HYPOTHESIS: FOOD FOR THOUGHT

Essential Fatty Acids as Possible Enhancers of the Beneficial Actions of Probiotics

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OBJECTIVES: I investigated whether there is a common link between essential fatty acids and probiotics, which have similar actions and benefits in atopy.

METHODS: I made a critical review of the literature pertaining to the actions of essential fatty acids and probiotics on immune response and the interaction between them with particular reference to atopy.

RESULTS: Colonization of the human gastrointestinal tract occurs in the first months and years of life. Probiotics are cultures of beneficial bacteria of healthy gut microflora, which reduce dietary antigen load and thus protect against atopy. A significant reduction in the risk of childhood asthma and other atopic conditions was reported in children who were exclusively breast-fed for at least 4 mo after birth. This beneficial action can be attributed to the immunomodulatory, nutritional, or other components of human milk. Human breast milk is rich in long-chain polyunsaturated fatty acids (LCPUFAs), which have immunomodulatory actions. Probiotics and LCPUFAs modulate T-helper 1 and 2 responses, show antibioticlike actions, and alleviate changes related to allergic inflammation. LCPUFAs promote the adhesion of probiotics to mucosal surfaces, which augments the health-promoting effects of probiotics.

CONCLUSIONS: In view of the similarity in their actions and because LCPUFAs promote the actions of probiotics, I believe that a combination of LCPUFAs and probiotics offer significant protection against atopy. It is likely that breast-feeding and probiotics are two naturally occurring, appropriate events in early human life that have significant health benefits. Nutrition 2002;18:786–789. ©Elsevier Science Inc. 2002

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INTRODUCTION

Allergy in the form of atopic eczema, allergic rhinitis, and asthma is common. In a study done in Finland, 10% to 20% of the children had asthma, 15% to 23% had allergic rhinitis, and 15% to 19% had atopic eczema. Allergy is a chronic disorder with increasing maternal age, maternal smoking, and early cessation of breast feeding may increase the susceptibility to asthma. Improved hygiene and reduced family size is believed to increase the incidence of atopy. Early life factors such as low birth weight, preterm birth, young maternal age, maternal smoking, and early cessation of exclusive breast feeding may increase the susceptibility to asthma. Exposure to respiratory infections in childhood may protect against the development of atopy in later life, although this has been disputed.

One critical process in developing immune responses is the differentiation of naive CD4+ T cells into T-helper 1 (Th1) or Th2 cell subsets. Th1 cells secrete interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin (IL)-2, and IL-12 and are central to cellular immunity against intracellular pathogens. Th2 cells, in contrast, produce IL-4, IL-5, IL-6, IL-9, and IL-13 and promote antiinflammatory actions. The differentiation of naive Th cells into Th1 and Th2 cells is influenced by the cytokines present during and after antigen presentation. The appearance of IFN-γ-producing cells is promoted by IL-12 signaling, which is mediated by signal transducer and activator of transcription (Stat) 4. Stat4-deficient mice do not respond normally to IL-12 and show marked impairment in Th1 differentiation and a propensity for Th2 differentiation. Conversely, IL-4 promotes Th2 differentiation by means of Stat6. Stat6-deficient mice show impaired IL-4 signaling and diminished Th2 responses. This suggests that during the early years of life the balance between Stat4 and Stat6 may play a pivotal role in determining the ultimate balance between Th1 and Th2 responses and their cytokines. Recently, the T-box transcription factor, T-bet, was shown to promote Th1 development and IFN-γ production. Moreover, IFN-γ can suppress Th2 differentiation. It was also reported that IFN-γ induces T-bet expression in monocytes, macrophages, and dendritic cells. In mice that lack T-bet, CD4 T cells failed to produce IFN-γ, the hallmark Th1 cytokine, and instead produced the Th2-specific cytokines IL-4 and IL-5. These T-bet–deficient mice failed to control a Th1-dependent protozoan infection and showed spontaneous changes consistent with human asthma. This suggests that Th1 cells have a role in autoimmune and a protective function in asthma.

GUT MICROFLORA AND ATOPY

The human gastrointestinal tract is sterile at birth. During the first months and years of life, gut microflora become established. Colonization of the human gastrointestinal tract occurs in the first months and years of life. Gut microflora playa dominant role in the development of gut-associated lymphoid tissue. This in turn determines the balance between Th1 and Th2 responses. Infection with Schistosoma hematobium in children was negatively associated with atopy. Epidemiologic studies showed a similar inverse association between Mycobacterium tuberculosis, Helicobacter pylori, viral or protozoan infections, and atopy. This negative association between childhood...
infections and subsequent development of atopy has been related to shift in the balance of immune responses toward Th1. As a consequence, Th2 responses will be suppressed, which are generally associated with atopy.15 However, this concept of a Th1/Th2 balance in the etiology of atopy may not always be true because in developing countries helminth infections are common, which results in high plasma concentrations of immunoglobulin (Ig) E, a Th2 response. The prevalence of allergic diseases is much less in these populations, suggesting that there may be other factors that determine the onset of atopy. It is interesting to note that the plasma levels of IL-10, an anti-inflammatory cytokine, were high in children infected with the parasite S. hematobium, so these children were protected from the development of atopy.12 IL-10 production by alveolar macrophages is diminished in patients with asthma.16 This suggests that, in addition to the balance between Th1/Th2 responses, IL-10 and other anti-inflammatory molecules play a role in the outcome of exposure to allergens.

In the newborn, Th2 responses are dominant. In those destined to develop atopy, the Th2 responses become intensified during the first few months and years of life. This intensification of Th2 responses depends, to a large extent, on the gut microflora. For instance, those who developed atopy showed a reduced ratio of Bifidobacteria to Clostridia in the gut flora in infancy.17 The other stimulus that determines the balance between Th1 and Th2 responses in the newborn is the dietary antigens. Animal studies indicated that dietary antigens provoke atopic-type immunity.18 This led to the suggestion that strategies aimed at preventing allergy should be instituted during early infancy, especially when the first encounters with dietary antigens or allergens is likely to occur. Probiotics seem to be immensely suited for this purpose because they can reduce dietary antigen and allergen load by degradation and modification of these macromolecules.19 A process that seems to be essential to render an individual non-responsive to antigens and allergens,20 and shift the Th1/Th2 response balance more toward Th1. The other actions of probiotics that aid in suppressing atopy include their ability to augment the production of transforming growth factor-β (TGF-β)21 and IgA production.22 TGF-β suppresses Th2 responses23 and induces oral tolerance,24 events that prevent the development of atopy. This is further supported by the observation that Lactobacillus GG decreased the incidence of atopy by almost half in at-risk infants.25 In that study, although the mean (standard deviation) time of exclusive breastfeeding and mean total time of breast feeding were closely similar between children given placebo for 2.7 (2.2-3.1) mo and 6.4 (5.4-7.5) mo, respectively, and those on Lactobacillus GG for 3.0 (2.6-3.4) mo and 7.2 (6.4-8.1) mo, respectively, not much significance was attached to that finding. I consider this a significant biological difference, if not a statistical one.

### POLYUNSATURATED FATTY ACIDS AND ATOPY

A significant reduction in the risk of childhood asthma was observed in children who were breast fed exclusively for at least 4 mo after birth.26 This protective effect can be attributed to exclusion of milk (and its potentially allergenic components) other than breast milk from the infant’s diet and the immunomodulatory, nutritional, and other components of human milk.27,28 A protective effect of breast feeding among children with a family history of atopy has also been demonstrated.29 It is interesting to note that this protective action of breast feeding was not confined to the period of feeding but lasted for the first 3 y of life. These results are supported by the observation that the occurrence of eczema and asthma was lower in breast-fed infants than in those given cow’s milk.30 Although the exact reason for this beneficial action of breast milk against the development of atopy is not clear, one strong possibility is its fatty acid composition. Breast milk is rich in long-chain polyunsaturated fatty acids (LCPUFAs),31,32 LCPUFAs, especially γ-linolenic acid (18:3, ω-6), arachidonic acid (AA; 20:4, ω-6), eicosapentaenoic acid (EPA; 20:5, ω-3), and docosahexaenoic acid (DHA; 22:6, ω-3) suppress immune response and the secretion of TNF-α.33-35 The inhibitory effect of EPA and DHA on the secretion of IL-1, IL-2, and TNF-α are much stronger than those exerted by γ-linolenic acid and AA. Kelley et al.26 reported that oral supplementation of AA (1.5 gm/d) significantly increases the secretion of leukotriene B4 and prostaglandin E2, but it did not alter the secretion of TNF-α, IL-1β, IL-2, IL-6, and the expression of the receptor for IL-2. Nor there were any changes in the number of circulating lymphocytes bearing markers for B, T, helper, suppressor, or natural killer cells. Intravenous administration of lipid emulsions rich in ω-6 and ω-3 fatty acids also showed that production of the inflammatory mediators thromboxanes B2 and P3 and TNF-α by peripheral blood monocytes was diminished by ω-3 lipid infusion and was unchanged or increased by ω-6 lipid infusion.36 Further, EPA/DHA supplementation enhanced TGF-β production and thus delayed autoimmune disease in experimental animals.37 TGF-β decreased the biosynthesis and release of TNF-α.38,39 Thus, TGF-β may serve as a negative controller of TNF production.40 In this context, it is interesting to note that some of the actions of TGF-β depend on the presence of LCPUFAs.41 Thus, depending on the type and amounts of LCPUFAs given, Th1 responses can be enhanced and Th2 responses are blunted, which favor suppression of atopy. This favorable action on Th1 and Th2 responses may depend on the ratio between ω-3 and ω-6 fatty acids. A higher ratio of ω-6 to ω-3 LCPUFAs favors an enhancement in the Th1 response, whereas a decrease in the ratio between ω-6 to ω-3 may favor the Th2 response. Because breast milk is rich in ω-6 LCPUFAs (see Table I for the fatty acid composition of breast milk), the protective effect of breast feeding against atopy might be attributed to its high content of these beneficial fatty acids. In addition, these LCPUFAs form precursors to various eicosanoids, which also have modulatory influences on Th1 and Th2 responses.38

### INTERACTION BETWEEN LCPUFAS AND PROBIOTICS

If LCPUFAs and probiotics offer significant protection against atopy, is there a breast-feast interaction between them? This is especially relevant because breast feeding and colonization of intestines by probiotics occur during the early days and months of infancy. Because gut flora play a significant role in the development of atopy and atopic disease, it is essential that events in early infancy should favor the growth of probiotics and inhibit the
proliferation of harmful bacteria. In this context, it is interesting to note that LCPUFAs show antiobioticlike actions, which are similar to those of probiotics. For example, linolenic acid rapidly killed cultures of Staphylococcus aureus, and hydrolyzed linseed oil (which contains α-linolenic acid) can inactivate methicillin-resistant S. aureus. In contrast, Lactobacilli suppressed the growth of Helicobacter pylori, Shigella flexneri, Salmonella typhimurium, Pseudomonas aeruginosa, Clostridium difficile, Escherichia coli, and Clostridium difficile-induced diarrhea. These findings suggest that LCPUFAs and probiotics have the ability to kill harmful bacteria that are likely to be present in the gastrointestinal tract. These results are interesting because the presence of large amounts of pathogenic bacteria in the gut microflora augments Th2 responses and precedes the development of atopy. Thus, adequate amounts of probiotics and possibly LCPUFAs help to restore normal and healthy gut microecology. Further, probiotics alleviate changes related to allergic inflammation by inducing the production of IL-12, IL-18, and IFN-γ. LCPUFAs also have anti-inflammatory actions. Based on these findings, during the first months and years of life when gut microflora are getting established, I believe that LCPUFAs derived from breast milk, aid in the rapid colonization of probiotics by suppressing the growth of pathogenic bacteria. In addition, LCPUFAs, especially α-linolenic acid, have been shown to promote adhesion of Lactobacillus casei to mucosal surfaces. Because adhesion to mucosal surfaces is pivotal in health-promoting effects by probiotics, LCPUFAs likely potentiate the beneficial actions of Lactobacilli. LCPUFAs by enhancing the adhesion of probiotic organisms to the gut mucosal cells may enhance the development of gut-associated lymphoid tissue by direct interaction between the probiotics and the lymphoid tissue and by the ability of LCPUFAs and probiotics to augment certain growth factors such as TGF-β and various cytokines. This ensures appropriate development of the gut-associated lymphoid tissue and the physiologic balance between Th1 and Th2 responses.

CONCLUSION

It is evident from the preceding discussion that probiotics and LCPUFAs have similar beneficial actions that aid in the prevention of atopy. The protective action of breast feeding against atopy may partly, if not exclusively, be attributable to their high content of LCPUFAs, especially of ω-3 fatty acids. LCPUFAs in turn have anti-inflammatory and antibioticlike actions that can potentiate the beneficial actions of probiotics. The growth inhibitory actions of LCPUFAs against pathogenic bacteria and their ability to enhance the adherence of Lactobacilli to mucosal surfaces will aid the probiotics in colonizing the gut. Once the gut microflora are established, probiotics enhance gut-specific IgA responses, Th1 immunity, TGF-β, and IL-10 production that protect against atopy. LCPUFAs, by virtue of their ability to alter the Th1/Th2 ratio, support these beneficial actions of probiotics. In this context, it is interesting to note LCPUFAs can influence the survival of cord-blood monocytes, which are crucial in the defense against invading pathogens and are involved in the lysis of infected or malignant cells, wound healing, repair, and remodeling of tissues. At a concentration of 50 µM, EPA, DHA, and AA showed no significant effect on monocyte cell death, but at 100 µM, only DHA induced monocyte death (60 ± 4%), whereas EPA and AA had no significant effect. At concentrations of at least 200 µM, all three LCPUFAs significantly increased monocyte cell death (AA: 70 ± 5%, DHA: 66 ± 2%, EPA: 70 ± 4%). These results suggest that LCPUFAs can influence monocyte cell survival and thereby modulate the immune response. It is essential that the gut epithelial cells should grow adequately in the newborn to meet the nutritional demands of the growing infant. It is interesting to note that signal transduction of certain growth factors such as fibroblast growth factor involves AA. In a similar fashion, even probiotics may have growth stimulatory actions on the intestinal epithelial cells because they enhance the production of TGF-β, a growth factor. Thus, there seems to be a close interaction between probiotics and LCPUFAs (Fig. 1). This suggests that breast feeding (in addition to its nutritional value, it is rich in LCPUFAs) and probiotics seem to be two naturally occurring early life events that potentiate each other’s actions and offer significant health benefit. A recent study reported that oral supplementation of probiotic Bifidobacterium lactis (5 × 10^10 or 5 × 10^10 organisms per day for 3 wk) to healthy elderly volunteers (63 to 84 y old) increases the proportions of total, helper (CD4+), and activated (CD25+) T lymphocytes and natural killer cells and increased the phagocytic activity of mononuclear and polymorphonuclear phagocytes and the tumoricidal activity of natural killer cells. This suggests that probiotics augment the immune response, which in turn can kill the harmful bacteria in the gut and elsewhere. Hence, a combination of LCPUFAs and probiotics may be beneficial not only to infants but also to individuals of other age groups. In view of the role of T-bet in Th1 responses, it will be
interesting to study the effects of various LCPUFAs and probiotics on T-bet expression.

REFERENCES

5. Saarinen UM, Kajosaari M. Breast feeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. Lancet 1995;346:1065
23. Chandra RK. Five-year follow-up of high-risk infants with family history of allergy who were exclusively breast-fed or fed partial whey hydrolysate, soy, and conventional cow’s milk formulas. J Pediatr Gastroenterol Nutr 1997;24:380
29. McCann ME, Moore JN, Carrick JB, Barton MH. Effect of intravenous infusion of omega-3 and omega-6 lipid emulsions on equine monocyte fatty acid composition and inflammatory media or production in vitro. Shock 2000;14:222
31. Tracy KJ. TNF and MxA protein expression after too much of a good thing. Lancet 1995;345:75
32. Das UN. Beneficial action(s) of eicosapentaenoic acid/docosahexaenoic acid and nitric oxide in systemic lupus erythematosus. Med Sci Res 1995;23:723