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The lessons of noninterventional and interventional prospective studies on the development of atopic disease during childhood

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Introduction

Over the last 30 years, the prevalence of atopic diseases in childhood (asthma, atopic dermatitis, and allergic rhinoconjunctivitis) has increased considerably in developed countries (1). Although several studies have shown this increase, it has been argued that increased awareness and improved ability to diagnose atopic diseases might account, at least in part, for this increase. However, population-based studies using identical methods of ascertainment at intervals of 10–15 years have also shown a significant increase in the prevalence of atopic diseases in children (2–5).

The development and phenotypic expression of atopic disease depends on an interaction between genetic factors, environmental exposure to food and inhalant allergens, and nonspecific adjuvant factors (e.g., tobacco smoke, air pollution, and infections). The expression of allergic diseases may vary with age, and symptoms may disappear and be replaced by other symptoms. In infancy, the main atopic symptoms are atopic dermatitis, gastrointestinal symptoms, and recurrent wheezing, whereas bronchial asthma and allergic rhinoconjunctivitis are the main problems later in childhood. Adverse reactions to foods, mainly cow's

milk protein, are most common in the first year of life, whereas allergy to inhalant allergens mostly occurs later. Correspondingly, specific IgE antibodies against milk and egg are most frequent during the first 2–3 years of life, whereas specific IgE against inhalant allergens is predominant later in childhood (6). Thus, it is very important to take into consideration the natural course of the disease when evaluating the results from interventional studies on prevention of allergic diseases.

At present, it is estimated that genetic factors account for around 50% of asthma and allergy (7). It is unlikely that a change in genetic factors can explain the increased prevalence of asthma and allergy seen during the last few decades. A further evaluation of the influence of genetic factors is beyond the scope of this review. Likewise, the development of the immune system and the immune response to allergens in fetuses and infants will be covered by other authors in this review series.

When evaluating possible risk factors for the development of allergic diseases and the possible effect of preventive measures, it is important to stress some factors. The study design of both noninterventional and interventional studies should be prospective (true) studies including well-defined diagnostic criteria, a

sufficient duration of follow-up, and a proper sample size for adequate statistical evaluation. Besides, interventional studies should include proper randomization, double blinding, and a control for confounders, as well as proper registration of compliance and follow-up of dropouts.

Due to recall bias and selection bias, retrospective studies should not be used for evaluation of predictive/risk factors for the development of allergic diseases. Likewise, cross-sectional studies are not suitable for assessment of cause-effect relationships between exposure to allergens/adjuvant factors and development of allergic diseases. Generally, it should also be borne in mind that prospective noninterventional studies can be used to generate hypotheses on the relationship between cause and effect in the development of allergic diseases. However, proper confirmation of a possible cause-effect relationship requires demonstration of the causative mechanism and effect of elimination/prevention of the suspected causative factor. Therefore, in the following discussion, mainly the results of proper prospective studies will be considered.

Noninterventional studies

From prospective studies, many possible predictive factors of the development of allergic diseases have been identified. Although it is well documented that atopic heredity is associated with an increased risk of the development of allergic diseases (8–10), it has also been demonstrated that most children who develop atopic diseases during the first years of life come from families without an atopic heredity. Thus, the majority of children who become atopic do not belong to groups at high risk of developing atopic disease (8).

Atopic heredity

It has been shown that around 30% of neonates have at least one parent or older sibling with previous or current atopic disease, around 5% have double parental atopic heredity, and around 10% have one sibling and one parent with atopic heredity. Around 60–65% are without atopic heredity (8–11). In children without atopic heredity, around 10% develop allergic disease, whereas 20–30% of children with single atopic heredity (parent or sibling) and around 40–50% of infants with double parental heredity have been shown to develop atopic disease (8, 11). However, it should be emphasized that the extent of exposure to environmental factors, such as allergens and tobacco smoking, as well as the extent of possible preventive measures, such as breast-feeding and pharmacologic measures, will always heavily influence such estimates.

Infants with a pronounced atopic predisposition may have a primary immunoregulatory defect, which can be identified by various methods, such as elevated

cord-blood IgE, low numbers of T cells, disturbed ratio of T-helper/T-suppressor cells, and decreased function of T-suppressor cells (12). Unfortunately, none of the tests are suitable for general allergy risk screening. However, elevated cord-blood IgE may help to define high-risk groups suitable for allergy-prevention measures (9, 10, 12). At present, the combination of atopic heredity and elevated cord-blood IgE seems to be the best method to define infants at “high risk” of developing atopic disease (8, 9, 12) who may benefit from allergy prevention.

In several studies (13–15), male sex has been associated with an increased risk of asthma, particularly before puberty.

Dietary factors

Breast-feeding. More than 60 years ago, Grulee & Sanford (16) reported that undiluted cow's milk caused a sevenfold increase in the risk of eczema at 9 months of age in a huge cohort of children compared with babies that were breast-fed and fed mixed food. One cohort study has demonstrated an association between early feeding of cow's milk formula and development of cow's milk protein allergy (17). In a Finnish study of nonselected, non-high-risk neonates followed until 17 years of age, breast-feeding was associated with lower rates of eczema and food allergy at 1 and 3 years, as well as a lower “score of respiratory allergy” up to 17 years of age, than in those fed cow's milk formula (18). Another prospective study (19) found that nonatopic children (negative skin prick test) at the age of 6 years who had not been breast-fed had increased risk of wheezing recurrently as compared with breast-fed children (odds ratio 3.03, 95% CI 1.06–8.69), but the same association was not found for atopic children. In a recent Australian study (20), introduction of milk other than breast milk before 4 months of age was a significant risk factor for all asthma and atopy outcomes in children up to 6 years (odds ratio 1.25, 95% CI 1.02–1.52), and for current asthma (odds ratio 1.31, 95% CI 1.05–1.64). Breast-feeding has also been shown to reduce the risk of wheezy bronchitis during infancy (21, 22) as well as the need for hospital admission due to bronchitis or asthma up to 4 years of age, as shown in a case-control study (23).

A possible protective effect of breast-feeding on the development of atopic diseases may be due to either 1) the protective effect of human milk (the constituents) or 2) avoidance of a “high dose” of cow's milk proteins.

The question of whether breast-feeding helps prevent allergy remains controversial due to the lack of evidence in some studies and because infants cannot, ethically, be randomly assigned to breast-feeding or formula feeding to enable a definitive study (24, 25). However, common factors apparent in studies reporting a benefit

of breast-feeding were as follows: 1) prolonged breast-feeding (>4–6 months) and 2) late solid food introduction (after 4 months).

Recent studies on the possible allergy-preventing effect of breast-feeding indicate that variations in the composition of human milk – e.g., low levels of α -linolenic acid and disturbed relationship between the n-3 and the n-6 fatty acids (26), or varying concentrations of cytokines (27) – may explain some of the controversies regarding the protective effect of breast-feeding against allergy. No association between nucleotide and polyamine levels in human milk and atopic development during the first year of life has been found (28). However, prospective long-term follow-up studies with a proper sample size are needed to confirm these possible relationships.

Solid foods. As for the introduction of cow's milk proteins before 4 months of age, the introduction of complementary foods (solid foods) before 4 months of age has been associated with a higher risk of atopic dermatitis until the age of 10 years (29).

Other dietary factors. The results of cross-sectional studies (30–32) suggest that dietary factors such as low intake of fresh fish and omega-3 fatty acids, high sodium intake, or inadequate intake of antioxidants may influence respiratory symptoms and asthma. However, prospective studies are needed to elucidate and confirm these possible causal relationships.

Environmental factors

Inhalant allergens. Airway hyperresponsiveness in children is mostly associated with allergic sensitization. Several studies have demonstrated a relationship between the risk of sensitization and the level of exposure to allergens. There is no generally safe minimum level of exposure. A dose-response relationship between exposure to house-dust mite and development and severity of asthma has been demonstrated (33, 34). In a recent German study, sensitization to allergens of cat and house-dust mite was found to be related in a dose-dependent manner to the level of exposure during the first 3 years of life (35). Other longitudinal studies have shown that sensitization to house-dust mites and animal dander antedates and is a risk factor for development of asthma in children (36, 37). Chronic asthma is mainly associated with indoor allergens, which are more important than the outdoor allergens, probably because we spend most of our time indoors. In a longitudinal follow-up of 881 children to 18 years in New Zealand, children born in winter exhibited a greater prevalence of sensitization to cats and mites (13). Several studies indicate a positive correlation between exposure to pets, especially

during the first year(s) of life, and sensitization and subsequent development of atopic disease (for review, see ref. 38).

The allergens associated with asthma depend on climatic, seasonal, and social factors and housing conditions. In temperate and humid regions, allergy to house-dust mites shows the strongest association with asthma, followed by allergy to furred pets (especially cats). In arid regions of the USA, allergy to the fungus *Alternaria* is important; in urban communities, allergy to cockroach may be important, especially in inner cities. These relationships have been reviewed by Platts-Mills et al. (39).

A clear association between exposure and sensitization, as well as a clear association between sensitization and development of asthma, has been documented. It has been disputed whether or not there is a causal relationship between exposure to allergens and asthma. Is it possible that the inflammation of the airways occurs before sensitization? Our knowledge of the allergy march, wherein the development of atopic dermatitis and food allergy is followed by development of tolerance to foods, and sensitization and clinical allergic symptoms to inhalant allergens, clearly indicates the opposite order of reaction in prospective studies (14, 15, 40–42). Likewise, in infants with atopic dermatitis and wheezing, early sensitization to house-dust mites and/or egg at 6 months of age was found to be associated with active allergic disease (asthma or atopic dermatitis) at 5 years of age (43).

Tobacco smoke. Several studies have shown a significant association between parental (particularly maternal) smoking and increased wheezing and asthma in children (13, 44–46). This association is strongest up to 6 years of age. The severity and frequency of symptoms were found to be related to the extent of exposure in the home (44, 46). Furthermore, passive smoking has been associated with sensitization to indoor allergens in some studies (45–48), but not in others (44). Importantly, maternal smoking during pregnancy is significantly associated with reduced respiratory function in early infancy and recurrent wheezing during infancy and early childhood (49–54).

Domestic air quality. In countries with a so-called Western lifestyle, most people spend more than 95% of their time in well-insulated modern buildings with reduced ventilation. Consequently, an increased load of house-dust mite and mold has become a normal condition (55). Furthermore, an increased burden of particulate materials associated with bio-fuel combustion and smoking is possible, as well as an increased load of chemical vapors and gasses including nitrogen dioxide, formaldehyde, and volatile

organic compounds. There seems to be an increasing significance of such indoor pollutants, although further prospective studies on the possible cause-effect relationship, especially as regards development of atopic respiratory diseases, are warranted (for review, see ref. 56).

Outdoor pollution. There is convincing evidence of a cause-effect relationship between exposure to outdoor pollution and induction of atopic respiratory symptoms. On a possible relationship between outdoor air pollution and development of asthma/atopic airway disease, the findings of many studies are weak or contradictory (for review, see ref. 57) (58, 59).

Immune modulation. Although viral respiratory infections frequently trigger acute exacerbation of asthma, the relationship between such infections and asthma is not clear, in part because of the difficulty in defining asthma in young children (60). There is evidence supporting two different but not mutually exclusive hypotheses:

- 1) predisposed children are susceptible to asthma and severe respiratory tract infections
- 2) severe viral infections may have a long-lasting influence on the subsequent development of asthma.

Recent studies indicate that early viral infections are primarily associated with the so-called infectious type of asthma, which has a more favorable prognosis before 10–11 years of age (61–63). However, early viral infections do not seem to increase the risk of later atopic asthma (60, 63–65).

Family size (number of siblings) has been proposed to be inversely related to the risk of atopy (66), but prospective studies have not confirmed this hypothesis (13, 14).

Recently, it has been suggested that tuberculosis and even BCG (bacille Calmette-Guérin) vaccination might have a preventive effect on the development of asthma and atopy due to a change in the TH1/TH2 balance in favor of the TH1 response (67). However, recent investigations have shown that BCG vaccination does not prevent atopy in children (68, 69). Some authors have investigated the possible influence of other vaccinations, such as that for pertussis, on the development of atopic responses in children (70, 71). At present, there is no evidence of a causal relationship.

Prospective clinical and immunologic studies in infants and young children are urgently required in order to elucidate the possible influence of early infections (type of infection?) and vaccinations on the development of asthma and atopic diseases.

Intestinal microbial flora. It has been proposed that

the intestinal microbial flora influences the development of sensitization (72), but this hypothesis needs to be confirmed by prospective studies.

Other factors. Many other factors have been cited in hypotheses (and speculations) on possible cause-effect relationships or increased susceptibility to the development of asthma and other allergic diseases. Some of these factors, such as low birth weight, day care outside home, and low socioeconomic status, seem to be important (13, 73, 74). However, confirmation by prospective studies is needed. At present, data from cross-sectional studies are contradictory (65, 74).

Interventional studies

Dietary measures

Unselected/non-high-risk infants. Only a few prospective intervention studies have been performed in infants without a hereditary atopic predisposition. In premature unselected infants, Lucas et al. (75) found no difference in development of atopic symptoms or cow's milk allergy (CMA) whether the infants were fed *human bank-milk* or *cow's milk*-based formula. Other studies (76–78) have concluded that cow's milk-based formula given during the first few days of life did not increase the risk of atopic disease until the ages of 2 (78), 4–6 (76), and 14 years (77). However, these studies included intervention diet only for the first few days of life, one (76) included only low-birth-weight infants, and one (77) was not randomized, and its outcome was based on data from medical files and questionnaires obtained at 7, 11, and 14 years. In one study (78), the diagnostic criteria were unspecific, and outcome measures were mainly based on questionnaires. A recent randomized study including a large number ($n=6209$) of full-term, unselected neonates (79) indicated that feeding of cow's milk-based formula at maternity hospital increased the risk of CMA when compared with feeding an extensively hydrolyzed whey formula, but exclusive breast-feeding for 8 weeks did not eliminate the risk of CMA. However, the dietary intervention included only the first 4 days of life, and no data on the diet after that period were given.

High-risk infants. As reviewed in a position paper of ESPACI (80) and a recent joint statement of ESPACI and ESPGHAN (25), prospective studies on high-risk infants have shown a preventive effect of different dietary allergy-prevention programs on the cumulative incidence of food allergy (FA), especially CMA and atopic dermatitis. An effect of dietary allergy prevention has been demonstrated only in high-risk infants, i.e., infants with at least one first-degree

relative (parent or sibling) with documented atopic disease (doctor-diagnosed), possibly combined with elevated cord-blood IgE in the case of single atopic predisposition. Due to great variations in study design and diagnostic criteria, it is difficult to compare the effect of these programs.

Breast-feeding. Previous studies (75, 81–86) have presumed that breast-feeding has an allergy-preventive effect compared with feeding cow's milk formula, but the extent of the preventive effect remains controversial. In high-risk infants, exclusive breast-feeding for at least 4 months in combination with avoidance of solid foods has resulted in a significant reduction of the cumulative incidence of CMA and atopic dermatitis during the first 4 years of life. Ethically, infants cannot be randomly assigned to breast- or formula feeding to enable a definitive study. Thus, confounding factors may highly influence the results of comparisons. There is no conclusive evidence for a protective effect of a maternal exclusion diet during pregnancy (87–89). A few studies indicate that the preventive effect of breast-feeding on the development of atopic dermatitis may be enhanced by maternal avoidance of potential food allergens (milk, egg, and fish) while breast-feeding, but other studies do not confirm this finding (89, 90).

Formulas. Some prospective studies have shown that soy formulas are as allergenic as conventional cow's milk-based formulas, and therefore should not be recommended for the prevention of food allergy (80, 84), but contradictory views have been expressed (14, 91–94). Further studies may clarify the allergenicity of soy formula in infants who are at risk of developing allergy. There is no evidence that formulas based on whole proteins other than cow's milk protein are less allergenic. A few prospective intervention studies (14, 84, 91) have compared the preventive effect of soy formula with cow's milk-based formula in high-risk infants, but none of these included controlled elimination/challenge procedures in order to diagnose food allergy. One such study (14) found no preventive effect of soy formula, whereas another (91) indicated a possible preventive effect of soy formula but only as regards antigen-unspecific atopic symptoms.

Extensively hydrolyzed formulas (eHF) have been investigated in studies on prevention of FA in high-risk infants. Several prospective studies (84, 86, 95–101) show a preventive effect of extensively hydrolyzed formulas in combination with avoidance of cow's milk proteins and solid foods for at least 4 months in high-risk infants on the cumulative incidence of atopic dermatitis and FA, especially

CMA, until the age of 4 years. In two prospective studies (100, 101), the cumulative incidence of FA and CMA was significantly reduced until the ages of 5 and 7 years, respectively. Thus, a real prevention, and not only a postponement of the onset of the disease, was documented.

Partially hydrolyzed formulas (pHF) (with moderately reduced allergenicity) have been investigated in randomized prospective studies (83, 85, 102–106) in high-risk infants, and an allergy-preventive effect has been reported. Because of great variations in study design and diagnostic criteria, the relative efficacies of the different interventions tested in the various studies cannot be compared directly with each other. At present, clinical trials are comparing directly the relative preventive effects of products with highly reduced allergenicity (extensively hydrolyzed) with those of formulas with moderately reduced allergenicity (partially hydrolyzed). Only one such study (107) has been published, and it reported a lower cumulative incidence of atopic symptoms up to the age of 18 months with both an extensive and a partial protein hydrolysate diet, compared with a cow's milk protein-based formula; a greater effect was reported with the extensive hydrolysate. More studies on this issue are needed.

Solid foods. The introduction of complementary foods during the first 4 months of life has been associated with a higher risk of atopic dermatitis (108). A preventive effect of breast-feeding or hydrolyzed formulas has been shown only in studies including avoidance of complementary foods during at least the first 4 months of life.

Duration of the diet. The duration and the dietary restrictions vary in different studies. All studies showing a preventive effect have included solely breast-feeding or extensively hydrolyzed formula and avoidance of cow's milk and solid foods for at least 4–6 months. Studies including restrictive diets for over 12–24 months and studies with dietary restrictions for only 4–6 months have shown comparable results. In one recent study (20), no significant effect of supplement with hydrolysate formula (eHF) compared with cow's milk-based formula after the age of 6 months in breast-fed high-risk infants was found. Controlled studies of the possible preventive effect of avoidance of other potential food allergens such as egg and fish after the age of 4–6 months of life have not been published. Thus, there is no evidence of restrictive diets having an allergy-preventing effect after 6 months of age.

Environmental measures

Exposure and sensitization to indoor allergens are

important risk factors for asthma (for review, see ref. 1). Many studies have tested and shown the efficacy of indoor allergen-avoidance measures, especially as regards house-dust mites in asthmatic children (for review, see refs. 1, 110, 111). Only a few preventive intervention studies have been performed, but, at present, some large environmental intervention studies in infants are underway in Europe. The results of these studies are warranted.

So far, only one prospective, randomized study (112) has investigated the efficacy of the avoidance of indoor allergens (house-dust mites) in high-risk infants followed from birth until the age of 4 years. The intervention included dietary avoidance measures and mite-avoidance measures including encasing of mattresses and acaricides. A significant reduction in sensitization and allergic symptoms at ages 1, 2, 3, and 4 years was found, as well as a significant reduction in the number of children who became sensitized to house-dust mites. A reduction (not statistically significant) was found in the incidence of asthma at 4 years. However, the small number of children and the difficulty of diagnosing asthma in this age group may explain this finding.

Early intervention in atopic infants

A recent prospective, randomized but open study (113) included infants less than 1 year of age with atopic dermatitis and sensitization to egg/milk/soy, but not house-dust mites. This study found that reduction of exposure to house-dust-mite allergens with encasing of mattresses significantly reduced the risk of sensitization to these allergens after 1 year of age.

Recently, a few studies (114, 115) of pharmacologic intervention in young children with atopic dermatitis have reported a significant reduction in the develop-

ment of asthma. In one of these studies (115), this effect was documented only in a subgroup of young children sensitized to grass pollen or house-dust mites.

Conclusion

Prospective noninterventional studies have shown atopic predisposition to be associated with a significantly increased risk of development of atopic disease in childhood. However, the majority of children who develop atopic disease, particularly recurrent wheezing and asthma during early childhood, do not belong to high-risk groups for development of atopic disease. In this age group, male sex has been shown to be an independent risk factor for recurrent wheezing and asthma. There is compelling evidence of at least two different phenotypes of asthma – an infectious type triggered by viral infections, and an atopic type associated with atopic predisposition and sensitization particularly to indoor allergens. The latter type of asthma is also associated with a more unfavorable prognosis.

Breast-feeding and late introduction of solid foods (>4 months) are associated with a reduced risk of food allergy, atopic dermatitis, recurrent wheezing, and asthma. A clear association between exposure to indoor allergens and sensitization, as well as a clear association between sensitization and development of asthma, has been documented, especially in high-risk infants. Several studies have documented a relationship between prenatal, as well as postnatal, exposure to tobacco smoke, and the development of asthma, as well as increased morbidity in asthmatics. The first year of life seems to be an especially vulnerable period, during which several risk factors, such as tobacco smoke and

Table 1. Recommendations based on present knowledge

Infants without a special risk of allergic disease

- No special diet during pregnancy or for the lactating mother
- If breast milk is not sufficient during the first 3–4 days, water is recommended
- Exclusive breast-feeding for at least 4 months; if supplement is needed, conventional cow's milk-based formula is recommended
- Avoidance of solid foods until 4 months of age
- Avoidance of exposure to tobacco smoke – also during pregnancy

Infants with a high risk of allergic disease

Dietary measures

- No special diet during pregnancy or for the lactating mother
- If breast milk is not sufficient during the first 3–4 days, water is recommended
- Exclusive breast-feeding for at least 4 months; if supplement is needed, extensively hydrolyzed formula is recommended
- Avoidance of solid foods until 4–6 months of age

Environmental measures

- Avoid exposure to tobacco smoke – also during pregnancy
- Reduce allergen exposure early in life – house-dust mites, furred pets, cockroaches
- Avoid damp housing conditions
- Increase ventilation

indoor allergens (house-dust mites and molds [often associated with high indoor humidity], and animal danders [especially cat]), seem to act synergistically.

Many other factors have been implicated, mostly from cross-sectional and/or case-control studies. These factors include early infections, vaccinations, intestinal flora, family size (number of siblings and sibling order), and dietary factors such as low intake of n-3 fatty acids and antioxidants, or high sodium intake. At present, there is no convincing evidence of the possible influence of these factors on the development of atopic disease. As regards the possible influence of "indoor pollution" (chemical vapors, gasses, formaldehyde, and volatile organic compounds) and outdoor pollution, there is evidence of a cause-effect relationship between exposure to pollutants and induction of respiratory symptoms, whereas the possible role of pollutants in the development of asthma and atopic airway disease seems less important and needs further elucidation.

Generally, the increased prevalence of atopic diseases in childhood has particularly been found in countries with a so-called Western lifestyle, and there has been an eager search for "the responsible factor". However, atopic diseases are multifactorial, and it appears unlikely that one or a few factors are responsible for the changing prevalence and pattern of atopic diseases. According to a cross-sectional study, a so-called anthroposophic lifestyle (116) seems to be associated

with a lower prevalence of atopy in children. However, prospective studies on the possible influence of such protective anthroposophic lifestyle factors are needed.

At present, data show evidence of the efficacy of dietary allergy-preventive measures as regards FA, especially CMA, and atopic dermatitis. There is no evidence of the efficacy of prenatal dietary intervention during pregnancy.

In planning intervention to prevent the development of allergic diseases, we need to target the factors for which there is valid evidence of their effect, and for which the preventive measures can be controlled. Obviously, an allergy-prevention strategy should include environmental as well as dietary measures. Moreover, it seems reasonable to act on the present evidence and advise parents accordingly, while we continue to test intervention strategies both to evaluate their efficacy and to determine the components which are necessary and which confer the greatest benefits.

In all infants, breast-feeding should be encouraged for 4–6 months; likewise, exposure to tobacco smoke should be avoided during pregnancy as well as during early childhood. In high-risk infants, a documented extensively hydrolyzed formula is recommended if exclusive breast-feeding is not possible for the first 4 months of life. In homes of high-risk infants, current evidence supports measures to reduce the levels of indoor allergens such as those of pets and house-dust mites. Table 1 summarizes our recommendations.

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