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Immunomodulatory effects of probiotics in different stages of life

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The immunomodulatory properties of lactic acid bacteria (LAB) and foods containing them (e.g., fermented milks) is a topic currently under investigation. Individuals could potentially benefit from the inclusion of LAB in the diet at different times during the life cycle. One of the most accepted specific uses of probiotic bacteria is the prevention of atopic eczema in infants with family history of the disease who receive the probiotic bacteria early, through supplementation of the gestating mother and orally after birth. Immune enhancing effects have also been suggested to be beneficial in diarrhoea treatment, especially in children infected with rotavirus and in malnourished patients, infants and adolescents, whose capacity to produce IFN-γ can be increased after LAB-containing yoghurt intake. Regarding young people and adults, investigations have been conducted exploring the immunomodulation by LAB in subjects under stressful situations, in the prevention of urinary tract infections in fertile women and in the treatment of allergy. However, the beneficial effects of probiotics in these conditions remain controversial and the scientific evidence provided so far is not considered to be conclusive. The elderly population has been the focus of investigations aimed at identifying the capacity of probiotics to counteract the immunosenescence process by increasing phagocytic and natural killer (NK) cell activities and to protect against infection. The mechanisms involved in the different effects attributed to LAB remain to be clarified. Moreover, considering that the immunomodulatory properties are strain-specific, defining the optimal dose of a certain bacteria or combination of bacteria strains and the duration of treatment for a desired effect in a target population group is essential in order to substantiate health claims.

Probiotics: Immunomodulation: Lifespan: Diseases with underlying immune mechanisms

One differential characteristic of some strains of lactic acid bacteria (LAB) used as probiotics compared with other microorganisms is their ability to survive during gastrointestinal transit [1]. This allows them to interact with commensal microbiota and/or intestinal epithelial cells and also with mucosa-associated lymphoid cells [2,3]. The communications between these systems result in the induction or modulation of a number of biological activities that can provide beneficial effects for health [4]. It is accepted that not all LAB strains show probiotic effects and that, with respect to their effects on the host's immune system, a high variability among species and among different strains of the same species is to be expected.

Probiotics are thought to reinforce the intestinal barrier and help maintain normal permeability. On the other hand, LAB have been described binding to the luminal side of the M cells present in the epithelium over the intestinal Peyer's patches and providing antigen transport that facilitates the stimulation of the underlying lymphoid tissue. There is also the possibility that dendritic cells anchored between epithelial cells provide a sampling function of luminal bacteria [5]. Following capture by dendritic cells, the activation of IgA responses is triggered locally and at distant mucosal sites[6] which enhances immune exclusion of foreign antigens [7]. In addition, the interaction of probiotic strains with immune cells in the mucosal environment has a principal role in a number of processes directly dependent on the mucosa associated lymphoid tissue (MALT), such as oral tolerance induction, the modulation of cytokine and chemokine release (i.e. induction of regulatory T cell cytokines) and in general, the regulation of immune responses in the intestinal mucosa which are important in the pathogenesis of inflammatory bowel disease[8].

Exposure to different challenges during life, either internal or environmental, interferes with the normal development and balance of the healthy gut microbiota. These factors include the early encounter of environmental insults in the newborn, dietary issues such as infant formula feeding, antibiotic treatment, age-related changes in the intestinal physiology and hence in the microbiota, and gastrointestinal diseases and stress. These factors act upon the idiosyncratic immune characteristics of each individual, derived from their genetic background, and drive the development of the immune system and the immune responses triggered by variable stimuli. As the number of environmental insults increases, the damage to the microbiota increases, along with the risk of inflammatory and allergic diseases [9]. Probiotics might help restore gastrointestinal health and immune responses in such circumstances. Physiological and pathological conditions occurring at different stages in life that might benefit from probiotic therapy will be addressed below.

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Gestation and newborns

The effect of *Lactobacillus* GG on the prevention of atopic disease has been studied in a group of pregnant women from atopic families[10]. A *Lactobacillus rhamnosus* GG (LGG) supplement was consumed by the mothers for 4 weeks at the end of their pregnancy and during the breast-feeding period until the child was 3 months old. The potential immunoprotection provided by breast milk was increased as assessed by the concentration of anti-inflammatory transforming growth factor β2 (TGF-β2) in the milk of mothers who received probiotics compared with a placebo group. Moreover, the risk of developing atopic eczema during the first 2 years of life in the infants whose mothers received probiotics was significantly reduced in comparison with that of infants whose mothers received the placebo (15 % and 47 %, respectively). These results support the protective effect of this probiotic strain against atopic eczema during the first 2 years of life. A more recent study by Kukkonen *et al.*[11] was carried out in pregnant women carrying children at high-risk of allergic disease. Mothers received capsules containing LGG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS during the 2 to 4 weeks before delivery. Their infants received the same combination of probiotics plus galacto-oligosaccharides or a placebo for 6 months. At 2 years of age the probiotic treatment showed no effect on the cumulative incidence of allergic diseases but significantly prevented eczema and especially atopic eczema (a 34 % relative risk reduction) supporting the inverse association between atopic diseases and colonisation of the gut by probiotics.

The potential use of probiotics in allergic asthma has been investigated in murine models after oral-allergen sensitisation and development of airway inflammation and hyper-reactivity[12,13]. In these models, the protective effect of probiotics has been assessed through an early administration given either to newborns[12] or perinatally, to the pregnant and lactating mother[13]. Significant effects have been observed in relation to the suppression of inflammation (i.e. reduced eosinophil influx and allergy related cytokines such as IL-5 and IL-10) and airway reactivity with strains such as LGG or *Bifidobacterium lactis*.

Infants and children

Immunosuppression is usually secondary to malnutrition and has negative health effects. In an attempt to correct these situations, different studies have been focused on the immunological effects of LAB in malnourished children. The effect of yoghurt (milk fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was studied in the refeeding of hospitalised malnourished children showing a weight for height value between 70 and 80 %, compared with a control group with similar characteristics, receiving the same amount of milk without probiotics in their diet. An increase in serum IFN-γ was found in the group consuming yoghurt[14], which might indicate maintenance of an improved resistance against pathogens.

The efficacy of LAB on the treatment of infantile diarrhoea (especially that due to rotavirus infection) has been assessed in many studies. LGG is one of the most extensively studied strains[15–18]. Two meta-analyses have reported that LGG is associated with moderate clinical benefits in the treatment of acute diarrhoea in children, namely a significant reduction in diarrhoea duration (approximately 1 day), particularly of rotavirus aetiology (about 2 days shorter), and duration of hospitalisation [19,20]. However no significant reductions in the number of stools or total stool volume were found. Sometimes the reduced duration of diarrhoea has been accompanied by an increase in rotavirus-specific IgA-secreting cells[16,17]. *Lactobacillus acidophilus* might provide similar effects[21,22] but not all studies have confirmed these results[23]. LGG might also be effective in the prevention of antibiotic associated diarrhoea[24,25], nosocomial infection diarrhoea[26] and diarrhoea secondary to malnutrition[27]. Some species of bifidobacteria, such as *B. infantis* and *B. bifidum*, in combination with lactobacillus have been evaluated in the treatment and prevention of diarrhoea with good results[22,28].

Adolescents

Eating disorders

Anorexia nervosa (AN), which is a relatively frequent eating disorder in modern society during adolescence, causes patients to present a very undernourished status with peculiar immunological alterations[29,30]. We studied the effect of yoghurt intake (375 g/day) over 10 weeks in a group of AN patients recruited at hospital admission[31]. The findings suggested that the inclusion of yoghurt in the refeeding therapy of AN patients may exert positive effects on the immunological markers related to the nutritional status of these patients, as shown by the higher ratio of $CD4^+$ to $CD8^+$ cells in the blood of patients consuming yoghurt compared to those consuming milk and by the increased production of IFN-γ by PHA-stimulated peripheral blood mononuclear cells (PBMC) both in anorexia nervosa patients and healthy adolescents receiving yoghurt treatment[31].

Adults

Healthy youth

A variety of experimental approaches *in vivo* and *in vitro* have been followed in order to test the effects of LAB on cytokine production by immune cells. Firstly, the incubation of PBMC from healthy young adults with *Lactobacillus casei*, *Lactobacillus acidophilus* or *Bifidobacterium*, *in vitro*, induced an enhancement of IL-1, TNF-α and IFN-γ production[32]. In addition, a number of *in vivo* or *ex vivo* studies have demonstrated that LAB consumption has a positive effect on IFN production in humans[33–35] possibly leading to an improved capacity of macrophages and NK cells to kill virus-infected cells or tumour cells. An increase in the 2' 5' adenylate synthetase activity (an enzyme which is induced by IFN-γ) in PBMC has been observed in subjects 24 hours post-consumption of a single dose of yoghurt containing 10^{11} bacteria[36] and also after yoghurt consumption during 15 days compared with milk consumption in a cross-over study[32].

Stressful situations in youth

The effects of psychological and physical stress on health are an increasingly relevant topic of research due to the current lifestyle in our society. Although a certain amount of moderate stress is even considered beneficial, surpassing the threshold has some negative effects on the immune system and causes an impaired resistance to infection. The effect of milk fermented with yoghurt cultures plus *Lactobacillus casei* DN-114001 (10^8/ml) on the immune system of subjects under academic examination stress was evaluated in a study with university students consuming either 200 ml/day of the fermented product or 200 ml/day of semi-skimmed milk. Although both groups showed increased cortisol and anxiety levels during the exam-taking period, the control group showed a decrease in NK cells that was prevented in the group consuming the fermented milk, who, in addition, showed an increase in numbers of lymphocyte cells[37]. This result could influence susceptibility to infection, which is believed to be higher under stressful conditions.

Infections

Probiotics are also considered beneficial in the prevention of urinary tract infections (UTIs) in fertile women as observed in a case-control study comparing dietary and lifestyle habits[38]. However, oral ingestion of LGG did not display any clinical efficacy in the protection against UTIs in a controlled intervention trial, which might indicate differences between strains[39]. Regarding microecology of the vagina, in a randomised, double-blind, placebo-controlled study of 64 healthy women, daily intake of *L. rhamnosus* GR-1 and *L fermentum* RC-14 resulted in significantly less yeast and fewer coliforms in the vagina[40]. Administration of the probiotic organisms even normalised flora in some cases of bacterial vaginosis, making it feasible to study this as an approach to long-term therapy for pregnant women and those susceptible to bacterial vaginosis and urinary tract infections. Adhesion and colonisation of the vaginal epithelium by the strain for days or even weeks may be necessary. Insertion of lactobacilli into the vagina via a pessary or capsule is an effective means of boosting the content of the flora and overcoming some pathogens or reducing their ability to dominate[41].

Allergies

The possible utility of probiotics in allergy has received a considerable amount of attention during the last fifteen years. However the evidence is still not strong enough to define any one strain as clearly beneficial in allergy treatment; neither are the mechanisms leading to the observed effects well defined. Remission of nasal allergy symptoms has been described in 42 young subjects and 56 adults consuming 200 g of yoghurt/day for one year in two separate studies[42,43]. No differences, however, were found in the immunological parameters measured. On the other hand, a study in adults with asthma taking 225 g of milk fermented with *L. acidophilus* twice a day for one month showed a tendency towards increased concentrations of IFN-γ and a decrease in eosinophil counts[44]. In this last study, however, no improvement was observed in the clinical symptoms or quality of life. A putative capacity of probiotics to direct immune responses towards the production of Th1 profile cytokines could be a possible mechanism to substantiate the use of LAB in the treatment of allergic diseases.

A study with *L. rhamnosus* administered for 3·5 months to young adult patients suffering from birch allergy showed no effect of the probiotic treatment on the allergy symptoms or the use of medication compared to the placebo group[45]. In summary, the evidence for a positive effect of probiotics in allergic diseases is still weak except in the case of atopic eczema.

Phagocytic activity

One of the immune functions for which more evidence of susceptibility to modulation by probiotic consumption exists is phagocytic activity by peripheral blood leukocytes. It has been documented in healthy volunteers that dietary deprivation of fermented foods for 2 weeks decreases the phagocytic activity of leukocytes[46]. In addition, the consumption of fermented milk containing *Lactobacillus acidophilus* (7×10^{10} cfu/d)[47] or *Lactobacillus johnsonii* La1 (10^7 cfu/d)[48] for 3 weeks increased the phagocytic capacity of healthy adults. Likewise, the consumption of *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711 or yoghurt alone for 2 weeks increased the phagocytic activity of monocytes and neutrophils in healthy subjects[49]. The dose of bacteria consumed daily has been proven important in achieving the desired effect[48]. Other effects of probiotic consumption have also been documented in placebo-controlled intervention trials, such as increases in IgA concentration, increased numbers of NK cells and activity, and increased oxidative burst capacity of monocytes[49,50]. However, not all the trials have yielded significant results. A probiotic product containing *Bifidobacterium lactis* and *Lactobacillus paracasei* assayed at 5 different concentrations, from low (10^8) to high (10^{11}), in groups of young healthy adults failed to demonstrate any significant modification of phagocytic activity, faecal IgA concentration or production of IFN-γ and IL-10 by blood cells[51]. Finally, a combination of probiotic bacteria (*Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5; 5 x 10^7 cfu/d) in a dietary vitamin and mineral supplement offered to healthy adults for 3 or 5 months in winter/spring seasons, respectively, significantly decreased the incidence, and also the symptoms, of common cold infections in comparison with the vitamin mineral preparation given to the control group[52].

Elderly

Aging is accompanied by a reduction in the functional capacity of all the organs in the body and accordingly the activity of the immune system also declines with age. The senescence of the immune system especially affects cell-mediated immunity with a decrease in lymphocyte proliferation capacity and IL-2 production. However, IgA concentration and antibody titers following immunisation are decreased as well[53]. A decrease has also been observed in the ratio of mature to immature T lymphocytes[54] and an increase in proinflammatory cytokine and reactive oxygen species (ROS) production[55]. Perhaps associated with these

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Table 1. Summary of some of the significant effects of different species of LAB shown for different conditions during the lifespan

Life stage	Condition	Effect	LAB species assayed	References
Gestation and infancy	Atopic disease	Prevention of atopic eczema at 2 years of age	*Lactobacillus* GG alone or in combination with other species or with prebiotics	10,11
Infancy and childhood	Malnutrition	↑ serum IFN-γ	*L. bulgaricus* + *S. thermophilus*	14
	Diarrhoea	Reduction of diarrhoea duration and prevention of diarrhoea of different aetiologies	*L. rahmnosus* GG, *L. acidophilus, B. infantis, B. bifidum.*	15–28
Adolescence	Anorexia nervosa, Healthy	↑ CD4+/CD8+ ratio and ↑ IFN-γ production	*L. bulgaricus* + *S. thermophilus*	31
Young adulthood	Healthy	↑ IFN-γ production	*L. bulgaricus* + *S. thermophilus*	32,36
	High stress	Prevention of peripheral NK cell depletion and increase in lymphocytes	*L. casei* + *L. bulgaricus* + *S. thermophilus*	37
	UTI in fertile women	Beneficial effect on the vaginal microflora	Lactobacilli	40,41
	Allergy	Amelioration or remission of nasal symptoms	*L. bulgaricus* + *S. thermophilus*	42,43
Adulthood	Allergy	Amelioration or remission of nasal symptoms	*L. bulgaricus* + *S. thermophilus*	42,43
	Healthy	↑ phagocytic activity	*L. acidophilus, L. johnsonii, L. gasseri* + *L. coryniformis*	47–49
	Healthy	↓ incidence of common cold infections	*L. gasseri* + *B. longum* + *B. bifidum*	52
Old age	Healthy	↑ Phagocytic activity, ↑ NK cell activity, ↑ IFN-α production	*B. lactis, L. rhamnosus*	56–59
	Healthy	↓ days with disease, but no effect on incidence of winter infections	*L. casei* + *L. bulgaricus* + *S. thermophilus*	60

immunological changes, and certainly with other physiological and environmental factors, the bifidobacteria numbers in the gut decrease markedly after 55-60 years of age. Functional foods such as probiotic products may have a particular application in this high-risk group, especially in terms of protection against entero- and urogenital pathogens, and perhaps also in the prevention of several age-related diseases.

Two 3-week intervention trials have shown that *Bifidobacterium lactis* HN019 supplementation (10^9-10^{10} cfu/d) increases the phagocytic capacity of monocytes and polymorphonuclear cells and the NK cell tumoricidal activity in elderly subjects [56,57]. The same bacteria used in a 6 week intervention trial significantly increased the phagocytic activity and the IFN-α production capacity by PBMC in elderly volunteers [58]. Similar results regarding phagocytic and NK cell activities have been also described following the supplementation of middle aged and elderly subjects with *Lactobacillus rhamnosus* HN001 in similar doses for 3 weeks[59].

A randomised, controlled pilot study has been carried out to assess the effect of milk fermented with yoghurt cultures and *L. casei* DN-114001 on the incidence and severity of winter infections (gastrointestinal and respiratory) in elderly people[60]. 360 free-living elderly subjects took part in the study and those in the treatment group consumed two doses of 100 ml fermented product per day for 3 weeks. Although no difference was found in the incidence of winter infections between groups, the duration of all pathologies was significantly lower in the treatment group than in the control group (19·5 % reduction in days), as was maximal temperature (mean 38·3 s.d 0·5°C treatment group vs. mean 38·5 s.d 0·6 °C control; $P=0·01$). Further studies with a double-blind placebo-controlled design should be performed for a better assessment of the usefulness of the fermented product in the enhancement of immune defence in the elderly population.

Conclusion

This review has summarised studies that, at different stages during the lifespan, have used probiotics to provide benefits regarding immune responses and health maintenance (Table 1). There is, however, a need for basic and applied research providing new insight into the nature of the interaction established between the probiotic bacteria and the intestinal mucosa, the mechanisms involved and the active component(s) responsible for the effect, whether bacteria wall components, end products of bacteria metabolism or milk proteins which might be immunologically relevant. In addition, increased knowledge about the specific strains that are the most adequate for each specific purpose, as well as the definition of the recommended dose and duration of supplementation is essential in order to substantiate health claims, and to be able to make specific recommendations to individuals and population groups.

Conflict of interest statement

AM has had research funding from Danone and Phergal, received consultancy fees from Danone and speaking fees from Danone and Phergal. EN, SGM, LED have received consultancy fees from Danone. JW and JR have no conflicts of interest to declare. All authors co-wrote the manuscript.

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