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REVIEW

IMMUNONUTRITION IN PEDIATRICS

The role of probiotics and postbiotics in modulating the gut microbiome-immune system axis in the pediatric age

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ABSTRACT

The complex microbial community of the gut microbiome plays a fundamental role in driving development and function of the human immune system. This phenomenon is named the gut microbiome-immune system axis. When operating optimally, this axis influences both innate and adaptive immunity, which orchestrates the maintenance of crucial elements of host-microorganisms symbiosis, in a dialogue that modulates responses in the most beneficial way. Growing evidence reveals some environmental factors which can positively and negatively modulate the gut microbiome-immune system axis with consequences on the body health status. Several conditions which increasingly affect the pediatric age, such as allergies, autoimmune and inflammatory disorders, arise from a failure of the gut microbiome-immune system axis. Prenatal or postnatal modulation of this axis through some interventional strategies (including diet, probiotics and postbiotics), may lead to a positive gene-environment interaction with improvement of immune-modulatory effects and final positive effect on human health. In particular probiotics and postbiotics exerting pleiotropic regulatory actions on the gut-microbiome-immune system axis provide an innovative preventive and therapeutic strategy for many pediatric conditions.

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KEY WORDS: Immunity, mucosal; Homeostasis; Dysbiosis.

The gastrointestinal tract harbors trillions of microbial inhabitants that are collectively known as the gut microbiota. All microorganisms and their total genome capacity, when considered together, constitute the gut microbiome (GM).[1] These complex communities of microbes that include bacteria, fungi, viruses and other microbial and eukaryotic species, play a pivotal role in modulating most aspects of host physiology.[2] Over the past few years, the field of immunology has been revolutionized by the growing understanding of the fundamental role of GM in the development and function of the human immune system.[3] This phenomenon is called the gut microbiome-immune system axis. When operating optimally, the gut microbiome-immune system axis influences both innate and adaptive immunity in a dialogue that modulates responses

in the most beneficial way. These beneficial effects can be observed not only locally but also in distant organs, due to systemic distribution of GM-derived metabolites and cells activated in the gut.[4, 5] The gut microbiome-immune system axis is a lifelong, complex and dynamic interaction that starts during intrauterine life. In particular, the period between conception and child's 2nd birthday is a critical window of opportunity in which are set the basis for a healthy status for the child and for his future life.[6]

It is now clear that several conditions that increasingly affect the pediatric age (such as allergies, autoimmune and inflammatory disorders) arise from a failure of the gut microbiome-immune system axis. Alteration of the structure and function of the GM as a result of negative influence of many environmental factors could transform our microbial allies into potential adversaries.

On the other hand, exposure to factors that positively influence GM composition and function (such as probiotics and postbiotics) leads to a positive modulation of the immune system with consequent powerful protective action against several diseases, not only in the pediatric age but also in future life (Figure 1).[7-10]

Here we review the current knowledge about the mechanisms involved in the GM modulation and how these changes can drive the immune sys-

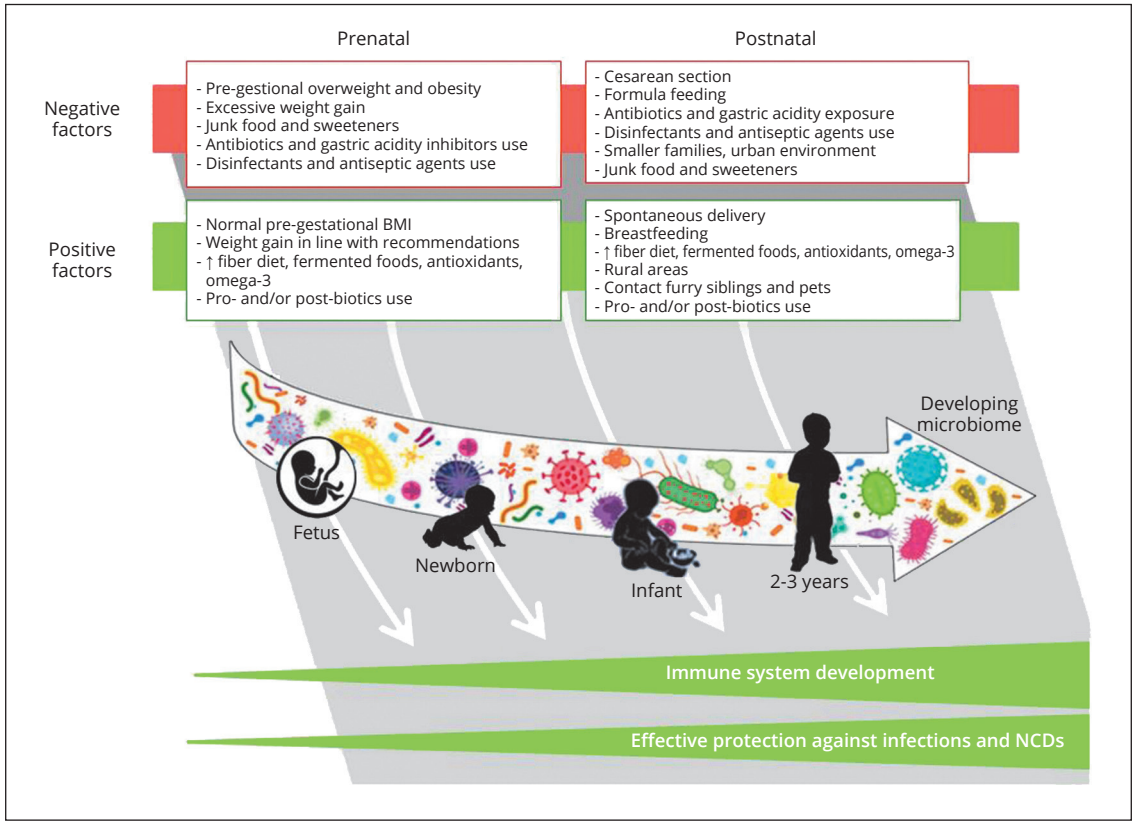

Figure 1.—Environmental factors able to influence the gut microbiome-immune system axis. The establishment of the gut microbiome (GM) drive the immune system development and function. Several factors can positively and negatively affect the GM function and composition. The optimal road begins with a mother with a normal BMI during and after pregnancy, which follows a Mediterranean diet (full of fibers, fermented foods, antioxidants, and omega-3 fats) and continue with a term gestational period, a spontaneous delivery, and breast-feeding. The alteration of a healthy balance of the GM (also called dysbiosis) caused by various factors (*i.e.* C-section, formula feeding, antibiotic therapy, smaller families, less contact with nature, etc.) has been associated with loss of immune tolerance and an increased risk of infections and non-communicable diseases (NCDs) occurrence. Pro- and postbiotics exert an important modulatory effect in this axis. Indeed, they promote and maintain the gut homeostasis (eubiosis) and thanks to their pleiotropic functions, these compounds are able to restore a gut eubiosis also in a dysbiosis condition.

tem development and function with a particular focus on the role of probiotics and postbiotics in influencing this active and critical relationship.

We conducted extensive search of electronic databases of MEDLINE [PubMed] using keywords and MeSH terms based on the gut microbiome, immune system, probiotics and postbiotics. We included review, meta-analysis, RCT, cohort, case-control, cross-sectional and *in-vitro* studies, published in English language in a peer-reviewed journal.

The gut microbiome and the immune system axis

For more than a century, the intrauterine environment was thought to be sterile and the GM of the neonate had been thought to be colonized during birth and early postnatal life. The use of modern sequencing technologies has enabled the detection of microbial DNA in the placenta, amniotic fluid, umbilical cord, and infant meconium during normal pregnancy, indicating that the microbiota influence on the immune system could start in the fetal life. The neonatal GM is characterized by a low microbial diversity and a relative dominance of the phyla *Proteobacteria* and *Actinobacteria.* Subsequently, the GM evolves, resembling the adult composition by the age of approximately 2-3 years.[11] The bacterial establishment is related to the immune homeostasis development. The interactions between the GM and host immunity are complex, dynamic and context-dependent.[3] In line with the "*in-utero* colonization hypothesis", the "first 1000 days" of the child's life, starting from conception to the first 2 years of life, are crucial for both colonization of GM and for the development and function of the immune system. This time frame in early life, according to the World Health Organization, is considered a "window of opportunity" and is crucial for the development and the health status both in childhood and adulthood.[12] Microbial community composition during this critical period is dynamic, unstable, and susceptible to alterations. During intra-uterine life, the fetal immune system is downregulated, making neonates particularly susceptible to infection and aberrant immune responses. The gut epithelial barrier, mucosa, and environmental conditions, such as pH, provide most of the protection against pathogens in the neonatal period.[13] Spatial and temporal interactions between the microbiome, microbial metabolites and gut epithelial cells in the lumen, on the surface of epithelial cells, and in the interior components of the gut associated lymphoid tissues (GALT), such as dendritic cells (DCs), modulate balanced immune development, immune response, homeostasis and diseases occurrence. Healthy immune development in infants is characterized by a transition from innate immunity, dominated by non-specific macrophages and neutrophils, to adaptive immunity, characterized by specific T cells and B cells, which are fundamental to the establishment of tolerance status: the ability to distinguish between beneficial commensal bacteria and harmful pathogens.[13] Alteration of the normal balance of the GM (also called "dysbiosis") has been associated with loss of immune tolerance and an increased risk of non-communicable diseases (NCDs) development, such as asthma, obesity, type 2 diabetes, cancer and autoimmune conditions (*e.g.* Crohn's disease).[14, 15] The infant GM is a highly dynamic organ, whose diversity and architecture is sensitive to various maternal and environmental factors. The impact of these determinants during "the first 1000 days" can affect the pattern of bacterial colonization and possibly result in "dysbiosis".[16] Considering that the mother is the primary source of the fetal and neonatal GM, it is plausible that several maternal factors, before and during pregnancy, are able to influence the composition of the infant's GM during the first years of life and, in turn, host immune system.[17] An important and well-established factor influencing fetal/neonatal GM composition is the gestational maternal diet, particularly fat intake and caloric density.[18, 19] Moreover, various maternal diseases, such as gestational diabetes[20] or allergy,[21] may affect the microbiota composition of the mother and the newborn.

Exposure to antibiotics is another important factor that shapes the GM. It has been demonstrated that the antibiotic administration during pregnancy alters the commensal microbiota of the birth canal in pregnant women and consequently the bacterial ecosystem of the off-

spring.[22] Exposure to antibiotics is common in early life mainly due to the use of intrapartum prophylaxis or to the administration of antibiotics in C-section deliveries. The use of intrapartum antimicrobial prophylaxis alters the natural establishment of the GM in the newborn with an effect that seems to persist at least for the first months of life, a very critical time frame for the correct development of the host's homeostasis induced by the GM.[23] Similarly, antibiotic exposure in early postnatal periods even for a short period leads to less microbial stability and biodiversity.[24]

At birth, the microbic colonization of the newborn is different according to the type of delivery. The vaginally delivered babies, acquire a microbiota composition resembling their mother's vaginal microbiota.[25] On the contrary, the infants born by cesarean section are deprived of exposure to the mother's vaginal microbiota and acquire bacteria derived from hospital environment (air and medical staff) and mother's skin. These differences in bacterial profiles can persist until the age of 2 years, *i.e.* when GM development has been completed.[26, 27] Birth gestational age is one of the major factors of the GM colonization. The GM composition of preterm infants (<37 weeks of gestation) is characterized by a great imbalance in the bacterial profile, with a low diversity and an increase of potentially pathogenic bacteria compared with full term newborn.[28] The profile of the GM is altered by organ immaturity and by the fact that these infants are more frequently born by cesarean section, are separated very quickly from their mother, receive first care in a highly sanitized neonatal intensive care unit, and are often treated with antibiotics.[29]

Breastfeeding is a major postnatal factor in influencing the establishment of the GM and the development of the immune system during infancy.[30] Most studies show that both heterogeneity and abundance of the microbiome are lower in formula-fed infants than breastfed.[31, 32] Human breast milk is the gold standard nourishment for newborn infants. Breastfeeding appears to moderate the detrimental effects of cesarean delivery, intrapartum antibiotics and prematurity, on GM development.[33] Breast milk is composed of vast amounts of biologically active components that have a significant impact on the development of the infant's GM and on the promotion of the maturation of the immune system.[34] The third most abundant component of human milk, after lactose and lipids, are human milk oligosaccharides (HMOs).[35] HMOs are complex glycans that resist to digestive enzymes and when reach the colon they serve as a substrate for fermentation of beneficial commensal bacteria, such as *Bifidobacterium* and *Lactobacillus*. This fermentation results in sub-products, such as lactate and short chain fatty acids (SCFAs) and other metabolites able to modulate immune system function.[36] The HMOs can be considered as prebiotic agents, as their influence is obvious when GM of breastfed and infant formula infants are compared.[37] In addition to being a rich source of nutrients, breast milk contains high concentrations of various protective factors, such as enzymes (lysozyme, lactoferrin etc.), immunoglobulins (secretory IgA, IgM), cytokines, complement system components, leukocytes, nucleotides, lipids, microRNA (miRNAs) and hormones that interact with each other and with the mucous membranes of the digestive and respiratory tracts of infants, providing passive immunity as well as stimulation for the development and maturation of the infant's immune system and GM.[38]

Recent studies have shown that human milk is not sterile, but contains commensal bacteria, and potentially probiotic bacteria.[39, 40] Therefore, we could consider breast milk as a natural synbiotic, containing both probiotics and prebiotics. It is estimated that around 25-30% of the infant bacterial microbiota come from breast milk.[30] The origin of the microorganisms present in the breast milk is unclear, but it appears that they are transferred from the maternal gut to the mammary gland through an entero-mammary pathway.[41]

From 6 months of life onwards, the nutritional requirements of the infant are no longer satisfied by exclusive breastfeeding; therefore, the introduction of solid foods is strongly recommended.[42, 43] The GM development during complementary feeding period seems to be influenced by the food quality and composition. Specifically, intake of high-fiber and animal protein foods, may provide selective advantages for

specific microbes, which increases microbial diversity.[44] After birth, the structure of the infants' GM is also influenced by environmental factors. In particular, infants who grow up near rural areas, with furry animals and pets, have an abundance of beneficial bacteria when compared to children who grow up without them.[45, 46] It has been observed that children living in rural areas of Africa (fed a diet high in fiber and low in fat and protein) have a different composition of the GM when compared with European children (fed a diet rich in lipids and animal proteins).[47] The geographical location, diet and lifestyle could be responsible for this effect considering that the influence provided by each of these factors may be different depending on the country where the study was carried out.[48] Taken together, these data support the importance of a "health axis" connecting maternal and newborn environmental factors with infant GM and immune system development and function (Table I). The optimal road begins with a mother with a normal BMI during and after pregnancy, which follows a Mediterranean diet (full of fibers, fermented foods, antioxidants, and omega-3 fats) and continue with a term gestational period, a spontaneous delivery and breast-feeding.[49] Therefore, intestinal "dysbiosis" caused by various factors (*i.e.* C-section, artificial feeding, antibiotic therapy, smaller families, less contact with nature) in the first 1000 days, can negatively affect the development of immune system with an increased risk of allergy and other communicable and NCDs during later life.[16]

Starting from this evidence, the modulation of GM using pro- and/or postbiotics, particularly in early life, represents a new possible strategy to prevent or repair any early dysbiosis and to provide benefic effects on infant health status and on future life.

The mechanisms of action of probiotics in modulating the gut microbiome-immune system axis

Probiotics are live microorganisms that confer a benefit to the host health when administered in adequate numbers.[50] Moreover, probiotic actions are dependent upon the specific strain and doses. The beneficial effects of selected probiotic strains on host health have been documented in the prevention and treatment of numerous conditions, such as infections, antibiotic-associated diarrhea,[51, 52] functional bowel disorders,[53] and immune-mediated diseases.[54-58] These health benefits derive from several modulatory actions elicited by selected probiotic strains able to influence the gut microbiome-immune system axis (Figure 2). The probiotic section is mainly focused on the regulatory action elicited by these micro-organisms on the two most relevant defense mechanisms against allergy and infectious diseases: the gut barrier and the immune system.

Direct mechanisms on the gut barrier

The gut barrier is a physical and functional protection against environmental harmful stimuli. The integrity is provided by a dynamic interaction between epithelial cells, immune cells, mucus layer, secretory IgA, epithelial junction adhesion complex, and antimicrobial peptides.[59] Studies *in vitro* have demonstrated that selected *Lactobacillus* strains could reinforce the intes-

TABLE I.—*The environmental factors influencing the infant gut microbiome.*

Negative factors	Positive factors
Pregestational overweight and obesity	Normal Pregestational BMI
Excessive weight gain	Weight gain in line with recommendations
↑Fat diet during gestation	↓Fat diet during pregnancy
Maternal gestational diabetes or allergy	Maternal exercise
Cesarean section	Spontaneous delivery
Preterm gestational period	Term gestational period
Formula feeding	Breastfeeding
Antibiotics and antiseptic exposure	↑Fiber diet, fermented foods, antioxidants, omega-3
Smaller families, urbane environment	Rural areas, contact furry siblings and pets
↑Lipids and animal proteins in the first years	Pro- and/or postbiotics

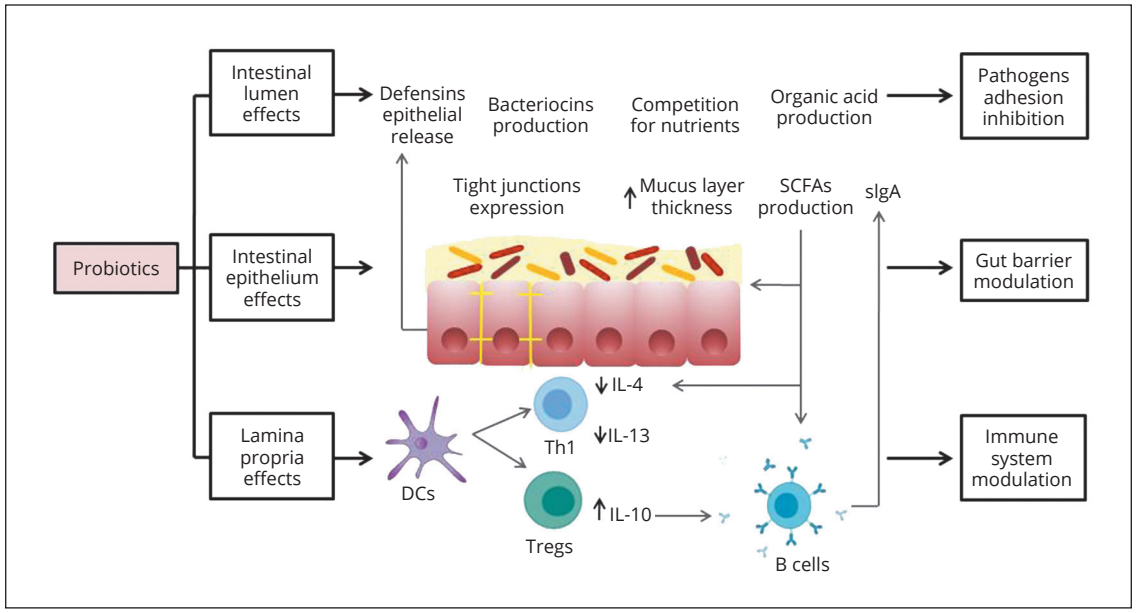

Figure 2.—How probiotics modulate the gut microbiome-immune system axis. From intestinal lumen to lamina propria, probiotics modulate all around the gut barrier. In the lumen these compounds directly produce antimicrobial peptides (bacteriocins), organic acids (lactic and acetic acids), and induce the release of defensins and cathelicidins from epithelial cells. All these mechanisms alter the intestinal lumen pH and creating a hostile environment, inhibit pathogens adhesions. Moreover, probiotics interacting with epithelial cells, can modulate the gut barrier increasing the tight junction adherence, the mucins genes expression and regulating the transepithelial ion fluxes. One of the main abilities to modulate the immune system, is related to the capacity of probiotics to directly interact and polarize the immune cells in the lamina propria, including dendritic (DCs), T and B cells. These differentiated immune cells influence each other and thanks to the production and release into the mucus layer of secretory IgA (sIgA) and regulatory cytokines, they induce a state of immune tolerance and prevent infectious diseases. Finally, probiotics metabolites such as short chain fatty acids (SCFAs) can act in all compartments of the gut barrier, and modulating its permeability and immune system function, resulting in an anti-inflammatory response.

tinal barrier integrity through different mechanisms, including induction of genes involved in tight and adherence junction expression, such as occludins, E-cadherin and B-catenin.[60, 61] The thickness and composition of mucus layer is very important for maintenance the gut barrier. Mucin, one of the main components of the mucus, is secreted by epithelial cells and play a pivotal role in the inhibition of pathogenic bacteria adhesion. Selected *Lactobacillus* strains (such as *L.rhamnosus* GG), thanks to the surface expression of adhesins bind the mucus layer and increase expression of mucin genes, with consequent reduction of pathogens adhesion.[62-64]

Moreover, probiotics can inhibit the growth and the adhesion of pathogens with consequent competitive exclusion, using other mechanisms, including establishment of a hostile environment, alteration of luminal pH, reduction of bacterial receptor exposure, competition for nutrients, and production of antimicrobial substances.[63]

Production of antimicrobial substances

Antimicrobial substances are several compounds that through different mechanisms are able to inhibit proliferation of pathogens. These compounds can be divided in two big categories based on their molecular weight, including low molecular weight (LMW) (<1000 Kda) and high molecular weight (HMW) (>1000 Kda) substances. Among LMW molecules, we find organic acids such as lactic and acetic acid, that cause alter lumen pH, creating a hostile environment and by reducing microbial intracellular pH, resulting in death of pathogens.[65] Bacteriocins are a large group of antimicrobial peptides with HMW that kill pathogens through creation of pores on the bacterial cytoplasmic membranes or inhibition of cell wall creation, binding cell wall precursors.[66] Moreover, probiotics, thanks to their bile salt hydrolase activity, interact with bile acids in the lumen and are able

to produce de-conjugated bile acids that have a powerful antimicrobial activity and hypocholesterolemic effect.[67] The antibacterial effects are not only due to direct release of antimicrobial substances; in fact, probiotics interacting with epithelial cells can induce the release of defensins. These small molecules are key effectors of innate immunity, implicated in host defense against infection (bacteria, viruses, fungi). They stabilize the gut barrier and promote repair of epithelial damage.[68, 69]

Probiotics and immune system modulation

With 70-80% of resident immune cells, the gut is considered the largest lymphoid organ of the human body.[70] The GM modulates immune system development and function through the production of immuno-modulatory molecules and direct interaction with epithelial and resident immune cells. This section is focused on the direct mechanisms of probiotics action on the GM and on the immune cells; the role of the immuno-modulatory molecules such as short chain fatty acids (SCFAs), will be discussed in the next section.

Probiotics directly interact with innate immune cells including DCs, monocytes/macrophages and adaptive immune cells such as B and T lymphocytes. The innate immune system represents the first defense line against pathogens, and thanks to the surface expression of pattern recognition receptors (PRRs), immune and epithelial cells recognize micro-organisms binding the pathogen associated molecular patterns (PAMPs). The PRRs include extracellular and intracellular receptors such as Toll like receptors (TLRs), nucleotide-binding oligomerization domains (NOD), adhesion molecules, lectins, and NOD-like intracellular receptors.[71] Among various pathways, TLR signaling represent the most important pathway used by probiotics to exert their anti-inflammatory and tolerogenic effects. Selected probiotic strains can reduce gut inflammation through down regulation of TLR expression, TNF-α secretion and inhibition of NF-kb signaling in enterocytes. Moreover, probiotics influence Th1/Th2 balance toward a Th1 response, and modulate the DCs maturation, driving the differentiation of Th0 into Treg. The Treg cells induction is a critical step to induce and maintain immune tolerance status and is responsible for inhibitory cytokines release such as IL-10 and for the increase of mucosal IgA levels.[66, 72] The role of probiotics in immune tolerance acquisition has been demonstrated by preclinical and clinical studies. In a murine models of cow's milk allergy (CMA), the administration of *Bifidobacterium infantis* CGMCC313-2, was related to the reduction of IgE, IL-4 and IL-13 concentration.[73] Our group recently demonstrated that supplementation of an extensively hydrolyzed casein formula (EHCF) with *Lactobacillus rhamnosus* GG (LGG) induced higher tolerance rates acquisition after 6 and 12 months compared with EHCF alone and other formulas.[74, 75] Moreover, at the 3-year follow-up of 220 infants with CMA, those treated with EHCF+LGG showed a greater rate of immune tolerance acquisition and a lower occurrence of other allergic manifestations incidence, compared with CMA children treated with EHCF alone.[76] To demonstrate the role of GM and their metabolites in these clinical outcomes, we showed that CMA infants treated with EHCF+LGG, resulted in an increase in the number of strains producing butyrate.[58] With regards of allergic diseases, the probiotics effects on Th1/Th2 balance and on increase of inhibitory cytokines, play a role in preventing and treatment atopic dermatitis and allergic rhinitis.[56, 77] A recent meta-analysis evaluating 17 trials, showed that when infants were treated with probiotics along with their mothers, children had a significant reduction in RR for developing atopic dermatitis compared to controls (RR, 0.78 [95%CI, 0.69-0.89]; P<0.001).[78] Taken together, these data about allergic diseases, suggest a possible role of probiotics in the prevention of allergy and atopic march through the modulation of GM[79] (Figure 2).

The role of probiotics in immune system modulation it is well known also in the prevention on the infectious diseases. As previously mentioned, probiotics increase the production of secretory IgA (sIgA) levels in the gut, but also in the airways and in the mammary glands. sIgA antibodies are a mainstay of protective humoral mucosal immunity against infections. In the gut, sIgA bind to commensally, pathogens, and tox-

ins, inhibiting them through a non-inflammatory process also known as "immune exclusion."[80] A recent randomized trial show that when stimulated with some lactic acid bacteria (LAB), peripheral blood mononuclear cells (PBMCs), produce sIgA, induced by IL-6 and IL-10, which are secreted by DCs in response to LAB.[81]

The postbiotics mechanisms of action in influencing the gut microbiome-immune system axis

The concept of postbiotics is based on the observation that numerous positive actions elicited by GM are mediated by the production of metabolites. Despite a precise definition is still lacking, we can define postbiotics as any substance released by or produced through the metabolic activity of GM-derived microorganisms which exerts beneficial effects on the host and do not meet the preprobiotic definition.[82]

Postbiotics exerts beneficial health effect through similar mechanisms that are characteristic of probiotics. When administered separately, the absence of live microorganisms minimizes the risks associated with their intake. Postbiotics are also known as metabiotics, biogenics, probiotics cell fragments or metabolites and cells-free supernatants, and can be classified by their bioactivity (immunomodulatory, anti-inflammatory, antimicrobial, antioxidant, antiproliferative, hypocholesterolemic, antiobesogenic, antihypertensive, etc.) or by their composition, which can be derived both from microbial compounds and from microbial action (synthesis of metabolites and products from microbial enzymatic activity upon the food matrix).[83] Microbial components include peptidoglycan, polysaccharides, lipoteichoic acids, cell surface proteins, while microbial metabolites consist of lactic acid, peptides/proteins, bacteriocins, enzymes, polysaccharides, organic acids, and SCFAs. In addition, the products of microbial enzymatic activity refer to peptides released by milk casein hydrolysis.[83]

Most known postbiotics are derived from the probiotics *Lactobacillus* and *Bifidobacterium* strains, but also *Akkermansia muciniphila*, *Eubacterium hallii, Streptococcus* and *Faecalibacterium* species and some fructophilic lactic acid bacteria and specific yeasts.[84]

The processing production methods used to obtain postbiotics involves cell disruption techniques. The cell-rupture may be achieved by chemical and mechanical techniques, including heat, high pressure, UV rays, ionizing radiation, ultrasound treatments, pH modification as summarized in Table II; acquisition methods include extraction and cleaning steps, used to isolate and identify postbiotics molecules, which can be performed by centrifugation, dialysis, chromatography, dehydratation and column purification.[85, 86]

Postbiotics display pleiotropic properties: a series of beneficial effects that can be observed not only locally in the GM, but also in distant organs through the connection called gut-organ axis.[82] The number of biochemical reactions that take place within the microbiota significantly impacts many aspects of host health. The immune system–gut cross talk is necessary for the proper development and functioning of immunity, so in this section about postbiotics we also discuss the immunomodulatory role of SCFAs.

The SCFAs regulate functionality and differentiation of T cells (Th17, Th1, and Tregs) through different specific pathways. Most regulatory activity in modulating immune response are mediated by the binding of SCFAs with G-protein- receptor (GPR) (*e.g.*, GPR41, GPR43,

TABLE II.—*Cells disruption techniques for postbiotics production.*

Technology applied	Involved mechanisms
Heat	Damage to cell membrane, loss of nutrients and ions, ribosome aggregation, rupture of DNA filaments, essential enzyme inactivation and protein coagulation
High pressure	Damage to cell membrane, protein denaturation and reduction of the intracellular pH value
UV rays	Protein denaturation and DNA photoproducts production
Ionizing radiation	Nucleic acids damage
Ultrasound	Cell wall rupture, damage to cell membrane, DNA damage, production of free radicals
pH modification	Damage to cell membrane, chemical denaturation of DNA and ATP and enzyme inactivation
Lyophilization	Ruptures in cytoplasmic membranes, changes in protein, nucleic acids, and ribosome structures

GPR109A, and Olfr78), inducing the activation of intracellular signal cascade. The SCFA butyrate is the ligand for GPR109A in the gut: its signaling through the macrophages and DCs activation induces differentiation of *Foxp3* Treg cells as well as the secretion of its key suppressive effector cytokine IL-10.[87, 88] Moreover, SCFAs can induce effector T cells and Tregs differentiation, through epigenetic mechanisms including histone deacetylase (HDAC) inhibitory activity.[89] The HDAC inhibition in Tregs cells leads to the transcription of *Foxp3* factor, improving their suppressive and regulatory properties[90] and suggests one of the mechanisms by which SCFAs regulate Tregs differentiation.[91] The SCFAs epigenetic regulation of gene expression (inhibition of HDAC) also leads to the acetylation of specific genes involved in plasma B-cell differentiation, facilitating the production of class-switched antibodies (IgG and IgA).[92] Taken together, all these mechanisms can modulate immune-system function with beneficial effects on human health not only in the immune-mediated disease such allergies, but also in the protection against infections.

Role of postbiotics in preventing infectious diseases

Evidence shows that postbiotics have a role in preventing infectious diseases. The potential postbiotic mechanisms of action in the prevention/treatment of infections are due to the stimulation of the immune and non-immune defense mechanisms. The inhibition of pathogen adhesion is a defensive characteristic of some postbiotics; these compounds interacting with epithelial cells are able to induce the release of defensins, well known as antimicrobial peptides.[93] Among postbiotics, SCFAs and bacteriocins are the main ones responsible for the antimicrobial effects, including bacteriostatic/bactericidal properties against pathogenic germs and reduction of relevant toxins production.[94] In addition, SCFAs maintain a hypoxic intestinal environment by triggering epithelial PPAR-γ signaling which, stimulating the energy metabolism of human enterocytes to β-oxidation, drive the microbial community towards a dominance of obligate anaerobes. These bacteria are responsible for SCFAs production, thereby closing a virtuous circle that maintain the healthy gut homeostasis (eubiosis) in defense of pathogens.[95] Finally, SCFAs, as previously mentioned, exert their protective effects against infections increasing IgA levels secretion and decreasing susceptibility to pathogens.[92]

Data derived from preclinical studies showed that one of the potential postbiotic mechanisms against infections, is based on the fermentation of food matrix, in general milk or dairy substrates, with microorganisms. In two double blind, randomized, placebo-controlled trials, the dietary supplementation of fermented foods with *Lactobacillus paracasei* CBA L74, was associated with a reduction of common infectious diseases in healthy Italian children aged 12 to 48 months. This protective effect was associated with a significant stimulation of both innate (α- and β-defensins and cathelicidin) and acquired (secretory IgA) immunity.[96, 97] Corsello *et al.*[96] have shown that children who consumed daily 7 grams of cow's skim milk fermented with *L. paracasei* CBA L74 for 3-month had a significantly lower number of acute gastroenteritis and upper respiratory tract infection compared to the counterpart that took placebo (maltodextrins). These data confirmed those previously published by our group in which we demonstrated that the fermentation with *L. paracasei* CBA L74 of cow's milk or rice was useful in preventing the onset of the aforementioned infections compared to the consumption of placebo.[97]

An *in-vitro* study showed that the prevention against infectious diseases elicited by fermented milk with *L. paracasei* CBA L74 on human enterocytes (Caco-2 cells) was due to the positive modulation of the gut mucosa integrity. In particular *L. paracasei* CBA L74 induced the up regulation of tight junction proteins expression, the increase of MUC2 expression and mucus layer thickness, and the release of innate immunity peptides with antimicrobial properties.[98]

Our group has recently shown that fermenting milk with *L. paracasei* CBA L74 resulted in a fecal increase of certain bacterial genera and oligotypes involved in butyrate synthesis, providing several non-immune and immune defense mechanisms against infections, and underlining

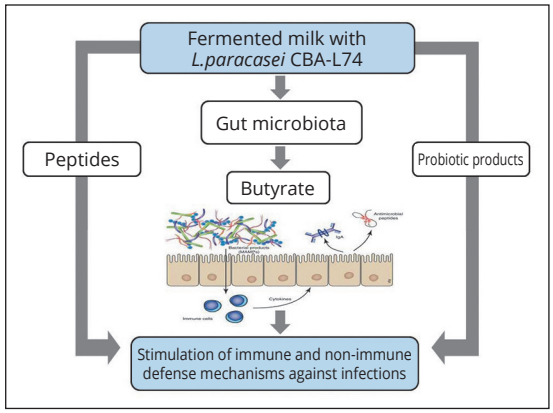

Figure 3.—The postbiotics mechanisms of action against infections. A postbiotic stimuli induce defense responses against invading pathogens through immune and non-immune action mechanisms. A postbiotic example is cow's milk fermented product containing the heat-killed probiotic strain *L. paracasei* CBA L74: the fermentative action can change the nutritional and bioactive properties of food matrices with possible beneficial consequences for human health; some of these could derive also from their impact on gut microbial composition and function. This postbiotic stimulus results in an increase of butyrate synthesis. Butyrate can prevent infectious through the positive modulation of gut mucosa (involving mucus layer, epithelial cell integrity, epithelial junction), the modulation of the inflammatory response and the stimulation of innate and acquired immunity. The detection of bacterial presence through Microbe-Associated Molecular Patterns (MAMPs) induce an innate immune response and activate common signaling pathways to induce antimicrobial cytokine and peptides production. Another contribute is the T and B cells activation, the main cells of the acquired immune system to boost antibody responses during infection, increasing IgA levels secretion and decreasing susceptibility to pathogens.

the role of the gut-microbiota composition in the prevention action against infectious diseases (Figure 3).[99]

Another randomized, double-blind, placebo-controlled clinical trial assessed the effect of a daily administration for 5 months of a fermented infant formula with non-live *Bifidobacterium breve C50* and *Streptococcus thermophilus 065* than a standard infant formula on the incidence of acute diarrhea. The study population consisted of 913 infants, age 4 to 6 months. Although the authors did not observe any reduction in the incidence and duration of diarrhea episodes in the group consuming fermented infant formula than standard infant one, the fermented formula had effects on severity indicators as shown by a lower number of dehydration cases and medical consultations.[100]

Conclusions

Growing evidence suggest that maintaining a healthy balance in the gut microbial ecosystem in early life is a crucial step for optimal immune system development and function. In this way, probiotics and postbiotics, thanks to their pleiotropic effects, are able to induce, to maintain and to restore the gut eubiosis, positively modulating the gut microbiome-immune system axis. These immune-modulatory effects are reflected in the infant health status, setting the basis for a long-lasting protection against infections and non-communicable diseases.

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