

## Breast milk – immunomodulatory signals against allergic diseases

Breastfeeding holds a key position with regard to the increasing burden of allergic diseases in the industrialized countries. Not only does it provide the infant with nutrients for growth and development, it also confers immunological protection during a critical period in life, when the infant's own defense mechanisms are immature. A delicate balance of stimulatory, even inflammatory, maturational signals, together with a myriad of anti-inflammatory compounds, is transferred from mother to infant via breastfeeding. Breastfeeding mothers, however, do not constitute a uniform group. The composition of breast milk shows marked individual variation and so, consequently, does the success of breastfeeding in reducing the risk of disease. Recent clinical studies indicate that the potential of breastfeeding to counteract allergic disease may be promoted by dietary means. While uncoordinated elimination diets result in a risk of general nutritional inadequacy or deficiency of essential single nutrients, a balanced diet following current dietary recommendations, specifically containing fresh fruits and vegetables (antioxidants) and fat of predominantly vegetable origin, may be associated with a lower incidence of atopy in the infant. As early nutrition appears to program the subsequent health of the child, the importance of the maternal dietary composition during breastfeeding should be emphasized. In future, an improved understanding of the mechanisms of this programming may offer specific therapeutic modalities for the prevention of allergic disease.

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The development of new allergy prevention strategies calls for a better understanding of the processes which initiate sensitization and regulate the maturation of immune defense mechanisms against allergic disease. The underlying denominators in atopic predisposition may be outlined as genetic susceptibility, aberrant barrier functions of skin epithelium and gut mucosa, and dysregulation of the immune response to ubiquitous environmental antigens.

The principal immunological feature distinguishing atopics from nonatopics is the propensity to mount IgE antibodies to common environmental antigens. IgE responses are under the control of cytokines produced by competing signals from distinct subsets of T helper cells. Interleukin-4 is essential for the development of T helper 2 cells, which induce IgE production, eosinophilia and, with sustained generation, the progression from sensitization to atopic disease. This responder phenotype is universal at an early age, as polarization of the cytokine profile away from cell-mediated T helper 1-type immunity toward humoral immunity of the T helper 2-type prevails *in utero*. Consequently, a significant overlap in the concentrations of interleukin-4 and IgE antibodies exists between atopics and non-atopics at an early age (1, 2).

Instead of being a simple consequence of a T helper 2-polarized responder type, allergic disease could thus

be ascribed to a lack of physiological counter-regulatory processes. Such signals are required for the priming of host-protective mechanisms. The earliest and most substantial driving forces for the development of the defense mechanisms are derived from microbial antigens, in particular from the establishment of the normal microflora. Specific strains of the intestinal microflora have been shown to contribute to the generation of anti-inflammatory cytokines, thus creating optimal conditions for redirection of the polarized immunological memory of the infant to a healthy balance (3).

Apart from essential nutrients, human milk provides several types of bioactive compounds which support infant growth and contribute to the development of the host defense mechanisms, particularly in the gastrointestinal tract, where the major load of new antigens is encountered. Breast milk-derived secretory IgA contributes to immune exclusion of antigens in neonates, whose endogenous intestinal IgA production is defective. Breastfeeding not only confers passive protection but also actively stimulates the development of the infant's own immune system. This creates a dilemma in breastfeeding – a delicate balance between stimulatory and suppressive signals, which may exert contrasting effects for the development of the intestinal barrier functions (Fig. 1).

### Breast milk promotes the development of an anti-inflammatory intestinal milieu

Breast milk-derived immunomodulatory and protective properties have been linked to several components: immunoglobulins, of which 90% are secretory IgA; cell surface homologues: glycoconjugates and oligosaccharides; nutrients such as antioxidants and fatty acids, glutamine and dietary nucleotides; lactoferrin; and other bioactive agents such as hormones, growth factors and cytokines.

Controversial results regarding the possible beneficial effects of breastfeeding on atopic diseases have been reported, and a unifying immunological evidence of breastfeeding and allergic disease is lacking. The question remains beyond final clinical demonstration since, for obvious ethical reasons, infants cannot be randomly assigned to breast- or formula-feeding. Several recent prospective clinical studies, however, have suggested that breast milk may provide the infant with protection, possibly extending beyond childhood, against atopic sensitization and atopic disease (4–6).

Oral tolerance mechanisms, a systemic state of hyporesponsiveness to orally administered antigens, allow the use of breast milk as a single source of nutrition in neonates. Nonetheless, local production of secretory IgA may ensue. Transforming growth factor (TGF)- $\beta$  has been demonstrated to have an impact on both IgA production and oral tolerance induction. Endogenous TGF- $\beta$  production in the intestine is sparse during the first days of life and gradually increases thereafter. In contrast, the exogenous supply, i.e. TGF- $\beta$  in maternal milk, peaks immediately after birth in colostrum and decreases significantly in mature milk, suggesting a shift from an exogenous to an endogenous supplier as the main source of TGF- $\beta$  production during early postnatal development. Interestingly, the pattern of TGF- $\beta$  receptor expression in the intestine changes with age in a manner which suggests a change in ligand from milk-derived TGF- $\beta$  to endogenously produced TGF- $\beta$  (7). A recent study has shown that high TGF- $\beta$  in maternal colostrum was associated with the infant's ability to produce specific IgA in response to  $\beta$ -lactoglobulin, casein, ovalbumin and gliadin, the dietary antigens most frequently responsible for sensitizing the infant (4). High TGF- $\beta$  in maternal colostrum was further associated with prevention of atopic disease during exclusive breastfeeding. This clinical demonstration tends to substantiate how breastfeeding may guide infants through the critical period of transition from ineffective and inexperienced immune response to mature barrier functions.

More recently, research interest has focused on breast milk oligosaccharides with prebiotic properties to promote the growth of lactobacilli and bifidobacteria in the intestinal microflora; these evince powerful anti-pathogenic capabilities and are mainly responsible for

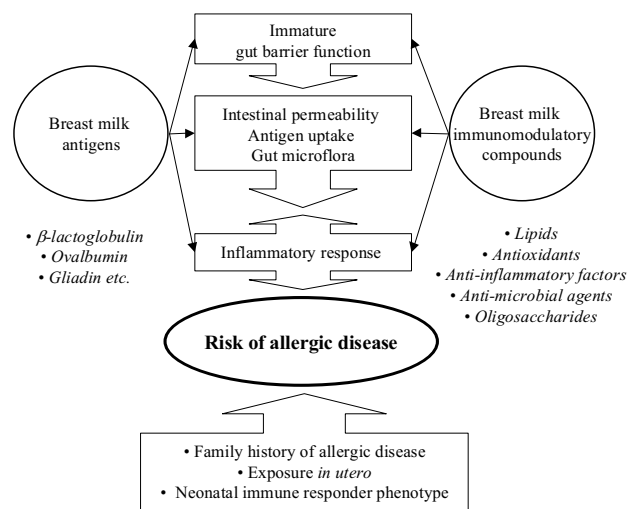


Figure 1. Dilemma of breastfeeding: breast milk provides anti-inflammatory protective factors and immunoinflammatory signals which impact on the risk of allergic disease in infant.

colonization resistance in the intestine. A recent study has shown that a reduced ratio of bifidobacteria to clostridia in early microflora precedes the subsequent development of atopy and atopic disease (5), which suggests that breast milk might provide its protection against atopic disease by modulating the intestinal microflora.

### Allergenic proteins are transferred to the infant via breast milk

Food antigens such as  $\beta$ -lactoglobulin, ovalbumin and gliadin are transferred to breast milk from the mother's diet. For instance,  $\beta$ -lactoglobulin has been detected in 95% of breast milk samples, with great intra- and interindividual variation, ranging from 0.9 to 150  $\mu\text{g/l}$ , after ingestion of the same amount of milk (8). Ovalbumin concentrations have ranged from 0.2 to 4.0  $\mu\text{g/l}$  after egg challenge (9). Determination of the concentrations of food antigens in breast milk in different populations and research protocols has yielded conflicting findings. Antigen levels do not seem to be associated with maternal atopy (8), or with the incidence and symptoms of allergy in the infant or positive skin-prick test reactivity (9).

Antigens in breast milk may have both harmful and beneficial effects (Fig. 1). Because the intestine's barrier functions are immature during the neonatal period, increased antigen uptake may lead to an inflammatory response. Antigens in breast milk, processed by the mother's intestinal mucosa and presented in conjunction with specific antibodies, may be associated with tolerance induction rather than sensitization. Presentation of dietary antigens so modified by the mother's mucosa may be ideal for the maturation of the

infant's immune system. In particular, intraluminal and mucosal degradation of the antigens has been shown to contribute to this end (10).

Despite the inconclusive association between antigen exposure and atopic disease, elimination of allergenic foods, e.g. cow's milk, egg and fish, from the breast-feeding mother's and infant's diet has been a common approach in attempts to prevent and treat food allergy and atopic disease in high-risk infants. Results of different elimination procedures with dietary restrictions alone or in combination with some other environmental control measures have been inconclusive regarding the prevention of atopic diseases, as reviewed by Zeiger (11). Elimination diets during pregnancy and breastfeeding have especially proved unsuccessful in long-term prevention of atopic disease. Maternal elimination diets may entail a risk to the nutritional status and health of both mother and infant and should not be followed unnecessarily.

Taken together, the failure of elimination diets to prevent atopic disease may be attributable to different factors. Firstly, total elimination of antigenic proteins is difficult in practice and maternal compliance with restricted diets may be poor. Secondly, there is great inter- and intraindividual variation in the amount of antigens in breast milk irrespective of the dietary regimen. Thirdly, antigens may contribute to tolerance induction rather than sensitization. Fourthly, besides allergenic proteins in the diet, other dietary factors such as dietary fatty acids and antioxidants could also be associated with the subsequent development of atopic disease (Fig. 1).

#### **Immunomodulatory properties of lipids and antioxidants**

The environmental determinants underlying the rising incidence of allergic diseases include changes in Western dietary composition over the past decades. Dietary lipids and especially polyunsaturated fatty acids exert various effects upon the immune system (12). Dietary antioxidants, in turn, counteract the endogenously generated oxidative stress (13). These nutrients may therefore influence immunoinflammatory responses and atopic sensitization.

Both cellular enzyme-based antioxidants and diet-derived antioxidants counteract oxidative stress and damp the inflammatory response (13). Dietary antioxidants may thus be important in implementing the ability of the individual to restrain the inflammatory response and in avoiding injury to tissues. Low concentrations of  $\beta$ -carotene, ascorbate and  $\alpha$ -tocopherol have been measured in plasma in wheezing illness, suggesting that antioxidant deficiencies may be associated with symptoms of the allergic disease (14). However, the role of antioxidants in breast milk in the onset of the allergic disease remains for the present obscure.

Polyunsaturated n-6 series fatty acids derived from dietary linoleic acid (18:2, n-6) result in the production of eicosanoids, which are considered to have proinflammatory properties since they can cause clinical symptoms such as bronchoconstriction (15). The best-known immunomodulatory functions are of arachidonic acid (20:4, n-6)-derived eicosanoid prostaglandin (PG) $E_2$ . PGE $_2$  results in elevated IgE synthesis due to the induction of B cell differentiation in the presence of interleukin-4 but it has been suggested that PGE $_2$  may suppress inflammatory response by inhibiting B cell proliferation (16). The effects of PGE $_2$  in allergic disease may also be exerted via IgE-binding receptors (Fc $\epsilon$ RII), as PGE $_2$  reduces the surface expression of the receptor, resulting in increased synthesis of IgE (17).

Dietary n-6 fatty acids may also have beneficial effects in the prevention of allergic disease. It has been shown in mice that antigen stimulation upregulates the expression of cyclo-oxygenase-dependent PGE $_2$  production from arachidonic acid, with ensuing suppression of antigen-specific T cell proliferation in gut-associated lymphoid tissue (18). It may be postulated that an enhanced synthesis of prostaglandins contributes to an anti-inflammatory intestinal milieu through suppression of T helper 1-type cytokines, stimulation of anti-inflammatory interleukin-10 and TGF- $\beta$  and maintenance of the IgA environment.

Compared to n-6 series fatty acids, n-3 series fatty acids derived from dietary  $\alpha$ -linolenic acid (18:3, n-3) result in the production of eicosanoids, which appear to have a less potent biological function. For example, 3-series thromboxanes derived from eicosapentaenoic acid (20:5, n-3) are less active than 2-series thromboxanes derived from arachidonic acid in aggregating platelets and constricting blood vessels (12). The anti-inflammatory properties of n-3 fatty acids also arise from their capacity to inhibit the release of arachidonic acid from membrane phospholipids. Furthermore, n-3 fatty acids are able to act as a substrate both for cyclo-oxygenase and lipo-oxygenase pathways, thereby reducing the production of arachidonic acid-derived eicosanoids, whereas the synthesis of n-3 fatty acid-derived eicosanoids increases. Various interactions between lipids and immune response may partially explain the development of atopy and the severity of the symptoms in allergic disease.

#### **Could we modulate the potential of breast milk to prevent allergy by maternal diet?**

The quality of the maternal diet during pregnancy and breastfeeding is important for both the health and the nutritional status of the mother herself and of her infant. Early nutrition has been shown to have an important impact on the subsequent health of the child (19). Maternal stores and diet during breastfeeding determine the quantity and quality of breast milk, especially the

fatty acid supply to the infant. The fatty acid composition of breast milk has been shown to be associated with atopic sensitization (20). The interindividual variation in fatty acid concentration, antioxidants and other immunomodulatory compounds in breast milk may be considerable, and could thereby explain the inconclusive evidence on the effect of breastfeeding on atopic sensitization.

Our prospective study on the diet of breastfeeding mothers in atopic families showed that the energy intake of the mothers was relatively low and the duration of exclusive breastfeeding was shorter than the recommended 4–6 months (21). The mean intakes of total fat and saturated fatty acids exceeded the current dietary recommendations (22) and atopic mothers had a higher intake of total fat and saturated fatty acids than non-atopic mothers. Moreover, maternal fat intake was inversely associated with vitamin C intake. Intake of saturated fatty acids was found to be associated with atopic sensitization of the infant.

Considering the interrelated effects of dietary antioxidants and fatty acids, their proportionate intake may

be important in controlling allergic inflammation. We therefore suggest that the approach in attempts to prevent food allergy and atopic disease could be shifted from maternal empirical elimination diets to ensuring a balanced diet. Sufficient energy and nutrient intake is essential for successful breastfeeding and for the optimal protection provided by breast milk. The importance of its composition culminates in young infants whose sole source of nutrients is breast milk when the immune responder phenotype becomes consolidated. In diet counseling, moderate fat (<30% of energy intake) and sufficient antioxidant intake should be promoted and counseling should preferably begin during pregnancy. The primary goal could be that the diet of mothers in atopic families should meet the current dietary recommendations (22) for pregnant and breastfeeding women. For more detailed recommendations, further studies are needed on the effects of maternal dietary composition and the possible benefits of dietary supplementation for the maturation of the immune defense mechanisms required to keep the infant in a nonatopic state, in view of the environmental pressure in Western civilization.

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