and try to emulate those conditions [19].

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Conflict of interest: Authors declare nothing to disclose.

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