Chapter

Immune System, Gut Microbiota and Diet: An Interesting and Emerging Trialogue

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Abstract

The present chapter provides a comprehensive overview of the multifaceted links connecting the immune system, the intestinal microbiota, and the diet, covering also some recent, less explored, and emerging topics such as the "trained immunity" and the immune cell metabolic activity. The main characteristics of the innate and adaptive immune system are described, as well as the gut-associated lymphoid tissue (GALT). Gut microbiota structure and function are also presented. Particular emphasis is given to the diet as a modulator of the microbiota-immune system crosstalk, focusing on the impact of the three main dietary components (carbohydrates, proteins, and fats) and the different dietary profiles on the gut microbiota, by shaping its composition and the deriving microbial metabolites that influence host health, also through interaction with the immune system. Western and Mediterranean diets are described and chosen as representative models of detrimental and beneficial dietary patterns, respectively.

Keywords: innate immunity, GALT, intestinal microbiome, Western diet, Mediterranean diet, metabolic inflammation

1. Introduction

The immune system is fundamental to protect the organism from pathogens and toxic exogenous agents, by discriminating between "self" and "nonself" antigens, and in normal physiological conditions it is programmed to react against "nonself." At the intestinal level, the gut-associated lymphoid tissue (GALT) is a key component of immune defense, protecting the body from foreign antigens and pathogens, while allowing tolerance to commensal bacteria and dietary antigens [1]. The intestinal microbiota, defined as the complex microbial community residing in the host's digestive tract, is recognized as an effective integral component of the host immune system, capable of finely tuning both the innate and adaptive immune responses during the entire lifespan. Indeed, the intimate relationship set up between microbiota and immune cells in the intestine is crucial for the maintenance of immunological homeostasis and, mostly, for the "education" of the immune system during the early

stages of life [2]. Diet has a strong influence on the gut microbiota, acting both as a modulator able to select specific microbial groups and providing substrates that can be metabolized by the microbiota producing metabolites that impact host health also through interaction with the immune system [3, 4]. Therefore, there is a close connection between diet, gut microbiota, and immune system, orchestrated by a fine-tuning of the complex mechanisms underlying this cross-talk.

2. The immune system

The immune system is a complex network designed to react against harmful foreign agents as well as pathogens. The immune system is immature during fetal and neonatal life. The fetus receives passive protection from the mother *in utero*, thanks to maternal immunoglobulins (Ig)G, which can cross the placental barrier. This protection continues until the first months of the life of the newborn, as maternal IgG are transferred also through breastfeeding. The development of the immune system begins early in life and is greatly influenced by the type of feeding and environmental exposure (including factors such as the presence of domestic animals, antibiotic use, and timing of introduction of different foods from weaning). In the elderly, a progressive decline of the immune function is observed, a process known as immunosenescence [5]. Two types of immune response exist: the nonspecific or innate response, which is the first line of defense and operates in a nonselective way against foreign antigens, and the specific or adaptive one, which is triggered after exposure to a particular antigen. Both responses have a cellular and a humoral component. The two responses are interconnected for several reasons: 1—The cytokines (soluble mediator molecules) secreted in the early stages of the innate response influence the type of adaptive immune response that will develop; 2—Macrophages and dendritic cells, activated during the innate response, act as antigen-presenting cells (APC) for naive (i.e., having not yet encountered the antigen) T lymphocytes, inducing their differentiation into effector T lymphocytes; 3 - In some cases, the phagocytosis performed by innate immune cells is more efficient, if the microorganism to be cleared has been previously bound and surrounded by antibodies (opsonization). The nature of the antigen determines which of the two responses is preferentially activated; however, a complete immune response requires the coordinated participation of both types, and it ends once the trigger is resolved (self-limiting capacity) [6].

2.1 Innate immune response

The innate immune response is less specialized and generally less effective than the adaptive one. The cellular mechanisms of innate immunity are characterized by phagocytic and cytotoxic activities, while the humoral component is based on the complement system.

2.1.1 Cellular component

Neutrophil granulocytes are normally found in the bloodstream. During the acute phase of inflammation, they are among the first inflammatory cells migrating from blood vessels to the inflamed site, recruited by chemical signals such as interleukin-8 (IL-8), through a process called chemotaxis. Similarly, monocytes migrate from the bloodstream to tissues in response to chemokine release at infection sites, become

activated, and differentiate into macrophages. Macrophages constitute, together with neutrophils, the largest group of cells endowed with phagocytic activity, they carry out their defense action by surrounding foreign microorganisms with pseudopodia, i.e., extroversions of the plasma membrane, forming the phagosome. The phagosome then merges with the numerous granules present in the cytoplasm, containing various compounds toxic for microorganisms, such as defensins, cathelicidins, lysozyme, and lactoferrin, forming the phagolysosome. Alternatively, the so-called respiratory or oxidative burst is activated, resulting in the formation of reactive oxygen species finally producing hypochlorite, hypobromite, and hypoiodite that kill microorganisms. In addition to the phagocytic function, macrophages are also responsible for the processing and presentation of antigens to T cells, as mentioned above [7]. Natural killer (NK) cells eliminate virus-infected and tumor cells through a cytotoxic activity, mediated by perforin-containing granules and granzymes. The former form pores in the plasma membrane and the latter, entering through these pores, induce the caspase cascade, leading to apoptosis of target cells. NK cells can also kill target cells through another mechanism, referred to as antibody-dependent cellular cytotoxicity (ADCC), in which NKs recognize target cells to which IgG has been previously bound [7, 8]. Many cells of the innate immune system are activated through receptors expressed on their membrane, namely pattern recognition receptors (PRR), with a long evolutionary history, which are able to recognize conserved structural patterns expressed by microorganisms, such as the microbe- and pathogen- associated molecular patterns (MAMP and PAMP, respectively). In particular, the type of PRR recognizing MAMP and PAMP is represented by the toll-like receptors (TLRs), a family of transmembrane proteins primarily expressed on the surface of immunocompetent cells, i.e., monocytes, macrophages, and dendritic cells, but also on intestinal epithelial cells. When a TLR recognizes a MAMP, a complex protein signal transduction cascade is triggered generating the appropriate immune response for that microorganism [9]. In most cases, the inflammatory response activated by TLRs leads to the activation of the nuclear factor-kappaB (NF-κB), which induces the transcription of numerous pro-inflammatory genes, including IL-8 [10]. TLRs can also be activated by endogenous danger signals such as the damage-associated molecular patterns (DAMP), molecules that are released in the intracellular or extracellular space following tissue injury, cellular stress, or apoptosis. Some innate responses can activate the inflammasome, a multiprotein complex resident in the cytosol as an inactive form, particularly in macrophages. A 2-hit-theory has been postulated, stating that for inflammasome activation two distinct signals are required. The first signal, triggered by PAMPs or DAMPs, activates the TLR signaling cascade, leading to the expression of some pro-inflammatory cytokines in an inactive form, such as proIL-1β and proIL-18. The second stimulus activates the inflammasome and generates caspase-1. Only thereafter proIL-1β and pro-IL-18 are cleaved by caspase-1 to mature IL-1β and IL-18, which can be secreted by macrophages and promote the inflammatory response [11]. Dendritic cells (DCs) are specialized to "sample" the entry sites of potential infectious agents, so they are found as immature cells in nonlymphoid tissues where antigens can be encountered, such as skin and other mucosal sites. The antigen recognition, through TLRs' activation, initiates the maturation process of DCs, which are induced to secrete various pro-inflammatory cytokines. After the encountering, the antigen is internalized through phagocytosis or pinocytosis and processed by the DCs, which migrate to secondary lymphoid organs (lymph nodes, spleen), where the exposed antigens are presented to populations of T lymphocytes, both naive and memory cells [6]. Mast cells and basophil granulocytes, similarly to monocytes, circulate in the

blood as immature progenitor cells, differentiating into mature cells in different tissues in response to cytokine secretion. Mast cells and basophils are particularly found in association with blood vessels and nerves, in close proximity to mucosal surfaces that interface with the external environment, where they are able to detect infectious agents through TLRs. Upon activation, mast cells and basophils immediately extrude histamine from granules and, within a few minutes, release lipid mediators (such as prostaglandins, leukotrienes, and thromboxane), promoting vascular permeability, vasodilation, and rapid recruitment of eosinophils, neutrophils, and other immune cells [7]. Eosinophils are another type of circulating granulocytes that can be recruited to sites of inflammatory reactions, where their numbers can be 100-fold higher than in the blood. When activated, eosinophils release the contents of their granules (numerous enzymes, major basic protein, eosinophilic cationic protein), which act primarily on extracellular helminthic parasites. Eosinophils also actively participate in allergic diseases [8]. Innate lymphoid cells (ILCs), although lacking antigen-specific receptors, play an important role in the inflammatory response and the maintenance of immune homeostasis, particularly in mucosal tissues. Based on their phenotypic and functional features, ILCs have been grouped into three major subsets. Among them, group 3 ILC (ILC3) are implicated in intestinal homeostasis as they produce IL-22, a key regulator of the intestinal barrier [12]. Recent studies have shown that some myeloid cells of the innate immune system, essentially macrophages and NK cells, can develop a nonspecific immunological memory, i.e., these cells, after a first stimulus, acquire the ability to respond effectively to a subsequent stimulus, different from the first. Effector stimuli for such "innate memory" are represented by various components of bacteria or fungi, such as lipopolysaccharide (LPS) or β-glucans, as well as viruses, and such innate memory is called "trained immunity" [13]. Following activation, the cells involved in this phenomenon undergo processes of chromatin unfolding, which thus becomes more accessible for gene transcription. These processes, globally referred to as "epigenetic reprogramming," include methylations, acetylations, and phosphorylations at specific chromatin sites. The activation of gene transcription following the first stimulus is therefore accompanied by the acquisition of specific "epigenetic profiles," which are only partially lost after the elimination of the stimulus. In this way, a kind of nonspecific "memory" is developed, which makes some innate immune cells more easily and rapidly activated, following a subsequent heterologous stimulus. Trained immunity has gained increasing scientific relevance in recent years, for the hypothesis that previous infections can induce a metabolic and epigenetic reprogramming of some cells of innate immunity, leading to an improved defense response during subsequent infections of various types, at the same time trained immunity could also be negatively involved in hyperactivation of the immune system leading to chronic inflammation, as in atherosclerosis [14].

2.1.2 Humoral component

The complement system represents a set of plasma and membrane proteins endowed with enzymatic activity that can result in direct lysis of the foreign agent. These proteins circulate in the blood as functionally inactive molecules, called components. Complement activation occurs by cascade mechanism events, leading to sequential activation of the various inactive components. There are distinct pathways of complement activation: classical (activated by antigen–antibody binding), alternative, and lectinic, which are triggered by different mechanisms, but then converge in a common pathway leading to the formation of the membrane attack complex, which,

by binding to the microorganism membranes, determines their osmotic lysis, through the formation of pores on the membrane itself [7].

2.2 Adaptive immune response

The adaptive immune response has the ability to recognize specific antigens and to remember those antigens in case of a subsequent exposure: this immunological memory allows a very rapid response, as the particular antigen has been already previously encountered and recognized. T and B lymphocytes, mediators of this response, undergo clonal expansion when they encounter the specific antigen they are programmed to recognize. At that moment, lymphocytes experience a real metabolic switch, increasing their metabolic needs for glucose and aminoacids, and passing from the normal oxidative phosphorylation typical of naive cells to aerobic glycolysis, in which pyruvate produced by glycolysis is reduced to lactic acid, with the simultaneous generation of NAD+ molecules, which promote the continuous production of 2 ATP molecules for each metabolized glucose molecule. This process, which occurs in the presence of oxygen, is less efficient than oxidative phosphorylation, but much faster, and thus able to meet the high ATP demand required to rapidly increase the biosynthesis of lipids, proteins, and nucleic acids of activated lymphocytes [15]. T lymphocytes exclusively recognize antigenic peptides exposed on the membrane of APCs via the major histocompatibility complex (MHC), whereas B lymphocytes recognize soluble, circulating antigens. Lymphocytes are present in an immature form in the primary lymphoid organs (bone marrow and thymus), where they differentiate into mature lymphocytes through a particular process of nonhomologous genetic recombination in the genes coding for antigen receptors (antibodies for B lymphocytes and T-cell receptors (TCR) for T lymphocytes). This somatic gene rearrangement accounts for the vast heterogeneity of lymphocytes, allows each individual to have a large and unique immunological repertoire, able to recognize a very large number of molecular configurations present in foreign agents, and thus counteract the majority of infections encountered during life [6].

2.2.1 T lymphocytes

T lymphocytes, effectors of the cell-mediated adaptive response, are divided into two major populations: T helper (Th) lymphocytes, bearing the CD4 receptor, which recognizes antigens presented by the MHC type II molecules, expressed on so-called "professional" APCs (dendritic cells, macrophages, and B lymphocytes), and cytotoxic T lymphocytes (Tc), with the CD8 receptor, which recognizes antigens presented by the MHC type I molecule, expressed on all nucleated cells [7]. CD4 and CD8 are co-stimulatory molecules, which bind to the MHC complex together with the TCR, contributing to T lymphocyte activation, which triggers different signaling cascades acting via various molecules and second messengers [16]. Th cells are critical in coordinating the immune response of other T cells and assist B cells in antibody secretion, whereas Tc cells are involved in the direct removal of damaged, pathogen-infected, or tumor cells. Th cells can develop into T helper 1 (Th1) or T helper 2 (Th2) cells, depending on the context in which antigen presentation occurs. Indeed, the cytokine secretion profile by APCs determines the "fate" of Th lymphocyte differentiation (**Figure 1**). The Th1 response is established in microenvironments where APCs produce essentially IL-12. This cytokine induces T lymphocytes to secrete IL-2 and interferon (IFN)-γ, through which cell-mediated responses, such as the antiviral

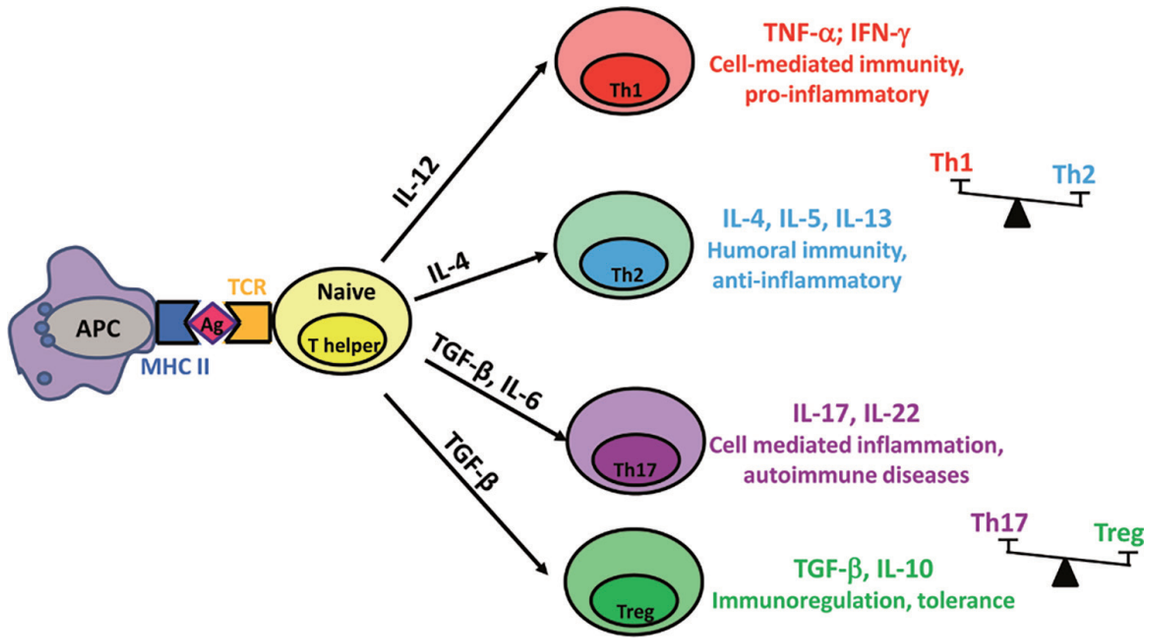

Figure 1.
Different differentiation fates of Thelper cells. Based on the context of antigen presentation and cytokine secretion, Thelper cells can differentiate in different subpopulations with pro- or anti-inflammatory features. The maintenance of the correct balance between them plays an important role for immune homeostasis. Ag, antigen; APC, antigen presentig cell; IFN, interferon; MHC, major histocompatibility complex; TCR, T cell receptor; TGF, transforming growth factor; Th, T helper; TNF, tumor necrosis factor.

response, are triggered. In particular, IFN-γ activates macrophages, inducing respiratory burst. Other important cytokines activated in this cascade, classically defined as pro-inflammatory, are IL-1, tumor necrosis factor (TNF)-α, and IL-8. In contrast, the Th2 response is associated with the production of IL-4, IL-5, and IL-13 cytokines, which mainly attract eosinophils and mast cells. The Th2 response, particularly involved in parasitic infections and allergies, is also associated with the expression of cytokines, such as IL-10, defined as anti-inflammatory, as they inhibit or reduce inflammatory-type responses. The maintenance of the correct balance between Th1 and Th2 subpopulations plays an important role in inflammation resolution [6]. Other relevant Th lymphocyte subtypes are the Th17, producing IL-17 and IL-22 cytokines, that are important in the response to extracellular pathogens such as fungi and bacteria, but also in some intestinal inflammatory responses; and the regulatory T (Treg) lymphocytes, involved in immune homeostasis especially in the intestine, as they contribute to the maintenance of oral tolerance to nonself harmless antigens, derived from food, but also environmental, such as pollen (**Figure 1**). The correct Th17/Treg balance is now recognized as fundamental for the maintenance of health status, in fact, this equilibrium results altered in many diseases with an autoimmune component, such as inflammatory bowel disease (IBD) [17].

2.2.2 B lymphocytes

B lymphocytes, expressing the surface receptor CD19, are responsible for the production of antibodies (or immunoglobulins), mediators of the adaptive humoral response. Upon encountering antigen, B lymphocytes can differentiate into short-lived antibody-producing plasma cells or long-lived memory cells. Plasma cells produce one of the five classes of immunoglobulins: IgG, IgM, IgA, IgD, and IgE,

each with a specific role. IgM, representing about 10% total Ig in serum, are the first Ig produced in response to a foreign antigen. After 5–6 days from infection normally IgM reach their peak concentration and, thanks to the specific profiles of cytokines released, the so-called "isotypic switch" to IgG occurs. IgG are the predominant and most important class of immunoglobulins, in fact, they represent about 70–75% total Ig in serum. IgG reach the peak of secretion about 14 days after infection and persist for long periods. Normally IgG are the most effective in foreign antigen removal, through the above-mentioned opsonization process, as well as through activation of the complement system [6, 8]. IgD are found in serum at low concentrations (representing less than 1% of all plasma Ig) and their biological functions, related to the regulation of peripheral tolerance to self-antigens and in the maintenance of mucosal homeostasis, involving also host-microbiota interactions, have been elucidated only recently [18]. IgA represent 15–20% serum Ig, but their concentration is higher in secretions (saliva, breast milk, tears, sweat, respiratory, and intestinal secretions) and in the mucosa, i.e., those tissues covering the hollow organs and therefore in contact with the external environment (digestive, respiratory, and genital apparatus), where IgA contribute to preventing microorganisms from adhering to and penetrating inside the body through epithelial cells. In intestinal mucosa, IgA are found in dimeric form (secretory IgA, sIgA), particularly important for the protection against bacteria and viruses from the lumen, but also for the maintenance of oral tolerance to harmless food antigens, as detailed below. IgE, present in serum only in trace amounts, play a role in the removal of extracellular parasites (such as helminths) by opsonization, but are also important mediator in allergic responses. Indeed, IgE bind to a receptor expressed on the membrane of basophils and mast cells, stimulating the degranulation and release of histamine and lipid mediators into the intercellular space, triggering the allergic reaction, as described above [8].

2.3 Soluble mediators of the immune response

Soluble mediators, called cytokines, low molecular weight "messenger proteins" secreted by many cell types, both immune and nonimmune, are involved in both innate and adaptive immune responses. Cytokines send intracellular signals by binding to specific membrane receptors present on the same cells that produced them, or on other target cells, that can be in proximity or not, thus acting in an autocrine, paracrine, or endocrine manner. In general, it is possible to distinguish four different types of cytokines, on the basis of their biological effect: 1 - cytokines produced by leukocytes, having effects on the leukocytes themselves: interleukins; 2 - cytokines with chemoattractant properties, i.e., with a positive effect on cell motility: chemokines; 3 - cytokines that induce differentiation and proliferation of stem cells: colony-stimulating factors; 4 - cytokines that interfere with viral replication: interferons. Cytokines can exert a pro- or anti-inflammatory action, but often the outcome depends on the context of the microenvironment where they are secreted, and on the cells involved [7].

3. The intestinal immune system

The intestinal immune system is the most extensive lymphoid tissue, given the enormous surface area of the intestinal mucosa with which it is associated. It is called gut-associated lymphoid tissue (GALT) and is mainly composed of:

organized lymphatic follicles, called Peyer's patches (PPs); mesenteric lymph nodes (MLN); *lamina propria* lymphocytes (LPLs); intraepithelial lymphocytes (IELs) (**Figure 2**) [1].

3.1 Inductive sites

PPs represent the main sites where antigenic presentation occurs, called inductive sites, where the intestinal immune response is triggered. The PPs are covered by an epithelial layer, containing specialized membranous cells, the M cells, responsible for the transport of antigens, bacteria, and macromolecules from the intestinal lumen into the patches. These specific characteristics on the one hand make M cells designated for the transepithelial transport of antigens, and on the other hand make them more easily accessible by pathogens. In fact, many pathogens use M cells as a "gateway" to cross the intestinal barrier. M cells do not have brush border nor glycocalyx, but they have an extensive system of endocytic vesicles and a large intraepithelial pocket, where vesicles, containing antigens from the lumen, are released. In the pocket are APCs, which acquire the material carried by M cells and present the antigens to naive lymphocytes, present in the underlying subepithelial layer, organized in lymphatic follicles. In such follicles, B lymphocytes are located in the germinal centers, whereas T lymphocytes preferentially occupy the periphery and interfollicular spaces. DCs are also able to expose luminal antigens through various mechanisms: in the *lamina propria*, they can take antigens directly from the lumen, as they are able

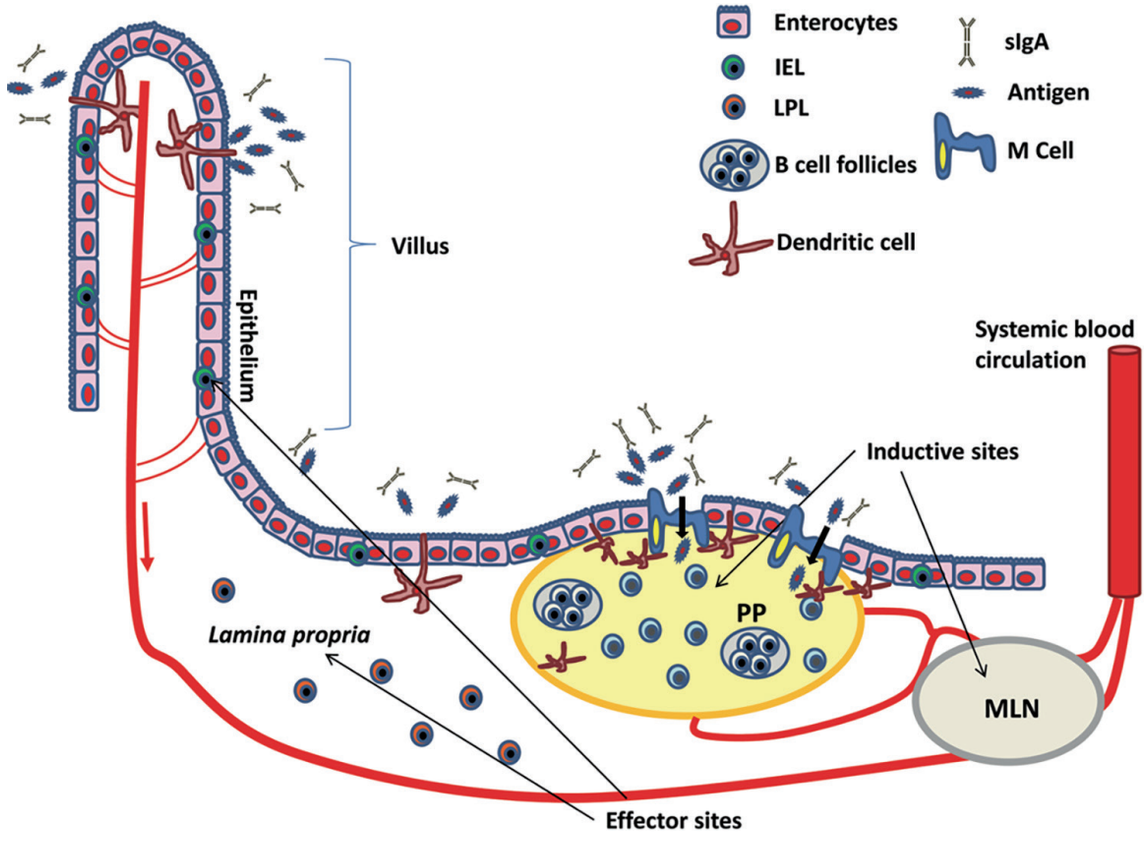

Figure 2.
Schematic representation of gut-associated lymphoid tissue (GALT) in small intestine. GALT is composed of organized lymphoid tissues of the Peyer's patches (PPs) and mesenteric lymph nodes (MLNs), the principal sites for induction of immune responses, while the lamina propria and epithelial layer are the effector sites. IEL, intraepithelial lymphocyte; LPL, lamina propria lymphocyte; MLN, mesenteric lymph nodes; PP, Peyer's patches.

to interdigitate between epithelial cells; or they can take luminal antigens that cross the intestinal barrier through transient "openings" [19]. After being primed, naive T and B cells become memory/effector cells and migrate from PP to MLN via efferent lymph and then via the thoracic duct to peripheral blood for subsequent extravasation at mucosal effector sites, both intestinal and extraintestinal, where the immune response will take place [20]. Other than a large number of lymphocytes, MLN also contain macrophages and APCs, which can themselves initiate immune responses against incoming antigens.

3.2 Effector sites

In the intestine, activated B and T lymphocytes essentially target two different lymphoid compartments: the *lamina propria* and the mucosal epithelium. The B lymphocytes of the *lamina propria* essentially produce IgA, the main class of antibodies secreted in the intestinal mucosa in large quantities, as mentioned above. In fact, it is estimated that 80% of plasma cells secreting antibodies reside in the intestinal *lamina propria*. The main function of IgA is to contribute to the intestinal barrier as the first line of defense, binding to antigens, neutralizing them, and removing them from the mucosa. IgA, unlike IgG, do not trigger an inflammatory response, as they do not bind to the complement system. As previously mentioned, IgA are found in the mucosal secretion as dimers, associating with a polypeptide present on the basement membrane of enterocytes, the secretory component (SC). Through the SC, IgA are transported through enterocytes and released into the intestinal lumen, becoming sIgA. This component gives sIgA resistance to proteases in the lumen, rendering them well-designated to perform their function in the intestine. Antigens able to bypass this first line of defense reach the *lamina propria*, where they encounter IgG, and the resulting immune complexes activate the complement system and trigger the inflammatory response. T lymphocytes in the *lamina propria* are effector cells, essentially CD4+ (helper/inducer phenotype) [21]. In the spaces between enterocytes, above the basement membrane (subepithelial space), there are populations of resident IELs, essentially CD8+ (suppressor/cytotoxic phenotype), acting as "sentinels", being the first components of the intestinal immune system exposed to food and microbial antigens. Indeed, IELs are among the most abundant lymphocyte populations in the body and play a key role in host defense against pathogens, wound repair, and intestinal homeostasis maintenance. IELs are composed of various cell subtypes bearing different TCRs, that can recognize antigenic peptides presented by conventional MHC molecules or by nonclassical MHC molecules, meaning that these cells are able to respond to some bacterial antigens in the absence of antigenic presentation by APCs [22].

4. The gut microbiota

4.1 Structure and function

Body surfaces facing the external environment, namely the skin and all mucosal surfaces (nasal, oral, gastrointestinal, etc.) are colonized by a huge number of microorganisms, collectively called microbiota. Most of them reside in the gut, in a *continuum* of extremely dynamic microbial communities. In terms of microbial density, it is estimated that approximately 10^{12} microorganisms per gram of content

are present in colon and feces. These microorganisms belong to all three domains of life: Bacteria, which predominate, Archaea (methanogens, essentially belonging to *Methanobrevibacter* and *Methanosphaera* genera), and Eukarya (fungi and protists) [23]. The evolution of the intestinal microbiota starts at birth and is completed during the first years of life until it stabilizes in the adult phase. Immediately after birth, the gastrointestinal tract is rapidly colonized by a microbial consortium whose composition varies depending on several factors, such as the mode of delivery (vaginal or caesarian), the diet during infancy (breast or formula milk), and during adulthood (for example, vegetable or meat-based), the use of antibiotics. In particular, breastfeeding stimulates the maturation of the intestinal microbiota, as breast milk contains bifidogenic oligosaccharides (HMO, human milk oligosaccharides), which have a prebiotic action [24]. The maturation is then completed within the first years of life and occurs in parallel and synergistically with the development of the immune system. Perturbations of gut microbiota composition are associated with aging, and these changes favor the growth of pathogens and increase the susceptibility to gut-related diseases [25]. In this complex ecosystem, the collective genomes of bacteria and other microorganisms have been the focus of increasing interest over the past two decades, facilitated by the rapid development of culture-independent genomic approaches and advanced computational technologies. The gut microbiota is characterized by an enormous phylogenetic diversity, with more than 1000 bacterial species found in the entire human population, among which about 150 are present in a single individual. At higher phylogenetic levels this biodiversity is reduced, in fact, the human gut microbiota is composed of two main populations belonging to the Firmicutes and Bacteroidetes phyla, which collectively constitute over 90% of the known phylogenetic taxa. Other less abundant, but not less important phyla, such as Actinobacteria, Proteobacteria, and Verrucomicrobia, whose relative abundances are often below 1%, are also present. The advent of culture-independent methods, although detecting a high inter-individual variability in the composition of the intestinal microbiota, has allowed to identify a common "microbial core", with shared metabolic activities, characterizing healthy individuals [26]. Indeed, the relative proportions of the various phyla are maintained in balance under physiologic conditions (eubiosis), whereas changes in microbial composition and function, termed dysbiosis, associated to a lower overall microbial diversity, often occur in immune-mediated and metabolic disorders, thus proving the important role of the gut microbiota in maintaining host health status, which goes far beyond the initial experimental observations about relevance in regulating body fat tissue accumulation and energy balance [27]. The microbiome, defined as the collective genome of the gut microbiota, contains approximately 3.3 million genes, a number about 150-fold higher than that of the genes of the human genome, most of which are involved in both the metabolism of carbohydrates, amino acids, cofactors, and vitamins, and the biosynthesis of secondary metabolites. Thanks to this enormous genetic heritage, intestinal microorganisms exert a profound influence on the nutritional, metabolic, and immune responses of the host, so that the intestinal microbiota is considered an "accessory organ" and the higher organisms, with their associated microbial communities, are defined as "holobionts" [28]. As mentioned, the main function of the gut microbiota concerns metabolic activity. Intestinal bacteria are, in fact, able to produce essential nutrients such as vitamins and, mostly, to extract energy from complex polysaccharides, which are not digestible by the human enzymes present in the gastrointestinal tract. Indeed, the microbiota possesses the metabolic capacity to degrade a wide range of substrates that reach the colon. In particular, the fermentation of complex polysaccharides

produces, among other substances, the short-chain fatty acids (SCFA), essentially acetate, propionate, and butyrate, which play a key and multifactorial role in the physiology of the host. Microbiota also contributes to the barrier effect, counteracting colonization by enteropathogens and opportunistic pathogens. The main mechanisms involved are both direct, such as competition for nutrient resources and adhesion sites to the intestinal mucosa, the inhibition of bacterial growth through the creation of microenvironments at acidic pH, and the production of bacteriocins (such as colicins, microcins, and nisin), and indirect, through stimulation of the host immune system and of maturation and growth of enterocytes [29]. Moreover, it is now universally recognized the existence of a gut-brain axis that envisages an active contribution of the intestinal microbiota in the regulation of anxiety, pain, and behavior by acting on the synthesis of neurotransmitters, and a possible contribution to the pathophysiology of disorders of the central nervous system. Finally, the gut microbiota is also able to interact and modulate the endocrine system, strongly influencing the levels of stress-related hormones and insulin, as well as appetite [30].

4.2 Influence of gut microbiota on the immune system

The intestinal microbiota is recognized as an effective integral component of the host immune system, capable of finely tuning the immune responses, innate and adaptive, in the different phases of life. Indeed, the close relationship established between bacteria and immune cells in the gut is crucial for the maintenance of immunological homeostasis and, mostly, for the "education" of the immune system during the early stages of life [2]. In fact, according to the most recent theories, the interaction between microbiota and the immune system is necessary to "train," first, and "keep trained," then, the various functions of the latter. Thanks to the continuous contact with the gut microorganisms, with the molecules they synthesize, with those they produce from undigested food components, the immune system satisfies two apparently conflicting needs: to defend the organism from real threats, and to tolerate microbes and molecules not harmful to the organism. Indeed, the large variety of microorganisms constituting the microbiota can be functionally distinguished into symbionts and pathobionts, also referred to as opportunistic pathogens, both fundamental, as the former educate the immune system to tolerance, while the latter train it to pathogen recognition and attack [31]. In the physiological condition of eubiosis, symbionts and pathobionts are present in equilibrium. If this balance is altered, for example, due to an excessive antibiotic treatment, one of the two groups becomes predominant, leading to the onset of one of two possible extreme conditions: hyperstimulation of the immune system (inflammation) or hypostimulation (immunosuppression) [32] (**Figure 3**). It is worth noting that pathobionts, that are not harmful and are even necessary to educate the immune system in physiological conditions, become dangerous when the equilibrium is altered, as in dysbiosis. The immunological surveillance of the intestinal microorganisms involves the above-mentioned TLRs, which recognize MAMPs and PAMPs [9, 33]. These receptors differently act in distinct cellular compartments. Indeed, recognition of these receptors on the apical surface of the epithelium (i.e., the one in contact with the intestinal lumen) generally promotes tolerance towards commensal bacteria and foodborne antigens, and low (basal) inflammatory tone; conversely, activation of these same receptors on the basolateral side, in contact with the underlying mucosa, promotes strong inflammatory responses. Numerous microbial stimuli activate inflammatory cascades through signal transduction pathways that essentially involve the nuclear

transcription factor NF-κB, with consequent production of pro-inflammatory cytokines, such as IL-1 and IL-6, or anti-inflammatory factors more directly related to the extinguishing of inflammation/immune response, such as IL-10, thus playing a crucial role in maintaining intestinal homeostasis [10]. The different communities of the intestinal microbiota, characterized by metabolic specialization, complementarity, and cooperation, constitute a very complex network of microbe-microbe and microbe-host interaction, in the form of a symbiotic or mutualistic relationship, resulting in a continuous cross-talk. The host derives substantial immunological and metabolic benefits from the physical proximity of microbial populations in the gut and underlying tissues, but at the same time, this proximity poses an ongoing threat to health. In fact, although the immune system is designed to establish the proper balance between tolerance to the gut microbiota, maintaining a low level of basal inflammation and surveillance against infectious agents and opportunistic pathogens, the disruption of this balance, for example, due to inflammatory diseases or following the excessive use of antibiotics, induces a malfunction of the intestinal barrier with consequent opening of the junctions between enterocytes. The assembly and maintenance of tight junctions are regulated by several signaling pathways, that can be altered by pro-inflammatory cytokines, in particular TNF-α, IFN-γ, and IL-1β. Thus, an increase of these cytokines due to an inflammatory status can induce a decrease in the expression of tight junction proteins, or alter their phosphorylation status, causing a "loosening" of tight junctions [34]. This condition, referred to as a leaky gut syndrome, facilitates the translocation of pathogenic bacteria or harmful antigens from the intestinal lumen to the underlying mucosa (**Figure 3**). This process determines the establishment of endotoxemia, i.e., the presence of LPS in the circulation. The LPS, present on the cell wall of Gram-negative bacteria, is one of the microbial components able to act as an immune activator, therefore, representing a MAMP that

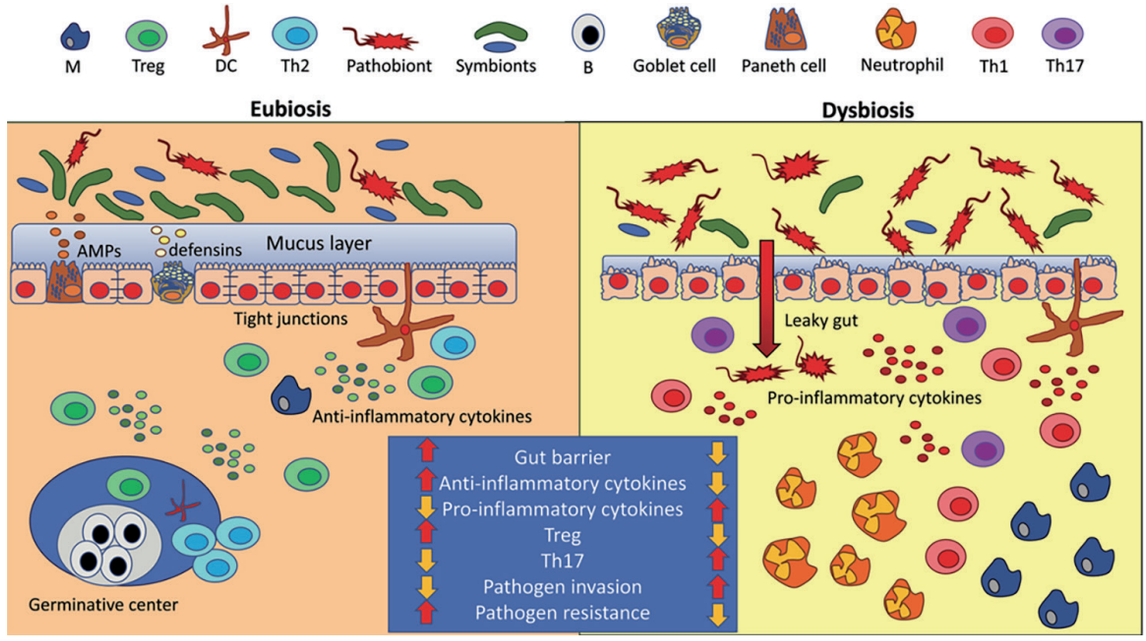

Figure 3.
Schematic representation of eubiosis and dysbiosis conditions. Gut immunological homeostasis is the result of a continuous cross-talk between microbiota and immune system. In eubiosis, the commensals predominate over pathobionts, maintaining the integrity of the intestinal barrier and an anti-inflammatory milieu. In dysbiosis, pathobionts take over and cross epithelial barrier inducing inflammation. AMPs, antimicrobial peptides; B, B lymphocyte; DC, dendritic cell; M, macrophage; Treg, regulatory T cell.

binds to TLR4 and triggers an inflammatory response, which from local becomes systemic. The polysaccharide A of *Bacteroides fragilis*, on the other hand, triggers an anti-inflammatory response, by stimulating IL-10 production and Treg proliferation [35]. Other bacteria, such as segmented filamentous bacteria (SFB), is pathobionts present exclusively during the first years of life, and play an important role in the immune system training, by inducing IL-17 secretion in the intestine and stimulating the production of IgA in the mucosal membranes of the oral and respiratory cavity [36]. Moreover, some evidence shows that SBF can promote IL-22 production by ILCs [37]. Fundamental to the maintenance of intestinal homeostasis is the proper balance of the different T lymphocyte subpopulations, mentioned in the 2.2.1 paragraph of the present chapter. In particular, the Th17/Treg balance appears crucial, and this balance is also modulated by the microbiota. It is worth noting that the interactions between microbiota and the immune system can have different outcomes, depending on the context of eubiosis or dysbiosis. Recently, the advent of high throughput molecular sequencing techniques has allowed the isolation from the human intestine of some bacteria with anti-inflammatory activity, which are of particular interest, especially for their possible applications in counteracting obesity and inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. Among the most important are *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which have been defined as "next generation probiotics," as they are not yet commercially available, but candidates to be used as "biotherapeutics" [38].

5. Diet as a modulator of the microbiota-immune system cross-talk

An adequate and appropriate nutritional status and composition of the diet, in terms of foods, nutrients, and bioactive substances, are critical for the proper functioning of the immune system, which in turn is a fine sensor of the nutritional status of the individual [39]. When the immune system is activated to respond to nonself antigens, the demand for energy and nutrients increases and cells undergo the metabolic switch [40], as previously mentioned in the 2.2 paragraph. The dependence of the immune response on energy, and therefore the onset of immune deficits as a result of undernutrition, is known for a long time, but recently it has been observed that also the excessive consumption of food and excessive intake of calories alter the immune system. In fact, if on the one hand a serious caloric restriction impairs immune system functionality and increases the risk of infections (as observed in childhood malnutrition, still widely spread in developing countries), on the other hand, an unbalanced diet rich in high-calorie foods leads to negative consequences, inducing an inflammatory state and metabolic disorders. Many metabolic diseases are in fact characterized by a chronic low-grade systemic inflammation, called metaflammation (metabolic inflammation). Obesity and overnutrition are both associated with this inflammatory state leading to an increased risk of cardiovascular disease, heart attack, type 2 diabetes, and nonalcoholic steatohepatitis [41].

5.1 Impact of diet on gut microbiota

Diet has a profound influence also on the gut microbiota, acting both as a modulator able to select specific microbial groups, and as a provider of substrates that can be metabolized by the microbiota producing metabolites that impact on host health status, also through interaction with the immune system. Therefore, there is a close

connection between diet, gut microbiota, and immune system, orchestrated by a fine tuning of the complex mechanisms underlying this cross-talk. The influence of diet in modulating gut microbiota composition is related to the concept of "enterotype." Indeed, although a wide inter-individual variability is observed among the bacterial groups present in the gut, the microbiota of most individuals can be classified into one of three variants or enterotypes, based on the dominant genera (*Bacteroides*, *Prevotella*, or *Ruminococcus*), which constitutes a relatively stable "core" [3, 26]. These enterotypes are associated with long-term dietary regimens [42]. In particular, enterotype 1, characterized by a predominance of the genus *Bacteroides*, able to extract the maximum energy from the fermentation of carbohydrates and proteins and to produce high amounts of vitamins B2 (riboflavin), B7 (biotin), and ascorbic acid (vitamin C), is associated with a diet rich in animal proteins and fats and low in fiber and vegetables, typical of the "Western Diet" profile. This enterotype may be related to increased intestinal inflammation and consequently to an increased state of general inflammation. Enterotype 2, dominated by the *Prevotella* genus, able to degrade complex polysaccharides and to produce high levels of vitamin B1 (thiamine) and vitamin B9 (folic acid), is instead correlated with a diet profile rich in fiber and carbohydrates. Finally, enterotype 3 is characterized by a predominance of bacteria of the genus *Ruminococcus* and is associated with a dietary profile rich in simple sugars [43]. Although most published papers demonstrate how long-term dietary regimen affects the structure and activity of the gut microbiota, there is still evidence suggesting the ability of the microbiota to respond to short-term dietary change in terms of macronutrients. For example, short-term consumption of diets composed entirely of animal or plant products has been shown to alter the structure of the gut bacterial community, minimizing inter-individual differences. Specifically, the most pronounced effect has been found for diets based on animal products, resulting in increased levels of bile-tolerant microorganisms (*Alistipes*, *Bilophila*, and *Bacteroides*), and decreased levels of Firmicutes capable of metabolizing plant polysaccharides (*Roseburia*, *Eubacterium rectale,* and *Ruminococcus bromii*) [44].

5.1.1 Effect of macronutrients

Among macronutrients, the effect of carbohydrates on the microbiota is the most described, while for proteins and lipids the mechanisms are less defined. Micronutrient intake is also critical for gut well-being; in fact, vitamin deficiencies have been associated with alterations in barrier function and GALT immune response. However, it is important to emphasize that modifications to the immune system and microbiota are primarily associated with the composition of the diet as a whole, and not with specific foods or nutrients [4]. Many complex carbohydrates are known to act as prebiotics, selectively stimulating, in the intestine, the growth of microorganisms beneficial to human health, such as bifidobacteria. Dietary fiber is a heterogeneous and complex mixture of different combinations of monosaccharides, with a minimum of 10 monomeric units or oligosaccharides containing from 3 to 9 monomeric units. A further classification of dietary fiber is related to its water solubility, viscosity, and fermentability. Polysaccharides are further categorized in non-starch polysaccharides and resistant starch, while oligosaccharides include resistant oligosaccharides. Soluble fiber is typically fermented to SCFA by the intestinal microbiota. A growing body of literature shows that dietary fiber has the potential to change the gut microbiota and alter metabolic regulation in humans. Most findings supporting the fiber hypothesis are based on short-term dietary interventions, while only sparse data

evaluating the impact of long-term dietary fiber on the gut microbiome exist. Specific sources of dietary fiber were differentially associated with the gut microbiome. Fiber from fruit and vegetable intake was related to the gut microbiome composition, characterized by an increased abundance of Clostridia, an important class of dietary fiber fermenters producing SCFA. Other evidence showed an association between legume fiber intake and Actinobacteria abundance, particularly Bifidobacteriales [45]. A recent systematic review demonstrated that the most consistent results can be related to an increased abundance of SCFA-producers, alterations in microbiota diversity, and in the *Prevotella*/*Bacteroides* ratio. However, to what extent a dietary intervention with fiber may affect the human gut microbiota and hence metabolic regulation is currently not well described, due to differences in methodologies and lack of standardization that hamper the interpretation of the results [46]. It is known that also proteins can shape gut microbiome, and that different protein sources differently impact its profile. As an example, a diet rich in pea protein has been shown to increase *Bifidobacterium* and *Lactobacillu*s levels [47]. Approximately 12–18 g of dietary protein reaches the human colon daily. Several gut microbiota species such as *Clostridum* spp., *Bacteroides* spp., and *Lactobacillus* spp. can metabolize proteins through different proteases [48]. Microbial metabolites deriving from dietary protein fermentation by gut microbiota include short branched chain fatty acids, sulfur-containing products, aromatic compounds, polyamines, and ammonia. Interestingly, several neuroactive compounds including neurotransmitters such as GABA, norepinephrine, dopamine, serotonin, and histamine are produced from amino acids by gut microbiota, and this is one of the most attractive topics to understand the role of microbiota in gut-brain axis [48]. On the other hand, the pro-atherogenic metabolite trimethylamine-N-oxide (TMAO) is produced by the combined activity of microbial and host enzymes after consumption of animal proteins, with a negative impact on health. Most of the ingested fatty acids are absorbed in the human small intestine, but a small fraction (about 7%) reaches the colon. With respect to carbohydrates and protein, the impact of dietary fats on gut microbiota profile is less reported. The most characterized effect of a high-fat diet is related to a decreased Bacteroidetes/Firmicutes ratio [49].

5.2 Effect of microbial metabolites on the host immune system

Most of the physiological effects of the microbiota are mediated by metabolites produced by the bacteria themselves or derived from the microbial transformation of host molecules. In fact, the gut microbiota has a high potential to synthesize bioactive compounds by acting on molecules of endogenous origin or derived from the diet. As previously mentioned, SCFAs are the principal metabolites derived from the microbial fermentation of complex polysaccharides. Acetate and propionate are mostly produced by Bacteroidetes, while Firmicutes are the principal butyrate-producing microorganisms [50]. While propionate and acetate reach the liver through the portal vein, where they contribute to gluconeogenesis and lipogenesis, respectively, butyrate, mainly produced by Firmicutes, plays a fundamental role in the intestine and represents the major fuel for enterocytes. SCFAs, especially butyrate, are molecules fully capable of transducing signals, as they are ligands of G-Protein Coupled Receptors (GPCRs). This interaction activates various molecular signaling pathways in the different intestinal cells, resulting in strengthening the intestinal barrier and exerting an anti-inflammatory action. In particular, Paneth cells are stimulated to release antimicrobial substances; intestinal endocrine L cells release satiety peptides, glucagon-like-1 (GLP-1) and peptide YY (PYY); goblet cells are stimulated to produce

mucin, while in epithelial cells butyrate exerts a trophic effect, promoting the expression of junction proteins and cell regeneration. SCFAs also have important actions on both innate and adaptive immune cells present in the intestine, increasing IL-10 expression levels and promoting Treg cell differentiation. SCFAs are also epigenetic modulators, as they act as inhibitors of histone deacetylase enzymes, resulting in transcriptional activation of several genes, including a Treg cell-specific transcription factor, Foxp3, that leads to an anti-inflammatory phenotype, through inhibition of NF-κB. In other contexts, however, it has been observed that SCFAs may have opposite, pro-inflammatory effects, especially in the presence of LPS or TNF-α. This observation demonstrates how the same molecule can have beneficial or detrimental effects, depending on the concurrent conditions of eubiosis or dysbiosis [4, 51]. The microbiota plays an essential role also in the metabolism of bile acids, influencing their profile with over 20 different secondary bile acids produced. Such diversity of bile acids composition differently affects the physiology and metabolism of the entire body. Cholesterol-derived primary bile acids, essentially cholic and chenodeoxycholic acid, are first conjugated with taurine and glycine in the liver to form the corresponding conjugated bile salts which are stored in gallbladder. Released into the duodenum after an abundant meal, most bile salts (95%) are reabsorbed from the terminal ileum and colon and delivered back to the liver via the portal vein in a process known as enterohepatic circulation [52]. A small percentage of bile salts, estimated at around 5%, reaches the colon, where they are deconjugated in a reaction catalyzed by bile salt hydrolase (BSH), and mediated by a broad spectrum of aerobic and anaerobic bacteria (Gram-positive *Bifidobacterium*, *Lactobacillus*, *Clostridium*, and *Enterococcus*, and Gram-negative *Bacteroides*). Then bacterial dehydrogenase enzymes convert primary bile acids into the secondary bile acids deoxycholic and lithocholic acids. This reaction is mediated by a limited number of bacteria belonging to B*acteroides, Clostridium, Eubacterium, Lactobacillus*, and *Escherichia* genera. [53]. Thus, gut microbiota composition determines the profile of secondary bile acids that are produced. The secondary bile acids are absorbed into the colon, return to the liver and after being conjugated enter the enterohepatic circulation. Secondary bile acids can undergo epimerization, sulfation, glucuronidation, and conjugation with N-acetylglucosamine in the liver, kidneys, and gut to form tertiary bile acids [52]. Bile acids exert multiple physiological functions, which are: 1 - intestinal detergent activity that solubilizes dietary lipids and fat-soluble vitamins promoting their absorption; 2 - hormone- like properties by acting as signaling molecules via two independent pathways, farnesoid X receptor (FXR) and G protein-coupled bile acid receptor (TGR5) signaling. Binding FXR, bile acids can regulate their homeostasis, as well as lipogenesis, gluconeogenesis, tumor suppression, and intestinal barrier function; while through TGR5, they regulate glucose homeostasis, energy expenditure, and anti-inflammatory response. Different bile acids have different affinities towards these receptors, with secondary bile acids preferentially activating TGR5; 3- antibacterial properties providing protection against invasive microorganisms, and acting as mediators of gut innate defense. However, it is important to note that bile acids can become cytotoxic at high concentrations, and excessive accumulation can lead to oxidative stress, apoptosis, and liver damage [54]. In this context, any dietary component, which could influence gut microbiota composition, may also modulate bile acid homeostasis and the ability to impact host health. High dietary fat intake is known to increase primary bile acids release into the small intestine and stimulate secondary bile acid synthesis mediated by various bacteria, including *Lactobacillus*, *Bifidobacterium,* and *Bacteroides* [55]. Milk fat has been shown to induce shifts in hepatic conjugation of bile acids in mice,

from glycocholic to taurocholic acid, compromising barrier integrity and resulting in increased abundance of *Bilophila wadsworthia*, a bile-tolerant pathobiont able to trigger a Th-1 immune response [56]. Carbohydrate intake affects bile acids metabolism, in particular, the role of soluble and insoluble dietary fiber in binding bile acids is well-documented in several studies. In a recent randomized cross-over clinical study, the consumption of a diet rich in whole grains, legumes, vegetables, and fruits was compared with a refined grain diet (high glycemic load) for the effect on circulating bile acids. The results showed a significant increase in the concentrations of specific bile acid ligands of FXR and TGR5 associated to a reduction of insulin resistance [57]. However, the role of the diet on bile acids composition and health is still partially known and needs to be confirmed and expanded in order to translate these findings into clinical settings. Lastly, tryptophan metabolites represent another important class of bacterial metabolites. Tryptophan is an essential amino acid and an important precursor of both microbial and host metabolites. Tryptophan can follow three different metabolic pathways, leading to the formation of serotonin, quinurenine, or indole and its derivatives, which represent the ligands of the aryl hydrocarbon receptor (AhR). In particular, the main microbial metabolite is indole, although the metabolic processes and pathways are complex and multiple. Indole derivatives are considered key mediators of intestinal homeostasis, as they act on epithelial renewal and barrier integrity through the activation of AhRs, which are expressed on many immune cell types, such as IELs, Th17 cells, ILCs, macrophages, dendritic cells, and neutrophils. The main effect is the production of IL-22 by ILC3, which in turn regulates metabolism by improving insulin sensitivity, modulating lipid metabolism in adipose tissue and liver, and promoting intestinal barrier integrity. Indole metabolites may also promote Th17/Treg reprogramming [4, 51].

5.3 Western diet and Mediterranean diet: examples of detrimental and beneficial dietary profiles

The scientific literature describes the diet as the most characterized factor capable of shaping gut microbiota and immune system. Indeed, the nutritional status of an individual and the composition of the diet, in terms of foods, nutrients, and bioactive substances, influence immunity. A recent analysis by Rinninella and colleagues [58] highlighted the effects of different dietary habits on gut microbiota composition, by comparing vegetarian/vegan, gluten-free, ketogenic, low FODMAP (i.e., low in highly fermentable but poorly absorbable carbohydrates and polyols), Western and Mediterranean diets. Overall, restrictive diets (gluten-free, ketogenic, low FODMAP) have been shown to exert negative effects on the intestinal microbiota, in terms of reduction of biodiversity and alteration of eubiosis, impacting also on the integrity of the intestinal epithelium (especially in the case of ketogenic diet), and on inflammatory status. Among the different dietary profiles, the most consistent evidence concerns the Western Diet and the Mediterranean Diet, indeed Western Diet was shown to negatively impact gut microbiota composition and diversity, and to reduce the intestinal mucus layer, thus favoring bacterial translocation and endotoxemia, while Mediterranean Diet was associated to increased bacterial diversity and improved gut barrier function [58]. The Western Diet is typically described as a diet high in calories and rich in ultra-processed foods with high levels of sugars, saturated and trans fats, salt and food additives, while complex carbohydrates, fiber, vitamins and minerals, and other bioactive molecules (such as polyphenols and omega-3 fatty acids) are scarcely present. The main effects of this diet

concern the elevation of plasma glucose and insulin levels, with a consequent increase in the accumulation of lipids in adipose tissue, which induces a rapid weight gain compared to more balanced diets. Furthermore, recent rodent and human studies have established that the Western dietary pattern is associated with elevated levels of inflammatory biomarkers, suggesting a direct or indirect action on the immune system [59]. It is noteworthy that macronutrients in food are part of a complex microstructure from which the physical, sensorial and nutritional properties, and health implications derive. "The complex assembly of nutrients and non-nutrients interacting physically and chemically, that influences the release, mass transfer, accessibility, digestibility, and stability of many food compounds" has been described as food matrix [60]. Therefore, diverse food matrices can differently affect the digestion and absorption processes of food compounds and play a role in the microbial fermentation of unabsorbed components. Ultra-processed foods and beverages are considered an important hallmark of the Western Diet, and high consumption of these foods appears to be correlated with an increased risk of morbidity. Food processing involves applying controlled procedures in order to preserve, destroy, transform, and create edible structures, whose aim is to prolong the shelf -life of foods. Ultra-processed foods are microbiologically safe, highly palatable, ready-to-eat, and highly profitable products composed primarily of ingredients not routinely found in "real foods" (e.g., hydrogenated/de-esterified oils or additives designed to provide the previously mentioned characteristics). The poor and uncomplex matrix of these foods, together with their low fiber content, generates an unfavorable environment in the gut and microbiota, thus leading to dysbiosis and immune alterations. Therefore, the Western Diet, intended also as an incorrect lifestyle, would induce low-grade inflammatory processes, which are a risk factor for the development of various chronic inflammatory diseases, predisposing the individual to metabolic inflammation, through various mechanisms, acting at both levels of microbiota and intestinal permeability [61]. The action on the microbiota leads to the onset of dysbiosis, intended both as taxonomic (shifts in microbial groups composition), but especially as metabolic (changes in microbial function). Moreover, the decreased bacterial diversity makes the microbial ecosystem less resilient and more susceptible to external stressors. The increase in pathogenic bacteria also causes an increase in pro-inflammatory metabolites, which can influence the response at the level of GALT, with which they are intimately linked. When abnormalities occur in these interactions, intestinal permeability can increase and the leaky gut phenomenon occurs, leading to metabolic endotoxemia, as described previously. But metabolic inflammation arises primarily at the level of white adipose tissue, where adipocytes, cells almost entirely formed by a single large lipid droplet, release numerous adipokines, cytokine-like molecules, in response to changes in lipid accumulation and in local and systemic inflammation. Adipokines can be either pro- or anti-inflammatory and play a key role in linking metabolism with immune function [62]. In individuals with normal metabolic status, pro- and anti-inflammatory adipokines are correctly balanced, and Th2 lymphocytes, Treg cells, and macrophages with an anti-inflammatory phenotype predominate in adipose tissue. Treg cells secrete IL-10 and also stimulate IL-10 secretion by macrophages. Eosinophils secrete IL-4 and IL-13, further contributing to the anti-inflammatory and insulin-sensitive phenotype. A long-term hypercaloric diet causes an increase in the number and size of adipocytes, which become hypertrophic and dysfunctional, starting to secrete pro-inflammatory adipokines, especially TNF-α. In addition, circulating immune cells, mainly monocytes, are recruited from the bloodstream in

response to chemotactic signals (particularly monocyte chemoattractant protein 1, MCP-1) produced in adipose tissue, transmigrate there, and differentiate into macrophages secreting high amounts of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6. These cytokines act in a paracrine manner, inducing changes in T lymphocyte populations, with a decrease in Treg and an increase in Th1 cells, which in turn secrete pro-inflammatory cytokines, thus generating a vicious circle, where the inflammatory state becomes systemic. Indeed, cytokines and chemokines from adipose tissue can act in an endocrine way and promote inflammation in other tissues, also causing the onset of insulin resistance and other metabolic disorders associated with obesity [63]. Adipocytes in visceral adipose tissue are metabolically very active and very sensitive to lipolysis, so following a prolonged positive caloric balance, very high amounts of free fatty acids (FFAs) are generated and released into the portal system. Insulin resistance results from an excess of circulating FFAs and excess TNF-α in adipose tissue, as both molecules result in functional blockade of the insulin receptor and its associated signal transduction. In particular, FFAs and TNF-α block insulin receptor signaling by activating phosphorylation of the insulin receptor substrate (IRS)-1 at a serine residue. Serine phosphorylation of IRS-1 causes it to detach from the insulin receptor, resulting in functional blockade of the receptor and of insulin signal transmission itself. In addition, TNF-α, secreted by adipocytes and adipose tissue macrophages, also acts by another mechanism, namely by inducing dephosphorylation of IRS-1 at tyrosine residues. Tyrosine-dephosphorylation has the same effect as serine-phosphorylation, thus IRS-1 is inactivated and detached from the insulin receptor [64]. It is known that several components characterizing the Western Diet determine an inflammatory state through the activation of the innate immune response, for example, excess cholesterol is considered the main cause of inflammation in the atherosclerotic process. In addition, an excess of free cholesterol crystals causes damage to lysosomes, with subsequent release of the pro-inflammatory cytokines IL-1β and IL-18 through activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, resulting in a systemic response characterized by a chronic low-grade inflammatory state, associated to insulin resistance and the onset of some related diseases, including colorectal cancer [65]. Saturated fatty acids carried through excessive consumption of animal-derived foods also have cytotoxic effects and can activate endoplasmic reticulum stress as well as the NLRP3 inflammasome. More recent theories suggest that saturated fatty acids induce dysbiosis and subsequent release of metabolites that alter intestinal permeability, inducing metabolic endotoxemia [59]. From a taxonomic point of view, an excess of fat causes an increase in the Firmicutes/Bacteroidetes ratio, while some unrefined oils, such as palm oil, may cause a decrease in *A. muciniphila* and *Clostridium leptum*. Through the consumption of red meat, eggs, and high-fat dairy products, dietary introduced L-carnitine and phosphatidylcholine are converted to the pro-atherogenic metabolite TMAO through a two-stage process, including first a fermentation process by the intestinal microbiota in an anaerobic environment, and then an oxidation catalyzed by the hepatic enzyme Monooxygenase containing Flavin 3. TMAO is a metabolite involved in the activation of inflammatory macrophages and the formation of atherosclerotic plaque foam cells, thus contributing to increased cardiovascular risk [59]. Consumption of excessive amounts of red meat also leads to elevated amounts of iron-eme, which has been associated with alteration of certain bacterial groups including *Escherichia coli* and *B. fragilis*. In general, levels of bacterial genera capable of metabolizing plant polysaccharides such as *Roseburia*, *Eubacterium*, *Ruminococcus,* and *Prevotella* are

underrepresented in individuals on the Western Diet, whereas the relative abundance of bile-tolerant microorganisms increases [44]. The concept of Mediterranean Diet has been developed to describe the eating habits followed by the populations of the Mediterranean basin, mainly Greece and Southern Italy. The Mediterranean Diet is based on the consumption of fruits, vegetables, legumes, dried fruits, fish, olive oil, and whole grains which together provide a combination of complex carbohydrates, polyunsaturated fatty acids, and bioactive molecules such as polyphenols and other antioxidants. It is also characterized by a low consumption of proteins of animal origin. A large number of epidemiological studies have shown that the Mediterranean Diet is associated to increased life expectancy, improved quality of life, and lower prevalence of diseases related to chronic inflammation, such as coronary heart disease, type 2 diabetes, and some forms of cancer. These beneficial properties are mediated by different mechanisms, including lipid-lowering, anti-inflammatory, and anti-oxidant effects. Accumulating evidence suggests that such activities are not ascribable to single foods or nutrients, but to interactions and synergistic activities of different nutrients and bioactive compounds from a variety of diverse foods with intact matrices [66]. Among the many molecules found in these foods, omega-3 polyunsaturated fatty acids, polyphenols, as well as fiber, can be mentioned. In particular, omega-3 contributes to balancing the Firmicutes/Bacteroidetes ratio and to increase bifidobacteria and Lachnospiraceae [67], while some polyphenols, e.g., resveratrol or hydroxytyrosol, have been described as modulators of bacterial groups beneficial for human health [68]. Some nutritional intervention trials based on Mediterranean Diet have been proposed as a therapeutic approach to improve the composition of the microbiota and the state of the immune system, opening the perspective of a possible use of this dietary habit to modulate the microbiota, directing it towards a healthy profile. In fact, it has been demonstrated that adherence to the Mediterranean Diet correlates with a state of eubiosis, in which members of the phylum Bacteroidetes and beneficial bacteria belonging to the clostridia group increase, while Proteobacteria and Bacillaceae decrease. In addition, increased levels of lactic acid bacteria (mainly lactobacilli and bifidobacteria) have been observed, together with a more general increase in biodiversity and stability of the intestinal microbiota, suggesting a greater resilience to possible perturbations. A study focused on obese subjects also showed that an intervention with Mediterranean Diet increased the abundance of SCFA-producing gut bacteria *Roseburia* and *Oscillospira* [69]. In conclusion, the Mediterranean Diet, rich in foods of plant origin, provides polyphenols, high-quality fats (monounsaturated such as oleic acid and polyunsaturated with high content of omega 3), micronutrients, such as vitamins and trace elements, and dietary fiber that, carried by an adequate and complete dietary matrix, exert their beneficial properties in maintaining the eubiosis of the intestinal microbiota and its metabolites, together with the integrity of the intestinal barrier and immune tolerance. In contrast, the Western Diet and ultra-processed foods, characterized by low levels of dietary fiber or micronutrients, have a plethora of nutritional components, including refined carbohydrates, poor quality fats (trans fatty acids and an excessive omega 6/omega 3 ratio due to refined oils), unhealthy salt and additives (mainly sweeteners), and finally excessive consumption of red and processed meats. In addition, they comprise a poor food matrix that causes detrimental effects on the intestinal barrier, leading to increased permeability, dysbiosis, and altered metabolite profiles, resulting in local inflammation, systemic endotoxemia, and chronic inflammation. **Figure 4** summarizes these observations.

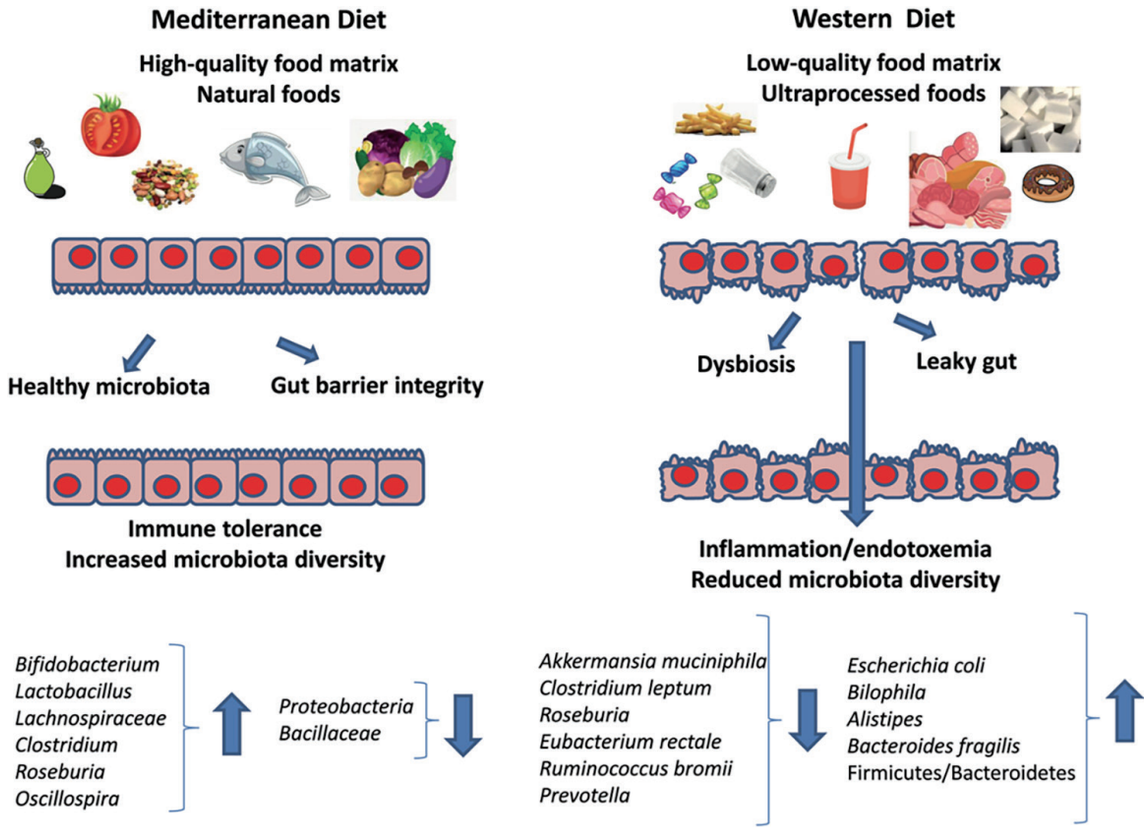

Figure 4.
Comparison of "Mediterranean" and "Western" dietary profiles. The main effects on gut integrity, immune status and microbiota composition are schematically represented.

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Conflict of interest

The authors declare no conflict of interest.

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