Clinical efficacy and mechanism of probiotics on allergic diseases

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Abstract

A complex interplay between genetic and environmental factors partially contributes to the development of allergic diseases through immune development during prenatal and early life. To explain the dramatic increase in the prevalence of allergic diseases, hygiene hypothesis was proposed that allergic diseases were prevented by early exposure to infection. This hygiene hypothesis has been changed to microbial hypothesis, which is closely linked to the development of early immune system and allergic diseases. The intestinal flora may be a contributor to allergic disease due to its substantial effect on mucosal immunity. On the basis of the findings that exposure to microbial flora early in life allows for a change in the Th1/Th2 balance, favoring a Th1 cell response, probiotics may be beneficial in preventing allergic diseases. However, evidence to prove its efficacy is lacking from both clinical and basic researches. To date, studies have yielded inconsistent findings on the usefulness of probiotics in allergic diseases. Due to limitations such as different first supplementation period, duration, different strains, short follow-up period, and host factors, it is difficult to demonstrate an exact effect of probiotics in asthma, allergic rhinitis, and food allergy. However, there are many literatures that demonstrate a significant clinical improvement in atopic dermatitis with the use of probiotics. An accurate understanding of the development of human immunity, intestinal barrier function, intestinal microbiota, and systemic immunity is required to comprehend the effects of probiotics on allergic diseases.

Key ward: Allergic disease, Hygiene hypothesis, Immunity, Microbiota, Probiotics
Introduction

The Developmental Origins Hypothesis for Health and Disease (DOHaD) proposes that all organ systems undergo developmental programming in utero that predetermines subsequent physiologic and metabolic adaptations during adult life\(^1\). Exposure to environmental factors during prenatal and early life can contribute to the development of allergic diseases, which affect human health later in life\(^2\). The rapid increase in the prevalence of allergic diseases and the high burden requires the development of new strategies for more effective prevention, diagnosis, and treatment. In this regard, the hygiene and microbial hypothesis may be helpful. Because exposure to microbial flora early in life allows for a change in the balance of immune development, probiotics may be beneficial in preventing allergic diseases. In this article, we review the evidence supporting the hygiene hypothesis and the mechanisms of action of probiotics based on this hypothesis. We summarize recent clinical and basic researches to better understand the effects and mechanisms of probiotics on allergic diseases.

From the hygiene hypothesis to the microbial hypothesis

The hygiene hypothesis was proposed more than 20 years ago by Strachan to explain the dramatic increase in the prevalence of allergic diseases over the past few decades\(^3\). The original hygiene hypothesis proposes that allergic diseases are prevented by early exposure to infection introduced by contact with older siblings\(^3,4\). Later, the hygiene hypothesis was extended to explain the great increase in the prevalence of Th1 diseases, such as inflammatory bowel diseases and autoimmune diseases, that occurred over the same period\(^5\). However, the exact scientific underpinning for the hygiene hypothesis, for example, a specific infection that can prime the immune system to prevent disease, is an unsolved puzzle. Several studies report a consistently low prevalence of allergies in farmers’ children\(^4\);
however, it remains unclear which specific factors are most important, but microbial exposures may play a role through inhalation of endotoxin\(^6\). Endotoxin, a lipopolysaccharide present in the outer membrane of Gram-negative bacteria, downregulates Th2 cytokine production\(^7\). However, in inner-city communities, domestic endotoxin exposure was positively associated with wheeze in children at 2 years of age\(^8\). One unanswered question is whether the greater levels of endotoxin associated with an inner-city lifestyle can explain the higher prevalence of asthma relative to that observed in rural areas.

Some studies have recently revisited the hygiene hypothesis, suggesting that the decline in childhood infections is less important than how modern societal practices caused the disappearance of ancestral indigenous microbiota species that might confer benefits beyond our current understanding\(^9\). Initial studies of allergic diseases focused on gut microbiota. Allergic diseases among children is partially associated with differences in their intestinal microflora in countries with a low (Estonia) and high (Sweden) prevalence of allergies\(^10\).

Over the last few decades, evidence from human and mouse studies indicates that colonization of the gut early in life has a substantial role in directing immune system development. Therefore, we need to understand the importance of gut colonization early in life and to determine whether altering microbial exposures during the perinatal period promotes lifelong dysregulated immune responses. In a mouse model, the intestinal bacterial flora was shown to play a crucial role in oral tolerance induction, probably by affecting the development of the immune system at neonatal stage\(^11\).

After birth, the diversity of the gut microbiome increases with age\(^12\). A healthy human fetus is thought to develop within a bacteria-free environment. Upon birth, a neonate is exposed to a wide variety of microbes, many of which are encountered during and after passage through the birth canal, the latter comprises an ecosystem heavily colonized by a relatively limited set
of bacterial taxa. Vaginally delivered infants acquire bacterial communities resembling their mother’s vaginal microbiota, which is dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp., while infants delivered by Caesarian section harbor bacterial communities similar to those found on the skin surface, which are dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.

Microbial exposure during the perinatal period is linked to the epigenetic regulation of genes involved in allergic inflammation, and alters susceptibility to allergic diseases. The emerging understanding of the importance of microbial contact during the fragile periods of fetal life, delivery, and infancy in healthy immune and metabolic programming, creates new opportunities to improve infant health and reduce the risk of disease in later life. On the basis of the hygiene hypothesis, the microbial inhabitants of the human body are proposed to affect early development of the immune system. This concept led us to investigate a probiotics-induced modulation of mechanisms underlying the development of allergic diseases.

**Clinical efficacy of probiotics on allergic diseases**

1) Asthma

A. What is known

Antibiotics enhance allergic airway responses in experimental animals by altering the intestinal microbiota, and probiotics modulate allergic responses in the lower respiratory tract. In a double-blind, placebo-controlled study, 1223 mothers with infants at high risk for allergy received a probiotic mixture (two lactobacilli, bifidobacteria, and propionibacteria) or placebo during the last few months of pregnancy, and their infants also received the same mixture from birth until they were 6 months of age. However, a preventive effect of probiotics on asthma was not observed up to 5 years of age. In two other double-blind, placebo-controlled randomized trials with infants at risk of allergy, probiotic
supplementation failed to decrease the frequency of wheezing at 1 year and influence asthma prevalence rates at 2 years\(^{21,22}\). In contrast, supplementation with probiotics is associated with an increased rate of recurrent episodes of wheezy bronchitis\(^{23}\) but not with a reduced prevalence of inhalant allergen sensitization\(^{24}\). Probiotics prevent asthma-like symptoms in infants with atopic dermatitis\(^{25}\), whose pulmonary function and peak expiratory flow rates decreased significantly. In addition, the clinical symptom scores for asthma and allergic rhinitis decreased in the probiotic-treated patients\(^{26}\). Oral administration of probiotics attenuated the symptoms of allergic asthma in a mouse model and induced immune regulation by a CD4(+)CD25(+)Foxp3(+) regulatory T (Treg) cell-mediated mechanism\(^{16}\), and effectively suppressed airway hyperresponsiveness\(^{2}\).

B. Future studies

Although the possibility of asthma prevention and treatment is indicated by research on animal models, no primary prevention study demonstrates an effect of probiotic supplementation in humans. Despite numerous studies, demonstration of an effect of probiotics has been impeded because of limitations, such as different first supplementation periods, duration of supplementation, and short follow-up periods. To overcome these limitations, future studies should be conducted on larger numbers of subjects and for longer duration.

2) Allergic rhinitis

A. What is known

Immune responses in the gut may modulate immune responses in distant target organs, including the nose\(^{18,27}\). Probiotics alleviate nasal symptoms and prevent the pollen-induced infiltration of eosinophils into the nasal mucosa\(^{28}\) and modulate Th2-skewed immune
responses in allergic rhinitis\textsuperscript{29}).

Probiotics alleviate perennial and seasonal allergic rhinitis. In a study of preschoolers treated with probiotics or placebo for 12 months, there was a difference in the cumulative incidence of rhinitis episodes\textsuperscript{30}). Probiotic taken by adult patients with seasonal allergic rhinitis modulated immune responses and might have the potential to alleviate the severity of symptoms\textsuperscript{31}). However, other studies showed that probiotics provided few clinical benefits and did not alleviate the symptoms or reduce the use of medication\textsuperscript{32,33}).

**B. Future studies**

The heterogeneity of studies on the effects of probiotics in allergic rhinitis precludes meta-analysis. Unlike other allergic diseases, the therapeutic effect of probiotics in allergic rhinitis has been primarily demonstrated, whereas their preventive effects have not been conclusively defined. Evidence indicates that allergic rhinitis may be subdivided into several phenotypes (perennial allergic rhinitis, seasonal allergic rhinitis, and Japanese cedar pollen-induced allergic rhinitis). Well-designed future studies that consider theses phenotypes of allergic rhinitis can help us to understand the effects of treatment on allergic rhinitis.

3) **Atopic dermatitis**

**A. What is known**

There is an abundance of studies on the efficacy of probiotics for prevention and treatment of atopic dermatitis\textsuperscript{34,35}) (Fig. 1). A Cochrane Review showed that there was insufficient evidence to recommend the addition of probiotics to infant diets for the prevention of atopic dermatitis\textsuperscript{36}). In contrast, there are convincing evidences that probiotics effectively prevent and treat adult and childhood atopic dermatitis\textsuperscript{37,38}).

In most studies reporting beneficial effects, mothers received probiotics during pregnancy
and their babies continued with the same product after birth. The preventive effects of probiotics on eczema were greater in infants with a family history of allergic diseases\(^{22,39,40}\). A Korean population based study showed that prenatal and postnatal supplementation with a probiotic mixture effectively prevents the development of eczema in infants at high risk of allergy during the first year of life\(^{41}\). A review article indicated that various meta-analyses and systematic reviews show that probiotics can prevent the development of atopic dermatitis, particularly in infants who were administered probiotics perinatally\(^{42}\). A study of probiotic supplementation in Korean adults with atopic dermatitis showed that modified production of cytokines from peripheral blood mononuclear cells of these patients\(^{35}\).

Oral application of probiotics prevented the development of atopic dermatitis in a novel mouse model by suppressing the production of the inflammatory cytokines; interleukin (IL)-4 and thymic stromal lymphopietin (TSLP) in the skin through a mechanism that may involve CD4(+) CD25(+) Foxp3(+) Treg cells\(^{43}\). Oral administration of *Lactobacillus* strains suppressed house-dust mite dermatitis in the NC/Nga mouse, a representative animal model of human atopic dermatitis\(^{44}\). A meta-analysis of the effect of probiotics on atopic dermatitis suggested that probiotics may be effective for preventing atopic dermatitis in high-risk infants. There is, however, insufficient evidence for giving probiotics to all pregnant women and their babies to prevent allergic diseases.

**B. Future studies**

The authors of the Cochrane review advised caution in interpreting the results of their meta-analysis because of heterogeneity and high rates of follow-up loss (17–61 %) in individual trials\(^{45}\). There is insufficient evidence that probiotics are clinically effective for treating atopic dermatitis; however, the suggestion that probiotics may be effective for preventing atopic dermatitis can be accepted under limited conditions. An accurate understanding of the
development of human immunity, intestinal barrier function, intestinal microbiota, and systemic immunity is required to evaluate the effects of probiotics on allergic diseases such as atopic dermatitis. Further basic research into the roles and functions of probiotics will be required.

4) Food allergy

A. What is known

There are very few studies of probiotic treatment for food allergy, and no systemic review seems possible at present. Moreover, most of the clinical information regarding probiotics and food allergy is derived from studies of patients with atopic dermatitis. The therapeutic oral administration of probiotics suppresses the skewed Th2 response in allergic mice and protects against anaphylactic reactions in recent murine models of food allergy. In several clinical trials, however, oral supplementation with probiotics did not significantly affect allergic inflammatory markers (total and specific IgE) or cytokines; and there were no positive therapeutic outcomes (improvement of symptoms and acquisition of tolerance). In studies reporting beneficial therapeutic effects, most infants were exclusively breast-fed or provided an extensively hydrolyzed formula, and the effects reported were related to atopic dermatitis rather than food allergy. The most significant improvements were observed when probiotics were used in conjunction with breastfeeding or a hypoallergenic formula.

B. Future studies

To date, studies have yielded inconsistent findings on efficacy of probiotics for preventing and treating food allergy. These discrepancies may be attributed to variation among the studies regarding multiple factors related to the probiotics (type, dose, mixtures, and duration) or to the recipient (birth method, diet, and age). Therefore, further studies are
required to overcome these limitations.

**Immunological mechanisms involved in the effects of probiotics on the prevention and treatment of allergic diseases**

Accumulating data suggest that certain bacterial strains may provide protective signals, while others may stimulate aggressive and damaging immune responses. The effects on an individual’s microbiota of the continuous administration of probiotics from an early age, which stimulates the immune system, has been considered beneficial by many clinicians and researchers (Fig. 2). However, the results of many studies of allergic diseases are not consistent. In particular, studies of allergic diseases in humans have not revealed any definite beneficial effects of probiotics. In the section discussing the mechanism of action of probiotics, we summarize recent clinical data and animal studies concerning the effects of probiotics on allergic diseases (Table 1).

1) Initial research on the mechanisms of probiotics in allergic diseases

The pathogenesis of allergic diseases was first described as an imbalance of Th1/Th2 cells. Probiotics inhibit allergic diseases by suppressing the Th2 response. IL-4, IL-5, and IL-13 are representative cytokines released by Th2 cells that are suppressed by probiotics\(^{16,52}\) (Fig. 3). Further, probiotics increase IL-10 and tumor growth factor (TGF)-β levels by inducing Tregs in allergic diseases\(^{52}\). However, the results of many studies differ and cannot be fully explained by an imbalance of Th1/Th2 responses in the development of allergic diseases. Therefore, there have been disputes among researches about the mechanism of probiotics.

2) New insight into the mechanisms of probiotics in allergic diseases
The previously discussed mechanisms of probiotics reached its limit following the discovery of a novel paradigm in the development of allergic diseases. Th17 cells induce allergic inflammation in a mouse model of asthma\(^{53}\), and this inflammation is suppressed by probiotics\(^{52}\). Probiotics suppress interferon-γ and IL-4 (Th1 and Th2-related cytokines) and Th17 cells in the spleen CD4 T cells and increased the expression levels of IL-10 and TGF-β (Treg–related cytokines) in mesenteric lymph nodes\(^{54}\). Our own studies using a mouse model of atopic dermatitis demonstrate the suppression of the Th17 response in the skin following the administration of probiotics (Fig. 4).

The influence of probiotics on T cell differentiation suggests that dendritic cells (DCs) play an important role in the mechanism of action of probiotics in allergic diseases\(^{54,55}\). Thus, it is reasonable to conclude that probiotics inhibit naïve T cell differentiation to Th2 cells. Moreover, probiotics inhibit the differentiation of mature DCs \textit{in vitro}\(^{54,55}\). However, very few \textit{in vivo} studies of allergic diseases demonstrate a relationship between mature DCs and probiotics. In addition, some studies have shown this effect only in tumors\(^{19}\). Our research confirms that DCs are involved in the suppression of asthma by probiotics following the transfer of mature DCs in mice\(^{56}\).

TSLP plays an important role in the DC-mediated differentiation of T cells\(^{55}\). We are focusing on how Th2, Th17, and Treg cells along with TSLP influence the effects of probiotics on allergic diseases. However, further studies are needed to identify the additional mechanisms of probiotics on allergic diseases in humans and animal models.

\textbf{Conclusion}

Evaluation of the interactions between microorganisms in the environment, in the mucosa, and humans, and how these microorganisms modulate immune responses, may be a new
interesting approach in studying human diseases caused by chronic inflammation. The interactions between probiotics and the immune system might be beneficial for preventing allergic disease; however, evidence is lacking to support this conclusion. Moreover, the advent of the “microbial hypothesis” has raised new concerns about the association of the immune responses with human gut microbiota and the impact of this association on the development of allergic diseases. Future research based on extending this concept may help to improve the efficacy of probiotics for preventing and treating allergic diseases, particularly atopic dermatitis. Confirmation of the association between the gut microbiota and the development of the allergic diseases in humans awaits future research.

Acknowledgement

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References


18. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy 1998;53:20-5.


37. Drago L, Iemoli E, Rodighiero V, Nicola L, De Vecchi E, Piconi S. Effects of


**Fig. 1.** Usefulness of probiotics in the prevention of atopic dermatitis. The preventive effect of probiotics on atopic dermatitis (A) and IgE-associated atopic dermatitis (B) (Adapted from Pelucchi C et al., 2012)
Fig. 2. Effect of probiotics on the intestinal microbiota
Fig. 3. Immune mechanisms of probiotics (Adapted from Lacono A et al., 2011)
**Fig. 4.** Proposed mechanism of probiotics in an animal model of atopic dermatitis. Exposure to allergens on atopic skin increases the expression of TSLP in the skin. TSLP-stimulated DCs induce naive T cells to differentiate into Th2 cells and Th17 cells, which induce allergic inflammation in the skin. Oral probiotics might inhibit these processes by increasing the number of Tregs in mesenteric lymph nodes. Tregs then migrate to the skin from the lymph nodes through lymphatic drainage, and suppress Th2 and Th17 responses (red bar) in the skin. Tregs also suppress the expression of TSLP in the skin (red bar).
Table 1. Recent clinical studies and animal model studies evaluating the efficacy of probiotics in Korea

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Title</th>
<th>Subjects/Model</th>
<th>Jurnal name</th>
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<tr>
<td>2010</td>
<td>Ji GE et al[19]</td>
<td>Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial</td>
<td>112 pregnant women with a family history of allergic diseases and their 68 infants at 1 year</td>
<td>Pediatr Allergy Immunol</td>
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<td>2010</td>
<td>Hahn YS et al[27]</td>
<td>Effect of Lactobacillus sakei supplementation in children with atopic eczema-dermatitis syndrome</td>
<td>88 children with AEDS</td>
<td>Ann Allergy Asthma Immunol</td>
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<td>2008</td>
<td>Hong SJ et al[56]</td>
<td>The Immunologic Effects of Lactobacillus rhamnosus (Lcr35) Supplements in Adult Patients with Atopic Dermatitis</td>
<td>5 adults with AD</td>
<td>Korean J Asthma Allergy Clin Immunol</td>
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<td>2007</td>
<td>Pyun BY et al[57]</td>
<td>The effects on treatment of atopic dermatitis with oral Lactobacillus casei supplements in Korean children</td>
<td>44 children with AD</td>
<td>Pediatr Allergy Respir Dis</td>
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<td>2012</td>
<td>Hong SJ et al[16]</td>
<td>Asthma Prevention by Lactobacillus Rhamnosus in a Mouse Model is Associated With CD4(+)CD25(+)Foxp3(+) T Cells</td>
<td>Asthma mouse model</td>
<td>Allergy Asthma Immunol Res</td>
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<td>2012</td>
<td>Hong SJ et al[42]</td>
<td>A novel mouse model of atopic dermatitis with epicutaneous allergen sensitization and the effect of Lactobacillus</td>
<td>AD mouse model</td>
<td>Exp Dermatol</td>
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<td>Year</td>
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<td>2011</td>
<td>Hwang KW et al</td>
<td>Oral administration of Lactobacillus strains from Kimchi inhibits atopic dermatitis in NC / Nga mice</td>
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<td>J Appl Microbiol</td>
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<td>2010</td>
<td>Hong SJ et al</td>
<td>The Effects of Lactobacillus rhamnosus on the Prevention of Asthma in a Murine Model</td>
<td>OVA-induced asthma</td>
<td>Allergy Asthma Immunol Res</td>
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<td>2010</td>
<td>Kim TS et al</td>
<td>Differential suppression of heat-killed lactobacilli isolated from kimchi, a Korean traditional food, on airway hyper-responsiveness in mice</td>
<td>OVA-induced asthma</td>
<td>J Clin Immunol</td>
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<td>2008</td>
<td>Ji GE et al</td>
<td>Effect of oral probiotics (Bifidobacterium lactis AD041 and Lactobacillus acidophilus AD031) administration on ovalbumin-induced food allergy mouse model</td>
<td>OVA-induced allergy mouse</td>
<td>J Microbiol Biotechnol</td>
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<td>Kook H et al</td>
<td>The Effect of Lactobacillus acidophilus on the Primary Prevention of Asthma in a Murine Asthmatic Model</td>
<td>OVA-induced asthma</td>
<td>Pediatr Allergy Respir Dis</td>
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<td>2008</td>
<td>Park YH et al</td>
<td>New functional probiotic Lactobacillus sakei probio 65 alleviates atopic symptoms in the mouse</td>
<td>AD mouse model</td>
<td>J Med Food</td>
</tr>
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AEDS; atopic eczema-dermatitis syndrome, AD; atopic dermatitis, OVA; ovalbumin
Figure 2

**Microbiologic action**
- Modulation of microbiota composition
- Competitive adhesion to receptors → Prevention of potential pathogen invasions
- Production of bacteriocin → Prevention of potential pathogen growth

**Epithelial action**
- Modulation of epithelial cell barrier
- Tight junction protein expression
- Production of SCFA (short chain fatty acid) like butyrate, acetate → Improvement of epithelial barrier & Potent antiinflammatory action

**Immunological action**
- Modulation of Innate immunity (dendritic cell maturation)
- Modulation of Th1/Th2 balance (↑ Th1, ↓ Th2)
- Increase in Treg number and function
Specific mechanisms

- Cell-mediated immune response balancing: DC and Treg modulation
  \( \uparrow \) Th1, \( \downarrow \) Th2
- Humoral response modulation:
  \( \uparrow \) IgA
  \( \downarrow \) IgE

Aspecific mechanisms

- Competitive exclusion of bacteria along epithelium
- Modification of the local microenvironment: antimicrobial peptides, SCFA, pH
- Improvement of barrier integrity
  \( \uparrow \) TJ, \( \downarrow \) mucus
- Reduction of intestinal inflammation:
  \( \downarrow \) NF-\( \kappa \)B activation
  \( \downarrow \) cytokine production
  \( \downarrow \) ROS

- Poly- saccharides
- SCFA
- Mono- saccharides

- Mucus
- Pathogen
- Probiotic
- Enterocytes
- DC
- Naive T cell
- Immature DC
- Treg
- IL-10
- TGFB
- IL-4
- IL-5
- TNF\( \alpha \)
- IFN\( \gamma \)
Figure 4