**MICROBIOME AND IMMUNE SYSTEM**

***Microbiome***

The microbiome is a complex ecosystem of microorganisms that inhabit the human body, primarily the gastrointestinal tract, but also the skin, oral cavity, and other organ systems. This ecosystem includes bacteria, viruses, fungi, and parasites, which play an important role in maintaining the health of the host.

The origin of the term *microbiota* probably dates back to the early 1900s. At that time, it was discovered that a large number of microorganisms, including bacteria, fungi, and viruses, coexist in different places in the human body (gut, skin, lungs, oral cavity). In addition, the human microbiome, also known as the “hidden organ,” contains over 150 times more genetic information than the entire human genome. Although *microbiota* and *microbiome* are similar, there are certain differences between these two terms. Microbiota describes the living microorganisms that are found in a defined anatomically localized region, such as the oral and intestinal microbiota. The microbiome refers to the set of genomes of all microorganisms in an environment, which includes not only the community of microorganisms, but also their structural elements, metabolites. In this sense, the microbiome encompasses a broader spectrum than the microbiota.

Members of the normal microbiome are defined as microorganisms found on or in the bodies of healthy individuals. Some of these organisms are found only in the bodies of humans or animals, while others can live freely in the environment. The exact number of microorganisms that make up the normal microbiome is usually not possible to specify. In fact, we all share a "core microbiome" (a certain number of microorganisms that are present in everyone), and some of us also have other microorganisms that may be transient. For example, *meningococcus* or *pneumococcus* are true pathogens that can cause meningitis, pneumonia, or septicemia, but in most people they only colonize the throat for a while without causing disease, so they can be called transient members of the normal microbiome of that individual. However, the disease caused by these microorganisms does not develop without prior colonization. Therefore, colonization is necessary, but not sufficient, for the development of meningococcal or pneumococcal disease. Long-term colonization is not a general prerequisite for all infections, many of them develop soon after the infectious agent enters the body. An example is the common cold.

Which parts of the body are inhabited by microorganisms?

Among the body parts colonized by the normal microbiome, the following typically contain

large amounts of microorganisms:

• Skin: especially moist areas, such as the groin and the area between the toes

• Respiratory tract: nose and oropharynx

• Digestive tract: oral cavity and large intestine

• Urinary tract: anterior parts of the ureter

• Genital system: vagina

Bacteria, and to a lesser extent fungi and protozoa, live and actively reproduce in these sites. Other parts of the body contain small numbers of microorganisms, which are usually transient. These sites include, for example, the rest of the respiratory and digestive tracts, the bladder and the uterus. The finding of pathogenic microorganisms in these sites indicates the possible presence of an infectious disease but is not proof of it. At the other extreme are certain tissues and organs that are normally sterile. The presence of microorganisms in these sites is usually of diagnostic significance. These sites include blood, cerebrospinal fluid, synovial fluids, and all deep tissues.

The composition of the microbiome varies from site to site (Figure 1). The gut microbiome is considered the most important in maintaining our health. Gut bacteria have several functions, such as fermenting food, protecting against pathogens, stimulating the immune response and producing vitamins. In general, the gut microbiome consists of 6 phyla of microorganisms including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* dominating. The most studied fungi (gut mycobiota) are *Candida*, *Saccharomyces*, *Malassezia*, and *Cladosporium*.

A diagram of a person's body

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**Figure 1**. Composition of the human microbiome in different locations

Although less well established than the gut, the microbiome is also localized in other regions including the oral cavity, lungs, vagina, and skin. The oral microbiome is considered the second largest microbiome community in humans. The oral cavity can be further divided into multiple microbiota habitats, including saliva, tongue, tooth surfaces, gums, buccal mucosa, palate, and subgingival/supragingival plaque, which can exhibit significant and rapid changes in composition and activity, due to factors such as changes in pH, gene mutations, and interactions among bacteria. The composition of the microbiomes at all seven locations shares general similarities but with minor differences. In general, the major bacteria present in the oral microbiome are *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*.

Although healthy human lungs have long been considered sterile, numerous studies have shown that the microbiome is also present in lung tissue. The basis of the lung microbiome is made up of: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. The composition of the lung microbiome is primarily determined by three factors: 1) migration of microorganisms, 2) elimination of microorganisms and 3) reproduction rate of microorganisms.

In human skin, the distribution and diversity of hair glands and follicles vary by geographical region. Physical and chemical differences between different skin regions create a distinct composition of the microbiome. The skin microbiome is primarily composed of: *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes* and *Proteobacteria*.

***A “healthy” gut microbiome***

The microbiome of different anatomical parts of the body plays a significant role in the physiological processes of the organism, and its composition is dynamic and influenced by various factors, including genetics, diet, and social interactions. The balance of the gut microbiota is closely relevant to human disease and health. Compared to other parts of the body, the human gastrointestinal tract contains an abundant microbial community that brings together ~100 trillion microorganisms. The human microbiome is directly involved in nutrient extraction, metabolism, and immunity. The microbiome can influence biological processes through several mechanisms. For the extraction of energy and nutrients from food, the microbiome plays a crucial role due to the diverse metabolic genes that provide independent unique enzymes and biochemical pathways. Moreover, the biosynthesis of bioactive molecules such as vitamins, amino acids, and lipids is also dependent on the gut microbiome. In terms of the immune system, the human microbiome not only protects the host from external pathogens by producing antimicrobial substances but also serves as an important component in the development of the intestinal mucosa and the local immune system. Under healthy conditions, the gut microbiome exhibits stability, resilience, and symbiotic interactions with the host. The gut microbiome is composed of bacteria, fungi, and viruses. A healthy microbiome community often exhibits high taxonomic diversity. However, it has been noted that the relative distribution of microorganisms is unique between individuals and is subject to individual variation. In humans, the gut microbiome can vary depending on age and environmental factors (e.g., drug use). In addition, the gut microbiome varies in different anatomical regions of the body. For example, *Proteobacteria* such as *Enterobacteriaceae* are found in the small intestine but not in the large intestine. Instead, Bacteriodetes such as *Bacteroidaceae*, *Prevotellaceae*, and *Rikenellaceae* are often found in the large intestine. Such variations are mainly due to different environments. In the small intestine, transit time is short and bile concentration is high, while in the large intestine, which has a slower flow rate and milder pH, microbial communities, especially anaerobic species, are larger. In addition to spatial distribution, the gut microbiome also differs “by age”. In general, microbiome diversity increases over time from childhood to adulthood and decreases in old age (over 70). Before the formation of a relatively stable gut microbiome composition, the diversity of the infant microbiome is dominated by *Akkermansia muciniphila*, *Bacteroides*, *Veillonella*, *Clostridium coccoides spp*., *Clostridium botulinum spp*.

By about 3 years of age, the infant gut microbiome becomes comparable to the adult microbiome, with three major microbial phyla including *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. Later, in older age, diet and immune system changes potentially influence the composition of the human gut microbiome. Specifically, older people tend to exhibit reduced numbers of *Bifidobacteria* and increased numbers of *Clostridia* and *Proteobacteria*. Decreases in anaerobic bacteria *Bifidobacterium* are thought to be relevant to the worsening inflammatory status due to their role in stimulating the immune system. Since the microbiome plays an important role in maintaining human health, it also proactively participates in several biological processes and the development of diseases.

***The impact of the microbiome on health***

The microbiome influences a range of physiological processes, including weight regulation, inflammation, and mental health. Dysbiosis, or an imbalance in the microbiome, has been linked to diseases such as obesity, type 2 diabetes, autoimmune diseases, and neurodegenerative disorders.

***Microbiome as a cause of infection***

Despite the benefits of the microbiome, when some members of the microflora are found in parts of the body where they are not normally found, they can cause disease. For example, anaerobic bacteria, usually of the genus Bacteroides, do not cause damage in the intestines of healthy people, where they help digest complex polysaccharides. But they can cause abscesses if they penetrate deeper tissues due to trauma or surgery. If fecal contents containing bacteria of the genus *Bacteroides* spill into the peritoneal cavity, as in the case of a ruptured appendix, the consequences can be fatal. *Staphylococci* from the skin and nose, or streptococci and Gram-negative cocci from the throat and oral cavity can also cause this type of infection. *Staphylococcus epidermidis*, a species that is predominantly present on the skin, has a strong tendency to adhere to the plastic surfaces of prostheses and can thus cause serious blood infections in patients with intravenous catheters. Likewise, *Escherichia coli*, a normal inhabitant of the gastrointestinal tract, is the most common cause of urinary tract infections. In fact, physicians see patients with diseases caused by members of the normal microbiome more often than by pathogenic microorganisms. These facts indicate that virulence is difficult to define and that no microorganism is essentially benign or pathogenic. Under certain circumstances, any microorganism that can survive and grow in the body can cause disease. Virulence depends not only on the properties of the microorganism, but also on the immunocompetence of the host. Members of the microbiome invade organs and tissues in immunocompromised patients. Thus, the yeast *Candida*, a harmless commensal found in about one-third of healthy people, is a common cause of blood infections in patients with various tumors and those receiving intensive chemotherapy. *Pneumocystis carinii*, a common inhabitant of the lungs of healthy individuals, can cause specific types of pneumonia and is one of the leading causes of death in AIDS patients.

***The impact of the microbiome on elimination (retention) of pathogens***

In some places in the body, the microbiome helps eliminate pathogens. Commensal bacteria have a physical advantage because they have previously occupied the site, especially on epithelial surfaces. Some commensal bacteria produce substances such as antibiotics or lethal proteins called bacteriocins that inhibit the newly arrived bacteria. In contrast, some intestinal pathogens stimulate an inflammatory response that is not harmful to them, and even grows in such conditions, but which has the effect of reducing the microbiome. This reduces the occupancy of the intestinal mucosa and allows colonization by pathogens. When the microbiome is reduced by antibiotics, both exogenous and endogenous microorganisms are given the opportunity to cause disease. For example, the infectious oral dose of *Salmonella* is reduced by a million-fold if the bacteria are given after the administration of antibiotics. Patients treated with antibiotics that are particularly effective in the stomach can suffer from diarrhea caused by toxins produced by the over-producing bacteria *Clostridium difficile*. A severe infection caused by this bacterium is called pseudomembranous colitis. This microorganism is present in a small percentage of the normal microflora of the digestive tract, but the presence of this bacterium can increase dramatically if other members of the microflora are suppressed.

***The role of the microbiome in human nutrition and metabolism***

The gut microbiome plays a role in human nutrition and metabolism. The vast and metabolically active biomass present in the large intestine is known to play a role in the nutritional balance of the host. Digestion of complex polysaccharides is carried out by members of the normal microflora, and several intestinal bacteria, such as *E. coli* and *Bacteroides*, synthesize vitamin K and are important sources of this vitamin for both humans and animals. The metabolism of several key components involves their secretion from the liver to the large intestine and their return to the liver. This enterohepatic circulatory loop is particularly important for the secretion of sex steroid hormones and bile salts. The compounds are excreted in the bile conjugated with glucuronides or sulfates, but cannot be absorbed in this form. Members of the gut microflora are a source of glucuronides and sulfates. The physiological significance of this activity is not yet fully understood, but some have referred to the colon as a second liver.

Compounds that we ingest can be chemically transformed by various metabolic activities of the intestinal microflora. Some compounds become carcinogenic only after modification by the colonic microflora. For example, the artificial sweetener cyclamate (cyclohexamine sulfate) is converted by bacterial sulfatases into the active cyclohexamine, a bladder carcinogen. On the other hand, members of the microflora detoxify some potential carcinogens by degrading them.

Nitrosamines present in processed foods, or formed from nitrites used to preserve food, can be inactivated by the action of the microflora. Similarly, carcinogenic heterocyclic amines that may be present in cooked meat become less toxic by the action of normal microflora.

***Factors that shape the microbiome***

The composition of the microbiome is influenced by genetics, diet, medications (especially antibiotics), stress, and lifestyle. A diet rich in fiber and fermented foods supports a diverse microbiome, while overuse of antibiotics can lead to a reduction in beneficial bacteria and an increased risk of infection. Microbiome modification through probiotics, prebiotics, and fecal microbiota transplantation is an increasingly popular therapeutic approach. Probiotics, live beneficial bacteria, can improve digestive health and immunity. Prebiotics, which serve as food for beneficial bacteria, also play an important role in maintaining a balanced microbiome.

***The microbiome as a stimulator of the immune system***

The microbiome plays a key role in the maturation and functioning of the immune system. Certain bacterial species stimulate the production of anti-inflammatory cytokines, while others can induce a pro-inflammatory response. Disturbances in the composition of the microbiome can contribute to the development of autoimmune diseases and chronic inflammatory conditions.

Our repertoire of antibodies (immunoglobulins) partly reflects stimulation by antigens from members of the normal microbiome. In principle, we do not have high titers of antibodies to bacteria, viruses, and fungi that normally inhabit our bodies. However, even at low concentrations, these antibodies serve as a defence mechanism, which is an obvious benefit of the microbiome. Among the antibodies produced in response to bacterial stimulation are IgA antibodies secreted through the mucosa, which are likely to be an important first line of defence and interfere with the colonization of deeper tissues by commensal microorganisms. Antibodies produced by stimulation with microbiome antigens sometimes cross-react with normal tissues. A relevant example is the antibodies of the ABO blood group system. People who belong to type A have anti-B antibodies, and conversely, members of type B have anti-A antibodies. People who belong to type O have both anti-A and anti-B antibodies. Neither of these antibodies is found in the newborn. What antigens are the stimulus for the production of these antibodies? At first glance, the source is not obvious. Why would one person produce antibodies to blood group antigens of another individual? Few of us come into contact with red blood cells that have different antigens through transfusion of the „wrong” blood. The answer to this puzzle is that bacteria of the normal intestinal microflora contain antigens that are similar to the A and B antigens. These members are the source of the antigenic stimulation. We produce antibodies to foreign blood group antigens, but not to our own blood group antigens, because we have mechanisms of self-tolerance. This type of cross-reactivity does not usually cause disease. In fact, some findings suggest that cross-reactivity to bacterial antigens may be protective. For example, antibodies against various bacteria commonly found in the intestines cross-react with the polysaccharide capsule of *meningococci* that cause meningitis; the presence of antibodies protects against this form of bacterial meningitis. Conversely, it is possible that antibodies that cross-react with microbial antigens may also be harmful to health. For example, the serious disease systemic lupus erythematosus has been associated with the production of antibodies to DNA. Some evidence suggests that the antigens that trigger the production of these antibodies are not nucleic acids but rather bacterial lipopolysaccharide with which there is cross-reactivity.

In addition to antigenic stimulation, microorganisms of the normal microflora play a key role in other aspects of the development of the immune system. Much of our knowledge in this area comes from the analysis of germ-free animals (usually mice). These animals have poorly developed spleens and lymph nodes. Furthermore, the differentiation of specific T helper lymphocytes Th17, which are responsible for regulating the mucosal immune response to microorganisms, depends on the presence of microorganisms. Finally, the importance of a single molecule member of the normal microbiome has been demonstrated by experiments with germ-free mice. Animals colonized with the commensal microorganism *Bacteroides fragilis* expressing the wild-type polysaccharide develop a healthy immune system. However, animals colonized with *Bacteroides fragilis* expressing the mutant polysaccharide have reduced numbers of T lymphocytes in the spleen, atypical thymus development, and an aberrant Th1 cytokine response.

***The immune system and microbiome connection: Key aspects of the interaction***

The microbiome plays a fundamental role in the induction, education, and function of the host immune system. In turn, the host immune system has evolved numerous mechanisms to maintain a symbiotic relationship with the microbiota. Maintaining this “dialogue” allows for the induction of protective responses to pathogens and the use of regulatory pathways involved in maintaining tolerance to innocuous antigens. The ability of the microbiome to “tune the immune tone” of tissues, both locally and systemically, requires constant sensing of microbes and complex feedback loops between the innate and adaptive components of the immune system.

Due to the ubiquitous and dominant role of the microbiome in various physiological functions, and given its interdependence with the functions of the immune system, the microbiome can be considered a corresponding functional component of the immune system. This implies the idea of ​​an extended immunity that therefore belongs to a functional whole that goes beyond the human organism and instead includes the human body and its associated microorganisms: that is, the so-called **holobiont**. The holobiont concept refers specifically to the creation of functional units of biological organization that are composed of different living beings, even those belonging to different species and other taxonomic categories. Therefore, from this perspective, it also means that the microbiome should be considered as a constituent element and therefore generates a constant but dynamic immune signal. The quality and any differences in response to this signal may clearly relate to both internal variations in the composition of the microbiome (which, as we know, can undergo modifications due to exogenous factors, such as diet, and endogenous factors such as genetics) and the influence that the microbiome has on the modulation of other components of the extended immune system, such as leukocytes, but also other cell types, such as intestinal epithelial cells. However, this process can also occur in the opposite direction, that is, the microbiome can be modulated by the activities of the human component of the holobiont.

In humans, a reduction in the gut microbiome and the consequent reduction in its specific byproducts, short chain fatty acids (SCFA), leads to a condition known as “*leaky gut syndrome*”, which is common to several inflammatory disorders such as obesity, cardiovascular disease, tumors, but also to neurological conditions (amyotrophic lateral sclerosis, Alzheimer’s and Parkinson’s disease), immunological disorders (inflammatory bowel disease, multiple sclerosis) and behavioral disorders (e.g. autism).

Indirectly, dietary patterns and lifestyle play a key role in chronic inflammatory, tumor, autoimmune and neurodegenerative diseases by shaping the composition of the microbiome. For example, plant foods are the main source of dietary fiber, which is converted to short-chain fatty acids (SCFA) in the distal intestine. Processed and refined foods are fermented in the small intestine, leading to bacterial overgrowth and a microbial signature that negatively affects the immune response. In humans, priming of the immune system begins in utero. The maternal microbiome and high-fiber diet begin to shape perinatal immune factors such as cord blood IgA, immune cells, and cytokines. From birth, *Bifidobacterium* present in breast milk is directly associated with the programming and maturation of the immune system: it leads to the development of the intestinal epithelial barrier and an increase in the number of regulatory T-lymphocytes, circulating interleukin 10, and anti-inflammatory monocytes.

The microbiome is further involved in promoting angiogenesis and epithelial cell development in the intestinal barrier. This explains why humans are born with a relatively immature immune system and a tolerogenic environment that facilitates the coexistence of microorganisms with the host without inducing inflammatory responses. Commensal bacteria express flagellin, a structural protein that interacts with Toll-like receptors in the event of intestinal barrier disruption. Dendritic cells located in the lamina propria respond to flagellin by secreting antimicrobial peptides and cytokines (IL-23), which induce innate lymphoid cells to release IL-22, which enhances epithelial protection.

In general, the microbiome actively orchestrates the immune response with other immune cells and host-dependent factors: (1) by competing with pathogens for the same nutrient substrates (colonization resistance); (2) by making the ecological niche unfavorable for other newcomers by changing the pH; (3) by secreting antimicrobial peptides; (4) by using metabolites (e.g., SCFA) that are able to modulate the immune response through downregulation or upregulation of gene expression.

By altering cytokine levels, the gut microbiome effectively influences the migration of immune cells. In tumors, the efficacy of checkpoint inhibitor therapy likely depends on the composition of the gut microbiome, directly related to the presence of *Akkermansia muciniphila*. In addition, *Bifidobacterium fragilis* and *Bacteroides thetaiotaomicron* play a key role in the efficacy of CTLA-4 inhibition on tumor cells. IL-12-dependent Th1 immune responses are influenced by microbiome composition, and this response helps reduce tumors in mouse models and human studies, while preserving intestinal integrity. Furthermore, *streptococci* from the gut can migrate into the bloodstream, colonize intratumoral tissue, modulate immune infiltration within the tumor microenvironment, and enhance the efficacy of immunotherapy.

Consequently, the immune system no longer acts solely as a barrier, but as a finely tuned functional modulator of a larger interaction, where mechanisms (which will largely remain to be discovered) govern the designation of some elements as self and others as non-self, thus confirming the fluid and contextual nature of this distinction. The boundary between the immune system and the microbiome is therefore not an impenetrable wall but a porous zone with reciprocal exchanges and mutual influences. Moreover, this does not occur only in specific, localized areas associated with tissue, but affects the organism as a whole. Thus, on the one hand, microorganisms play a decisive role in determining the functional conformation of the immune system. On the other hand, the immune system itself is responsible for the ecological balance and diversity of activities of symbiotic populations of these microorganisms. Consequently, the relationships between organisms are far more complex than previously established and the immune system is much more than its name suggests. As already explained, holobionts challenge the traditional “individual boundaries” of those entities we call “organisms”. If living beings previously classified as animal and plant species are no longer uniform individuals, they can now be viewed as systems of interactions and functional assemblies of different components, namely the host and its heterogeneous associated microorganisms or holobionts. Since holobionts are proposed to represent a new level of biological organization (and therefore worthy of special research), they could also legitimately be considered potential new targets for specific therapeutic approaches, with a view to personalized medicine.

The microbiome and the immune system are inextricably linked, and this interaction is crucial for maintaining health and preventing disease. The new structure of the immune system would imply three interconnected pillars: adaptive immunity, innate immunity, and the **symbione** to better understand the diverse functionality of the extended immune system, the symbione. From this perspective, the immune system is expanded to a higher level of biological organization: the microbiome together with the traditional immune system and other cell types (i.e., those involved in the complex interactions and signaling of the extended immune response). Diet and lifestyle directly influence the composition of the microbiome and the repertoire of gut-associated immune cells by improving the efficacy of tumor immunotherapy and enhancing antitumor immunity or by suppressing inflammation and alleviating autoimmune diseases such as type 1 diabetes, multiple sclerosis, and inflammatory bowel disease. In addition to diet and lifestyle, microbiome-based therapy, such as the use of prebiotics and probiotics, or fecal microbiota transplantation, and even possibly helminth therapy, could, in light of a better understanding of the symbiome, become an approach within immunotherapy, which would also be directed towards the idea of ​​improved and prolonged immunity.