



## Probiotics and the skin

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**Abstract** A review of the relationships between probiotics and the skin is presented. After a brief historical introduction, the main pathophysiological data on intestinal microflora, the immune system and the skin are presented. Clinical studies with probiotics in atopic children are discussed in detail. Many experimental studies have found that probiotics exert specific effects in the luminal lumen and on epithelial cells and immune cells with antiallergic potential. Not all probiotics have the same immunological properties. Moreover, although rarely, complications of probiotic use can occur and must be known and taken into account. This review underlines the potential interest in probiotics for the management of skin pathology. © 2008 Elsevier Inc. All rights reserved.

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### A brief history from the Bible to probiotics

Probiotics were still far to come when in the Bible (Genesis 18, 1-8), “Abraham offered to God Father, in oak wood, fermented milk,” and also, Isaiah (7:15) said that “it will be eaten curdled milk and honey.” The consumption of fermented foods gets lost in the mist of times. Also, the term *yogurt* term goes back to four thousand years ago, and it has Turkish origin because the word *juggurt* in Turkish means “milk dense.” In ancient Greece and Rome, consumption of fermented milk was recommended for children and convalescents. In 1780, Italian abbot, naturalist, and biologist Lazzaro Spallanzani (1729-1799) from Modena refused a very widespread theory on microorganism generation or “spontaneous gemmation,” introducing a new microbiological concept concerning the genesis of diseases and the term *germ*. In 1907, Elia Metchnikoff, from Ukraine (1845-1916), who received later the Nobel Prize for the discovery of the phagocytosis, assumed that all microorganisms are not harmful to human health and that several intestinal bacteria “produce

useful substances against a premature aging,” favoring instead a “healthy aging.”<sup>1</sup> The slow scientific evolution became favorable to the development of the Metchnikoff theory in 1936, when Zobell and Andersen supported the theory of a “microbic film” in the large intestine. Zobell and Andersen postulated the existence of a layer made of many species of bacterial populations adhering to the intestinal mucosa, a complex ecosystem with intense metabolic activities. In 1965, during a period of full enthusiasm for antibiotics, Lilly and Stillwell,<sup>2</sup> veterinarians, introduced the term *probiotic*, the antinomy of antibiotic. In 20 years, scientific research improved, recognizing the existence of a dynamic equilibrium among nutrition, intestinal microflora, and host health.

The aim of this review is to focus on the relationship between probiotics and skin in pediatrics.

### Intestinal microflora and evolution of its knowledge

Early research on intestinal microflora demonstrated that the human intestine is sterile during fetal life, and this condition lasts approximately 24 hours after birth, when the

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newborn undergoes a fast intestinal colonization from several bacteria depending on different factors such as type of delivery, environment where the baby lives, and feeding.<sup>3</sup>

Because of the oligosaccharide content, breast-feeding stimulates intestinal increase of anaerobic microorganisms such as bifidobacteria, present in more than 30 species, and some typical of intestinal flora of the infant, such as *Bifidobacterium breves*, *B infantis*, *B pseudocatenulatum*, lactobacilli, and *Bacteroides*. Well-documented benefits from these microorganisms can be summarized as follows: digestive function modulation such as nutrient and mineral absorption, synthesis of some vitamins (B, K), immune system stimulation and reduction of allergy risk, barring of pathogenic flora development, better resistance to infections, reduction of diarrheic episodes, limiting development of several pathologic conditions, interfering with mutation of enzyme activity, and lowering cholesterol.<sup>4</sup>

Bottle-feeding develops a mixed bacterial flora in the infant intestine, with a reduction of *Bifidobacteria* and greater presence of other germs (*Bacteroides* species, clostridia species, staphylococci) that, in particular situations, can have pathogenic effects, too.

When breast-feeding is supplemented with bottle-feeding, the profile of intestinal microflora is similar to that of infants nursed with formula, and the bifidobacteria are not dominant.<sup>4,5</sup>

Beginning at weaning, intestinal flora is similar to that of the adult, with an increase in *Bacteroides* and anaerobic gram-positive number, such as peptococci, peptostreptococci, *Veillonella* species, and staphylococci. In adults, diet variations determine individual characteristics of intestinal microflora, according to age, feeding, lifestyle, interactions among numerous constituents of the same flora, as well as pathologic conditions, in particular, gut infections and antibiotic treatments; ethnic and climatic environment are less influential.

Adult intestinal mucosa contains, in a surface estimated to be approximately 250 m<sup>2</sup>, a population of thousand of billions of microorganisms, equal to 10<sup>14</sup> members of 17 families, 45 types, and 500 species.<sup>6</sup> Most bacterial species (97%) are anaerobic; only 3% are aerobic (anaerobic facultative). Most of the anaerobic species are *Bacteroides*, bifidobacteria, eubacteria, fusobacteria, clostridia, and lactobacilli. Among aerobes, in the gram-negative group, *Escherichia coli* and *Salmonella* species are most represented; in the gram-positive group, enterococci, staphylococci, and streptococci; and among fungi species, *Candida albicans*.

Intestinal mucosa separates the internal environment from the external one and absorbs nutrients, but it is an immunologic barrier against pathogenic microorganisms. In fact, it contains the major lymphoid organ, gut-associated lymphoid tissue, which develops during fetal life.

In the newborn, Peyer patches are small, made by primary lymphoid follicles, including T lymphocytes, a g dome region, and follicle-associated epithelium follicles with germinated centers, which appear, later on, under antigenic stimulation, are absent.<sup>7</sup> Intestinal flora is a source of

antigenic stimuli for gut-associated lymphoid tissue; however, the goral tolerance phenomenon limits immunologic stimulation: lack of immunogenic activity, if many antigens are ingested and absorbed, allows our nutrition and our cohabitation with a part of the intestinal microflora, making it possible for the immune system of the intestinal mucosa to induce gtoleranceh to inhalant antigens.<sup>8</sup>

In Estonian children—with low prevalence of allergies—the intestinal flora is represented by lactobacilli; in Swedish children—with high prevalence of allergies—clostridia are prevalent, and further studies have found smaller enteric colonization of lactobacilli in allergic children compared with nonallergic ones, with a prevalence of aerobic bacteria (coliform and *Staphylococcus aureus*).<sup>9</sup> Moreover, in vitro and in vivo, various studies demonstrated that lactobacilli modulate the immune system against allergies. This is evident in early childhood, when the immune system can be moulded, perhaps definitively,<sup>10,11</sup> because it is immature. These data contribute to the hygiene hypothesis, although it is not completely accepted<sup>12-14</sup> that cutaneous, respiratory and gastrointestinal allergic pathologies are secondary to a reduced number of infections in early childhood, with secondary altered equilibrium of intestinal microflora that significantly influence the immune system. Elevated fecal levels of i-caproic acid (indicators of high level of *Clostridium difficile*) suggest that the enteric microflora could be altered in allergic babies. A confirmation is that children who develop allergies present low levels of bifidobacteria, gram-positive aerobic organisms, and enterococci, but more elevated levels of clostridia and *S aureus*.

Against the hygiene hypothesis, the alteration of gastrointestinal flora equilibrium would be secondary not only to genetic factors, diet, and infection frequency, but also to other factors, such as antibiotic therapies, passive smoke, number of relatives, pollution, vaccinations, psychologic stresses, reduction of immunologic activity, and development of allergic diseases and some inflammatory diseases.<sup>15-19</sup>

Antibiotic therapy leads to changes in intestinal, bacterial, and fungal components and also to overgrowth of *C albicans*, which can secrete potent prostaglandin-like immune response modulators. This can promote the development of allergic events in distal mucosal sites such as the lungs and the skin.<sup>20,21</sup>

The composition of the intestinal microflora would be the common element in allergic diseases, and species of anaerobic probiotics could play a role in the development of healthy immunity response, participating in enteric microecology, preventing and, prospectively, potentially treating allergic diseases.<sup>8,22-27</sup>

These data suggest that general living standards have changed, and lifestyle factors are important in the development of atopic and immunodysregulatory diseases. In the first years of life, our immune system, in addition to mitigating the allergic response to T helper type 2 (Th2), seems to develop a protection system against atopic diseases marked out from Th2 and against those characterized by a T helper type 1 (Th1) excess.

So, there is well-grounded evidence that simply bringing back balance in intestinal microflora can achieve a good and balanced immunoregulation.

## Immune system, gut mucosae, and the skin

Oral tolerance is present from the first hours after birth and not only gives way to the suppression of immune response against proteins, introduced with nourishment, but also acts against microflora that can modulate the immunologic aspect, favoring tolerance itself. Oral tolerance to an allergen can block immunologic response outside the gastrointestinal environment, such as in respiratory and cutaneous apparatus.<sup>28-31</sup>

Immunologic tolerance, including oral tolerance, is likely mediated by a large group of T cells originated from dendritic cells (DCs), which show antigen (antigen-presenting cell).<sup>32</sup> Immature DCs, which have not received inflammatory stimuli, mature also in the intestinal mucosa and originate regulator DCs, DC type 1, and DC type 2, with regard to the interactions among pathogenic, different, and/or microbial products.<sup>32,33</sup> They can originate also regulatory T cells, through interleukin (IL) 10 and/or transforming growth factor- $\beta$ , and type 1 (Th1) and type 2 (Th2), through the production of cytokine.<sup>8,32</sup>

The alteration of the intestinal microflora equilibrium may interfere in the specific DCs that stimulate the antigen Treg, favoring an altered oral tolerance, with a prevalence of the Th2 cells that predispose to atopic diseases. The tolerance, therefore, would be mediated by Treg cells that can regulate Th2 response against antigens in several organs, including the skin and respiratory apparatus.

It appears clear that, in "germ-free animals," bacterial colonization is associated with anatomical and functional intestinal alterations of all the immune system (decrease in immunoglobulin levels, production of natural antibodies, cellular infiltration of the gut mucosa, and formation of germinal centers in Peyer patches), alterations that are resolved with the introduction of nutrients as indispensable conditions for the normal development of the microflora.<sup>34</sup>

Human microflora composition is the same across many generations. Modern lifestyle negatively influences the intestinal ecosystem, and from one generation to the next, there may be cumulative degradation of the intestinal microflora. Linear changes in environmental conditions and lifestyle may lead to nonlinear changes in the gut flora possibly to an increasing susceptibility to atopic diseases.<sup>17</sup>

Also, the skin is an immunogenic organ that works as the first defense and biologic sensor against external allergens. Exposure to cutaneous allergens can favor the onset of allergic diseases, and the atopic response can be secondary to the cutaneous barrier disruption. They can be helped by the interaction of genetic factors for immunoglobulin (Ig) E production (chromosome 5) and for correlated cytokine Th2 production (chromosome 11).

The keratinocytes produce a large number of immunomediators such as IL-1, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, interferon (IFN)  $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , tumor necrosis factor (TNF)  $\alpha$ , TNF- $\beta$ , granulocyte macrophage colony-stimulating factor, macrophage colony-stimulating factor, chemokine, and growth factors.<sup>35-37</sup>

Langerhans cells, part of the great family of DCs, are found in the layer of the keratinocytes over the basal membrane, with a population of T lymphocytes.<sup>38</sup> These cells are specialized in showing antigen (antigen-presenting cells) and represent 2% to 4% of all epidermic cells. In dermal and perivascular areas, the greater number of cells are with immunity activities: mast cells, monocytes, macrophages, and the T cells.<sup>36</sup> Dermal DCs represent a major portion within the infiltrated portion of atopic dermatitis (AD) lesions. As antigen-presenting cells, they are able to regulate the quantity and quality of T-cell responses and thus are likely to play a key role in the pathogenesis of T-cell-dominated skin diseases such as AD.

When the antigen exceeds the corneous layer, it catches up with keratinocytes, and it can be assumed from the Langerhans cells. Then, because of the lack of organized lymphoid tissue inside the derma, with the migratory ability of dermal Langerhans cells and DCs, it is transported to the draining lymph nodes<sup>35,39</sup>; lymph node cells are activated, with remarkable production of IL-4, limited IFN- $\gamma$  production, and increased production of B cells and antigen-specific IgE and IgG1, but not IgG2a.<sup>35</sup>

The Langerhans cells exposed to an antigen protein induce the production of Th2, but when the antigen reaches the derma, it induces the production of Th1, and, migrating to the draining lymph nodes, it induces a potent immune response with a strong Th2 bias. In contrast, antigen delivery into the derma induces predominantly Th1-type immune responses.<sup>39-42</sup>

The allergen penetration through the skin can lead to a systemic sensitization involving the intestinal mucosa and respiratory apparatus, with intense response of Th2 type and specific production of IgE.

Because of different imprinting of cellular tropism, the T cells activated from the gut DC cells present an intestinal tropism for the gut, whereas those activated from the lymph nodes, peripheral DCs cell, presented a tropism for the skin.<sup>43-46,51,52</sup>

These data and recent advances on immunity of the skin probably represent a further confirmation that the switch toward a Th2 response does not increase the frequency of allergies.<sup>50</sup> Pregnancy and helminth infections are examples that prevalence of Th2 lymphocytes is not associated with an increase of allergy or atopic disease.<sup>47,48</sup> Moreover, the dramatic increase of allergic diseases in the western world is associated with autoimmune diseases, Th1-mediated, with unknown etiology such as inflammatory diseases of the gut.<sup>49</sup> The imbalance in the development of the responses of Th1 and Th2 seems to be insufficient to explain the increase in the allergic and autoimmune diseases; perhaps it would be explained by an altered mechanism of immunoregulation.<sup>18</sup>

## Clinical studies in atopic children

Scientific researches have elaborate intervention strategies to balance the intestinal microecology with oral administration of select bacteria called probiotics.<sup>52-54</sup>

Concerning cutaneous pathologies, the first double-blind placebo-controlled intervention to improve the atopic manifestations, dermatitis/eczema, and allergy to cow's milk (CMA) with probiotics was done in 1997.<sup>55</sup> The authors have estimated the immunologic and clinical effects of cow's milk elimination in children with or without *Lactobacillus rhamnosus* GG (LGG;  $5 \times 10^8$  colony-forming units [CFUs]/g) in hydrolyzed formula. *Lactobacillus rhamnosus* GG ( $2 \times 10^{10}$  CFUs) was administered to mothers of 10 breast-feed children with atopic eczema and CMA.

A significant improvement in AD assessed by the severity scoring of atopic dermatitis (SCORAD) score<sup>56</sup> was observed after 1 month of intervention only in those receiving LGG. The concentrations of fecal  $\alpha_1$ -antitrypsin and TNF- $\alpha$ , determined as markers of intestinal inflammation before and after dietary intervention, decreased significantly in the group receiving LGG. It was concluded that probiotic bacteria may be effective possibly by promoting endogenous barrier mechanisms in patients with AD and food allergy, and minimizing intestinal inflammation may act as a useful tool in the treatment of food allergy. An experimental and a follow-up study was not performed after treatment.

Isolauri et al<sup>57</sup> studied the effects of probiotics in atopic eczema in a randomized double-blind study. A total of 27 infants (mean age, 4.6 months) who presented with AD during exclusive breast-feeding were divided into 3 groups: probiotic-supplemented, *B. lactis* Bb-12 or LGG, and extensively hydrolyzed whey formulas or the same formula without probiotics. A significant reduction in SCORAD score was seen after 2 months in the probiotic-supplemented groups as compared with the unsupplemented group ( $P = .002$ ). A reduction in the serum concentration of inflammatory markers such as soluble CD4 (a marker of T-cell activation) and urinary eosinophil protein X, (a marker of eosinophilic inflammatory activity) was also observed, suggesting that probiotics may counteract inflammatory responses beyond the intestinal milieu.

Pessi et al<sup>58</sup> studied IL-10 generation in atopic children. Anti-inflammatory properties of IL-10 are well known for acting on IL-2, IL-6, IL-12, TNF- $\alpha$ , and IFN- $\gamma$  and IgE synthesis. The authors added LGG ( $2 \times 10^{10}$  CFUs) for 4 weeks to the diet of 9 children (mean age, 21 months) with AD and CMA. Levels of IL-10 rose in the blood of atopic children, demonstrating anti-inflammatory properties of specific probiotic bacteria strains.

Rautava et al,<sup>59</sup> in a double-blinded, placebo-controlled study of 62 mother-infant pairs administered LGG ( $2 \times 10^{10}$  CFUs) during the 4 weeks before birth and during breast-feeding (3 months), LGG increased the immunoprotective potential of breast milk, as assessed by the amount of anti-inflammatory transforming growth factor- $\beta_2$  in milk of mothers receiving probiotics vs placebo. The risk of

developing atopic eczema during the first 2 years of life in infants whose mothers received probiotics was significantly reduced. Infants who most likely benefit from maternal probiotic supplementation were those with an elevated cord blood IgE concentration.

Kalliomäki et al,<sup>60,61</sup> in a double-blind, randomized, placebo-controlled trial,<sup>60,61</sup> administered LGG ( $1 \times 10^{10}$  CFUs) during 4 weeks before birth and during breast-feeding for 3 months to mothers who had at least 1 first-degree relative or partner with atopic eczema, allergic rhinitis, or asthma, and to their infants postnatally for 6 months. Chronic recurring atopic eczema, which is the main sign of atopic disease in the first years of life, was the primary end point. The frequency of atopic eczema in the probiotic group was half that of the placebo group. The number needed to treat was 4.5. Thus LGG was effective in prevention of early atopic disease in children at high risk, and the preventive effect was still effective after 4 years.<sup>60</sup>

Rosenfeldt et al<sup>62</sup> investigated the use of probiotics as an adjuvant to topical steroids (hydrocortisone or hydrocortisone butyrate) in the treatment of established AD in a double-blind, placebo-controlled crossover study, in which subjects aged 1 to 13 years with severe chronic eczema had taken either a combination of 2 *Lactobacillus* strains (LGG and *L. reuteri* DSM) or placebo for 6 weeks. The decrease in mean SCORAD index was not statistically significant. Eczema extent decreased, and 56% of probiotic-treated patients experienced subjective symptom improvement compared with 15% of placebo-group patients. Serum eosinophilic cationic protein levels used to monitor disease activity in AD decreased with probiotic therapy, whereas no change was observed in the placebo group. The modest effect of the probiotics on improvement of AD may be attributed to the older age of the subjects and the severity of the eczema. The more pronounced effect of probiotics was observed in the subset of allergic patients (at least 1 positive skin prick test response and elevated IgE levels).

Kirjavainen et al,<sup>63</sup> in a randomized, double-blind research, analyzed 35 infants with atopic eczema and CMA. At a mean age of 5.5 months, they were assigned to receive either extensively hydrolyzed whey formula (placebo group) or the same formula supplemented with viable LGG or heat-inactivated LGG.

Heat-inactivated LGG was associated with adverse gastrointestinal symptoms, leading to premature termination of study cohort recruitment. Scores in SCORAD in the viable LGG group tended to be higher than within the placebo group, pointing to the possible benefits of probiotics as a primary intervention in eczema and CMA.

Hattori et al<sup>64</sup> selected 15 children with AD who had *Bifidobacterium*-deficient microflora. Eight subjects in the bifidobacteria-administered group were given oral administration of lyophilized bifidobacteria (*B. breves* M-16V strain). In the treated group, the proportion of *Bifidobacterium* in the fecal microflora was increased significantly, and the proportion of aerobic bacteria was significantly decreased

**Table 1** Probiotics and skin: experimental data

Authors	References	Methods	Remarks
Majamaa and Isolauri	55	Milk elimination in children with (n = 13) or without (n = 14) <i>Lactobacillus</i> GG in hydrolyzed formula. <i>Lactobacillus</i> GG was administered to mothers of 10 breast-fed children with atopic eczema and CMA.	Significant improvement of AD. Experimental and control groups were not compared after treatment, however.
Isolauri et al	57	27 infants with AD during exclusive breast-feeding divided into 3 groups: probiotic-supplemented; <i>B lactis</i> Bb-12 or <i>Lactobacillus</i> strain GG; and extensively hydrolyzed whey formulas or to same formula without probiotics.	Significant reduction in SCORAD score after 2 mo in probiotic-supplemented groups as compared with unsupplemented group; reduction in concentration of inflammatory markers.
Pessi et al	58	9 children with AD and CMA, treated with LGG.	Increase of IL-10 in sera demonstrates anti-inflammatory properties of probiotics bacteria.
Rautava et al	59	62 mother-infant pairs, treated with LGG, pre- and postnatally.	Significant reduction of atopic eczema in infants whose mother received probiotics. Benefit in those with elevated cord blood IgE.
Kalliomaki et al	61	LGG administered pre- and postnatally to mothers for 3 mo, and to their infants for 6 mo.	LGG was effective in prevention of early atopic disease in children at high risk and still effective after 4 y. Skin prick test reactivity, however, was same in both groups.
Rosenfeldt et al	62	LGG and <i>L reuteri</i> as adjuvant to topical steroid in children with AD.	Probiotics as primary therapy shows only modest improvements in AD.
Kirjavainen et al	63	35 infants with AD and CMA received either extensively hydrolyzed whey formula (placebo group) or same formula supplemented with viable (viable LGG group) or heat-inactivated <i>Lactobacillus</i> GG (heat-inactivated LGG group).	Probiotics as primary therapy demonstrated reduction in AD with viable probiotic treatment.
Hattori et al	64	15 children with AD and <i>Bifidobacterium</i> -deficient microflora treated with lyophilized bifidobacteria.	Modulation of intestinal microflora seems to be useful in management of AD. There was, however, no correlation between fecal microflora and allergic symptoms.
Pohjavuori et al	65	LGG and mixture of 4 bacterial species (MIX) or placebo administered for 4 wk to infants with suspected CMA and IgE-associated dermatitis.	Interferon- $\gamma$ -deficient responses in atopic children improved with <i>Lactobacillus</i> administration. No effect on IL-4, IL-5, and IL-12 levels.
Viljanen et al	67	230 children with AEDS or CMA treated with mix of probiotics strains.	Treatment with probiotics may alleviate AEDS and CMA. There are no data on cutaneous findings after treatment.
Weston et al	68	56 children with AD, treated with <i>L fermentum</i> .	SCORAD index was better than baseline at wk 16, compared with placebo group.

AEDS indicates atopic eczema/dermatitis syndrome.

after 1 month of administration. Significant improvement of allergic symptoms ( $P = .0176$  in cutaneous symptom score,  $P = .0117$  in total allergic score) was also observed in the bifidobacteria-administered group.

Pohjavuori et al,<sup>65</sup> in a randomized, double-blind study, concomitantly with elimination diet and skin treatment, have administered LGG and a mixture of 4 bacterial species or placebo for 4 weeks to infants with suspected CMA and IgE-associated dermatitis. Before the treatment, secretion of IFN- $\gamma$  was significantly lower in infants with CMA and in infants with IgE-associated CMA than in infants without CMA. Among the infants who received LGG, the level of secreted

IFN- $\gamma$  increased in those with CMA ( $P = .006$ ) and in those with IgE-associated dermatitis ( $P = .017$ ) when compared with the placebo group.

No significant effect was noted on IL-4, IL-5, or IL-12 levels.

Deficiency in IFN- $\gamma$  appears to be related to CMA, and production rises with the addition of LGG. Interferon  $\gamma$  rises in infants with CMA and IgE-associated dermatitis. This might reflect the beneficial Th1 immunomodulatory signals that probiotics can provide.

Viljanen et al,<sup>66,67</sup> in a randomized, double-blinded study, concomitant with elimination diet, a mixture of 4 probiotic

strains (MIX), or placebo for 4 weeks, treated a total of 230 infants (aged 1.4-11.9 months; mean, 6.4 months; 62% males) with atopic eczema/dermatitis syndrome (AEDES) and suspected CMA.

Four weeks after treatment, CMA was diagnosed with a double-blind, placebo-controlled milk challenge. Fecal samples of 102 infants, randomly chosen for analysis, were collected before treatment, after 4-week treatment, and on the first day of the milk challenge. After treatment, IgA levels tended to be higher in probiotic groups than in the placebo group, and  $\alpha_1$ -antitrypsin decreased in the LGG group but not in other treated groups. After the challenge in IgE-associated CMA infants, fecal IgA was higher for LGG than for placebo, and TNF- $\alpha$  was lower for LGG than for placebo, but insignificantly. Treatment with LGG may alleviate intestinal inflammation in infants with AEDES and CMA, but the authors did not report any data on cutaneous findings after treatment.

After, the mean SCORAD decreased by 65%, but no differences between treatment groups were observed. In IgE-sensitized infants, however the LGG groups showed a greater reduction in SCORAD.

Weston et al<sup>68</sup> recruited into a randomized, double-blind, placebo-controlled trial 56 children with moderate or severe AD. The children were given a probiotic ( $1 \times 10^9$  *L. fermentum* VRI-033 PCC) or an equivalent volume of placebo, bid for 8 weeks. A final assessment at 16 weeks was performed. Severity and extent of AD reduction at the end of the study, as measured by the SCORAD score, was significant in the probiotic group ( $P = .03$ ) but not the placebo group. Children with moderate or severe disease receiving probiotics ( $n = 24$ , 92%) had a SCORAD index that was better than baseline at week 16 compared with the placebo group ( $n = 17$ , 63%;  $P = .01$ ).

Flohr et al<sup>18</sup> recently reviewed the reliability of the hygiene hypothesis through Medline from 1966 until August 2004 to identify relevant studies.

A total of 1178 articles were identified, of which 64 studies were directly related to the topic, but few small, randomized controlled trials have suggested that probiotics can reduce AD severity and that probiotics may also be able to prevent AD to some degree.

The conclusion is that existing studies on the potential therapeutic effect of probiotics are generally promising, but all scientific researcher workers agree that there is not enough evidence to reliably assess the possible role of probiotics in the treatment of skin allergic disorders and that further studies should be done with a higher number of patients to get more reliable results.<sup>18,69,70</sup>

In a recent study performed on mouse DCs, LGG appeared to significantly induce the production of cytokines promoting Th1 response (ie, IL-12, IL-6, TNF- $\alpha$ ) without increasing the production of IL-10 (which promotes the Th2 response). This strain would therefore be of interest in treating AD.<sup>71-73</sup>

Smits et al<sup>74</sup> confirmed these data on human monocyte-derived DCs cultured in vitro with *L. reuteri* and *L. casei*.

This may explain their beneficial effect in the treatment of several inflammatory diseases including AD.

Prescott et al<sup>75</sup> demonstrated that probiotics administration (*L. fermentum* PCC trademark) was associated with a significant increase, from peripheral blood mononuclear cells, in T-helper type 1 (Th1-type) cytokine IFN- $\gamma$  responses to PHA and *S. aureus* enterotoxin B at the end of the supplementation period (week 8:  $P = .004$  and  $.046$ ) as well as 8 weeks after ceasing supplementation. The increase in IFN- $\gamma$  responses to *S. aureus* enterotoxin B was directly proportional to the decrease in the severity of AD, but there were no other effects on allergen-specific responses. These data suggest that the effects of probiotics may be mediated through other independent pathways.

## Conclusions

Many experimental studies have found that probiotics exert specific effects in the intestinal lumen and on epithelial cells and immune cells with antiallergic potential (Table 1). These effects include enhancement in antigen degradation, gut barrier function, and induction of regulatory and proinflammatory immune responses, the latter of which occurs more likely beyond the intestinal epithelium. Probiotics can stimulate systemic, cell-mediated immunity that approximates type 1 activity and may also down-regulate conditions linked to Th2 overactivation.<sup>71,72</sup>

Dietary supplementation using particular defined strains was found to increase IFN activity in the blood of human volunteers, and children born to families who consume traditional *Lactobacillus*-rich fermented foods experience fewer allergies than those from families who consume more sterile foods. Other epidemiological studies indicate lower incidence of atopic (IgE-mediated) skin and respiratory tract hypersensitivity complaints among children with stable gut populations of lactobacilli and bifidobacteria compared with those who had a paucity of gut bacteria.<sup>71,72</sup> Thus, the use of probiotics in the primary prevention of atopic disease is based on the ability to reverse increased intestinal permeability, a characteristic of children with atopic eczema and food allergy.

They also help to improve gut barrier function and restore a healthier gut microecology, to provide a microbial stimulus for the host immune system characteristic of the healthy infant gut microflora, and to antagonize the inflammatory alterations that have been shown to occur in allergic individuals.

Specific strains of indigenous gut microflora have been shown to have important effects on the physiology and immunology of the host: LGG and *B. lactis* Bb-12 are an effective adjunct to extensively hydrolyzed formula in treating infants with mild AD and CMA, and the combination of *L. rhamnosus* 19070-2 and *L. reuteri* DSM 122460 is effective in patients with moderate to severe AD. Extreme caution must be used given the small number of studies and patients evaluated.

We must remember that not all probiotics have the same immunologic properties, and we have yet to define the specific bacteria of the developing intestinal microflora that most influence infant health. It is of overriding interest to improve our knowledge of species composition within a healthy microflora. Specific deviations in intestinal microflora may predispose infants to allergic disease. Such aberrancies include decreased numbers of bifidobacteria and atypical composition of bifidobacteria. Aberrancies in clostridium content and composition also have been reported to be important. Last but not least, in our medical profession, we have to remember the famous Eschilo (525-456 BC) aphorism: "every thing brings its burden." So, complications of probiotic use can occur, and infections and invasive disease associated with probiotic strains of lactobacilli have been reported, although extremely rare. Predisposing factors to bacteremia were severe, fatal comorbidities or underlying diseases; immunosuppression; prior prolonged hospitalization; and prior surgical interventions. Especially in pediatric age, *Lactobacillus* bacteremia is a rare entity, and its clinical significance is poorly defined but should be kept in serious account. For this reason, it is very important to know the antimicrobial susceptibility of *Lactobacillus* strains for a more rational and aimed therapy.<sup>76-79</sup> Although further investigations should be done, in vitro and in clinical areas, with different types of probiotics, the importance of the improvement reported in this review underlines the potential interest in probiotics for the management of skin pathology.

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