UNIVERSITY OF KRAGUJEVAC
FACULTY OF MEDICAL SCIENSES

IMMUNOLOGY, INFECTION AND INFLAMMATION

Microbiome and immune system

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Microbiome

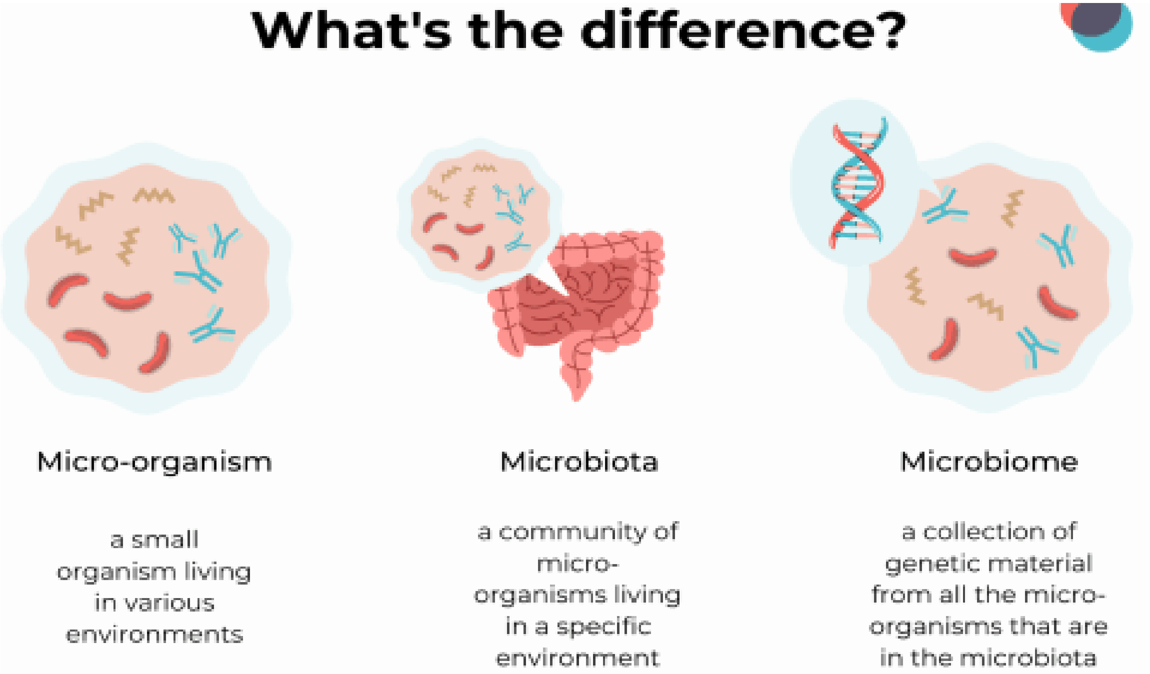
- The microbiome is a complex ecosystem of microorganisms that inhabit the human body, primarily the gastrointestinal tract, but also the skin, oral cavity, and other organ systems. This ecosystem includes bacteria, viruses, fungi, and parasites, which play an important role in maintaining the health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

- A large number of microorganisms, including bacteria, fungi, and viruses, coexist in different places in the human body (gut, skin, lungs, oral cavity).

- The human microbiome, also known as the “hidden organ,” contains over 150 times more genetic information than the entire human genome.

microbiota and microbiome

- Although microbiota and microbiome are similar, there are some differences between the two terms.
  - Microbiota describes the living microorganisms found in a defined anatomically localized region, such as the oral and intestinal microbiota.
  - 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.



normal microbiome

- 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.

- 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.

normal microbiome

Members of the

normal microbiomeare

defined as microorganisms

found on or in the body of

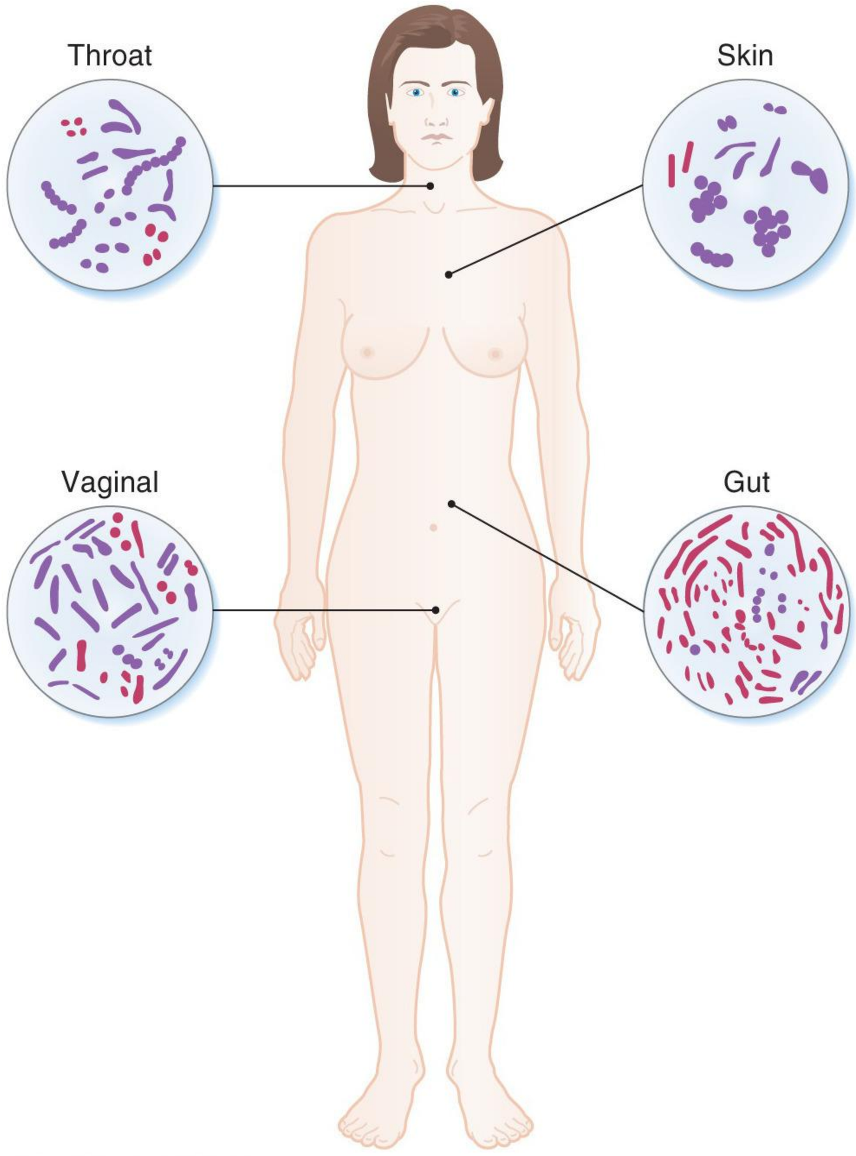
healthy individuals

Most coexist with humans,

without causing harm,

and in somecases they provide

great benefit (mutualism)



normal microbiome

Resident microflora (strains that form niches in certain areas of the body and remain there permanently)

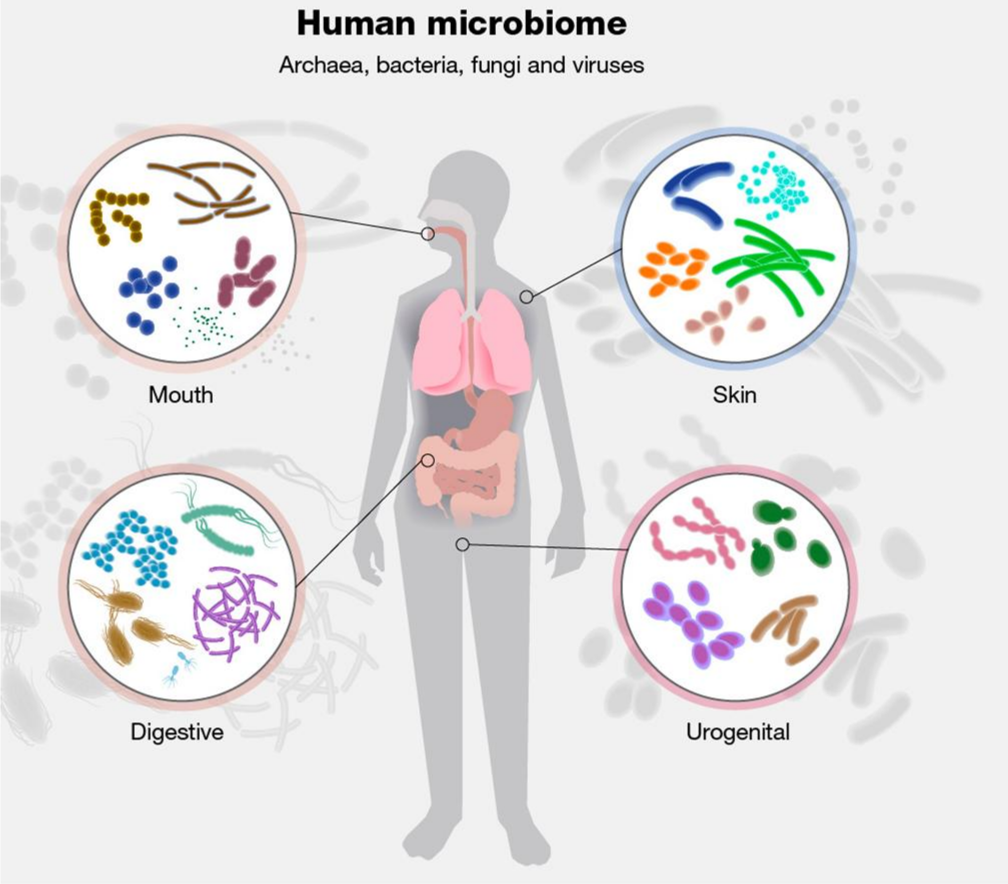
Transient microflora (acquired from the environment, microorganisms quickly occupy places, but are in competition with members of the resident flora or are in constant battle with the host's immune mechanisms)

The role of normal microflora

- Opportunistic infections
- Stimulation of the immune system
- Elimination (preventing colonization by pathogens)
- Role in human nutrition and metabolism

Microbiome

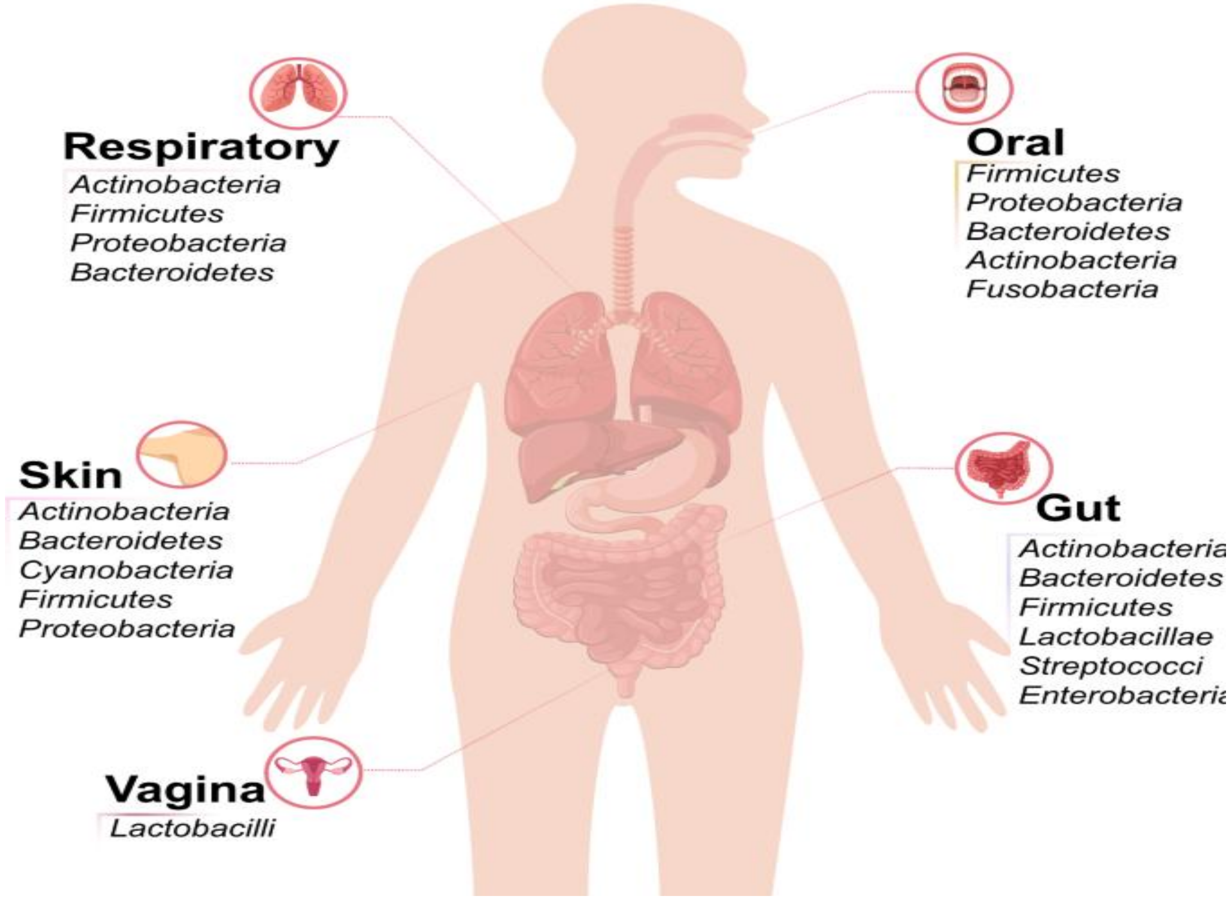
The human body (skin, gastrointestinal tract and other mucosal organs) is colonized by a huge number of microorganisms (10:1 relative to the number of host cells).This ecosystem consists of numerous species of bacteria and other microorganisms including fungi and viruses.



95% of the bacteria in the human body are found in the gastrointestinal tract:

- 100 trillion microorganisms
- 2 kg of mass
- 300-1000 species of bacteria
- 150 times more genetic information than the entire human genome

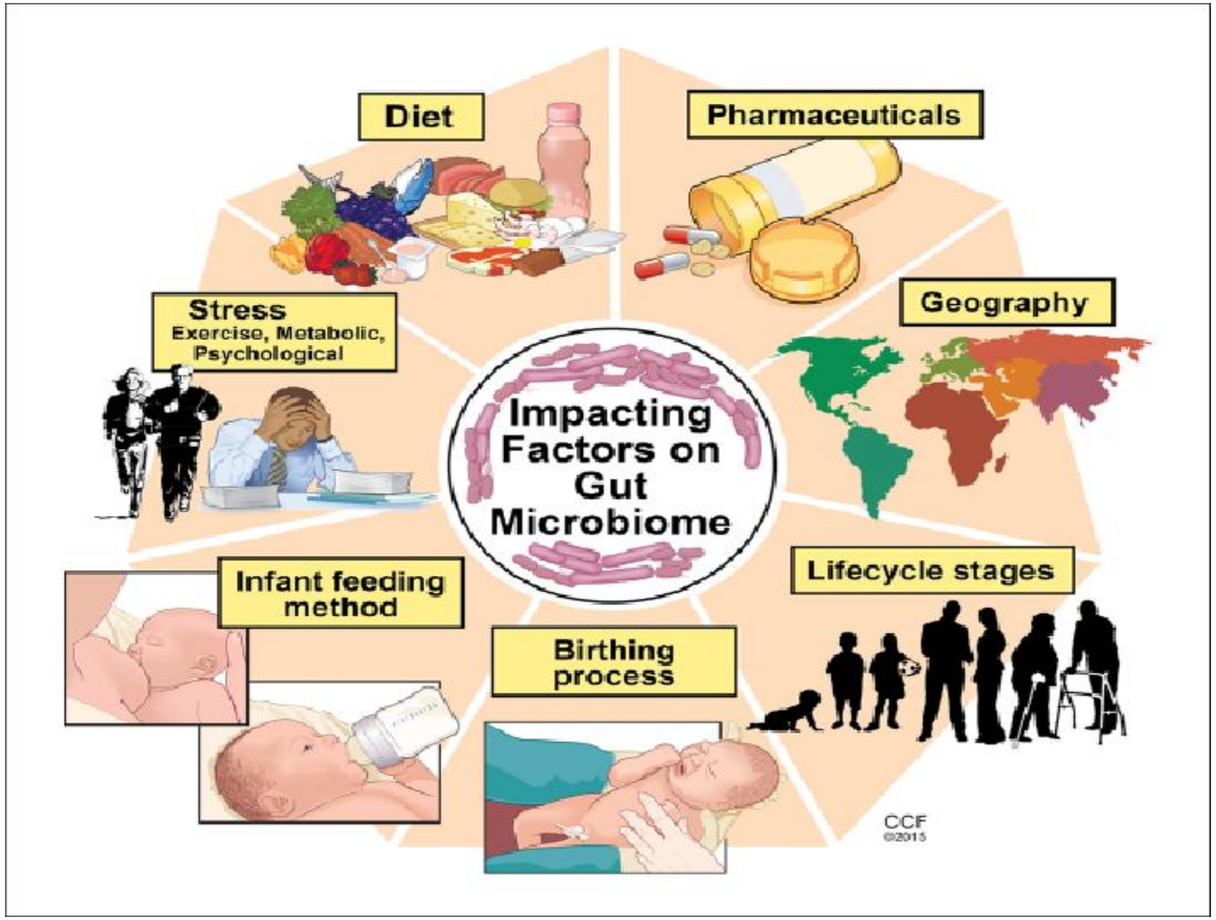
Microbiota composition in different anatomical regions



In addition to bacteria, the intestinal microbiota also includes various fungi (*Candida, Saccharomyces, Malassezia* и *Cladosporium*), viruses, bacteriophages and *archaea* (*Methanobrevibacter smithii*).

Factors that influence the composition of the microbiome

One third of the gut microbiota is common to most people,
while two thirds are specific to the individual –
the composition of the gut microflora is individual.



A "healthy" gut microbiome

- Compared to other parts of the body, the human gastrointestinal tract contains a rich microbial community, which includes ~100 trillion microorganisms.

- The human microbiome is directly involved in nutrient extraction, metabolism, and immunity.

- 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.

- Regarding the immune system, the human microbiome not only protects the host from external pathogens by producing antimicrobial substances but also serves as a significant component in the development of the intestinal mucosa and the local immune system.

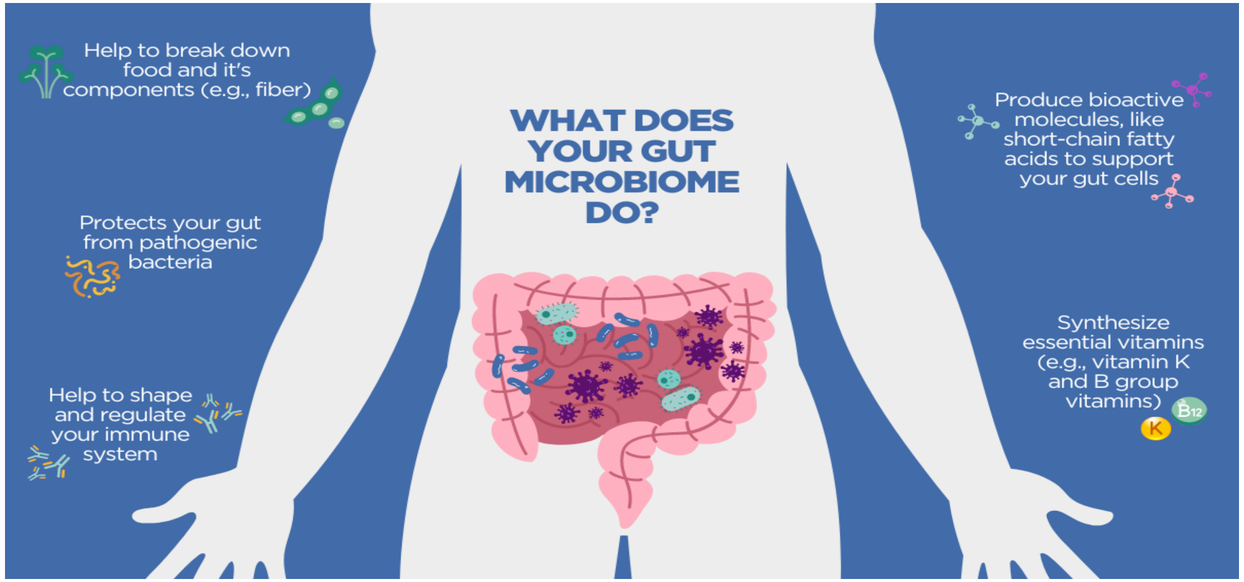
Microbiome-host interaction

Interaction between host and commensal microorganisms – mutualism and homeostasis.

The importance of microbiota-host interactions lies in the fact that members of the microbiota shape almost all aspects of host metabolism.

The gut microbiota is a "superorganism" within the host:

- participates in food digestion
- produces nutritional metabolites
- protects against infection
- maintains the function and morphology of intestinal epithelial cells
- regulates host immunity

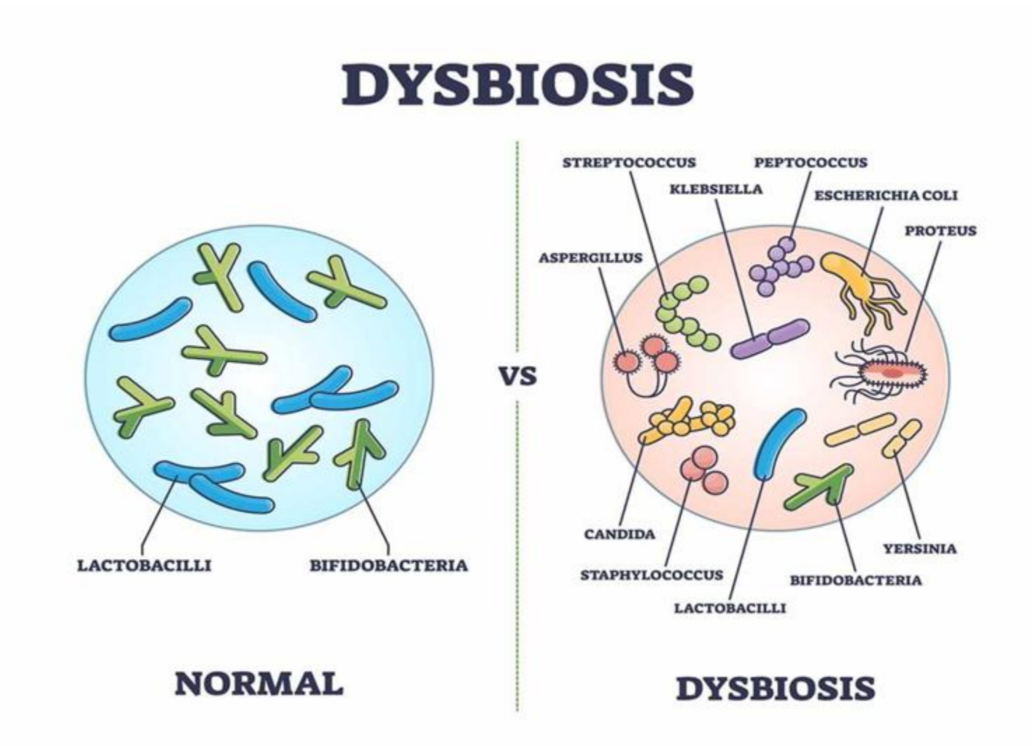


The impact of the microbiome on health

- The microbiome influences a range of physiological processes, including body 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.

Dysbiosis – imbalance of the microbiome

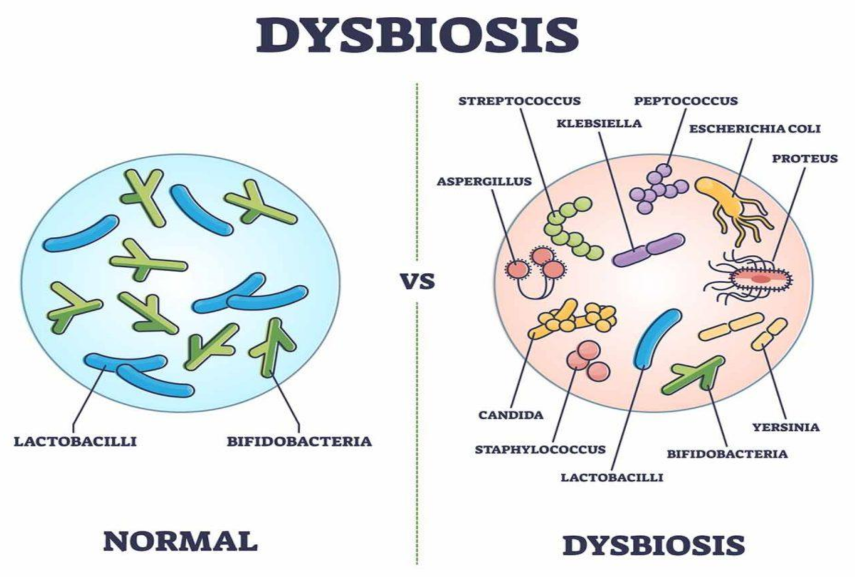
Dysbiosis – an increase in opportunistic pathogens or a decrease in the number of beneficial commensal microorganisms, or both.



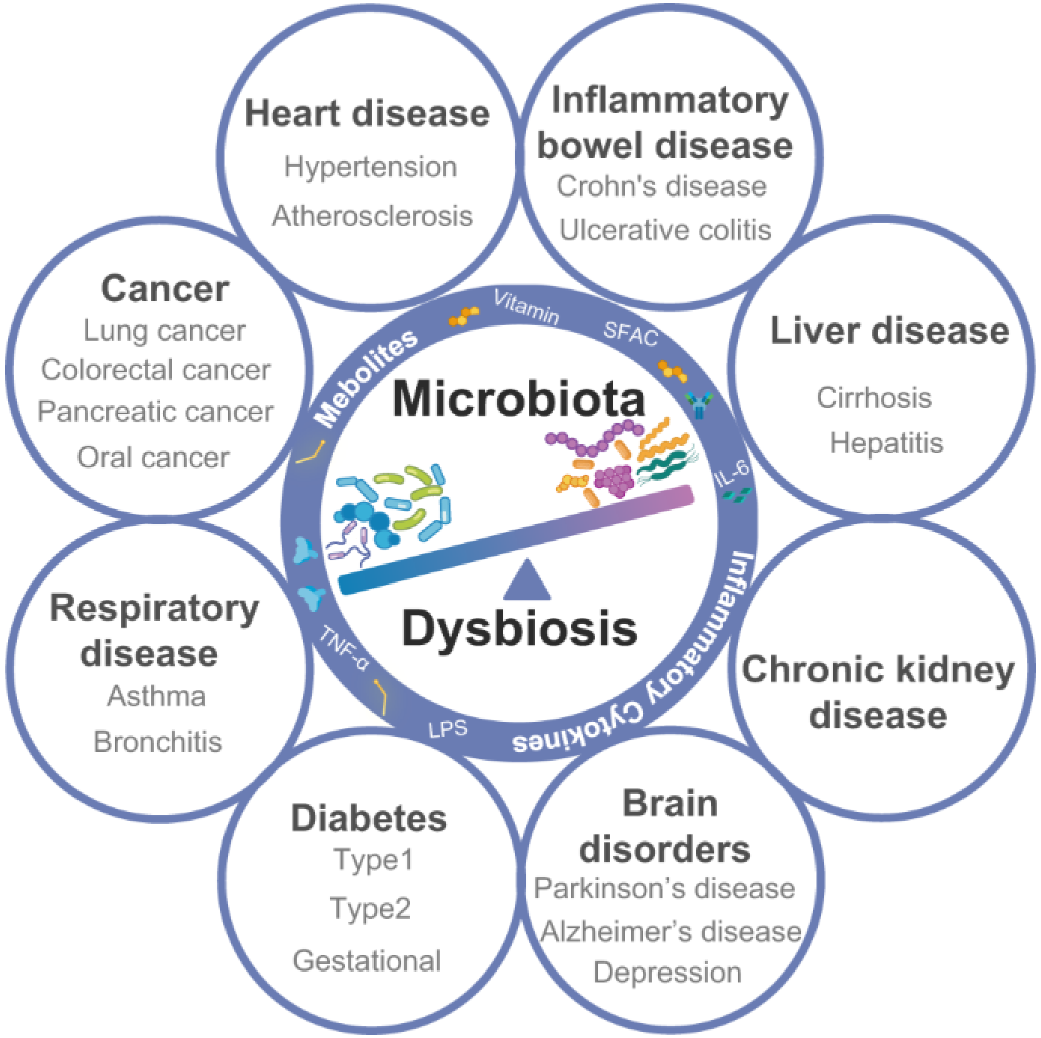
Dysbiosis – imbalance of the microbiome

- Disturbances of the microbiome (use of antibiotics, unbalanced diet ...) or the immune system can be the cause of the **dissemination of commensal microorganisms or infection with pathogenic strains**.

- In addition, interactions between the microbiome and the immune system are involved in the **development of "non-communicable" gastrointestinal diseases** (inflammatory bowel diseases, celiac disease), as well as **extraintestinal disorders** (rheumatoid arthritis, metabolic syndrome, neurodegenerative disorders, malignant diseases).



Microbiome dysbiosis contributes to the development of various diseases



Hou K, Wu ZX, Chen XY *et al*. Microbiota in health and diseases. *Sig Transduct Target Ther* 2022; **7**:135.

Microbiome as a cause of infection

- When some members of the microflora are found in parts of the body where they are not normally found, they can cause disease.
  - 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 tissue 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.
  - Escherichia coli, a normal inhabitant of the gastrointestinal tract, is the most common cause of urinary tract infection.
- We are increasingly seeing patients with diseases caused by members of the normal microbiome rather 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.
  - 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)

- 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 under 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 gain 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 bacteria is called pseudomembranous colitis.

Metabolites of commensal microorganisms

The gut microbiota has an enormous metabolic capacity. It converts lipids, carbohydrates and proteins, from food or host origin, into various metabolites that can have a positive or negative effect on the health of the host.

Bacterial metabolites have antimicrobial activity, i.e. they protect the organism from pathogenic bacteria:

- **short-chain fatty acids (SCFA)**
- **secondary bile acids**
- **bacteriocins**

The role of the microbiome in human nutrition and metabolism

- The gut microbiome plays a role in human nutrition and metabolism.
- A vast and metabolically active biomass is present in the large intestine and plays 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 into the large intestine and their return from there to the liver. This enterohepatic circulation 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 intestinal microflora are a source of glucuronides and sulfates.

- 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. 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.

Probiotics and prebiotics

Probiotics:

- live microorganisms
- bacteria or fungi
- available as dietary supplements or in foods such as yogurt, kefir, some cheeses, kombucha...

Prebiotics:

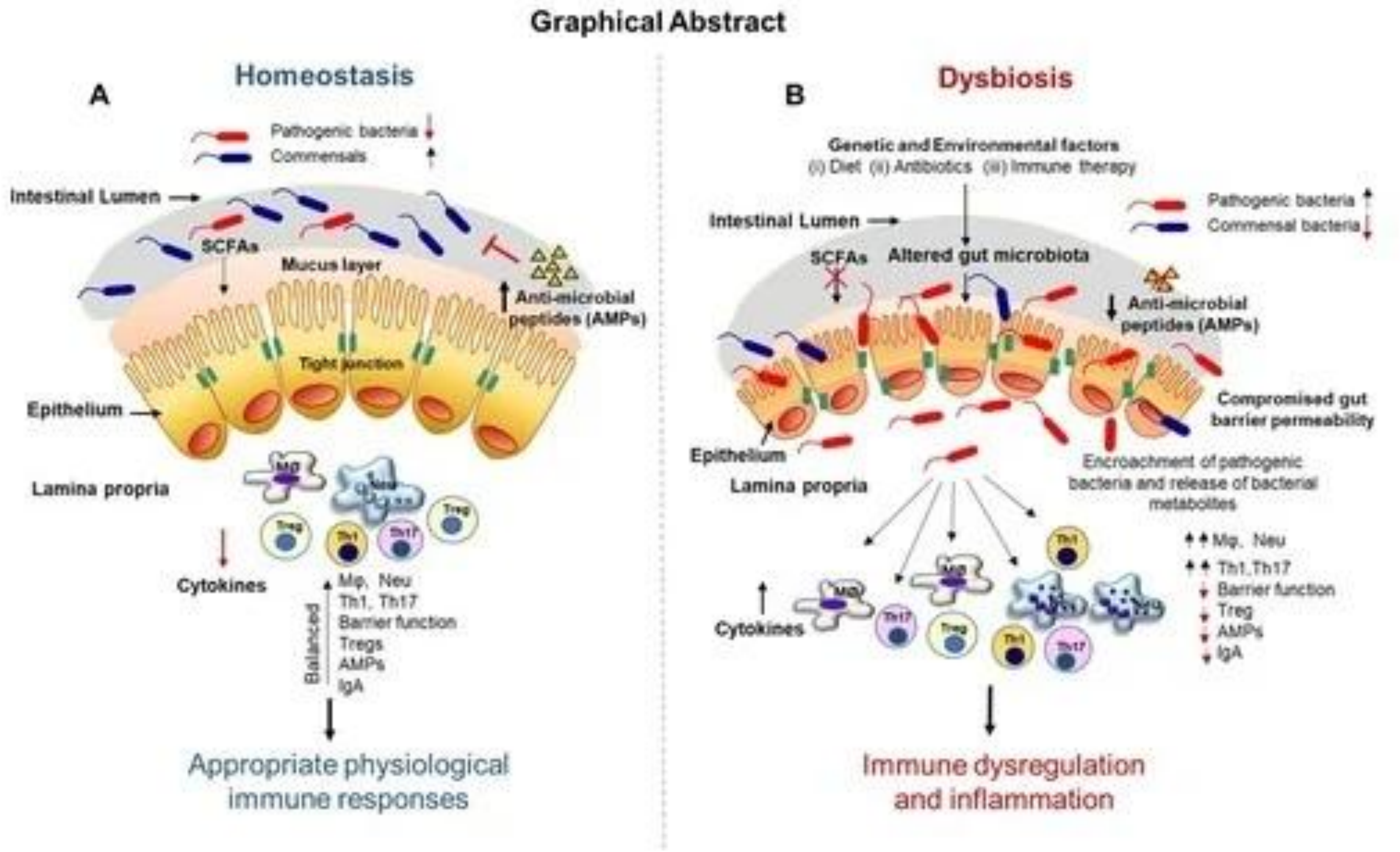
- are not living microorganisms, are not digestible (carbohydrates)
- serve as food for "good" intestinal bacteria
- available as dietary supplements or in foods such as bananas, onions and garlic, leeks, carrots, honey ...

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.

Interaction of the microbiome and the host immune system

The interaction of the microbiome and the host immune system is a complex and dynamic process – the immune system protects the organism from infection, while simultaneously tolerating the presence of commensal microorganisms.

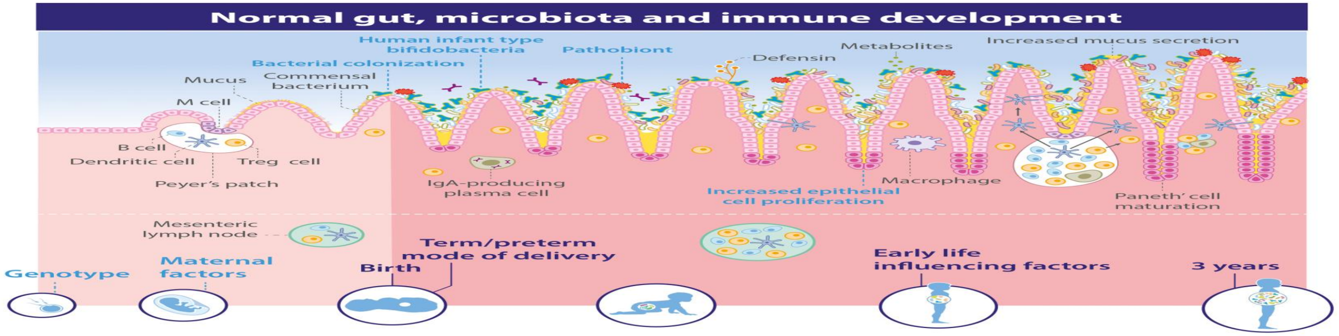


The microbiome as a stimulator of the immune system

- 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.

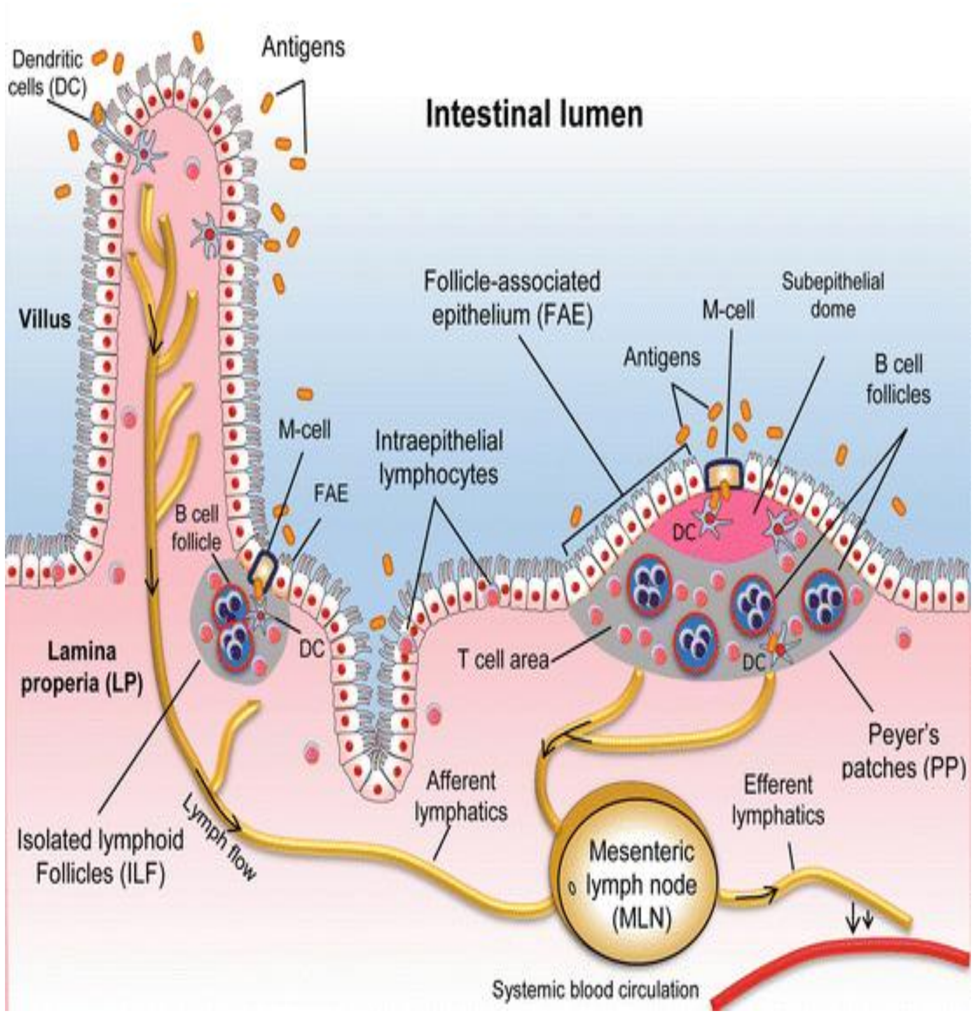
The absence of commensal microorganisms is associated with severe defects in the architecture and function of the lymphoid tissue of the gastrointestinal system:

- reduced number of αβ and γδ intraepithelial lymphocytes
- reduced IgA production
- reduced number of ILC3s and F4/80+CD11c+ mononuclear cells
- absence of Th17 lymphocyte development
- impaired Th1/Th2 balance
- impaired synthesis and regulation of the immunoglobulin repertoire (absence of regulation of IgE production – increased susceptibility to allergic diseases) ...



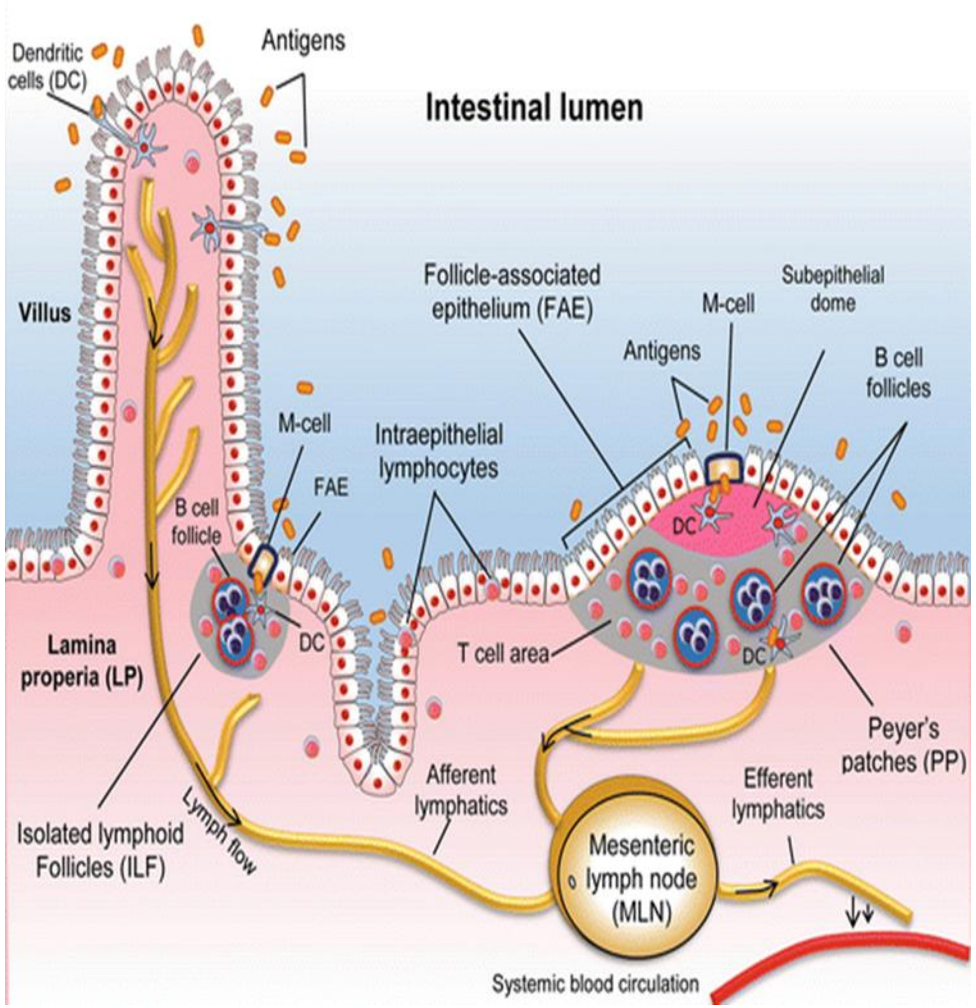
Interaction of the microbiome and host innate immunity

- First line of defence
- Epithelial barrier (mucus, tight junctions, antimicrobial proteins) – specialized epithelial cells (goblet and Paneth cells) produce mucus, defensins, cathelicidins, lysozyme
- PAMPs activate TLRs and NODs on macrophages and neutrophils (phagocytosis, inflammation) – TLR5 plays an important role in the development of the immune system and the configuration of the intestinal microbiota; NOD2 prevents infection and regulates the regeneration of the intestinal epithelium
- Dendritic cell activation and antigen presentation
- Innate lymphoid cells (ILCs) produce cytokines (ILC3 and IL-22 production), ...



Interaction of the microbiome and acquired host immunity

- Intraepithelial lymphocytes (IELs) and lamina propria lymphocytes (LPLs)
- γδ T lymphocytes inhibit bacterial dissemination by producing proinflammatory cytokines and antimicrobial peptides
- Helper (CD4+) (Th17) and cytotoxic (CD8+) T lymphocytes
- Regulatory T lymphocytes (Tregs) play a key role in immune tolerance to commensal microorganisms
- IgA secretion



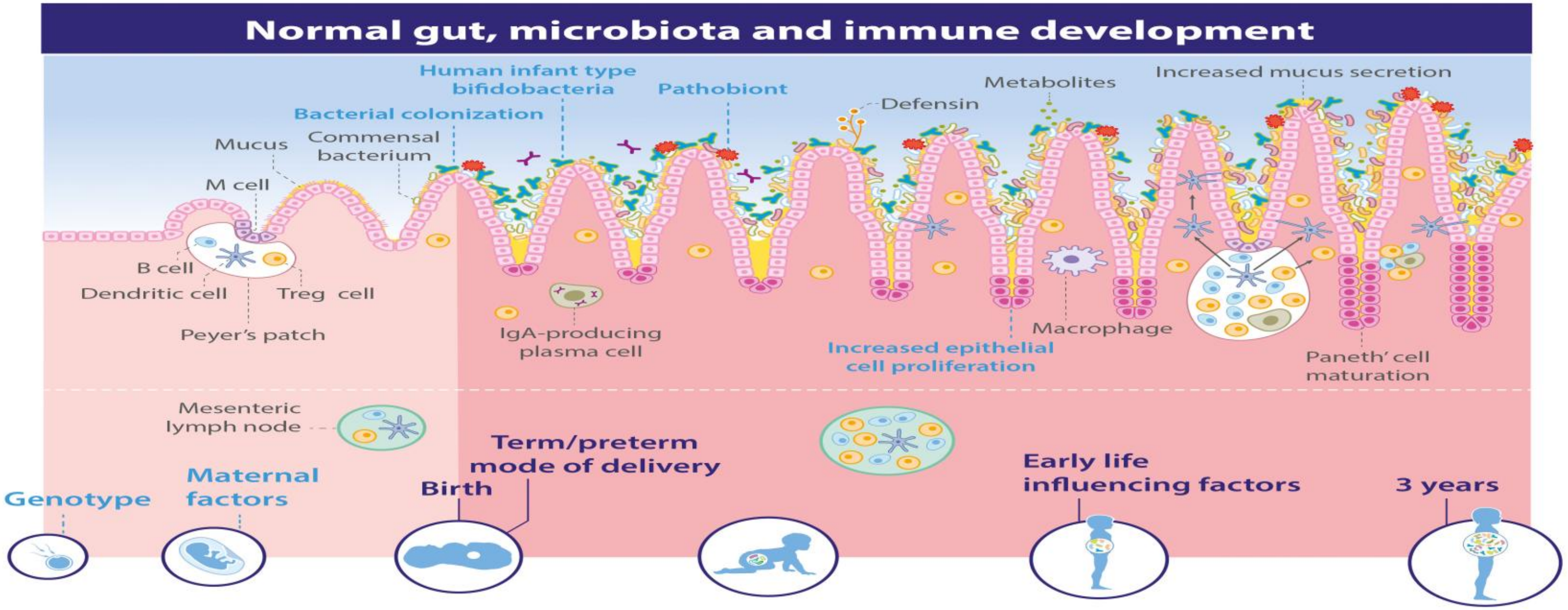
The microbiome as a stimulator of the immune system

- Our antibody (immunoglobulin) repertoire partly reflects stimulation by antigens from members of the normal microbiome. In general, we do not have high titers of antibodies to the bacteria, viruses, and fungi that normally inhabit our bodies. However, even at low concentrations, these antibodies serve as a defense mechanism, which is an obvious benefit to us from the microbiome.
- Among the antibodies produced in response to bacterial stimulation are IgA antibodies secreted by the mucosa, which are likely to be an important first line of defense and interfere with the colonization of deeper tissues by commensal microorganisms.

- Antibodies produced by stimulation by microbiome antigens sometimes cross-react with normal tissues. A relevant example is the antibodies of the ABO blood group system. People belonging to type A have anti-V antibodies, and vice versa, members of type B have anti-A antibodies. People who belong to blood group O have both anti-A and anti-B antibodies. Neither of these antibodies is found in newborns. What antigens stimulate the production of these antibodies? At first glance, the source is not obvious. Why would one person produce antibodies to the 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 antigens of foreign blood groups, but not to antigens of our own blood group, because we have mechanisms of self-tolerance.

The role of the microbiome in the maturation of the immune system

- The most critical events in the maturation of the immune system occur during the first years of life when the composition of the microbiota shows the greatest intra- and inter-individual variability (a stable composition of the microbiota is achieved at the age of ~3 years).
- Intensive colonization with microorganisms occurs after birth and is mainly of maternal microbiota origin. Initial colonization is influenced by the mode of delivery, nutrition, ...
- The immaturity of the immune system of newborns is reflected in increased susceptibility to infections, while at the same time there is an increased tendency to excessive inflammation (necrotizing enterocolitis).



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

- 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.

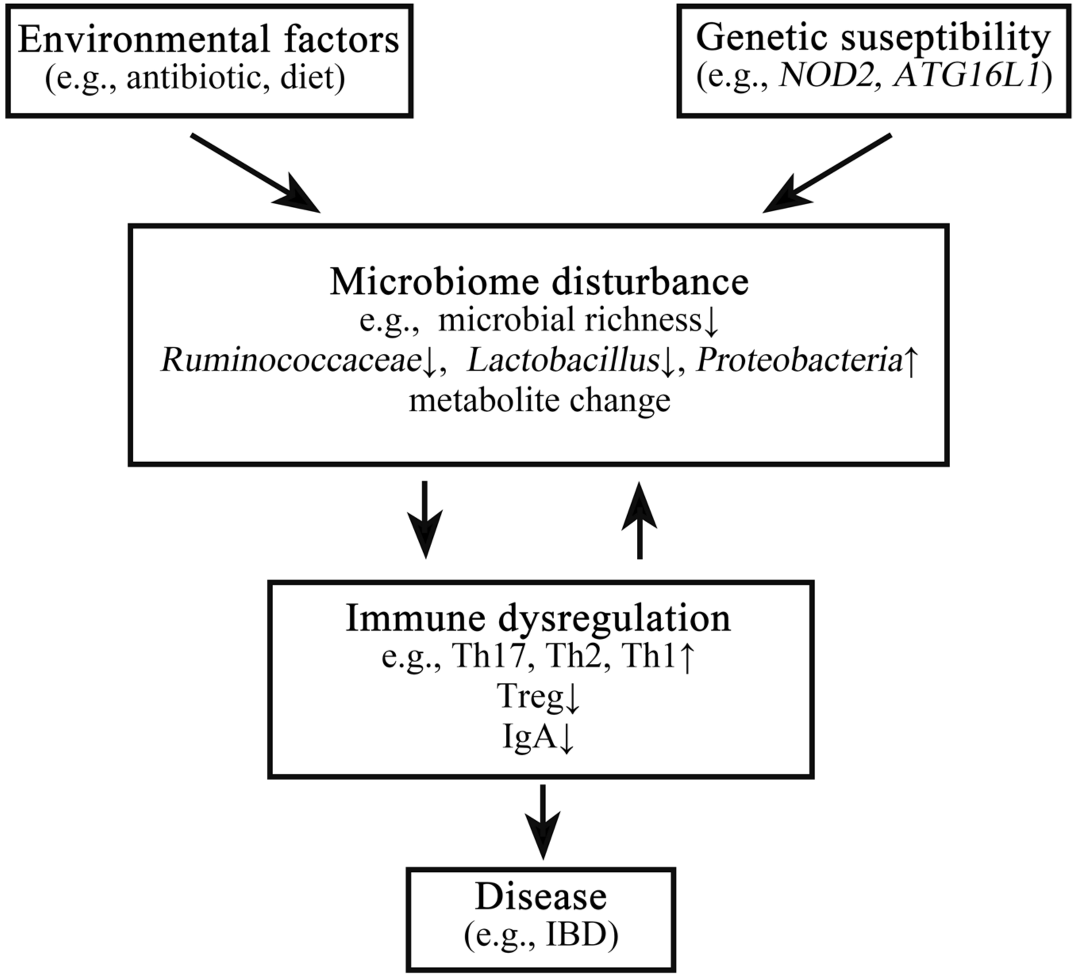
- This implies the idea of an extended immunity that therefore belongs to a functional whole that goes beyond the human organism and instead includes the human body and its associated microorganisms: that is, the so-called **holobiont**.

- 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.

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

- The reduction of the gut microbiome and the consequent reduction of 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 a major 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 impacts immune responses.
- 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 linked to 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.

Dysregulation of the interaction between the microbiome and the immune system in the development of inflammatory bowel diseases



Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res 2020; 30:492–506.

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

- 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 defense.

- In general, the microbiome actively orchestrates the immune response with other immune cells and host-dependent factors: (1) 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 decreasing or increasing gene expression.

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

- 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.

- The immune system itself is responsible for the ecological balance and diversity of activities of symbiotic populations of these microorganisms.

- 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 **simunobiont** in order to better understand the diverse functionality of the extended immune system, hence the simunobiont. From this perspective, the immune system is extended 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 signals of the extended immune response).