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Early Probiotic Supplementation for the Prevention of Atopic Disease in Newborns—Probiotics and the Hygiene Hypothesis—

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Environmental factors during early infancy could theoretically affect immune system development and subsequent risk of allergic disease. One potentially helpful exposure is early infant supplementation with specific probiotic strains. Unlike other exposures, probiotic supplementation is feasible and has a good safety profile. A review of recent randomized, controlled trials suggests that the effect of supplementation with probiotics on preventing the development of allergic disease is mixed. Further studies are needed to define potential mechanisms of action, such as effects on infant microbiota, as well as potential subgroups of patients that may benefit from these interventions.

Key words: The Hygiene Hypothesis; Allergic Disease; Probiotics; Infant Microbiota

INTRODUCTION

The hygiene hypothesis suggests that the absence of infectious exposure at a critical time in immune system development leads to a greater risk of the development of allergic disease. This idea has been applied to other environmental factors during early infancy which could theoretically affect immune system development and subsequent risk of allergic disease. One potential exposure is early infant supplementation with specific probiotic strains. This article reviews the evidence surrounding the hygiene hypothesis, as well as the potential role of supplementation with probiotics in preventing the development of allergic disease.

THE HYGIENE HYPOTHESIS

T-helper (Th) cells are part of a larger system that helps recognize foreign antigens and secretes cytokines to help activate other components of the immune system. Two subtypes of Th cells, Th-1 cells and Th-2 cells, are defined in general by the specific cytokines they produce *(1)*. At birth, a Th-2 system is predominant, and later, a Th-1 dominant balance is established. In the absence of infectious exposure at a critical time in an infant's immune system development, the hygiene hypothesis suggests that no shift occurs from the unfavorable Th2-dominated balance to the more favorable Th1-dominated balance, thereby enhancing the risk of the later development of atopic disease or asthma.

The hygiene hypothesis suggests that the presence of infectious exposures during childhood decrease the likelihood of allergic diseases (*2, 3)*. This idea has been developed to describe other environmental factors during early infancy which could theoretically affect immune system development and subsequent risk of allergic disease. In addition, the hypothesis has been extended to explain epidemiologic changes in autoimmune diseases, as well *(4)*.

One of the initial observations regarding risk of allergic disease and early infectious exposure was based on the follow-up analysis of a cohort of 17,414 British children. All of the children were born in the same week in March, 1958, and were followed until the age of 23 years. The outcomes of interest were parental reports of "hay fever or allergic rhinitis at 11 years of age" and parental recall of eczema in the first year (Strachan, 1989).

After controlling for socioeconomic status, housing tenure, breastfeeding, region of birth, smoking, and shared amenities, Strachan's analysis noted the relationship between birth order and the development of allergies. Children born first had a greater likelihood of developing atopic disease, while children with older siblings had a decreased likelihood of atopic disease. This exposure to other children was equated with exposure to early infections, which was thought to be negatively associated with the risk of allergic disease.

This observation has been strengthened by other studies, as a variety of different infectious exposures have

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been negatively associated with the development of allergic disease. A few of many examples are listed below. A cross sectional study of 5005 Italian naval recruits, 18 to 26 years of age, noted an association between the prevalence of hepatitis A antibodies and asthma. A history of a previous hepatitis A infection was negatively associated with asthma *(5)*. Data from the Third National Health and Nutrition Examination Survey was used to examine the association between allergic disease (asthma, wheeze, and hay fever) and antibodies to two strains of oral bacteria associated with periodontal disease. Consistent with the hygiene hypothesis, colonization of the oral cavity by pathogenic bacteria was protective in the development of allergic disease *(6)*. A history of pediatric *H. pylori* infection was negatively associated with physician diagnosis of allergy *(7)*. In relationship to exposures in developing countries, children with a history of *Trichuris trichiura* had a decreased likelihood of allergen skin test reactivity *(8)*.

The use of antibiotics has been positively associated with the development of allergic disease. For example, a cross-sectional study of 456 New Zealand children, 5 to 10 years of age, noted that exposure to antibiotics in the first year of life was associated with an increased risk for asthma, with an adjusted OR of 4.05 (95% CI: 1.55–10.59). In addition, the number of courses of antibiotics was also associated with an increased risk for asthma. These studies have been replicated in different settings with different populations allowing for pooling of results. A meta-analysis of 21 studies examining the association between antibiotic use and subsequent wheeze suggested a positive, but weak association (OR: 1.08; 95% CI: 0.93–1.23) when adjusting for confounding by indication *(9)*.

There are several observations that growing up on farms is associated with a decreased likelihood of development of allergies *(10)* In a cross-sectional survey of communities in rural Austria, Germany and Switzerland, children from farming families were less likely to have asthma, hay fever or atopic sensitization. Specifically, continual long-term exposure to stables until the age of 5 years had a protective effect on the development of asthma, hay fever and atopic sensitization *(11)*. A farming environment may have unique exposures and continual contact with large animals (e.g., cows and horses) which may in turn create exposure to high levels of endotoxin from Gram-negative bacteria.

Braun-Fahrlander *et al*. noted a dose-response relationship between endotoxin levels and decreased levels of hay fever and asthma *(12)*. Studies conducted in rural communities on other continents have not found associations as strong as described in Europe *(13,14)*; however, the protective effect of being raised in a farm environment may be due to distinct exposures which may reflect different mechanisms. The Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study analyzed a cross-section of 8263 school-age children from rural areas in 5 European countries. Pig keeping (OR: 0.57; 95% CI, 0.38–0.86), farm milk consumption (OR: 0.77; 95% CI, 0.60–0.99), and frequent visits to animal sheds (OR: 0.71; 95% CI, 0.54–0.95) were negatively associated with asthma *(15)*.

COUNTER-ARGUMENTS

Many of the studies that support the hygiene hypothesis are observational in design. With observational studies, the study subjects are not exposed randomly to the variable being studied. As a result, the potential for bias exists. For example, farm exposure or taking residence on a farm is based on a previous family decision. Those families that were predisposed to atopic disease may have been less likely to select a farming profession or live on a farm. As a result, families with atopic disease would be less likely to be represented in this study.

Another counter-argument is that the changes in hygiene do not match the change in the prevalence of atopic disease. Many of the advances in hygiene in the developed world occurred in the early part of the twentieth century. These include increased indoor plumbing, decreased dependence on horse transportation and migration to urban environments. However, the changes in asthma prevalence happened during the latter half of the twentieth century from 1960 to 1990 *(16)*.

The lack of a temporal association between the changes in hygiene and the changes in the rates of asthma does not seem to support the hygiene hypothesis.

PROBIOTICS

Despite the lack of conclusive evidence, the hygiene hypothesis may be a useful framework to devise strategies that can create a favorable Th1/Th2 balance, leading to a decreased likelihood of the development of allergic disease. Although there are many potential exposures linked to the hygiene hypothesis, few would be appropriate to test. Based on feasibility, safety and preliminary data, some probiotic strains provide potentially promising exposure that may be useful for the primary prevention of allergic disease.

Probiotics are live microorganisms, that when given in adequate amounts confer a health benefit on the host *(17)*.

In pediatric care, probiotics are most commonly used in the treatment of infectious diseases and the prevention of antibiotic-associated diarrhea *(18)*. The use of probiotics has become increasingly more common in consumer products as well as medical therapies.

In terms of feasibility, specific environments (e.g., farms) are associated with decreased risk of developing asthma. It would be difficult to conduct randomized controlled studies exposing children to drastic environmental changes or incorporate specific exposures (e.g. livestock, farm animals) to assess the effect on the development of allergic disease. Even if it were possible to maintain this exposure in a clinical trial setting, it would not be easy or cost-effective to translate this type of intervention to a clinical setting. In contrast, probiotic supplements are a practical and available exposure, which can be incorporated into the infant diet. In many countries, probiotics are available as a supplements for infant formulas, and such supplementation is characterized by minimal side-effects or issues with the use of probiotics in infants *(18)*.

Any exposure that is selected should present a justifiable risk in relation to the patient. For example, a previous history of hepatitis A infection has been associated with decreased risk of atopic disease *(5, 19)*. However, it would be unethical to encourage exposure to such pathogens. Similarly, although decreased antibiotic exposure has also been associated with a decreased likelihood of developing asthma (Wickens, 1999), trials that limited the use of antibiotics would also present practical and ethical challenges, as well. There are several probiotic strains with a long safety record and documented benefits. Although the potential risks of probiotic supplementation are low, care should be taken when probiotics are used with infants that are immunocompromised or being treated with central line access *(20)*.

One potential effect of probiotic supplementation is the effect on the infant microbiota. The enteric microbiota is a complex, dense ecosystem. Multiple factors can affect infant microbial colonization with potential implications for later life. For example, delivery by caesarean section may result in an altered pattern of intestinal colonization, compared to infants born vaginally *(21)*. Exposure to antibiotics during infancy, parenteral nutrition, delayed oral feeding and intubation also result in altered patterns of colonization that may have implications for the microbial populations which develop during childhood *(22, 23)*. Fluctuations in microbial populations in early life may affect intestinal physiology and subsequent development of atopic disease.

At birth, the infant gastrointestinal tract is rapidly colonized by a diverse set of microbes. Broad ecologic studies suggest links between differences in the early infant intestinal microbiota and the likelihood of developing chronic disorders, particularly allergic disorders. For example, infants who developed allergic diseases were less colonized with *Bifidobacteria* and more colonized by *Clostridia* at 3 months of age *(24)*. Differences in infant gut mircroflora have been associated with decreased likelihood of atopic sensitization and atopic diseases, such as rhinitis *(25, 26)*. Studies from Japan and Sweden also note that eczema is associated with lower infant colony counts of *Bifidobacteria (27, 28)*. The early neonatal period may potentially be a time when the evolution of an individual infant's microbiota can be influenced through probiotic supplementation.

PROBIOTICS FOR PREVENTION

There have been mixed results in the use of probiotic supplementation for allergic disease prevention. A double-blind, randomized, controlled trial by Wickens *et al*. suggested a potential effect of *Lactobacillus rhamnosus* HN001. Pregnant women were randomized to take *Lactobacillus rhamnosus* HN001, *Bifidobacterium animalis* subsp lactis strain HN019 or placebo daily. The intervention took place from 35 weeks gestation until 6 months, if breast-feeding. Postnatally, infants were randomized to receive the same treatment from birth to 2 years. No effect was found for *B. aminalis*, however, supplementation with *L rhamnosus* HN001 had a protective effect (hazard ratio: 0.51; 95% CI, 0.30–0.85) compared with placebo.

In a randomized, controlled, double-blind study of 159 newborns, Kalliomaki *et al*. found that early *Lactobacillus GG (LGG)* exposure as a probiotic supplement led to a decreased risk of atopic disease *(29)*. A follow-up study reported that the effect was sustained past infancy. Although the benefits of *LGG* exposure are only associated with the prevention of eczema, early development of this condition is associated with later development of asthma *(30)*.

Kopp *et al*. used a protocol similar to the Kalliomaki study with a variation in the dosing schedule. Pregnant women (n=105) with a family history of atopic disease were randomized to receive either placebo or 5×10^9 CFUs of *LGG* given twice a day. However, no significant difference in the development of atopic dermatitis (OR: 0.96; 95% CI: 0.38, 2.33) was found at 24 months of age *(31)*. Direct comparison with the Kalliomaki cohort is confounded by the differences in the

mean duration of breastfeeding for the infant populations in the two different studies.

In another randomized, controlled trial, 231 infants were given six monthexposure to *Lactobacillus acidophilus*. This early probiotic supplementation did not reduce the risk of atopic dermatitis *(32)*. In addition, a randomized, controlled trial involving prenatal and postnatal exposures to a combination of four probiotic strains and prebiotics for six months, reported there was no effect on the incidence of allergic disease at two years of age *(33)*.

At present , the effect of specific probiotic strains in the primary prevention of allergic disease is uncertain. Additional studies are ongoing and further studies need to be completed. For example, the Trial of Infant Probiotic Supplementation (TIPS) study is a randomized, controlled trial in the United States which has been designed to evaluate the effectiveness of *Lactobacillus GG* exposure in decreasing the likelihood of the development of early markers for asthma. The timing of the intervention, a daily, 6-month course of 10^{10} CFU of *Lactobacillus GG,* is during the post-natal phase, without prenatal supplementation to the mother in the third trimester. The causes of asthma are assumed to be multi-factorial and the analysis will take into account a number of familial and environmental exposures.

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