

The Impact of Probiotics and Prebiotics on Serum Levels of Allergy Associated Th1 and Th2 Cytokines

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Food sensitization happens early in life and is commonly the primary sign of future atopic disease. Thereby, interferences to forestall food allergies and also the development of the atopic phenotype are a best created early in life. A Probiotic is distinguished as a viable microorganism dietary supplement that beneficially affects the host through its effects within the intestinal tract. A prebiotic is known as non-absorbable food parts that helpfully motivate one or additional of the gut-beneficial microorganisms and so have a positive impact on health. Several literatures have strongly concentrated on the variations of cytokines levels resulted from the effects of probiotics and prebiotics. The current review study aimed to evaluate the results of different studies carried out on this subject. Most articles published during 2007 to 2012 selected based on PRISMA 2009 Diagram Flow. Data were extracted as matrix data and analyzed by in a subject subheading manner according to the matrix review article system. Gut microbes like *Lactobacillus* induce regulatory T cells that enhance Th1/Th2 ratio and systemic innate immunity. Probiotics in pregnancy increased the IFN- γ levels in sera while lowering the TGF β 2 levels. Genetically modified *Lactococcus lactis* induced antigen-specific IL-10-secreting CD3+ cells in Peyer's plaques and resulted in a mitigation of the allergic reaction to the model allergen. *Lactobacillus rhamnosus* GG reduces elevated concentrations of tumor necrosis factor in patients with atopic dermatitis and cow milk allergy while *Lactobacilli* in fermented milk products or as live attenuated bacteria enhances the production of IFN γ and TGF β and IgA by PBMC. Probiotics can enhance SIgA and decrease TNF- α and eosinophil cationic protein (ECP). Yoghurt can enhance the IFN γ and lessen the IL-4 levels. As a conclusion probiotics have several immunomodulatory effects such as adjuvant-like and anti-inflammatory properties mostly mediated by the balance between the proinflammatory and anti-inflammatory cytokines.

Key words: Allergy, Probiotic, Prebiotic, Cytokine, Immune response.

Probiotics are live microorganisms which, when recruited in an appropriate amounts confer a health benefit on the host¹. There is some literatures that shown specific probiotic strains could be effective in the prevention² and treatment¹ of atopic eczema, and also they could alleviate both local and systematic allergic inflammation. But in the present, there is insufficient and paradoxical

evidence of probiotic efficacy against allergic rhinitis and immunological sensitization predisposing to asthma is insufficient and in contradictory at present. The previously studied probiotic strains or combinations of these may not have targeted airway allergies, or the populations studied may not have been responsive to immune modulation¹. Many probiotic effects are mediated via immune regulation, and particularly through control of the balance between proinflammatory and anti-inflammatory cytokines². Such data show that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize the

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dysfunction of gut mucosal, and down-regulate hypersensitivity reactions²⁻³. More recent data show that differences exist in the immunomodulatory effects of candidate probiotic bacteria. Furthermore, distinct regulatory effects have been detected in healthy subjects and in patients with inflammatory diseases². Specific probiotics, selected from members of the healthy intestinal microbiota most of them belonging to *actobacillus* or *Bifidobacterium*, could be help in degradation or some structural modification of enteric antigens, and also they could have regulatory effects during critical period of life by regulation of inflammatory mediators, and direction the development of the immune system, specially when these functions are immature and inexperienced and the risk of allergic disease is heightened³. Colonization of the sterile gut by commensal bacteria is essential for tolerance induction to oral allergens. Commensals stimulate the gut's lymphoid tissue with their cell wall components, such as lipopolysaccharide from Gram-negative bacteria and peptidoglycan from Gram-positive and Gram-negative bacteria. Breast milk contains a pattern recognition receptor, CD14, to which commensals bind. As a result of binding, immune cells differentiate into the Th1 type, production of regulatory cytokines (i.e., IL-10 and TGF- β) is up-regulated, and Th2- type immunity as a promoter of allergy is down-regulated⁴. The current review aimed to evaluate the results of different studies carried out on the evaluation of role of probiotics on Th1 and Th2 related cytokines against the allergy.

Tumor Necrosis Factor- α (TNF- α)

Six different isolates of *lactobacilli* from mice without colitis significantly decreased the production of TNF- α ⁵. Also another reports evidenced the increased mRNA levels for IL-1 α , IL-6 and TNF- α from the colons of dextran sulfate sodium (DSS)-colitis mice treated with CCTCC M206119 strain or saline though it was evident that such increasing from colons of CCTCC-M206119 treated DSS-colitis mice were significantly higher than those from colons of saline treated DSS-colitis mice. The changes in expression at the protein level of such cytokines showed that DSS treatment can result in increased expression of pro-inflammatory cytokines, while DSS plus CCTCC M206119 strain treatment can result in significantly

higher expression of those cytokines⁶.

In other words DSS treatment increased the expression of pro-inflammatory cytokines and decreased anti-inflammatory cytokines, whereas DSS plus CCTCC M206119 treatment resulted in significantly higher and earlier expression of the pro-inflammatory cytokines and inhibited the expression of anti-inflammatory cytokine in DSS colitis mice⁶.

IL-6

The DSS-induced colitis has been reported to be less severe in IL-6 gene knockout mice, whereas transgenic mice with a mutant CIS/SOCS3 gene, encoding a negative regulator for the IL-6/STAT3 (signal transducer and activator of transcription) signaling pathway, had increased susceptibility to DSS-induced colitis^{6,7}.

Inflammatory and Anti inflammatory cytokines

There are some strong evidences supporting the view that an imbalance in the intestinal microflora triggers the intestinal inflammation so that a marked decrease in intestinal *Lactobacillus* spp. precedes the onset of colitis in IL-10 gene knockout mice as was mentioned earlier in this review⁸. IL-10 can inhibit the expression of IL-1 α , IL-1 β , IL-6, IL-12, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, M-CSF, TNF, leukemia inhibitory factor and platelet activating factor produced by activated monocytes or macrophages^{6,9}. It exerts strong immune inhibitory function and plays a main role in the immune tolerance of intestinal mucosa^{10,11}. Thus, it is possible that *L.crispatus* CCTCC M206119 strain administration could disrupt the anti-inflammatory and pro-inflammatory responses balance in colonic mucosa or lamina propria after DSS treatment, and leads to subsequently aggravated colonic inflammation. These processes are similar to those observed in UC patients⁶. Probiotic *B.infantis* 35624 has immunomodulatory effects in IL-10 KO mice prior to the onset of chronic inflammation and also on control mice of the same genetic background. The effects are not dependent exclusively on IL-10 and are most prominent within the Peyer's patch¹².

Chemokine changes

The recruitment and activation of leucocytes at sites of intestinal inflammation is mediated by chemokines and is the hallmark of inflammatory bowel disease (IBD)¹³. CCL2 and

CCL5 are down-regulated by IL-10 and elevated levels of CCL2 are seen in the inflamed mucosa of IBD patients¹². The chemokine responses are modulated differentially in Wild Type (WT) and IL-10 KO mice. CCL2 levels are decreased in IL-10 KO mice but enhanced in WT mice following *B. infantis* 35624 consumption and in vitro stimuli¹².

IFN- γ

Many studies have evaluated the different host-dependent cytokine responses following probiotic consumption. Healthy Wild Type (WT) mice can differentiate between stimuli, reducing IFN- γ levels in response to in vitro challenge with non-pathogenic probiotic bacteria while mounting an appropriate Th1-mediated immune response to pathogenic salmonella. This effect has also been seen in Balb/c mice, where mice fed with *B. infantis* 35624 and exhibited enhanced production of IFN- γ following in vitro stimulation with *Salmonella*¹². This was not the case in IL-10 KO mice, where IFN- γ was attenuated following in vitro challenge, with probiotic bacteria and pathogenic bacteria initiating the same response. In a study by Braat *et al* the probiotic bacterium *Lactobacillus rhamnosus* was shown to induce T cell hyporesponsiveness in healthy subjects through modification of dendritic cell function^{12,14-15}.

IL-4

An association has been reported to be between IL-4, IL-6, and IL-10 polymorphisms and Atopic Dermatitis (AD) so that a clinical shift in allergic phenotype indicating by these cytokines can be seen in the first 3 years of life. These data indicated that IL-4 and IL-10 polymorphisms may be considered as some important predictive factors of respiratory allergy in children with AD¹⁶. Associations between polymorphisms in IL-4, IL-6, and IL-13 and Atopic Dermatitis^{17,18}, asthma¹⁹, Allergic rhinitis, and elevated levels of IgE²⁰ have been described by some researchers¹⁶.

Allergen Specific Immunotherapy/ IgG responses

Allergen Specific Immunotherapy (SIT) significantly improves the symptoms of respiratory allergic diseases as well as reduce the need for symptomatic medication that are indicated by detection of the level of related cytokines, but SIT also has the capacity for long-term clinical effects and could have a protective effect against the development of further allergies and symptoms²¹. The allergic condition that are driven by a subset of T-

helper lymphocytes (Th2) are characterized by the production of cytokines like IL-4, and IL-5²¹. Immunological changes following SIT lead to potential curative effects. One mechanism, by which the immunotherapy can suppress the allergic response, as it was expressed in previous section, is through the increased production of IgG4 antibodies. Different immunological effector cells such as eosinophils, mast cells and basophils are responsible for allergic inflammation²²⁻²⁴ and also Th2 cells that can play an essential role in the promotion of allergen-specific IgE synthesis by B cells. In other words the allergic disease is driven by a subset of T-helper lymphocytes (Th2), which are characterized by the production of cytokines like IL-4, IL-5 and IL-13 among others. On the base of the above data many studies have revealed that symptomatic improvement was correlated with reductions in eosinophils and IL-5 expression in the nasal mucosa²⁵ during the pollen season, as well as with increased in INF- γ production²⁶. Immunotherapy based on IL-12-response is inversely related to IL-4 production promoting a Th1-response mostly the INF- γ production which promotes B-cells into IgG production. Some experiments indicated that immunotherapy induces a new regulatory T-cell response characterized by induction of IL-10^{21,26-28} which precedes the inhibitory IgG4 antibody activity. The *Bacteroides fragilis* group and *Bifidobacteria* were among the species that changed most with pollen dispersion. Real-time PCR analyses indicated that the cell numbers of the *B. fragilis* group increased considerably along with pollen dispersion in both BB536 and placebo groups. Cell numbers of *Bifidobacteria* were appreciably higher in the BB536 group in the comparison by the placebo group²⁹⁻³⁰.

The ratio of cell numbers of the *B. fragilis* group to *Bifidobacteria* increased significantly during the pollen season in the placebo group, but not in the BB536 group²⁹⁻³⁰. An in vitro study using peripheral blood mononuclear cells from JCPsis subjects indicated that strains of the *B. fragilis* group induced significantly more Th2 cytokines (IL-6) but fewer Th1 cytokines (IL-12 and interferon) compared with those of *Bifidobacteria*. These results suggest a relationship between the fluctuation in intestinal microbiota and pollinosis allergy. Intake yogurt appears to exert positive influences on the formation of anti-allergic microbiota²⁹⁻³¹.

Table 1. Chronologically table of selected studies

Researchers	year	Study design	Sample size	Aim of study	Results	Conclusion
Tanja Kuehnbacher, et al	2008	Clinical trial	42 Persons	Intestinal TM7 bacterial phylogenies in active inflammatory bowel disease	Results support recent findings that TM7 bacteria may play a role as promoters of inflammation in inflammatory gastrointestinal disorders with environmental influence.	In this study, we highlighted the presence of TM7 bacteria in the human colon. We could demonstrate that the composition of the TM7 bacterial division is altered in patients with active IBD compared to healthy controls.
P.C.konturekl, ofet al	2009	In vitro assay		Probiotic bacteria <i>Escherichia coli</i> strain Nissle 1917 attenuates Acute gastric lesions Induced by stress	Present study demonstrates for the first time that probiotic strain <i>E. coli</i> Nissle (EcN) 1917 dose-dependently attenuates the acute gastric lesions provoked by the exposure to stress. This remains in keeping with previous studies showing that probiotics exhibit the protective activity not only in the lower portion of GIT such as colon, but also protect gastric mucosa against acute mucosal lesions.	The pretreatment with probiotic strain <i>E. coli</i> Nissle attenuated the acute gastric mucosal lesions induced by stress through the anti-inflammatory actions, induction of protective factors such as ghrelin and HSP70 synthesis in the gastric mucosa and the enhancement of gastric microcirculation. Endogenous PG, NO and neuropeptides released from sensory nerves such as CGRP are also involved in the gastroprotective activities of EcN. Further studies are needed to clarify whether the gastroprotective potential of EcN and other probiotic bacteria exists also in human stomach and whether the prevention of stress-induced lesions can be also observed in human stomach.
Arthur C Ouweland et al	2009	Clinical trial	24Probiotic 23Placebo	Specific probiotics alleviate allergic rhinitis during the birch pollen season	Concomitantly with the differences in nasal symptoms the infiltration of eosinophils in the nasal mucosa increased in the placebo group but an increase was not observed in the probiotic group, where the fraction of	In conclusion, our study showed that consumption of a combination of <i>L. acidophilus</i> NCFMTM and <i>B. lactis</i> BI-04 could positively influence markers of respiratory allergy, especially in the mucosae, and also resulted in a tendency for a reduction in reported

Yuko Nagata, et al	2010	Clinical trial	35persons 17persons (placebo Group)	Improvements in Seasonal allergic disease with <i>Lactobacillus plantarum</i>	subjects with nasal eosinophil infiltration remained unchanged. Concentrations of IFN-γ and IL-2 were below the detection limit for both groups. No differences in the concentrations of TGF-b2, IL-4, IL-5 and CD14 were detected. In particular, the symptom score for itchy eyes was significantly lower in the LP14 group than in the placebo group (p=0.014). No significant differences were observed in the medication scores Furthermore, excluding the influence of allergy medicines, the Th1/Th2 ratio in the placebo group tended to decrease after 6 weeks intake period based on the increase in pollen dispersal, but in the LP14 group, the percentage of Th1 cells increased significantly.	nasal symptoms
Fu-Xi Zhou, et al	2012	Clinical trial	40 mice	Lactobacillus crispatus M206119 exacerbates murine DSS-colitis by interfering with inflammatory responses	In vitro assay demonstrated that CCTCC M206119 strain increased the nuclear translocation of NF-κB in epithelial cells. Proinflammatory exacerbation of intestinal inflammation in colonic cytokine (IL-1α, IL-6 and TNF-α) levels were clearly increased in CCTCC-M206119-treated animals, whereas anti-inflammatory colonic cytokine (IL-10) level was lowered compared with saline or 5-aminosalicylic-acid-treated DSS-colitis mice. Not all lactobacilli are beneficial for intestinal inflammation, and <i>L. crispatus</i> CCTCC M206119 strain is involved in DSS-colitis mice.	

TGF- β

TGF- β is also a T-regulatory cell mediator induced by immunotherapy which is responsible for the down regulation of the Th2 response - reducing IL-5 production and preventing allergen-exposure-induced eosinophilia and inflammation. Probiotics can enhance the production of TGF- β by which Th1 cytokines are upregulated²¹.

Bifidobacter effects

Yogurt supplemented with BB536 promotes a healthy intestinal environment by increasing counts and relative ratios of Bifidobacteria, in addition to defecation frequency after consumption for 2 weeks. A direct correlation between serum total IgE titer and Bacteroides counts in the gut microbiota of high-risk subjects with atopic disorders has been found³². IgG titers against *B. vulgatus* were significantly higher among school children with any 2 of the allergic symptoms of asthma, rhinitis, eczema or food allergy than among non-allergic groups³³.

These findings suggest a beneficial role of Bifidobacteria and a deteriorating effect of the *B. fragilis* group on allergic conditions³². Alterations in IFN- γ in the Peyer's patches of WT mice (enhancement) versus IL-10 KO (reduction) were observed following in vitro stimulation with salmonella. Differential IL-12p40, CCL2 and CCL5 responses were also observed in IL-10 KO mice and WT mice. The cytokine profile of IL-10 KO mice in early disease was similar to that of WT mice. The most pronounced changes occurred in

the Peyer's patch of IL-10 KO mice, suggesting a probiotic mechanism of action independent of IL-10¹².

Lactobacteria

Evidences do not support that all strains of Lactobacteria have an in vitro immunomodulatory activity but only some strains have shown such activity⁵. Some *Lactobacillus* strains such as *L. Casei* proved to strongly induced IL-12, IL-6, and TNF from bone marrow-derived dendritic cells DCs³⁵. Therefore, it is clear that changes in cytokine profile induced by probiotic strains may be site specific and dependent on the experimental system used. The immunomodulatory effects of intranasal versus intragastric administration of *Lactobacillus paracasei* NCC2461 in a mouse model of allergic airway inflammation and the specificity of different probiotics by comparing *L. paracasei* NCC2461 to *Lactobacillus plantarum* NCC1107 has been evaluated³⁴⁻³⁷. Results showed that intranasal *L. paracasei* NCC2461 efficiently protected sensitized mice upon exposure to OVA aerosols in a dose-dependent manner as compared to control mice. Inflammatory cell number, eotaxin and IL-5 were significantly reduced in BALF. Intranasal supplementation of *L. paracasei* NCC2461 was more potent than intragastric application in limiting the allergic response and possibly linked to an increase in T regulatory cells in the lungs. Finally, intranasal *L. plantarum* NCC1107 reduced total and eosinophilic lung inflammation, but increased

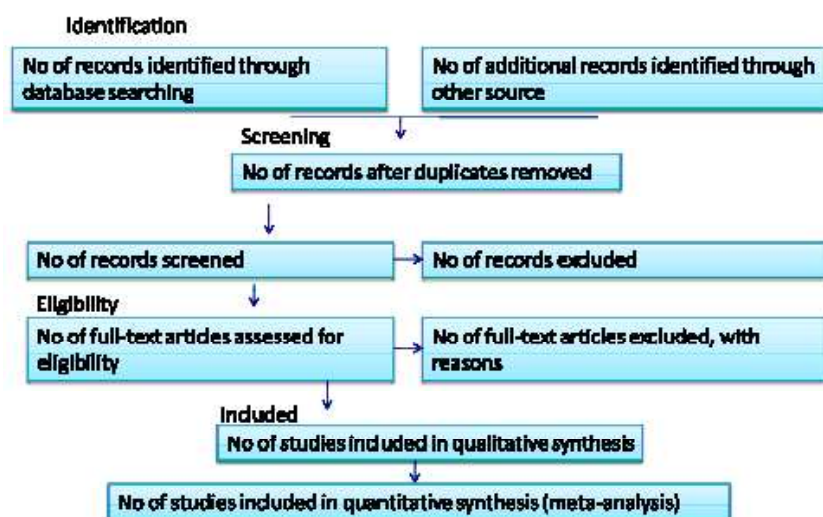


Fig. 1. Flow of information through the different phases of a systematic review

neutrophilia and macrophages infiltration³⁴⁻³⁶.

Evaluating the impact of *Lactobacillus plantarum* No. 14 (LP14) in female students with seasonal allergic diseases showed a significant improvement in ocular symptom-medication score³⁵. In the placebo group, the Th1/ Th2 ratio tended to decrease after a 6-week probiotic intake period, while in the LP14 group, the percentage of Th1 cells significantly increased. Post-intake eosinophil counts significantly increased in comparison to those at intake cessation in the placebo group, but it appeared to be suppressed in the LP14 group. LP14 strongly induced the gene expression of Th1-type cytokines³⁵. Oral administration of live *Lactobacillus reuteri* considerably attenuated the influx of eosinophils to the airway lumen and parenchyma and also reduced the levels of TNF, MCP-1, IL-5, and IL-13 in bronchoalveolar lavage fluid of antigen-challenged animals, but there was no change in eotaxin or IL-10³⁷. *L. reuteri* in contrast *L. salivarius* could decrease allergen-induced airway hyper-responsiveness³⁷.

Killed organisms could not mimic the ability of the live in this way³⁷. Different strains and subspecies of lactobacilli modulate the functions of CD4 T cells. It was shown that *L. Paracasei* B21060 directly inhibits the in vitro proliferation of human CD4 T cells, a finding consistent with the demonstration that T cells are

able to directly respond to bacteria^{36,37}. *Lactobacilli* or *Lactobacillus* subsp. modulate specific immune responses depending on the cell system considered. For example, lactobacilli are powerful inducers of Th1-type cytokines, such as IL-12 and TNF α , in blood cells, whereas they down-regulate TNF- α when used in *ex-vivo* organ cultures of IBD mucosal explants^{36,37}.

Lactobacillus reuteri is a promising therapy for many different conditions, including diarrheal disease, infantile colic, eczema, “episodes of workplace illness”, and *Helicobacter pylori* infection³⁸. *L. reuteri* is considered an endogenous organism of the human gastrointestinal tract and is present on the mucosa of the gastric corpus and antrum, duodenum, and ileum^{38,39}. It has been known that *L. reuteri* produces a potent antibacterial compound, reuterin, that is capable of inhibiting a wide spectrum of microorganisms. An anti-inflammatory action of *L. reuteri* has been shown by previous studies documenting inhibition of experimental colitis in transgenic IL-10-deficient mice^{38,40}, as well as reduction of levels of the proinflammatory cytokine TNF- α in mice with colitis⁵. Furthermore, studies have shown that live *L. reuteri* has a potent inhibitory effect on TNF- α induced IL-8 expression in human intestinal epithelial cells^{38,41}.

It is also evidenced that the modulation of TNF- α production by *L. reuteri* secreted factors is

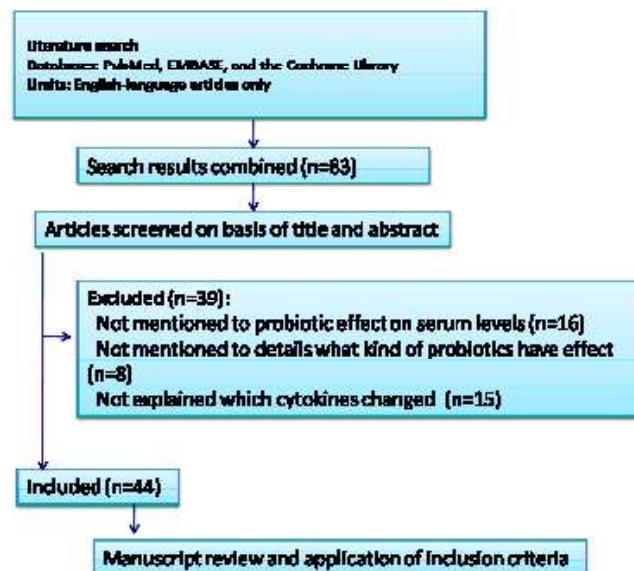


Fig. 2. Flow diagram of study selection

strain dependent⁴². Anti-inflammatory *L.reuteri* strains ATCC PTA 6475 and ATCC PTA 5289 suppressed TNF- α production by bacterial lipopolysaccharide (LPS)-activated monocytoïd cells. In contrast, immunostimulatory *L. reuteri* strains ATCC 55730 and CF48–3A did not suppress TNF- α production by LPS-activated monocytoïd cells^{38,42}.

E.coli

E. coli Nissle 1917 and several bacterial pathogens, including *Helicobacter pylori* and enteropathogenic and enterohemorrhagic *E. coli*, can activate the MAP kinase signaling pathways and epithelial proinflammatory responses such as IL-8 but in a noninvasive manner. In contrast to pathogens, *E. coli* Nissle 1917 might induce a protective response, including hBD- β as well as IL-8 but just below the threshold level of an active inflammation⁴³⁻⁴⁴. Probiotic bacteria *Escherichia coli* Nissle (EcN) was shown to prevent or heal acute murine colitis, but gastroprotective effects of EcN against mucosal injury have been studied by evaluating the effects of EcN on formation of stress-induced gastric erosions in rats⁴³⁻⁴⁴. In rats pretreated with EcN, a significant downregulation of mRNA and protein expression for IL-1 β , COX-2 and PPAR γ and increased expression of HSP70 without major change in activation of NF κ B were observed⁴⁴.

Gram positive and gram negative probiotics

The gram-positive probiotic bacteria *L. plantarum* and *B. adolescent* may have a role in controlling local inflammation in the gut as they do not activate DCs, but they could also be significant for systemic immunity activation after translocation as they preferentially trigger monocytes activation. Gram-negative bacteria, such as *E. coli* and *V.parvula*, are strong activators of DCs, which might lead to either tolerance or hypersensitivity to concomitantly, presented innocuous antigens depending on the level of bacterial stimulation⁴⁵.

CONCLUSION

Probiotics use their effects on immune system as immune regulators, particularly via control of proinflammatory and anti-inflammatory cytokines balance so that they can be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction,

and down-regulate hypersensitivity reactions. Specific probiotics, selected from members of the healthy intestinal microbiota most of them belonging to *Lactobacillus* or *Bifidobacterium*, aid in degradation/structural modification of enteral antigens, regulation of the secretion of inflammatory mediators, and direction of the development of the immune system during the critical period of life. Binding the probiotics to their receptors make the immune cells differentiate into the Th1 type by which the production of regulatory cytokines (i.e., IL-10 and TGF- β) is upregulated, and Th2- type immunity as a promoter of allergy is downregulated.

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