

# Probiotics: Use in Allergic Disorders

## *A Nutrition, Allergy, Mucosal Immunology, and Intestinal Microbiota (NAMI) Research Group Report*

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**Abstract:** The underlying denominators and treatment targets in allergic disorders may be outlined as aberrant barrier functions of the skin epithelium and gut mucosa and dysregulation of the immune response to ubiquitous environmental antigens. Dietary methods to control symptoms and reduce the risk of allergic disease have hitherto focused on elimination diets, alone or in combination with other environmental measures. The results have not been satisfactory regarding long-term prevention, and new approaches are urgently needed. Realization of this, together with the demonstration that the immunophysiologic regulation in the gut depends on the establishment of the healthy gut microbiota, has led to the introduction of novel modes of therapeutic intervention on the basis of the consumption of monocultures and mixed cultures of beneficial live probiotic microorganisms. The current aims of intervention are to avert deviant microbiota development, strengthen the gut barrier function, and alleviate abnormal immune responsiveness. 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 when these functions are immature and inexperienced and the risk of allergic disease is heightened. In humans, documented effects have been reported for alleviation of intestinal inflammation, normalization of gut mucosal dysfunction, and down-regulation of hypersensitivity reactions, thereby preferentially targeting allergic conditions with intestinal involvement. The probiotic performance of strains differs; each probiotic strain is a unique organism itself with specific properties that cannot be extrapolated from other, even closely related, strains. Moreover, it would seem simplistic to assume that a single supplementation would suffice to counter the plethora of allergic disease. First, it needs to be acknowledged that a more profound understanding of the complex nature of allergic disorders is needed, as it is likely that there are

distinct etiologic factors and pathogenetic mechanisms underlying the heterogeneous manifestations. Second, host-related factors influence the probiotic effects; the distinction in the antiallergic potential of probiotics can be explained by the age of the host and the habitual diet with other potentially active compounds and their conceivable joint probiotic effects. Therefore, research activities are currently focusing on identification of specific strains with immunomodulatory potential, and on the question how the food matrix and dietary content interact with the most efficacious probiotic strains or specific strain combinations.

**Key Words:** allergic disease, inflammation, microbiota, probiotics

(*J Clin Gastroenterol* 2008;00:000–000)

### THE ESTABLISHMENT OF HEALTHY INTESTINAL MICROBIOTA: A STEPWISE PROCESS

The gastrointestinal tract functions as a barrier against antigens derived from microorganisms and food, controlling excessive antigen transfer and immune responses.<sup>1</sup> The generation of immunophysiologic regulation in the gut depends on the establishment of indigenous microbiota. The term commensalism implies the impartial coexistence of the microbes with the host, where a risk of disease may be generated by translocation of intraluminal bacteria. Recent research in mucosal immunology demonstrates interactions between defined microbes and the host, which could be characterized as mutualism.<sup>2,3</sup> The significance of this host-microbe interaction culminates at an early age, when the mucosal barrier and immune system are still immature. Novel methods, again, have expanded the understanding of intestinal microbiota diversity and complexity in infants. DNA-based methods help to identify unculturable microbes and assess ecologic shifts in species composition. The new microbiota microarrays facilitate the assessment of whole microbiota composition and diversity, together with the temporal variations within the microbiota.<sup>4</sup>

The mode of delivery has a great impact on the acquisition of the intestinal bacteria, also beyond the immediate neonatal period. Vaginally born infants and

Received for publication October 26, 2007; accepted November 26, 2007. From the \*Department of Paediatrics; and †Functional Foods Forum, University of Turku, Finland.

The authors declare no conflict of interest.

Supported by the Academy of Finland.

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infants born by cesarean section evince major differences in culturable microbiota, still to be observed at 6 months of age.<sup>5</sup> Colonization seems to be associated with the maturation of humoral immune mechanisms. Interestingly, *Bacteroides fragilis* and *Bifidobacterium* species are important in this respect, as infants harboring these organisms had more circulating immunoglobulin (Ig) A-secreting and IgM-secreting cells.

The initiation of nutrition intake via the oral route further shapes the early gut microbiota, and the healthy breast-fed infant can be considered here as a model of infant feeding. Molecular methods indicate that lactic acid-producing bacteria may account for <1% of the total microbiota, whereas bifidobacteria can range from 60% to 90% of the total fecal microbiota in breast-fed infants. In fact, the greatest difference in the microbiota of breast-fed and formula-fed infants may lie in both the bifidobacterial numbers and species composition. *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Bifidobacterium longum* are species frequently found in fecal samples from breast-fed infants, whereas later in life *Bifidobacterium adolescentis* becomes more common.<sup>6,7</sup> *B. adolescentis* species may be associated with deviations in infant microbiota, and this species is also more common in infants with allergic diseases.<sup>8–10</sup> It has been recently demonstrated that allergic mothers may transfer *B. adolescentis* species to their infants more often than healthy mothers,<sup>11</sup> leading to aberrant compositional development of the microbiota, which may predispose infants toward allergic diseases.<sup>9</sup> The introduction of solid foods and withdrawal of breast milk coincide with the next major microbiota changes in the colonization process, although the impact of this phase is largely unknown.<sup>6,12</sup>

Recent discoveries suggest 2 new sources of intestinal microbes: bacteria in breast milk and microbes potentially present in the amniotic fluid before birth.<sup>13,14</sup> Hence, the differences constantly reported between the gut microbiota of breast-fed and formula-fed infants may also lie in differences in the microbiologic composition of human milk versus infant formula,<sup>6,15–17</sup> above the bifidogenic oligosaccharides, in particular its 2-linked fucosylated oligosaccharides, in breast milk.

### HOST-MICROBE CROSS TALK—PROMOTING THE MATURATION OF NONATOPIC IMMUNE RESPONSIVENESS

The dilemma of the host defense mechanisms in the gut-associated lymphoid tissue is to generate an efficient response to pathogens while concomitantly maintaining immunosuppression to avoid local and peripheral inflammatory responses to ubiquitous antigens such as food and gut microbiota bacteria. Host-microbe cross talk further appears critical in protection against gut injury by controlling expression of protective factors and epithelial cell proliferation.<sup>18</sup> As recently reviewed,<sup>19</sup> there are several molecular mechanisms within the gastrointestinal epithelium that reduce the inflammatory response to commensals

constantly present in the healthy intestine and ensure tolerance to indigenous gut microbiota. These include sparse surface expression of Toll-like receptors (TLRs), abundant expression of the TLR signaling suppressor Tollip and other negative regulators of the TLR signaling pathway, a lack of indispensable coreceptors for TLRs, and secretion of the suppressive cytokines interleukin (IL)-10 and transforming growth factor- $\beta$ . Probiotics interact with the mucosal immune system via the same pathways as commensal bacteria, specifically via interaction with epithelial and dendritic cells, to influence both innate and adaptive immune responses (reviewed in Ref. 20).

### ALLERGIC DISORDERS IN INFANCY: POTENTIAL TARGET FOR PROBIOTIC THERAPY

The prevalence of allergic disorders has been progressively increasing in Western societies, these conditions now comprising the most common chronic disease of childhood. Not only self-reports on symptoms of atopic disease but also relevant objective measurements have consistently suggested a true increase in the prevalence of atopic diseases,<sup>21</sup> which comprise a familiar group of conditions, including allergic rhinoconjunctivitis, asthma, and atopic eczema. These are frequently associated with the generation of T helper ( $T_H$ ) cell 2-type cytokines, including IL-4, IL-5, and IL-13, which promote IgE production and eosinophilia. The  $T_H$ 2-skewed immune type may be balanced by cytokines secreted by  $T_H$ 1,  $T_H$ 3, and T regulatory cells, partially as a result of stimulation by the gut microbiota.<sup>22</sup> In general, the hygiene hypothesis of allergy implies that recent advances in affluent societies have reduced the extent of microbial exposure at an early age.<sup>23</sup> The establishment of the gut microbiota provides an initial and massive source of microbial stimuli to the host.

The route for the first allergic responses frequently arises from the gastrointestinal tract, and food allergy is a common problem in infants with atopic eczema. Aberrant barrier functions in the skin epithelium and gut mucosa lead to greater antigen transfer across the mucosal barrier and the routes of transport are altered, thereby evoking aberrant immune responses and release of proinflammatory cytokines with further impairment of the barrier functions. Such increased inflammation would lead to further increases in intestinal permeability and in a vicious circle of increasing allergenic responses, and a more permanent dysregulation of the immune responses to ubiquitous antigens in genetically susceptible individuals (Fig. 1).

To take an example, an interesting hypothesis has been put forward; atopic eczema, the earliest of the atopic conditions, may act as a portal for the development of IgE-mediated atopic manifestations.<sup>24</sup> This hypothesis may thus provide a working target for reducing the risk of allergic disorders (Fig. 1): impaired barrier functions of the skin epithelium and gut mucosa, including the role of local microbiota in these functions. Probiotics, live microbial food ingredients with health benefits

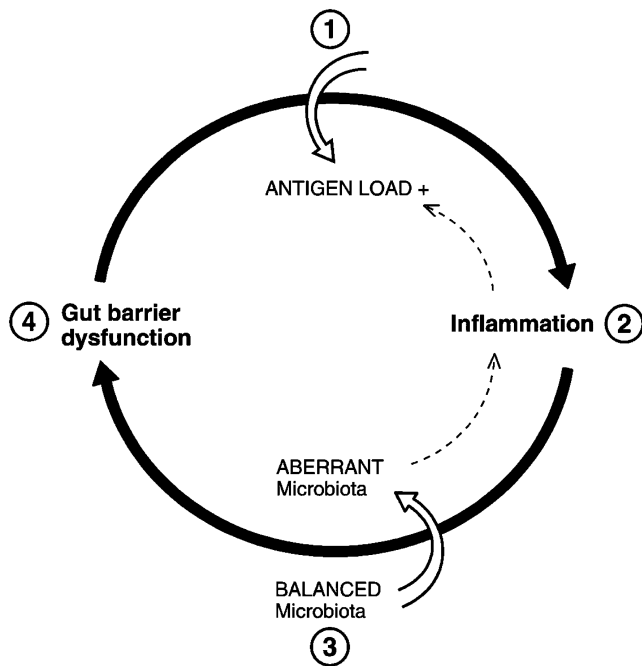


**FIGURE 1.** Intraluminal antigens induce an immunoinflammatory response that impairs the intestine's barrier function in a sensitized subject. Mucosal dysfunction may lead to aberrant antigen absorption. Probiotic therapy exploiting endogenous defense mechanisms that degrade antigens (1), terminate inflammatory response (2), avert deviant microbiota development (3), and normalize the barrier functions (4) may reduce the risk of allergic disease and offer novel ways of treating hypersensitivity disorders and of preventing the progression of sensitization to debilitating disease.

demonstrated in clinical intervention studies, may be a good candidate for this objective.

### THE ANTIALLERGIC POTENTIAL OF PROBIOTICS IS STRAIN-SPECIFIC, PRODUCT-SPECIFIC, AND HOST-SPECIFIC

In the first randomized double-blind, placebo-controlled clinical studies of probiotic use, *Lactobacillus rhamnosus* GG, or *B. lactis*, an improvement in the clinical course of atopic eczema and cow's milk allergy was observed in infants when given probiotic-supplemented hydrolyzed formula compared with placebo-supplemented formula.<sup>25,26</sup> In parallel, markers of systemic and intestinal allergic inflammation were reduced. The 2 strains in combination were successful in the management of food sensitized children.<sup>27</sup> In the same vein, subsequent studies in young infants and children with the condition have shown similar effects,<sup>28–30</sup> whereas others showed effects confined to children with evidence of IgE-associated allergic disease.<sup>31</sup> The preventive potential of probiotics in atopic diseases has been demonstrated in a double-blind, placebo-controlled study.<sup>32</sup> Specific probiotics administered prenatally and postnatally to children at

high risk of atopic diseases reduced the prevalence of eczema and/or atopic sensitization compared with infants receiving placebo.<sup>33–35</sup> Moreover, the clinical benefit extends beyond infancy.<sup>36</sup> However, in other allergic disorders, such as asthma, no differences were detected. Some studies have contradicted the effectiveness of probiotics in the treatment or prevention of allergic disorders. Here, the selection of the target population<sup>37</sup> and probiotic strain<sup>38</sup> may be seen as sources of conflicting data.

First, the importance of the age of the host in responding to microbial stimulation has been demonstrated in experimental animal models<sup>39</sup> and in humans.<sup>40</sup> The impact of the intestinal microbiota in oral tolerance induction was shown in germ-free mice, which, unlike the control mice, maintain a tendency to systemic immune responses, such as production of IgE antibodies, upon oral administration of ovalbumin. The aberrant IgE response in germ-free mice can be corrected by microbiota reconstitution at the neonatal stage, but not later.<sup>39</sup> Mice lacking a functional receptor for TLR4 again showed a heightened susceptibility to allergic responses to food antigens.<sup>41</sup> In like manner, the mucosa of young children tends to be, through a TLR-dependent pathway, particularly responsive to microbial stimuli. In an in vitro study by Tulic and colleagues,<sup>40</sup> explanted nasal mucosa was cultured with bacterial lipopolysaccharide and allergen. The lipopolysaccharide was able to prevent allergic inflammation in the nasal mucosa of atopic children, but not in adults, by transforming local immune responses from T<sub>H</sub>2-type to T<sub>H</sub>1-type and increasing the expression of anti-inflammatory IL-10. Indeed, in the case of allergic rhinitis, clinical results are conflicting.<sup>42</sup> For example, in a study on the effects of *Lactobacillus* GG in birch pollen allergy in adolescents and adults, no benefits were shown,<sup>43</sup> whereas supplemented *Lactobacillus casei* tended to reduce and delay symptoms in patients sensitized to Japanese cedar pollen with more severe nasal affection,<sup>44</sup> and *B. longum* has been shown to relieve ocular<sup>45</sup> and nasal symptoms<sup>46</sup> as compared with placebo. In addition, no advantages have been shown with probiotics in the treatment of asthma,<sup>47</sup> pointing to the hypothesis that the effects of probiotics are likely to be most marked in gastrointestinal disorders or in allergic conditions with intestinal involvement (Fig. 1).

Second, the targets of probiotic therapy in allergic disorders need to be evaluated in detail. Thus far, specific probiotics were shown to reverse the increased intestinal permeability<sup>48</sup> characteristic of children with atopic eczema and food allergy,<sup>49</sup> and to enhance specific IgA responses,<sup>50,51</sup> frequently defective in children with food allergy.<sup>52</sup> Promotion of gut barrier functions by probiotics also includes normalization of the gut microecology, alterations in which have been demonstrated in allergic subjects.<sup>9</sup> To take an example, Brouwer and colleagues<sup>37</sup> concluded that administration of *Lactobacillus* GG or *L. rhamnosus* strain had no effects on eczema severity when given in hydrolyzed formula compared with the same formula as placebo. The majority of the patients

were more likely to have intrinsic eczema, as their cow's milk challenges were negative; they, therefore, can be expected not responsive to treatment of cow's milk allergy with extensively hydrolyzed formula and probiotics. Thus, the population entirely differs from that of previous studies.<sup>25,26,28,29</sup>

Third, probiotics have indeed been shown to exert distinct effects on antigen transport, depending on the food matrix, for example the quality of protein in the usual diet. In a rodent model, mucosal transport of degraded macromolecules has been found to be stimulated when *Lactobacillus* GG is administered together with unhydrolyzed protein, but reduced when administered with hydrolyzed protein.<sup>53</sup> Such protein may thus stimulate the humoral immunity in the gut, but also affect on the induction of oral tolerance, as antigen degradation is an indispensable component in the acquisition of mucosal tolerance.<sup>22</sup>

Finally, the strain-specificity of probiotic effects was demonstrated already in the early clinical intervention studies.<sup>54</sup> For example, 2 *L. rhamnosus* strains acted in a totally opposite manner on the duration of rotavirus diarrhea, 1 *Lactobacillus* strain effectively shortened it, whereas the other had no clinical effect.

A previously undefined *Lactobacillus acidophilus*, again, did not reduce the prevalence of eczema<sup>38</sup>; this probiotic had no effects on the innate or allergen-specific<sup>55</sup> immune responses of the infants. The strain was later defined as *L. acidophilus* LAFTI L10, which has been used in meat starter cultures.

In several in vitro studies, closely related strains show significantly different adhesion, aggregation, competitive exclusion, and pathogen displacement properties.<sup>56</sup> Also, combining probiotic strains requires exact knowledge of these properties to achieve desired properties rather than combining strains that counteract each others' effects. Accumulating information on the genomes of specific probiotics confirms that the differences between even closely related strains are significant and explain the distinct clinical properties of each strain. To take an example, intervention with *Lactobacillus* GG in combination with *B. lactis* during pregnancy and breastfeeding reduced the risk of atopic eczema and allergic sensitization in child,<sup>35</sup> whereas a mixture of probiotics (*Lactobacillus* GG, *L. rhamnosus* LC705, *B. breve*, and *Propionibacterium freudenreichii* ssp. *shermanii* JS) failed to reinforce the beneficial effect of *Lactobacillus* GG alone in the management of atopic eczema,<sup>31</sup> substantiating the strain differences and interactions. The combination was later assessed in vitro for the impact on major model pathogens and, compared with *Lactobacillus* GG alone, demonstrated that some properties were enhanced and some counteracted.<sup>56</sup> Moreover, the importance of viable probiotics was demonstrated in a trial evaluating viable versus nonviable *Lactobacillus* GG, which was prematurely terminated because of adverse gastrointestinal effects in the group receiving the nonviable heat-inactivated strain,<sup>57</sup> whereas alleviation of gastrointestinal symptoms has been achieved with viable probiotics.

## NEONATAL PROBIOTIC INTERVENTION: SAFETY CONCERNS

The numerous immunologic properties of probiotics have, however, raised safety concern. For example, long-term colonization by probiotics or impairment of the natural diversity of the gut microbiota has been addressed with early or prenatal administration of probiotics. Permanent colonization has not been reported in clinical intervention studies.<sup>58</sup> Thus, the probiotic impact on gut microbiota succession is temporary, but it may guide subsequent colonization and immunologic development, conferring a clinical benefit. However, when comparing fecal and mucosal recoveries of probiotics administered, their colonization patterns differ.<sup>59</sup> In general, viable probiotics are present in mucosal samples even after fecal recovery has ceased. Thus, the exposure to a given probiotic, even though temporary by nature, may last longer than indicated by fecal recovery. This phenomenon should be considered when assessing the safety of new probiotics. Currently used probiotics are mainly species of Gram-positive bacteria that constitute common components of the healthy gut microbiota and also the microbial community of foods. These have a long history of safe use and a demonstrated safety record in human consumption, and no health concerns have been observed. Their safety has been assessed by European Food Safety Authority (<http://www.efsa.europa.eu/EFSA/DocumentSet/Annex3.0.pdf>).

## CONCLUSIONS

The intestine's mucosal surface provides a defense barrier against antigens encountered by the enteric route. In this system, a balance is generated and maintained between the host and the microbiota. In intestinal inflammation, the integrity of the barrier is disrupted, a greater amount of antigens traverses the mucosal barrier, and the routes of transport are altered, possibly evoking aberrant immune responses and release of proinflammatory cytokines with further impairment of the barrier function. An important function of probiotics is related to their immunomodulatory effects, beyond "colonization," as probiotics may exert their effects locally or during transient passage through the gastrointestinal system; abundant evidence implies that specific strains selected from the healthy gut microbiota exhibit powerful antipathogenic and anti-inflammatory capabilities. The targets for the probiotic approach in allergic disorders can be identified as degradation of enteral antigens, normalization of the properties of aberrant indigenous microbiota and of gut barrier functions, regulation of the secretion of inflammatory mediators, and promoting development of the immune system. The probiotic effects are strain-specific; different bacteria or components thereof have defined adherence sites, immunologic effects, and varied effects in the healthy versus inflamed mucosal milieu. These demonstrations call for detailed considerations when choosing probiotics or probiotic combinations for intervention studies, especially the

preclinical testing and selection of strains with confirmation of their viability to exert any anti-inflammatory influence is of utmost importance. The challenge in terms of reducing the risk of allergic disorders is to identify the mechanisms of the disease to detect accurate targets for probiotics with anti-inflammatory potential and the optimal combinations of probiotics and other active dietary compounds.

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