

Dietary Intervention during Pregnancy and Allergic Diseases in the Offspring

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Introduction

In 1903 Baron Clemens von Pirquet [1], an Austrian pediatrician, proposed the terms allergy and allergen after observing that some of his patients had 'altered responses' to certain substances. Hypersensitivity causes objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects. Atopy is a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis. Allergy is a hypersensitivity reaction initiated by immunologic mechanisms [2]. In atopy, genetic predominance is more important. In allergy, environmental factors are more relevant.

The prevalence of allergic diseases is increasing worldwide and represents one of the most frequent chronic diseases with relevant health care costs and impaired quality of life [3]. Almost 100 years ago Cooke and Coca [4] reported on the importance of genetic components in allergy. Genetic factors alone cannot explain the recent rapid increase in the incidence of atopic diseases (table 1). The genetic risk factor for atopy is associated with a delayed postnatal maturation of T-cell competence. Children at high risk of allergic disease have a slow maturation to adult levels of T-helper Th-1 and Th-2 due to possible polymorphisms in genes (table 2). Besides genetic factors, the amount and timing of the antigen presented to the immune system are likely to be determining factors causing an allergic reaction. Smoking and alcohol are two examples that (toxic) substances ingested during pregnancy by the mother influence the development of the fetus. Infectious diseases during pregnancy are another example.

Table 1. Genes and atopy

Locus	Candidate gene	Phenotype
1p36	Unknown	Asthma
2q21-23, q33	Unknown	Specific IgE, asthma
3q21	CD80-CD86	Total IgE, severe eczema
4q35	Endothelin receptor	Asthma, atopy
5q23-31, q33	IL-4, IL-13, MHC, CD14, glucocorticoid and β_2 -adrenergic receptors	Total IgE, asthma, eczema
6p21.3, 22-24	MHC class II, TNF- α	Asthma, atopy, specific IgE
7p15, q33	T cell receptor	Asthma, total IgE
8p21-23, q13	Unknown	Asthma, specific IgE
9q22	Unknown	Asthma
11q13	FC ϵ R1, Clara cell 16 gene	Asthma, IgE, atopy
12q15-q24.1	IGF1, SCF, IFN γ , NOS1	Asthma, total IgE, atopy
13q14	Unknown	Asthma
14q11.2, q32	TCR- α	Specific IgE, eczema, atopy
16p11.2-12.1	IL-4 REC	Total IgE, asthma
17	Rantes	Eczema
19p13	Unknown	Asthma
21	Unknown	Atopy

Intervention during Pregnancy?

The ideal situation would be to bring the individual in contact with levels of antigens capable of inducing tolerance but not causing allergic reactions. The question arises as to how relevant antigen exposure is during pregnancy for the development of later tolerance.

Allergy prevention may be directed at three potential stages: primary prevention, which inhibits immune sensitization; secondary prevention, which avoids disease expression subsequent to sensitization, and tertiary prevention, which suppresses symptoms after disease expression. As a consequence, intervention during pregnancy concerns primary prevention. A prevention strategy may be allergen-specific (antigen avoidance) or non-specific (environmental effect). The success of allergy-prevention strategies must be judged by the ability to predict the high-risk subject and the effectiveness of the intervention strategy using acceptable interventions with minimal adverse effects and cost-effective outcomes.

The intrauterine milieu is normally skewed towards Th-2 immunity, which seems the necessary pattern for successful pregnancy or the natural response to different allergen priming through the placenta [5, 6]. After birth, the neonatal switch from a Th-2 to a Th-1 pattern does not develop normally in atopic subjects who perpetuate IgE production. A Th-2-modulated response during pregnancy has a trophic effect on the placenta; Th-1 has an inhibitory

Table 2. Principal cytokines involved in the allergic cascade

Cytokine	Cell sources	Main functions
IL-2	Th1	Increase proliferation of Th and B lymphocytes
IL-4	Th2, Th0, mast cells	Increase production of IgE, development and recruitment of eosinophils, development of mast cells
IL-5	Th2, mast cells	Increase development and recruitment of eosinophils
IL-9	Th2	Increase production of IgE, development and recruitment of eosinophils, development of mast cells
IL-10	Th2, Tr1	Inhibit Th1, downregulate inflammation
IL-12	APC, Th0	Increase proliferation Th1 and production of IFN- γ , reduce IL-4
IL-13	Th2	Increase production of IgE, development and recruitment of eosinophils, development of mast cells
IFN- γ	Th1	Increase expression of HLA molecules, phagocytosis and NK cytotoxic functions, inhibit Th2
TGF- β	Th3	Downregulate inflammation, stimulate IgA production, ? induce tolerance
TNF- α	APC, Th1	Stimulate APC, Th, neutrophils, adhesion molecules expression, increase acute phase proteins...

APC = Antigen-presenting cells; NK = natural killer.

effect on the placenta. Children who develop later allergy have high IL-4 and low interferon- γ in the cord blood, and thus an attenuated Th-1 and Th-2 response. It is presently unknown which factors determine the strength of the Th-2 or Th-1 response during pregnancy. An attenuated Th-1- or Th-2-polarized situation during fetal life is switched off via microbial stimulation at the mucosal surface towards an adult equivalent adaptive immune function that is Th-1 skewed. Animal studies indicate that intestinal flora is necessary and sufficient to drive this maturation process.

Few studies have evaluated dietary restriction instituted solely during pregnancy. The rationale to consider a maternal dietetic role during pregnancy to prevent allergy is based on evidence that the fetus is exposed to circulating antigens through the placenta. However, it is very likely that fetal exposure has a tolerogenic effect in the vast majority of infants. Only part and probably a minority of the circulating antigens are of dietary origin. However, milk allergens are the best studied. Recognition of milk and other allergens (such as serum albumin, Der p1 and the rye grass allergen) by cord blood cells clearly indicates that allergen priming occurs prenatally but with a nonsignificant difference between atopy-prone and non-atopy-prone subjects [7].

Allergen exposure in utero may occur both via transfer of allergens in the amniotic fluid and via transfer across placental tissue. Human amniotic fluid at 16–18 weeks of gestation may contain intact IgE at levels that evolve proportionally with maternal circulating levels and that may bind to low-affinity IgE receptors (CD23+ cells) expressed in lymphoid follicles of the fetal gut from 16 weeks of gestation. Cytokine production within the fetoplacental unit may influence susceptibility to subsequent disease development. Neonatal rats, sensitized in utero through an intraperitoneal injection of β -lactoglobulin, show a reduced delayed-type hypersensitivity response compared to control rats, suggesting a tolerizing effect of exposure during fetal life [8]. This observation is a strong argument against dietary restrictions during pregnancy. However, it is very likely that dose and timing in combination with the genetic background may be detrimental for the outcome.

The fetus can mount (its own) specific IgE responses to foods and T-cell responses to cow's milk and egg proteins and aeroallergens. There is some evidence that sensitization to food allergens may occur in utero and result in raised specific IgE in cord blood [9]. The transplacental passage of common allergens such as β -lactoglobulin, ovalbumin and major birch pollen allergen Bet v1 is described to occur from before 26 weeks of gestation using an ex vivo model of transfer [10]. Cord blood mononuclear cell reactivity to allergens was observed as early as 20 weeks of gestation, indicating intrauterine exposure to allergens with subsequent development of memory function [11]. The proliferative response of cord blood mononuclear cells to specific inhalant allergens (birch rBet v1 and timothy grass rPhl p1) was much higher when maternal pollen exposure occurred during the first 6 months of pregnancy (especially at the end of the first trimester) than in the third trimester [12]. Because in humans the migration of T-cell precursors to the epithelial thymus takes place at 7–8.5 weeks of gestation, priming should not be obtained in the first 2 months of pregnancy. CD3+ and TCR β + cells were detected at 8.5 and 9.5 weeks of gestation, respectively. The importance of lymphocyte proliferation in the presence of a potential antigen in the clinical manifestation of allergic symptoms still needs to be fully elucidated. The ability of a molecule to traverse the placenta is associated with its molecular weight, lipid solubility, polarity and ionization, as well as the existence of specific transport mechanisms [10]. Most low molecular weight substances (<500 D) simply diffuse through placental tissue. However, amino acids require specific transport mechanisms. Materno-fetal IgG transport begins at about 16 weeks of gestation, rapidly increasing from 22 weeks of gestation with fetal IgG1 exceeding maternal levels. The transport of maternal IgG offers an ideal vehicle for allergen carriage to the fetus. The inhalant cat allergen, Fed d1 has been detected in IgG complex in up to 40% of infant umbilical cord sera [13]. The ex vivo transplacental transport of cow's milk β -lactoglobulin is enhanced by the addition of human immunoglobulin, and is essential for the transfer of the inhalant birch pollen allergen, Bet v1 [9].

In term newborns, the spontaneous cytokine response of cord blood mononuclear cells is dominated by IL-4, IL-5, IL-10 and transforming growth factor (TGF)- β . There is a clear tendency for infants of atopic mothers to have a higher Th-2 response than infants of non-atopic mothers. Only for TGF- β , the response of infants of atopic mothers is significantly lower than of non-atopic mothers. Spontaneous cytokine-secreting cells and cow's milk-induced responses are virtually absent in the cord blood of infants born at <34 weeks of gestation, but are clearly detectable in infants born at >34 weeks of gestation. Cow's milk antigen-specific IL-4 and TGF- β responses are preferentially observed in memory cells of infants with a maternal history of atopy, which strongly suggests a Th-2 skewing to dietary antigens in utero. This also suggests that there may exist some deficiency in Th-3 cells (the activity of Th-1 and Th-2 cells are regulated by Th-3 cells making TGF- β or Tr cells making IL-10) from children of atopic mothers [14]. Germ-free animals showed impaired tolerance mechanisms. Thus, genetic predisposition skews the immune system towards an inappropriate development in the absence of the right prenatal and/or perinatal and/or postnatal correcting factors such as a 'favorable' maternal gastrointestinal flora or chronic or repetitive infections during early life. The role of prenatal antigens is yet unclear, although today it is justified to emphasize that they may play a role.

According to data from Japan, allergic disease in infants is positively related to family history of atopy, to infant head and chest circumference, to maternal body mass index before pregnancy and at delivery, to maternal intake of lipids and vegetables; allergic disease in infants correlates negatively with birth order, maternal intake of milk, proteins and carbohydrates [15]. The results suggest that a high intake of energy and lipids (fat and vegetable oil) during pregnancy may accelerate allergic diseases in infants [15]. Feeding during the first weeks of life programs the risk of developing later atopic disease [16]. Atopic mothers have a higher intake of total and saturated fat and a lower intake of carbohydrate as a percentage of total energy intake than non-atopic mothers ($p = 0.017$, $p = 0.050$, $p = 0.004$, respectively). Maternal intake of saturated fat during breast-feeding is associated with atopic sensitization of the infant (OR = 1.16; 95% CI 1.001–1.36; $p = 0.048$), regardless of the maternal atopic status. The observation thus extends findings implying that early nutrition programs the subsequent health of the child to the risk of developing atopic disease [16].

Evidence for in utero sensitization to inhalant allergens is currently weak and controversial [9]. Lovegrove et al. [17] investigated the effect of a maternal milk-free diet during late pregnancy and lactation on the immune response and allergy incidence in at-risk and control infants. Atopic mothers were randomly allocated into an intervention group ($n = 12$) or an unrestricted-diet group ($n = 14$) and compared with non-atopic mothers following an unrestricted diet ($n = 12$). The intervention involved a maternal milk-free diet

during late pregnancy and lactation. Infants were followed up for 18 months postnatally. A significant fall in maternal serum β -lactoglobulin-IgG antibody levels ($p < 0.05$) was observed after a 7-week milk-exclusion diet. Single-blind allergy assessment by a pediatrician at 12 and 18 months showed that the infants born in the non-atopic group had a significantly lower allergy incidence compared with the infants born in the atopic group following an unrestricted diet ($p < 0.008$ and $p < 0.02$, respectively). The allergy incidence in infants born in the atopic diet group was significantly lower compared with that of the atopic group following an unrestricted diet ($p < 0.04$). It was observed that the atopic nature of the parents significantly affected the allergy incidence in their children. A trend towards a beneficial effect of a maternal milk-free diet during late pregnancy and lactation was also observed in infants born to atopic parents.

Several birth cohort studies on the avoidance of inhalant and/or food allergens commenced antenatally and continued postnatally [18–20]. Chandra et al. [21] observed a reduction in infant atopic eczema if the mothers followed a diet restricted in milk, dairy products, egg, fish, beef and peanut during pregnancy and lactation. The same group reported similar results if dietary restrictions were limited to lactation, and thus started at birth [22]. Attempts to prevent cow's milk and egg allergy with maternal avoidance during the third trimester failed to reduce food allergy, or any other atopic disorder up to the age of 5 years [23–26]. In the study by Falth-Magnusson and Kjellman [24], eczema, allergic rhinitis and asthma were equally common in all groups. Interestingly, food intolerance to egg was significantly more common in children of the mothers on an exclusion diet. However, there was no overall long-term difference in food intolerance [25]. Lilja et al. [26] could not find any difference in atopic disease, or positive skin prick test for ovalbumin, ovomucoid, β -lactoglobulin and cow's milk in infants born to mothers with a 'very low' dietary intake of cow's milk or daily consumption of 1 egg and 1 liter of milk. Atopic dermatitis and cow's milk and egg sensitization was not reduced in high-risk babies born to mothers who avoided cow's milk and egg during the third trimester of pregnancy [27]. Unfortunately, neither Lilja et al. [25] nor Falth-Magnusson and Kjellman [24] considered the genetic background. Also, it may be that intervention during the last trimester is too late. A cow's milk-free diet in the first or second trimester, or an increased exposure during pregnancy has not been tried. The elimination of early contact of the fetal immune system with (minute amounts of) antigens of dietary proteins may compromise the development of tolerance. A recent Cochrane Review [28], including 4 controlled trials involving 450 pregnant women, did not suggest any protective effect of maternal dietary antigen avoidance during pregnancy on the incidence of atopic eczema (relative risk (RR) 0.94, 95% CI 0.67–1.33) during the first 12–18 months of life in high-risk infants. Data on allergic respiratory manifestations were insufficient to draw meaningful conclusions. The two

Swedish trials considered in the review showed a lower incidence of positive skin prick tests to egg antigen at 6 months of age, but higher cord blood IgE levels in the intervention group. Additionally, atopic manifestations, prick test to cow's milk and prick tests at 18 months did not show any reduction [23, 29].

A diet with reduced allergenic content does not necessarily affect intake of calories, macro- and micronutrients. However, in the absence of professional dietary advice, the risk that a diet with reduced allergenicity during pregnancy would result in imbalanced feeding is real. A diet with reduced allergenicity during pregnancy can be considered as more disadvantageous for possible maternal and fetal malnutrition than eventually advantageous for allergic prophylaxis. However, the situation may be different if specific allergens are avoided such as eggs or peanuts. Grimshaw et al. [30] reported a significantly reduced sensitization rate to egg and inhalant allergens in 18-month-old infants who were included in a program on strict maternal egg avoidance during pregnancy and lactation. The reduced sensitization to egg in particular is encouraging as it represents a major risk factor for the development of asthma. However, it is not clear from the data how many mothers chose to avoid egg for their infants as well. In an epidemiological study of 622 adults and children with suspected peanut allergy Hourihane et al. [31] reported an association between a self-reported increase in maternal consumption of peanut products during pregnancy and lactation and an earlier age of onset of peanut allergy during childhood. It is possible that allergen avoidance may have to commence very early in pregnancy to be effective as a fetus is capable of producing IgE at 11 weeks and T-cell priming at 22 weeks of gestation. Mothers who report the consumption of peanuts more than once a week during pregnancy are more likely to have a peanut-sensitized child than mother consuming fewer peanuts [32]. Interestingly, there is no significant relation between peanut consumption during lactation and peanut sensitization. Peanuts and peanut butter are introduced into the child's diet from a significantly younger age in peanut-sensitized subjects [32].

Other maternal dietary factors such as fat and antioxidant intake may have changed over the years contributing to the raised prevalence of allergy. There is growing interest in the potential role of anti-inflammatory polyunsaturated fatty acids (n-3 PUFAs) in the prevention of allergic disease. Newborns at risk of atopic disease showed significantly lower levels of arachidonic and docosahexaenoic acids in umbilical cord blood than infants who are not at risk [33]. The breast milk of mothers whose children have atopic dermatitis contains reduced levels of prostaglandin E precursor fatty acids and it has been hypothesized that defects in n-6 fatty acid and prostaglandin E metabolism favor the development of atopy. Maternal fish oil supplementation in pregnancy reduces IL-13 levels in the cord blood of infants at high risk of atopy [34]. A diet intervention (oil supplement, margarines, and

cooking oils containing high levels of n-3 fatty acids) during pregnancy in an unselected population of 616 pregnant women resulted at the age of 18 months in a 9.8% absolute reduction (95% CI 1.5–18.1; $p = 0.02$) in the prevalence of any wheeze and a 7.8% absolute reduction (95% CI 0.5–15.1; $p = 0.04$) in the prevalence of wheeze of >1 week, but it had no effect on serum IgE, atopy, or doctor's diagnosis of asthma. The house dust mite avoidance intervention did not affect these outcomes but was associated with a lower use of oral steroids [33]. A randomized, controlled trial in 83 atopic, pregnant women receiving fish oil (3.7 g n-3 PUFAs/day) or placebo from 20 weeks of gestation until delivery showed lower cytokine responses (IL-5, IL-13, IL-10 and IFN- γ) in the supplemented group. Infants in the fish oil group were 3 times less likely to manifest a positive skin prick test to egg at 1 year of age (OR 0.34; $p = 0.055$) although there was no difference in the frequency of atopic dermatitis at 1 year of age [35].

There are only a few published data on the impact of a change in microbial exposure during pregnancy on the child's risk of developing allergic disease. The rationale to consider a possible benefit of probiotic and/or prebiotic supplementation in the maternal diet during pregnancy (and lactation) is based on the differences reported in the flora of atopic versus non-atopic subjects, on the immunological effects of the intestinal flora and on the promising results of the firsts interventional studies. An atopic population has a high prevalence of *Clostridia*, coliforms and *Staphylococcus aureus* versus *Lactobacilli* and *Bifidobacteria* (bifidum) [36–39]. Prenatal probiotics were only administered for 2–4 weeks during pregnancy in the study by Kolliomaki et al. [39]. An ideal 'good' maternal flora might thus influence the establishment of a similar neonatal flora through vaginal delivery and first nursing [40], thus conditioning subsequent colonization and decreasing the likelihood of allergy in later life. Cesarean section has an influence on immune development during the first months of life, but at the age of 5 years no difference can be observed.

Lactobacillus GG has been shown to induce a NF- κ B-mediated response in human macrophages [41]. Additionally, specific probiotics including *Lactobacillus* GG may generate anti-inflammatory IL-10 and TGF- β [42]. The exact components of probiotics exerting immunological effects still need to be clarified. Also, whether the change in maternal flora and immunological markers has an influence on the fetus still has to be demonstrated.

A Finnish placebo-controlled, randomized clinical trial was the first to attempt the prevention of food allergy through supplementation with *Lactobacillus* GG for 2 weeks before delivery and for 6 months to the at-risk infant (through maternal intake as long as breast-feeding, and directly to the infant when formula-feeding started). A 2-fold reduction (23 vs. 46%) in the incidence of atopic dermatitis by age 2 years was reported. No effects were noted, however, on the total IgE levels, specific IgE levels to foods and skin prick tests [39].

Potential immunological strategies for allergy prevention may include modulation of (atopic-prone) cytokines (downregulating IL-5, IL-13...) by competitive cytokines or by specific bacterial/probiotic stimulation or use of monoclonal antibodies against organ-specific integrin (blocking T cells or other inflammatory cells from trafficking to target organs) or against receptors for allergic chemokines (such as antibody against CCR3, the receptor for eotaxin, which is able to block eosinophil migration).

Conclusion

The development of atopic disease is influenced by postnatal, perinatal and very likely also prenatal factors. Of these, the prenatal factors are the least studied, but are likely to exist but only have a borderline impact. Avoidance of multiple food allergens during pregnancy severely restricts what mothers can eat, and causes potential problems with compliance and interpretation of results [23, 24]. Allergy may start during fetal life and the risk of such a development may be amplified by prenatal exposure to allergens. It can be hypothesized that the dietary antigens with which the fetus comes into contact are some of the antigens that may contribute to the development of tolerance or atopic disease. However, the genetic background is most likely to be overruling the impact of fetal contact with dietary or other antigens.

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Discussion

Dr. Vanhorick-Verloove: Do you happen to know if anyone studied these elimination diets starting at conception or even pre-conception? We heard a lot about the pre-conception period and I am not aware of any studies doing this kind of thing very early in pregnancy.

Dr. Vandenplas: Me neither, I think the only one that tried to do that fairly early is the study of Chandra et al. [1], but I don't know exactly when they started. I am not sure but I think they started almost at conception and with negative results. If you look at the development of the immune system, it also makes little sense for allergy to start before the development of the immune system, which is around 16 weeks.

Dr. Di Renzo: You said that the babies born by cesarean section are more prone to atopy compared to vaginal delivery, I presume at term. Can you speculate about the reason, or is it because after a cesarean section the mothers don't breast feed as much as the others for instance?

Dr. Vandenplas: The speculation is that the development of the gastrointestinal tract flora has much to do with that because the gastrointestinal flora of infants born by cesarean section is totally different from that of breast-fed or formula-fed infants. So actually there is a lot of research going on in that area and that is why there is such a lot of interest in pro- and prebiotics in infant feeding because there is a huge difference between gastrointestinal flora with regular formula and breast feeding, and so to mimic the gastrointestinal flora much better with artificial feeding than with breast feeding.

Dr. Di Renzo: So a way of prevention is to decrease the number of cesarean sections?

Dr. Vandenplas: That may be a good form of prevention, yes, I agree.

Dr. Uauy: You said that as far of the preventive scheme if a child was not breast feeding, you would offer hypoallergenic formula. Do you want to comment on that? Are you talking about standard hypoallergenic formula? In fact some recent findings have raised the question whether the best is extensive hydrolyzed hypoallergenic formula.

Dr. Vandenplas: There are two kinds of hypoallergenic formulae, extensive and partial hydrolysates. Amino acid-based formulae should be considered rather as non-allergic formulae. The residual allergenicity of extensive hydrolysates is, per definition, smaller than that of partial hydrolysates. However, the cutoff between 'extensive' and 'partial' is not clear. Extensive hydrolysates are indicated in treatment and prevention.

Partial hydrolysates are only indicated in prevention. The efficacy of hydrolysates used in prevention should only be based on well-designed clinical studies, thus on clinical data. In an at-risk population it has been shown that a reduction in cow's milk protein allergy and a reduction in atopic symptoms, mainly eczema, can be obtained with some hydrolysates by the age of 5 years [2]. However, not all commercialized hydrolysates have enough clinical data to support this claim. Today it is still unclear which is the best in prevention, extensive or partial hydrolysates. In Europe there is a consensus that both partial and extensive hydrolysates can be effective in prevention [3]. Both have been demonstrated to be effective, and both have their benefits and shortcomings. Breast feeding is in fact hypoallergic, it is regular cow's milk formula that is hyperallergic. So, if a breast-fed child needs, for whatever reason, an additional feeding, it would be a pity to stimulate an immunologic reaction with 'hyperallergic feeding'. What we need are hydrolyzed formulae with well-documented clinical efficacy.

Dr. Bleker: We looked for IgE in our first 50-year-old people from the Dutch famine, and just to inform you, there was no relationship with birth weight, there was no relationship with any period of exposure to the Dutch famine. This in addition to your comment on possible intervention in pregnancy.

Dr. Vandenplas: For preventive intervention measurements IgE has been abandoned. It is family history that is used as screening methods. In future what we should also consider is that 30% of the population is atopic. This is a high percent of the population. On the other hand, the best sensitivity and specificity of family history is only 75%, and in absolute numbers there are more allergic children in the not at-risk group. This raises the question whether in future prevention should continue to focus on at-risk groups, or should instead concern the entire population?

Dr. Korzhynskyy: Are there any data about the protective effect of even very short breast feeding. For instance if children are breast fed a very short period, 2 weeks for instance, is there any benefit compared to children who are never breast fed?

Dr. Vandenplas: There is always benefit from breast feeding, even if it is for 3 days. 'Breast is best', and prevention of atopic disease is only one of the benefits of breast feeding. Very short periods of breast feeding do not prevent atopic disease, but, of course, breast feeding is always better than formula feeding.

Dr. Uauy: Can I ask Dr. Lönnerdal a question related to antigen reduction? You studied some of the new protein derivatives removing antigens from foods, rice, etc. Can you comment on where we are in that field? Can we perhaps think about products that will have antigens removed?

Dr. Lönnerdal: Yes, but we haven't looked at atopic disease yet. We have been expressing the recombinant human milk proteins at fairly high levels in rice and we could achieve some of the benefits of the human milk proteins, not all of them but some of them perhaps, and also introduce them in a context, in this case rice, which would most likely have a relatively low degree of allergens. This combination of human milk proteins with the background of rice protein could possibly be a beneficial treatment but we have not done any clinical studies on that yet. I would like to emphasize another thing and I agree with what you said about the use of hypoallergenic formula for the breast-fed infants when there is a high parental history or risk of atopic disease. But we should be aware of the fact, which all formula manufacturers are aware of, that the products that are used and called hypoallergenic usually have been tested clinically with regard to their prevention of atopic disease. Very rarely have they received the same attention when it comes to all the other properties, that is the nutritional properties of those products. We have looked at 3 of those products and all of them are basically off when it comes to protein equivalents: they are usually very high, they are usually imbalanced in amino acid composition, they sometimes have an adverse effect on iron absorption and so on. In this case if such products are going to be used more commonly, I think we have to be a little bit more careful when it comes to the nutritional properties of those products.

Dr. Vandenplas: We fully agree, and I wanted to say exactly the same, that we need good clinical data because it is not the in vitro analysis of the hypoallergenicity of the product which will show clinical efficacy. Nutrition is of course even more important than the effect on the reduction of atopy because hydrolyzed formula is, before everything, food. Regarding prevention, I also think it is important to have a formula with reduced allergenicity but not with non-allergenicity because if an allergen is not introduced, tolerance will not develop. So the right balance between reduction of allergic reaction and induction of tolerance needs to be found. The induction of tolerance is probably the reason why mother's milk contains small amounts of everything the mother eats.

Dr. Uauy: What about the use of killed probiotics, killed bacteria, as one way of providing the immunogenic stimuli without the rest of the biological effects? I think that is somehow related to the rising figures of allergy that we see in developed countries, more than 30% if you go to Scandinavia, some people say 100% of children have allergic manifestation. Probably when we talk about prevention it is no longer an issue of the future but it may be that the common feeding would be an allergy prevention scheme.

Dr. Vandenplas: Your point is very well taken because with regard to probiotics it is clear that strain specificity is very important, and what is found with one strain of lactobacilli cannot be extrapolated to another strain. The most classical definition of probiotics is that they are living or viable microorganisms, but I completely agree with you that to study immunological mechanisms then dead microorganism may be just as useful as living microorganisms. But there are no data to support this hypothesis. This is certainly one of the interesting topics for future research.

Dr. Hornstra: I wonder whether you have an opinion with respect to γ -linolenic acid? It has been said to be involved in one way or another in the programming or deprogramming of atopic eczema. According to recent publications in the *British Medical Journal* [4, 5] and the *British Journal of Dermatology* [6] the case seems closed because vegetable oils rich in γ -linolenic acid were shown to have no benefit at all. On the other hand there are also studies showing that there may be some benefit from high amounts of γ -linolenic acid administered to small infants [7] because this fatty acid is converted into dihomo- γ -linolenic acid which is the precursor of prostaglandin E_1 . Do you have any opinion with respect to this particular possibility?

Dr. Vandenplas: My personal opinion is that this is an area with many contradictions that need to be studied. I don't think the last word has been said about this topic. The relation between the long-chain polyunsaturated fatty acid content of mother's milk and atopic disease in the baby is a very challenging one. So I think it needs to be studied, we cannot make conclusions today.

Dr. Kramer: I don't know this literature very well, but I wonder if you or someone else in the audience could comment on the strength of the evidence showing that so-called hypoallergenic formulas are really hypoallergenic? I would just like to know from the studies how good the evidence is and whether the recommendations to use hypoallergenic formulas are based on good science?

Dr. Uauy: Just on that issue, you probably have not seen the *British Medical Journal* had about 3 articles on this issue last year [8]. It is worth-while notifying other people to read the *British Medical Journal*, we don't need to credit or discredit, but the data on the problems of this literature are there.

Dr. Vandenplas: There have been meta-analyses published regarding the use of partial hydrolysates [9]. To be concrete, I think that in the literature of today there are two formulae which have been really well studied: Nutramigen is an extensive casein hydrolysate, and the partial hydrolysate from Nestlé. There is an ongoing German study, sponsored by the government, comparing the partial hydrolysate from Nestlé, Nutramigen and an extensive hydrolysate from Numico [10]. As far as I recall, the outcome is quite comparable for the partial hydrolysate and the extensive casein hydrolysate. In some subgroups, the extensive hydrolysate scores better, whereas in

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the other subgroups the partial seems best [10]. Both concepts of extensive and partial hydrolysate have advantages and disadvantages.

Dr. Uauy: I think I have a good final point regarding what is a hypoallergenic formula. The only discrepancy is that, based on the physiology, we used to say you need extensive hydrolysates to have an effect and we find that the clinical benefit of partial hydrolysates are identical if not better than the full hydrolysates.

Dr. Vandenas: The European Society of Pediatric Allergy and the European Society of Gastroenterology, Hepatology and Nutrition both clearly state that partial and extensive hydrolysates both have their indications in prevention [3, 11]. Treatment is of course a totally different situation.

Dr. Yin: Your guidelines recommend breast feeding for at least 6 months, but in China, especially in rural areas, women breast feed for 1 or 2 years. Regarding atopic disease, would there be some benefits if we postpone breast feeding and introduce complementary feeding?

Dr. Vandenas: The longer the mother can breast feed the better. Exclusive breast feeding for more than 1 year may cause nutritional deficiencies in the infant. Somewhere between 6 months and 1 year diversification should start. In my country it is common to breast feed for only 2 months, but if breast feeding could be prolonged up to 4 months that would be much better. But there is no problem with breast feeding for 1 year or longer, as long as it is not exclusive.

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