Immune System Modulation

Subjects: Biology
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The intestinal microbiota, together with other immune system components are involved in the development of the immune response. Considering the impact of intestinal microbiota on health and disease, scientific and commercial interest has increased in the use of probiotics that stimulate the modulation of the intestinal microbiota for improving health and diseases treating immune system. Despite the health benefits of probiotics, concerns about their use have also arisen since they are affected by various properties of the products they are used in (shelf life, food additives, product matrix, etc.

probiotics parabiotics postbiotics immune system

1. Introduction

Immune system modulation is one of the hot topics of today. The main function of the immune system is to defend us against pathogens by recognizing “stranger” (pathogen associated molecular patterns-PAMPs) and “danger” (damage-associated molecular patterns-DAMPs) molecular motifs according to the danger theory. In this way, it plays an important role in the pathogenesis of various diseases and health [1][2][3].

The immune system basically performs this defense function by integration of various host barriers and cellular and humoral agents such as immune system mechanisms [4]. It mainly performs this defense function by two mechanisms: the innate and adaptive immune system [2]. Physical barriers, which is served as the skin, mucous membranes, and endothelia throughout the body that prevent the entry of microbes into the host and reaching potential sites of infection, comprises the innate immunity [5]. Moreover, the physical barrier, which is the first line of defense, is composed of microorganisms that are hosted in our body and colonized outside the epithelial cells of the skin and gastrointestinal system [4][6]. These microorganism communities are defined as microbiota. The genetic material of the microorganisms that make up the microbiota is called the microbiome [7]. Current literature suggest that gut microbiome and/or a new organ system are especially commensal mainly due to the microorganisms’ specific biochemical interaction and systemic integration with their hosts [8].

Gut microbiota, in other words intestinal microbiota which is the intestinal flora of the human body, has a vital role in human health, especially in the development of the host immune system and the regulation of metabolic events [9]. Given the health effects of intestinal microbiota, there is an increasing interest in probiotics, prebiotics, and synbiotics, which are closely related to the microbiota for health promotion [10]. Probiotics are defined by the International Scientific Association for Probiotic and Prebiotic (ISAPP) as “living microorganisms that create health benefits in the host when taken in sufficient amounts” and prebiotics as “inanimate food ingredients that support

health in the host through microbiota modulation" [11][12]. The definition of synbiotics was updated with the consensus published in 2020 as "a mixture containing live microorganisms and substrate(s) selectively used by host microorganisms, beneficially affecting to the host" [13].

Although they have beneficial effects on health, the World Health Organization (WHO) and The Food and Agriculture Organization (FAO) reported that probiotics may have some side effects and safety issues may arise due to the use of living microbial cells [14]. There is evidence that the use of forms or metabolites of living microorganisms inactivated by various methods can eliminate safety problems and reduce the risk of infection in individuals with increased intestinal permeability and weak immune systems [15]. While terms such as paraprobiotics, parapsychobiotics, ghost probiotics, metabiotics, and postbiotics are used to refer to these probioactive compounds that do not fit the definitions of probiotics, prebiotics, or synbiotics, ISAPP proposed the use of the term "postbiotic" in the consensus of 2021 [16]. Literature investigating the efficacy of postbiotics and paraprobiotics report their potential, such as probiotics, in demonstrating various health benefits in the host and those involved in immune system modulation [17]. Moreover, postbiotics have mainly been associated with immunomodulatory activities by playing a role in maintaining the integrity of the intestinal mucosal barrier and antagonizing pathogens with antimicrobial compounds by stimulating the innate and adaptive immune system [18].That is why one of the emerging topic is the role of some metabolites of probiotics such as postbiotics or their different (non-living) forms, such as parabiotics, in the immune system modulation. This review aimed to focus on probiotics, parabiotics, and postbiotics and their involvement in the immune system.

2. Immune System

When pathogens cross physical barriers and the innate immune system, an inflammatory response is generated by the second line of defense and components [19]. For neutrophils, basophils, dendritic cells, eosinophils, Kupffer cells, tissue macrophages such as alveolar macrophages, and phagocytic cells called monocytes in the blood, the Fc (constant and crystallized part of Ig) part of IgG and IgA on their surface bind to the pathogen with special receptors they carry for various factors involved in the complement system and inflammation and they also bind and clear to pathogens with the proteins in their granules by phagocytosis [20]. For example, components such as bacterial lipopolysaccharides (LPS) found in the outer membrane of pathogenic bacteria are recognized by Toll-like-4 receptors (TLR4) on the surface of monocytes and macrophages. TLR4 and other TLRs such as TLR2 and TLR9 are involved in the formation of inflammation by stimulating the activation of genes responsible for cytokine and antimicrobial molecule production in the nucleus [21]. TLRs are involved in the formation of inflammation by stimulating the activation of genes responsible for cytokine and antimicrobial molecule production in the nucleus [21]. Natural killer cells (polymorphonuclear cell-NK) are non-phagocytic granular lymphocytes responsible for killing infected body cells [22].

Some substances secreted by pathogens cause chemotaxis in phagocytic cells [23]. Monocyte-macrophages and other cells secrete cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interferons (IFN), causing fever and thus increasing the severity of inflammation [24]. If the innate immune system against pathogens is inadequate, the adaptive immune system creates pathogen-specific immune responses with immunological

defense mechanisms and the severity of the response and intensity increases after the first encounter with the pathogen thanks to immunological memory [25][26].

B lymphocytes that develop from pluripotent hematopoietic stem cells in the bone marrow produce antigen-specific immunoglobulins (IgM, IgD, IgG, IgA, and IgE) and enable the formation of a humoral immune response by allowing the recognition of antigens by other cells by binding Igs' to the antigen [27]. Macrophages are activated by Th1 cells from the CD4+ T cell (Th) group [28]. Th1 also releases cytokines such as interleukin-2 (IL-2) and interferon-gamma (INF-γ), resulting in cellular immunity that protects against intracellular infectious agents such as viruses, mycobacteria, and fungi [29]. Th2, on the other hand, stimulates B lymphocytes, which are the most essential elements of humoral immunity, which enable a response to extracellular pathogens by producing cytokines such as IL-4, IL-10, and IL-6 that stimulate immunoglobulin production [30]. The task of Th17 cells is to stimulate tissue inflammation by producing IL-17 in cases where Th1 and Th2 mediated responses are insufficient. Research has reported that Th17-mediated response plays a role in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, psoriasis, and asthma [31]. Other members of the Th group, T regulator cells (Foxp3+ T regulator-Treg) are defined as T cells responsible for suppressing potentially deleterious activities of T and B cells [32]. Treg cells prevent the development of autoimmunity by controlling Th1 or Th2 responses via the immunosuppressive cytokine TGF-β [33].

TLRs allow microbial membrane components such as lipid, lipoprotein, protein to be recognized by binding to PAMPs found in microorganisms and plays a role in the formation of the immune response by stimulating the secretion of inflammatory mediators (**Table 1**) [34][35]. Antimicrobial molecules secreted by intestinal epithelial cells act by disrupting the cell wall structures of both pathogenic bacteria and the intestinal microbiota [36]. Antimicrobial molecules are secreted by the mechanism associated with pattern recognition receptor (PRR) and intestinal epithelial cells [37]. Activation of various signaling pathways required for mucosal barrier functions, antimicrobial molecules, mucin glycoproteins, and IgA production occurs through PRR-PAMP interaction [38]. In addition, TLRs activated by PAMP interact with MyD88 and TRIF pathways in the cell according to the localization of TLRs and prevents bacteria from adhering to epithelial cells [39]. TLRs are involved in the formation of the immune response, thus increasing the susceptibility to dysregulation, dysbiosis, and inflammation [40].

Table 1. Cells with TLRs, their ligands, and the cytokines they stimulate to be secreted.

TLR	Cell in Which It Is Located	PAMP	Cytokine with Stimulated Secretion	References
1	Monocyte/macrophages Dendritic Cells B lymphocytes	Triacyl lipopeptides	IL-6, IL-10, TNF-α	[41]
2	Monocyte/macrophages Dendritic Cells Mast Cells	Diacyl and triacyl lipopeptides Peptidoglycan Lipoteichoic acid	IL-6, TNF-α, IL-1β, IL-10	[42][43]

TLR	Cell in Which It Is Located	PAMP	Cytokine with Stimulated Secretion	References
3	Dendritic Cells B lymphocytes	Viral DNA	INF-γ	[44]
4	Monocyte/macrophages Dendritic Cells Mast Cells Intestinal epithelium	Lipopolysaccharide	IL-1β, INF-γ	[45]
5	Monocyte/macrophages Dendritic Cells Intestinal epithelium	Flagellin	IL-6, TNF- α, IL-10	[46]
6	Monocyte/macrophages Mast Cells B lymphocytes	Diacyl lipopeptides Lipoteichoic acid	IL-1β	[43][47]

PAMP: pathogen associated molecular pattern, IL-6: Interleukin-6, IL-10: Interleukin-10, TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin-1β, DNA: Deoxyribonucleic acid, INF-γ: Interferon-γ.

3. Microbiota and Immune System

The human body maintains a symbiotic life with a microorganism as much as its own cell number (1:1 ratio) according to current literature [48]. These microorganisms, which were previously called the flora of the region they colonized in the body, are defined as microbiota and the genetic material of microbiota is defined as microbiome [7][9]. The close relationship of microbiota with health, especially the immune system, has started to be understood with the Human Microbiome Project (IMP), which was initiated in 2007 as a continuation of the Human Genome Project (IGP) and aims to examine the interaction of the microbiome with genetics, age, gender, nutrition, drugs, environmental factors, and consequently its effect on human health [49][50].

Bacteria, fungi, viruses, and other microorganisms that make up the microbiota are mostly colonized in the intestine of the human body due to its large surface area and being rich in nutrient quantity diversity [51]. According to a microbiome theory, intestinal microbiota begins to develop in the prenatal (intrauterine) period and reaches the adult diversity at an average age of 2.5 years (In the intestinal microbiota, there are more than 10 phylum member bacteria including Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria and most of them belongs to Bacteroidetes and Firmicutes phyla.) and is influenced by many factors such as the mode of delivery; nutrition (Bifidobacterium phylum was found to be more dominant in the microbiota in the 20 following birth in infants born by normal birth and breastfed than in infants born by cesarean section and formula-fed); whether antibiotics are used or not (Antibiotic use in childhood have been associated with obesity, diabetes, inflammatory bowel disease, asthma, and allergies); and the geographic characteristics of the living environment [52][53][54]. On the other hand, there is not yet enough evidence whether the microbiome starts in the womb. There are two theories about the human microbiome formation process. Some scientists believed that the human fetal

environment was sterile and babies were born sterile while others have stated intestinal microbiota begins to form in the intrauterine period [55][56].

The intestinal microbiota, together with macrophage and dendritic cells of the innate immune system, T and B lymphocytes of the adaptive immune system (responsible for IgA production), and intestinal associated lymphoid tissue (GALT) are involved in the development of the immune response [57]. Bacteria forming the intestinal microbiota stimulates the production of TLR-MyD88 signal-related IL-1β and function in the formation of the Th1 response by inducing the formation of IL-17 from Th-17 cells. The polysaccharide A found in Bacteroides phylum bacteria and the butyrate SCFA produced by the bacteria forming the microbiota stimulate the immune system cells and release TGF-β and IL-10. TGF-β and IL-10 stimulate the production of Treg cells, preventing the formation of T lymphocyte response, and exhibits anti-inflammatory effects [58]. In addition, a study reported that butyrate can stimulate the conversion of monocytes to macrophages through histone deacetylase 3 (HDAC3) inhibition, thus enhancing antimicrobial host defense [59].

The ratio of Bacteriodetes and Firmicutes phyla with the largest colony of bacteria, which are the dominant microorganisms of the intestinal microbiota, varies between 1:1 and 1:3 in healthy individuals [60][61]. This condition is called “eubiosis” and the condition in which the balance is disturbed is called “dysbiosis”. It has been indicated that dysbiosis is associated with plenty of diseases, particularly rheumatoid arthritis, inflammatory bowel disease (IBD), cancer, irritable bowel syndrome (IBS), autism, liver disease, celiac, obesity, diabetes, and cardiovascular and respiratory diseases [62].

4. Probiotics and the Immune System

Today, probiotic microorganisms are known to mainly belong to groups of lactic acid-producing bacilli (phylum containing different genera including LAB- Streptococcus , Staphylococcus , Lactococcus , Lactobacillus , Enterococcus) and Bifidobacteria groups. For the beneficial effects of probiotic microorganisms to be seen, it is recommended that the number of viable cells reaching the intestine should be at least 10 6–10 7 colony-forming units (cfu)/g [63]. However, in most commercial probiotic products, many beneficial microorganisms, especially near the end of their shelf life, lose their “viability” [64]. Therefore, probiotic products are produced to contain more microorganisms (on average 2.5 times) than the number of live probiotic microorganisms written on the label. However, the health effects of these dead microorganisms are not clearly known since no studies have been conducted [65]. The viability of these microorganisms varies depending on many conditions, such as the characteristics of the microorganism, the acidity degree of the product, the storage temperature, and the characteristics of the packaging materials used [66].

In the last three decades, the therapeutic potential of probiotics has been evaluated in many times [67]. With the increasing use of probiotics to treat dysbiosis associated with many diseases, safety problems have also been raised [68]. Although many studies are reporting that the use of probiotics is generally safe, this situation has been questioned with current studies and it has been concluded that “probiotics should be applied in high-risk groups (older adults, hospitalized patients, cancer patients) after careful evaluation of the risk-benefit ratio” [69][70].

Despite various health benefits, research on probiotics have reported that issues such as unknown molecular mechanisms; strain-specific behavior; the difference in the response of probiotics of short-lived, autochthonous (resident or colonized in the host) and allochthonous microorganisms (externally applied such as probiotics); antibiotic resistance that can develop with horizontal gene transfer; maintenance of vitality and stability during the shelf life, although rare; problems such as infective endocarditis, sepsis, bacterial translocation into tissue or blood; and bacteremia in immunocompromised individuals may develop [70][71][72]. Moreover, it has been reported that live probiotics are affected by host-specific factors in the GIS, which activate various bacterial genes for the degradation and production of nutrients through different metabolic pathways [73][74].

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