

REVIEW

Immunological effects of probiotics with special reference to lactobacilli

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Introduction

The concept of lactic bacilli as health-promoting environmental factors was presented by Metchnikoff, who made 'a recommendation to absorb large quantities of microbes amongst which lactic bacilli have an honourable place'. Lactobacilli have been a natural component of the diet when fermentation has been used for improving the storage of food. Together with the 'hygiene hypothesis' linking a decreased exposure to microbial load and increasing incidence of allergic and other immune-mediated diseases, a century later, Metchnikoff's idea of the health-promoting lactic bacilli has induced an enormous scientific and economical interest to clarify the relationship between intestinal microbes and different immunological diseases. The definition of probiotics ('for life') is as follows: microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. The purpose of this review is to evaluate the immunological effects of probiotics including both mechanistic and clinical aspects.

Epidemiological and ecological studies – microbial load

Most of the epidemiological studies of the microbial load as a modifier of clinical diseases are related to allergic diseases. The prevalence of allergic diseases is increasing in Western countries. Rapid changes in the prevalence of allergic diseases and atopic sensitization are likely influenced by changes in environmental factors or in the host responses to environmental factors related to the disease process. During the past decades, indirect evidence has accumulated supporting the view that risk of allergic diseases is modified by the environmental microbial load, which plays a crucial role in the maturation of the host immune system during the first years of life. This view, known as the hygiene hypothesis and introduced by Strachan [1], is mainly based on epidemiological or observational data showing a link between the changes in the microbial load and risk of allergic diseases.

The intestinal microflora differs between different populations, and it may also differ between healthy individuals and those with immune-mediated diseases as suggested. The differences in the composition of intestinal bacteria between

Swedish and Estonian children have been indirectly linked to risk of allergic diseases [2, 3]. Lactobacilli are more commonly found in the intestinal microflora in Estonian than in Swedish infants; the incidence of allergic disease is lower in Estonian children than in Swedish infants. When intestinal microflora-derived fatty acids were studied in Swedish children, fecal levels of *Clostridium difficile*-derived caproic acid appeared to be higher in allergic than in non-allergic children [4]. Direct evidence of differences in fecal microflora has been reported; a prospective study in Swedish children demonstrated that children who developed allergic diseases were less often colonized by enterococci and bifidobacteria during the first year of life, but had higher counts of clostridia and staphylococci when compared with children who did not develop allergic diseases [5]. Also Finnish children who developed atopic sensitization had more clostridia and less bifidobacteria than children who remained non-atopic [6], and a similar profile of intestinal microflora has recently been reported in Japanese patients with atopic dermatitis [7]. These studies suggest that the composition of intestinal microflora modifies the quality of immune response, but it should be emphasized that the host immune system may modify the colonization of the intestinal tract, and these findings may reflect the characteristics of the immune system associated with allergic diseases. Thus, the mechanistic and clinical studies are needed to demonstrate the functional effect of intestinal microbes on the immune system and immune-mediated diseases.

It seems that the beneficial effect of microbial load in maturation of the immune system and prevention of allergic diseases is not restricted to intestinal Gram-positive bacteria, such as bifidobacteria and lactobacilli. Recent studies suggest that exposure to environmental lipopolysaccharide, an outer membrane structure of Gram-negative bacteria, in infancy correlates inversely with the risk of atopic diseases [8, 9]. Furthermore, exposure to foodborne and orofecal microbes, such as hepatitis A and *Helicobacter pylori*, may be protective for atopic diseases [10, 11]. The use of Gram-negative bacteria, lipopolysaccharide (i.e. endotoxin) or pathogenic enteral viruses for clinical trials is not, however, an attractive option due to safety problems. Instead, Gram-positive bacteria offer a safe means of modulating the human intestinal microflora. In particular, bifidobacteria and lactobacilli, which are acid- and bile-resistant and capable of colonizing the human gastrointestinal tract, have been widely used in clinical studies without safety problems. During the use of *Lactobacillus* GG in functional foods, no changes in the incidence of severe infections caused by *Lactobacillus rhamnosus* have been observed in Finland [12].

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Intestinal microbes and the gut immune system in animal models

Studies in germ-free animal models have suggested that colonization of the gastrointestinal tract plays a crucial role in the development of the gut immune system by mechanisms related to the maturation of the intestinal epithelium and immune compartment cells [13]. Intestinal microflora is responsible for the diversification of the antibody repertoire after birth. The colonization of the gastrointestinal tract has been found to influence the diversification and expansion of the IgM and IgA repertoire in mucosal lymphoid tissues in newborn piglets [14]. When microbial colonization was prevented after birth in rabbits, the majority of immunoglobulin VDJ genes in peripheral blood lymphocytes were undiversified [15]. These studies indicate that microbial load during the first days of life primes the immune system for the great variety of antigens encountered later in life.

Several studies in animal models suggest that intestinal colonization plays a role in the regulation of oral tolerance [16, 17], supporting the hypothesis that probiotics during infancy could be used for the prevention of food allergies. The effect of colonization is seen in the number of T cells in Peyer's patches (gut-associated lymphoid tissue), the T cell number being limited in germ-free mice when compared with mice in a pathogen-free environment. A recovery in the number of T cells is seen when mice are colonized by Gram-positive or Gram-negative bacteria, such as bifidobacteria or *Escherichia coli*. Furthermore, the insufficient number or absence of T cells in Peyer's patches was related to the failure to induce oral tolerance in germ-free mice. These findings suggest that intestinal microflora is important for the development of the gut immune system and the induction of oral tolerance.

The importance of intestinal microflora for immune protection has been studied intensively in animal models. It seems that colonization of the gastrointestinal tract of germ-free rodents with lactobacilli interferes with the colonization of enteropathogenic microorganisms [18]. In addition to the beneficial effect related to competition in colonization, the studies in animal models suggest that immuno-enhancing properties of probiotics may also be important for protection from infections. *Lactobacillus rhamnosus* HN001 was shown to reduce the severity of *E. coli* infection in a mouse model [18]. *Lactobacillus rhamnosus*-fed mice exhibited higher intestinal IgA responses to *E. coli* and showed increased blood leucocyte phagocytic activity than control mice, suggesting that immune mechanisms are important for the protection [18]. Immuno-enhancing effects may be mediated by components of lactobacilli, since heat-killed lactobacilli were shown to protect against candidiasis in germ-free mice [19].

Clinical studies of probiotics in humans

The gut is a major immune organ in humans. It has been estimated that the number of small intestinal intraepithelial lymphocytes is more than half of the T cell number estimated for peripheral lymphoid organs. The gut immune system can

be divided into three major compartments: organized gut-associated lymphoid tissue (GALT) such as the Peyer's patches (PP), the mucosal lamina propria and the epithelium. The activation of naïve T cells takes place in the GALT where differentiation of the activated lymphocytes occurs in PP from where the lymphocytes circulate to the peripheral circulation. The gut immune system has a dual role: it provides defence against infectious agents, but also induces tolerance to harmless food and microbial antigens encountered in the gut. Oral tolerance is a major compartment of peripheral tolerance, and its control of the immune response is not necessarily restricted locally but may include systemic effects [20].

The molecular mechanisms of the induction of oral tolerance are poorly understood. The nature of oral immune response is modified by several factors, including characteristics of the host and the administered antigen. It must be emphasized that most of the data on the development of oral tolerance are based on animal studies, and we do not know how the dose of antigen or the age of the host modifies oral tolerance in humans.

The clinical studies with probiotics have mostly focused on the prevention or treatment of atopic diseases or gastrointestinal infections in children. Food allergies are the most common manifestation of atopic diseases in infants and young children. Food allergies are a manifestation of failure of oral tolerance, and are usually spontaneously cured by the age of 2 years suggesting that the maturation of the gut immune system with age favours the development of oral tolerance. Animal studies suggest that the role of intestinal microbes in the regulation of the intestinal immune system and the induction of oral tolerance seems to be important. Oral tolerance can be enhanced or inhibited by microbial agents administered together with antigen. Oral administration of antigen with an invasive microbe induces systemic immunity instead of tolerance [21]. Instead, non-invasive microbes such as probiotics seem to support the development of oral tolerance. The nature of the oral immune response is modified by several factors, including characteristics of the host, influenced by genes and environment, and the administered antigen.

The effect of *Lactobacillus* GG was studied in the prevention of early atopic disease in children at high risk, i.e. with a family history of atopy [22]. *Lactobacillus* GG was given in capsules to mothers daily for 2–4 weeks before the expected delivery, and after delivery either to breastfeeding mothers or children (capsule content mixed with water and given with spoon) for 6 months. Most families gave capsules to infants. In a prospective study from birth, atopic eczema was diagnosed in 46 of 132 (35%) children aged 2 years. The frequency of atopic eczema in the probiotic group was half that of the placebo group (15/64 [23%] vs. 31/68 [46%]; relative risk 0.51 [95% CI 0.32–0.84]). The mechanisms of *Lactobacillus* GG treatment in the prevention of atopic eczema were, however, not clarified in this study. No differences in skin prick test positivity or total or specific IgE levels were observed between the placebo and probiotic group. Furthermore, this report did not include studies on the cell-mediated immunity. These children were studied also at 4 years of age, and atopic eczema was diagnosed on the basis of a questionnaire and a clinical examination [23]. Fourteen

of 53 children who received *Lactobacillus* GG had developed atopic eczema compared with 25 of 54 children in the placebo group, the relative risk being 0.57 and the 95% CI being 0.33–0.97. No differences in skin prick test reactivity were found between the groups.

The treatment of atopic eczema with probiotics was studied in infants who developed eczema during exclusive breastfeeding [24]. The infants were weaned to probiotic supplemented hydrolysed formula. After 2 months of treatment, the SCORAD score reflecting the extent and severity of atopic eczema was significantly decreased in the infants who received either *Bifidobacterium lactis* Bb-12- or *Lactobacillus* GG-supplemented formula when compared with infants who received unsupplemented hydrolysed formula. The levels of soluble CD4 in serum and eosinophilic protein X in urine reduced in parallel [24]. In a previous study by the same group [25], the clinical score of atopic dermatitis improved significantly during a 1-month study period in infants treated with *Lactobacillus* GG supplemented hydrolysed formula. The concentrations of fecal α 1-antitrypsin and TNF- α decreased also in these infants [25]. A recent study by Rosenfeldt et al. [26] demonstrated that a combination of *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460 given for 6 weeks to 1 to 13-year-old children with atopic dermatitis resulted in a more efficient improvement of eczema although the total SCORAD index did not change in the whole group [26]. During the treatment, serum eosinophil cationic protein levels decreased, whereas no changes in the production of cytokines from peripheral blood mononuclear cells were observed.

Several studies have reported that *Lactobacillus rhamnosus* GG alone or together with *Lactobacillus reuteri* are beneficial in ameliorating acute diarrhoea in children and reduce the duration of rotavirus excretion [27–30]. According to a recent meta-analysis of randomized, controlled studies, it was estimated that a reduction in diarrhoea duration of 0.7 days and a reduction in diarrhoea frequency of 1.6 stools on day 2 of treatment were achieved in subjects who received *Lactobacillus* compared with those who received placebo [31]. These clinical effects may be based on both immuno-enhancing and colonization effects of these probiotics. Interestingly, the long-term consumption of milk containing *Lactobacillus* GG was found to reduce respiratory infections in children attending daycare centres [32]. Thus, the immuno-enhancing effects of probiotics may not be restricted to local protection in the gastrointestinal tract but may also include other mucosal surfaces.

Mechanisms of probiotics as immune modulators

The mechanisms of microbial stimuli in the maturation of the host immune response and, further, in the modification of immune-mediated diseases are poorly understood. Several studies, performed mainly in animal models, show that microbial stimuli modify the immune response in the host, but studies in humans are mostly restricted to *in vitro* experiments. Intestinal microflora and probiotics, accordingly, may influence the immune mechanisms of the host by effects on mucosal barrier mechanisms and on the functional

maturation of the immune system. The primary effector arm of the immune system is the so-called innate immune system, which includes non-specific immune protection mediated by monocytes, macrophages and dendritic cells. The cells of the innate immune system have an important role as antigen-presenting cells. The innate immune system further regulates the function of the antigen-specific adaptive immune system, such as the functional balance of immune response related to cytokine and chemokine receptor profiles. Defective maturation of immune competence in association with poor microbial stimuli may thus be lead to dysregulation of both innate and adaptive immune systems.

Effect of probiotics on gut epithelium

Intestinal epithelial cells play an important role in the presentation of oral antigens and also in the regulation of intestinal immune responses. Thus, changes in the epithelium are reflected in the function of the immune system and *vice versa*. Activation of gut immune cells resulting in the secretion of cytokines may cause epithelial injury. Intestinal microbes have been suggested to be important regulators of the function and development of immune and epithelial cells. *Lactobacillus rhamnosus* GG given to germ-free rats increases the rate of mitoses in the small intestinal epithelia, resulting in an increased number of cells in the villi [33]. A beneficial effect of *Lactobacillus* GG on gut permeability was seen in suckling rats, in which cow's milk challenge induced gut permeability disorder [34]. These kinds of changes in the epithelial structure indirectly influence the gut immune system. Permeability controls the amount and quality of antigenic exposure, e.g. dose and size of antigens encountered in the gut immune system. Probiotics may also play a role in the recovery of intestinal epithelia as suggested by a report of prevention of cytokine-induced apoptosis in intestinal epithelial cells in the presence of *Lactobacillus rhamnosus* GG [35]. This kind of protection from inflammation-induced injury may be important in the regulation of the oral immune response when new antigens are encountered during infancy or in inflammatory conditions induced by pathogenic microbes.

Effect of probiotics on the innate immune system

Lactobacillus have been shown to activate monocytes and macrophages, which are important in antigen processing, presentation and activation of antigen-specific immune response, i.e. cell-mediated immunity. The functional nature of monocytes, macrophages or the most professional antigen-presenting cells, dendritic cells, has an important effect in the deviation of immune response to the so-called type 1 response with cytotoxic effector cells or towards type 2 response characterized by antibody response. The cytokine profile and up-regulation of surface markers with co-stimulatory function are different in type 1 and type 2 antigen-presenting dendritic cells or macrophages. IL-12 together with IFN- γ typically deviate the immunity towards type 1 response characterized by enhanced IFN- γ secretion. Type 1 response supports the development of cell-mediated and cytotoxic immunity. Instead, PGE2 together with IL-4 support the development of type 2 immune response. Type 2 immune response is related to the secretion of IL-4, IL-5, IL-9 and

IL-13 by T-helper 2 cells, which support the production of antibodies and especially contribute to the deviation of immune response towards the induction of IgE and allergic responses.

IL-10 secretion by monocytes has been linked to type 2 immune response, but it seems that the role of IL-10 is more complex and it is considered as an anti-inflammatory cytokine with a possible role in immune regulation.

Most of the published studies related to the effect of *Lactobacillus* on monocytes or macrophages have been performed as *in vitro* experiments. The stimulation of human mononuclear cells with *Lactobacillus rhamnosus* GG has been shown to promote the production of IFN- γ , IL-12 and IL-18, the latter two being monocyte-specific cytokines [36]. The production of IL-10 was weak, and IL-4 was not detected.

When the mechanisms of activation have been studied in human monocytes, the activation of NF- κ B and STAT have been shown after stimulation with *Lactobacillus* GG cell wall components [37]. These results indicate that Gram-positive bacteria activate transcription factors involved in cytokine signaling by two mechanisms: directly, leading to NF- κ B activation, and indirectly via cytokines, leading to STAT activation.

Dendritic cells (DCs) are the so-called professional antigen-presenting cells present in lymph nodes and in low frequencies in peripheral blood. DCs are present throughout the gastrointestinal tract. They regulate the development of T cell responses, especially the polarization of the T cell response. Exposure to killed *Lactobacillus* species induced the up-regulation of markers of maturation, such as MHC class II and B7-2 (CD86), in murine DCs [38]. These findings suggest that lactobacilli could enhance the mechanisms of antigen presentation. However, the different lactobacilli showed differences in their ability to induce cytokine expression. *Lactobacillus casei* was a strong inducer of IL-12, IL-6 and TNF- α together with maturation surface markers. *Lactobacillus reuteri* appeared to be a poor IL-12 inducer and inhibited cytokine induction and up-regulation of B7-2 induced by other *Lactobacillus* species. The results suggest that different species/strains of *Lactobacillus* may modify the function of DCs differently, and thus modify the characteristics of immune response triggered.

Lactobacillus rhamnosus GG in the presence of IFN- γ stimulation induced a low-level production of nitric oxide (NO) in macrophage cell line J774 [39]. Also lipoteichoic acid (LTA), a structural component of Gram-positive bacteria, induced NO formation in J775 cells in the presence of IFN- γ , suggesting that LTA may be the active component in *Lactobacillus* GG. Both cell wall and cytoplasmic extracts of lactic acid bacteria induced the activation of proinflammatory cytokines, such as IL-6 and TNF- α , and NO induction in *in vitro* experiments with murine macrophages [40]. Peritoneal macrophages showed a strong response to *Lactobacillus* species.

Lactobacillus rhamnosus HN001 have been shown to enhance the phagocytic activity of blood and peritoneal immune cells. Both live and heat-killed preparations of *Lactobacillus rhamnosus* HN001 enhanced the phagocytic activity of peripheral blood cells but only the live *Lactobacillus rhamnosus* phagocytic activity of peritoneal cells. Thus,

the enhanced mucosal response may be dependent on live bacteria [41].

In a clinical study with *Lactobacillus* GG, a down-regulation of cow milk-induced inflammatory response was seen in milk-hypersensitive subjects. In this study, the subjects with non-IgE-mediated milk hypersensitivity had up-regulation of receptors in neutrophils (CR1, Fc γ RI and Fc α R), and this was shown to be decreased after *Lactobacillus* GG treatment [42]. In healthy subjects, cow milk challenge did not influence receptor expression but *Lactobacillus* GG induced increased receptor expression in neutrophils. These results suggest *Lactobacillus* GG is able to induce neutrophil activation in healthy subjects, but in patients with milk hypersensitivity it inhibits cow milk-triggered activation of neutrophils.

Few studies have been performed in the elderly treated with probiotics. Functional aberrancies in cell-mediated immunity are associated with ageing. Natural killer cells are important for immune protection against viruses and tumours, and a decrease in the activity of natural killer cells has been reported in the elderly [43]. When age-related immunological changes were studied, dietary probiotic supplementation enhanced natural killer cell activity in the elderly [44]. Ingestion of *Lactobacillus rhamnosus* HN001 or *Bifidobacterium lactis* HN019 for a 3-week period resulted in an increase in the number of natural killer cells in the peripheral blood and increased *ex vivo* tumoricidal activity [44]. The interesting finding was that the most significant changes were seen in subjects over 70 years of age, suggesting that the immune changes in the elderly could be affected by probiotics.

Altogether the evidence from *in vitro* studies shows that both live lactobacilli and the components of lactobacilli, such as LTA, are capable of stimulating the innate immune system. The stimulation of the innate immune system seems to be directed to potentiate the function of cell-mediated immunity, possibly cytotoxic activity. On the other hand, this kind of immunoenhancing effect may result in the induction of regulatory mechanisms. Decreased activation of IFN- γ response has been associated with atopic diseases [45] as well as with autoimmune diseases [46, 47]. Physiological maturation of the immune system during childhood includes increased production of IFN- γ [48]. In addition, the recovery of impaired IFN- γ production is associated with clinical improvement of rheumatoid arthritis [49].

Effect of probiotics on B lymphocytes

The effect of probiotics on B lymphocytes and antibody responses has been evaluated in vaccination trials. The immunogenicity of oral rotavirus vaccination was reported to be enhanced in the group who received *Lactobacillus rhamnosus* GG [50]. Similarly, a trend towards increased *Salmonella*-specific IgA levels was found in subjects who received a combination of *Lactobacillus* GG and oral *Salmonella* vaccination [51].

In acute diarrhoea, the number of peripheral blood cells secreting IgA specific to rotavirus antigen increased in those children who received *Lactobacillus rhamnosus* GG [52]. The number of cells secreting IgA to cow milk β -lactoglobulin increased in children with Crohn's disease when treated with *Lactobacillus* GG [53]. The differences were specific to

children with Crohn's disease and no changes were seen in healthy children or children with juvenile chronic arthritis.

Based on these findings, it seems evident that *Lactobacillus* GG feeding in humans enhances the IgA response to the antigens encountered at the same time.

Also in a mouse model, the administration of yogurt supplemented with *Lactobacillus acidophilus* and *Bifidobacterium* species enhanced mucosal and systemic IgA responses to the cholera toxin which was used as an oral immunogen [54].

Effect of probiotics on T lymphocytes

The *in vitro* and clinical studies with *Lactobacillus* GG indirectly suggest that *Lactobacillus* GG could modify T cell response by attenuating the type 2 immune response. The *in vitro* studies of peripheral blood mononuclear cells showed that *Lactobacillus* species, among them *Lactobacillus* GG, were potent inducers of a type 1 cytokine profile and IFN- γ secretion, which is a down-regulator of IL-4. The increase in IFN- γ activation could offer a mechanistic explanation for the possible clinical effect in the prevention or treatment of atopic diseases in children. As discussed above, poor maturation of the IFN- γ response has been associated with atopic diseases, and the production of IgE is inhibited by IFN- γ and stimulated by IL-4.

Pochard et al. [55] studied the effect of *Lactobacillus* strains on the cytokine profile secreted by peripheral blood mononuclear cells stimulated with either Staphylococcal enterotoxin A (mitogen) or *D pteronyssinus* allergen. They found that pre-incubation of mononuclear cells with different lactic acid bacteria strains (*Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus casei* and *Lactobacillus rhamnosus*) inhibited the production of IL-4 and IL-5, so-called Th2 cytokines, whereas Gram-negative *E. coli* did not inhibit the induced Th2 cytokine secretion. In allergic patients, *D pteronyssinus* stimulated poor IFN- γ activation, which was recovered with pre-incubation with lactic acid bacteria combined with down-regulation of IL-4 and IL-5 response. The inhibitory effect of lactic acid bacteria was mediated by IL-12 and IFN- γ since neutralization of these cytokines restored IL-4 production. In animal models, a similar kind of cytokine pattern induced by lactobacilli has been reported: induction of the production of IFN- γ and reduced allergen-stimulated production of IL-4 and IL-5 [56].

However, the pathogenic mechanisms of atopic diseases or mechanisms of probiotics, on the other hand, are not fully understood. The effect of probiotics may be mediated by immune regulatory mechanisms not directly linked to increased IFN- γ secretion but to the induction of regulatory cells, such as cells secreting IL-10. In a clinical study, the ingestion of *Lactobacillus rhamnosus* GG was associated with high serum levels of IL-10, and a rise in mitogen-induced IL-10 from peripheral blood mononuclear cells preceded this [57]. IL-10 is secreted by several different immune cells such as monocytes and T cells.

It is also possible that probiotics may change the immune balance by indirect mechanisms as suggested by Pessi et al. [58]. When cow milk-derived caseins were treated with *Lactobacillus* GG, the presence of treated caseins decreased the mitogen-stimulated proliferation of lymphocytes as well

Table 1. Immunological effects and the possible clinical benefit of *Lactobacillus rhamnosus*

<i>In vitro</i> studies	<i>In vivo</i> studies	Possible clinical benefit
TNF- α ↓	Atopic dermatitis↓ Skin prick test 0 Total or specific IgE 0	
IL-12↑		Maturation of 'atopic' phenotype
IFN- γ ↑		Cell-mediated immunity↑
	IL-10↑	Immune regulation↓
IL-6↑	IgA↑ NKT cells↑ Phagocytosis↑ Tumoricidal activity↑	Maturation of mucosal immunity Cell-mediated immunity↑

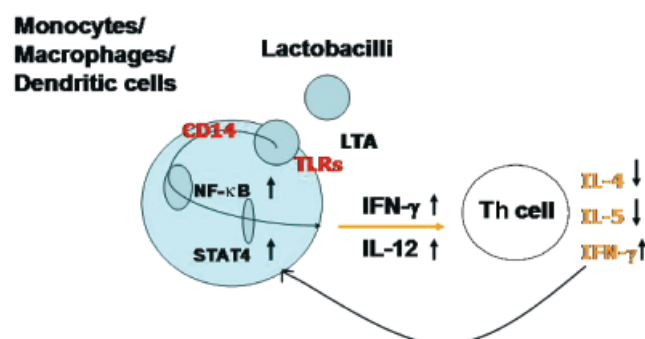


Fig. 1. Effects of lactobacilli on immune cells according to *in vitro* studies. Lactobacilli may modify the innate and adaptive immune response by activating IFN- γ and IL-12 production in monocyte lineage cells. This is mediated by activation of NF- κ B and STAT signalling pathways. The cytokines produced by monocytes/macrophages inhibit the production of IL-4 and IL-5, but support IFN- γ production by T cells. Thus, lactobacilli enhance the immune response and modify the polarization of T cell response by down-regulating Th2 immune response.

as the anti-CD3-triggered secretion of IL-4, a type 2 cytokine [59, 60]. This kind of change in the antigenic quality due to probiotics could be responsible for changes in the function of the immune system. This is however a very speculative mechanism and needs further evaluation.

Conclusions and future prospects

The role of intestinal microflora as a modulator of the immune response has been studied intensively during the last years. The immunological effects of *Lactobacillus* strains suggested by *in vitro* and *in vivo* studies, and the possible clinical importance of probiotics are described in Table 1. The results from *in vitro* studies show that bacteria of the intestinal microflora may modify the polarization of immune cells and/or mechanisms related to antigen processing, suggesting potential therapeutic effects in immune-mediated diseases, such as in atopic diseases. Probiotics, especially *Lactobacillus* and *Bifidobacterium* strains, modify *in vitro* the functional pattern of monocytes and lymphocytes towards increased production of IL-12 and IFN- γ , so-called type 1 cytokines (Fig. 1). The effects of probiotics on the immune response may

also be indirect and mediated by their effects on the intestinal epithelium, such as permeability and morphology.

Several studies indicate that probiotics are beneficial in the treatment of gastrointestinal infections due to their effects on the immune response and intestinal epithelium. An accumulating number of clinical studies suggest that probiotics, such as *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Bifidobacterium lactis* BB12, may be useful in the prevention and treatment of atopic diseases in children. However, the immunological effects of probiotics have not yet been clarified in these clinical studies. Thus, further prospective studies including immunological follow-up are needed. The reported *in vivo* effects of ingested probiotics have been observed in studies including only a small number of subjects, and no clear association between immunological changes and clinical effects has been reported. Some prospective follow-up studies are ongoing and the results of these studies will hopefully answer these questions. Whether different bacteria have different clinical effects as suggested by *in vitro* studies needs to be addressed further [61].

Although several *in vitro* studies suggest that cell-wall components, such as lipoteichoic acid from Gram-positive bacteria, are potent immune modulators, the effect of killed bacteria or bacterial components as immune modulators may be questioned. The colonization of the intestinal lumen with lactobacilli and changes in the composition of microflora may be needed. However, if the mechanisms of microbes as immune modulators are understood, then microbial components, instead of live bacteria, could be used as clinically effective probiotics.

It should be emphasized that host-related factors may influence the contact with microbial stimuli. The colonization of the gastrointestinal tract has been shown to be associated with MHC genotype of the host [62]. MHC gene products are responsible for antigen presentation, and MHC class I and class II gene alleles have been linked to several immune-mediated diseases. Polymorphisms in the recognition receptors of microbial structures, such as toll-like receptors and CD14, may influence the host's immune response to microbial agents, and modify the diversity of contact with microbial environment [63]. Also dietary factors, especially early nutrition during the first months of life, may modify the colonization of the gastrointestinal tract and thus the contact and interaction with microbial stimuli.

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