

Markers for early sensitization and inflammation in relation to clinical manifestations of atopic disease up to 2 years of age in 133 high-risk children

M. P. LAAN*, M. R. M. BAERT*, A. M. H. BIJL†, A. E. C. M. VREDENDAAL*, F. B. DE WAARD-VAN DER SPEK‡, A. P. ORANJE‡, H. F. J. SAVELKOUL* and H. J. NEIJENS§

*Department of Immunology, Erasmus University and University Hospital, Rotterdam, †Department of Allergology, University Hospital Rotterdam, ‡Department of Dermato-Venereology (Paediatric Dermatology Unit), University Hospital Rotterdam and §Department of Paediatrics, Sophia Children's Hospital, Rotterdam, The Netherlands

Summary

Background The combination of genetic susceptibility and environmental factors induce allergic sensitization and subsequently local inflammation, resulting in atopic manifestations.

Objective To examine whether immunological features reflecting sensitization (total and specific IgE levels, allergen-induced proliferative responses and skin tests) and markers of inflammation (plasma sE-selectin and blood eosinophils) are related to the clinical expression of atopy and whether they precede atopic disease in children up to 2 years of age.

Methods The development of these markers during the first 2 years of life was studied prospectively in 133 newborns at high risk to develop atopic disease.

Results The prevalence of atopic disease increased from 25% at 12 months to 32% at 24 months of age. The children with food allergy at 12 months, who all had atopic dermatitis (AD), turned out to have asthma-like disease in 40% and AD in 100% at the age of 24 months. Total IgE levels increased with time and from 12 months onward levels started to differ markedly between atopics and nonatopics. Food-specific IgE antibodies were significantly associated with AD (relative risk [RR] = 2.39), food (RR = 1.32) and upper-airway allergy (RR = 1.20), and house dust mite-specific IgE antibodies with upper-airway allergy (RR = 5.00). A positive skin test was significantly associated with AD (RR = 2.90) and food allergy (RR = 1.36). The inflammation markers investigated, were not related to the clinical expression or preceded atopic disease at 2 years of age in high-risk children.

Conclusion Positive skin tests and specific IgE to food or inhalant allergens were related to the clinical expression of different atopic diseases. The combination of AD and food allergy at 12 months reflected the strongest risk factor in this high risk cohort for the development of asthma-like disease at 24 months of age.

Keywords: children, eosinophils, prevalence atopy, sE-selectin, skin tests, total and specific IgE

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Introduction

The prevalence of atopic disease is increasing, particularly among children [1]. This creates a necessity to detect

Correspondence: H. F. J. Savelkoul, Department of Immunology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

children at risk of developing atopic disease and to initiate preventive measures. During the first year of life, atopic dermatitis (AD) is the most common atopic disease. Sixty percent of the children who will develop AD start to have symptoms during the first year of life and 85% during the first 5 years [2]. Approximately 20% of children with AD have allergic reactions to food constituents, mostly cow's milk, egg and peanut [3,4]. The natural course of atopic disease gradually evolves from AD with or without food allergy in the first years of life, to asthma and rhinitis in the years thereafter. Similarly, in infancy allergic sensitization occurs predominantly to foods, but in later childhood allergic sensitization to inhalant allergens, such as house dust mite (HDM) and pollen, is more prevalent [5,6].

The development of atopic disease is the result of complex interactions between genetic susceptibility and environmental factors. These interactions can induce allergic sensitization and subsequently lead to local inflammation resulting in clinical symptoms of atopic disease.

Early diagnosis of children at high risk for the development of atopic disease will allow measures to be taken to prevent or diminish the clinical expression of atopic diseases. Therefore, several *in vivo* and *in vitro* tests have to be evaluated for detection of early sensitization and for their role in the early diagnosis of atopic disease in children. IgE is highly associated with atopic disease, but only a minority of children who develop atopic disease are born with increased total IgE levels [7,8]. However, in 80–85% of children who had developed AD the serum total IgE levels were elevated [9,10], and 20% of these children had positive immediate skin tests and serum allergen-specific IgE antibodies to various food or inhalant allergens [3].

Several procedures can be used to test for IgE-mediated food sensitization or allergy. Skin tests and RAST are widely applied, while oral challenge is still the gold standard test for the diagnosis of food allergy [11–18]. The presence of IgE antibodies specific to hen's egg protein during the first year of life is proposed to precede the development of atopic disease during childhood [19,20].

Increased proliferative responses of peripheral blood mononuclear cells (PBMC) to food allergens were found in children who subsequently developed food-sensitized AD [21,22]. CD4⁺ T lymphocytes were found to be the predominant responding cells in the PBMC fraction [23]. Moreover, increased proliferative responses were considered to be a more accurate predictor for atopic disease than elevated cord blood IgE levels [21].

The RAST, proliferative responses of T lymphocytes and skin tests are all tests to detect sensitization to allergens at plasma, cellular and skin level, respectively. Allergic sensitization tends to result in inflammation of the target organs and subsequently in clinical expression of atopic disease. We and others have previously described soluble E-selectin

(sE-selectin) as a marker of skin inflammation related to the clinical severity of the AD [24,25]. In addition, the degree of the eosinophilia has been shown to correlate with the severity of inflammation and the degree of airway hyperresponsiveness [26].

In this longitudinal study we investigated whether the development of the above mentioned markers is related to the clinical expression of atopic disease and whether they precede the atopic manifestation at 24 months of age. To select children with high genetic predisposition for atopy, we included 133 newborns born to atopic mothers. We investigated the time course of total- and specific-IgE levels, allergen-induced proliferative responses, skin tests, sE-selectin levels in plasma and blood eosinophil counts in relation to the development of atopic disease up to 2 years of age.

Materials and methods

Study population

High-risk children were followed from birth until 24 months of age, in order to monitor the development of atopic disease, such as AD, asthma-like disease, food or upper-airway allergy.

All mothers of the newborns were atopic, as established by a questionnaire concerning the clinical history of atopy and current symptoms. Moreover, the atopic symptoms were confirmed by a positive skin test and/or specific IgE to common inhalant and/or food allergens.

The Medical Ethical Committees of the participating hospitals approved this study. Informed consent was obtained from the parents of all children prior to their participation in the study.

In total 133 newborns were included. For various reasons, 23 children dropped out the study before one year of age, and seven children between 1 and 2 years of age. The main reasons for drop out were transport difficulties to the hospital and nonacceptance by the parents of repeated venapunctures of their child.

Physical examinations were performed, questionnaires concerning environmental factors and histories of symptoms of atopic disease were completed and venous blood samples were obtained at ages 0, 6, 12, 18, 24 months. The clinical evaluation during the different visits was made without knowledge of the immunological or skin test results.

Definition of atopic disease

AD was based on Sampson's criteria: erythema, oedema, oozing and excoriation with evidence of itchiness lasting longer than 4 weeks [2]. The severity of the eczema was measured by the objective SCORAD [27].

Asthma is difficult to define in young children. Therefore, we have introduced the term asthma-like disease to classify the complaints that indicate asthmatic symptoms before 2 years of age. This group of asthmatic children include both children with ongoing and temporary respiratory problems after the age of 2 years. Based on criteria of the American Thoracic Society [28] asthma-like disease is diagnosed if children either have had at least three separate wheezing episodes each lasting 3 days or more, or have had more than two separate episodes of paroxysmal nocturnal cough with sleep disturbance for three consecutive nights, or required antiasthma therapy during the last 6 months.

Food allergy was defined as clinical manifestation of erythematous, papular, macrovesicular skin eruptions, diarrhoea, vomiting, or respiratory problems in relation to food ingestion. For the definitive diagnosis a positive skin test or specific IgE was necessary. All children suspected for food allergy were asked for an open oral provocation test to confirm the diagnosis.

Upper-airway allergy was defined as seasonal or perennial rhinoconjunctivitis, symptoms of sneezing, redness, itching and tearing of the nose or eyes and/or respiratory complaints. Diagnosis was confirmed by the presence of specific IgE in the plasma.

Sensitization to food or inhalant allergens was defined as a positive skin test or specific IgE without clinical manifestation.

The term atopic disease was used for high-risk children with clinical manifestations of allergy, such as AD, asthma-like disease, food or upper-airway allergy.

Total and specific IgE and eosinophil count

The total IgE concentrations in all cord blood samples were determined with a modified radio-immuno assay to result in a lower detection limit of 0.2 kU/L. At the other time points, in all plasma samples of the children total IgE was measured using the Pharmacia CAP RAST (Kabi Pharmacia, Uppsala, Sweden). Analysis of specific IgE antibodies directed towards the most common inhalant allergens (HDM, grass and birch pollen, cat, dog and moulds) and food allergens (peanut, chicken egg white (EW), cow's milk (CM), codfish, wheat and (soy) were performed using the Pharmacia CAP system Phadiatop[®] RAST and the Pharmacia CAP RAST (Kabi Pharmacia), respectively. Specific IgE was measured in all plasma samples of the children, whether or not having symptoms after food ingestion or after exposure to inhalant allergens at 12 (91/110 samples) and 24 (88/103 samples) months of age. Additionally, determination of specific IgE in plasma samples obtained from children of 6 (38 samples) and 18 (32 samples) months of age was considered to be relevant in those children with symptoms

of atopic disease, or specific IgE or a positive skin test before 24 months of age.

The percentages of eosinophils were counted in peripheral blood by leucocyte differentiation.

Proliferative responses

From birth up to 24 months of age heparinized blood samples were obtained every 6 months. After diluting 1 : 1 with PBS, PBMC were isolated by density gradient centrifugation over Ficoll-Hypaque (Pharmacia). Lymphocyte proliferation assays were performed as described previously [29]. Briefly, PBMC were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 50 µg/mL streptomycin, 1 mM pyruvate and 10% heat-inactivated human serum in 96-wells round bottom microtitre culture plates. PBMC were cultured in quadruplicate at 2×10^5 cells/well in the presence of *D. pteronyssinus* extract (HDM, 50 µg/mL, ALK-Benelux, Groningen, The Netherlands) or EW extract (50 µg/mL, ALK) or CM extract (25 µg/mL, ALK) or without additions in a total volume of 200 µL. After 7 days of allergen-specific stimulation, proliferation was measured using [³H]-thymidine incorporation. The results are expressed as stimulation index (SI), equal to the ratio of mean c.p.m. in allergen-specific-stimulated cultures over mean c.p.m. in unstimulated cultures. A SI greater than three is considered a positive proliferative response, as is generally accepted.

Determination of sE-selectin

After Ficoll centrifugation, plasma samples were obtained and stored at -70 °C until analysis. sE-selectin was measured at the different time points in plasma by ELISA as described previously [30].

Skin tests and oral provocation test

Between 9 and 12 months of age, two different skin tests, namely the skin application food test (SAFT) and the prick-prick test [17,31], were performed for cow's milk, egg and peanut in 97 children, independent of a history of AD. Only in selected cases other foods were tested in children, on account of a history of complaints to these foods. Children were asked for an oral provocation test, when the skin test was positive.

The SAFT is a reliable and child-friendly skin test, validated for AD in children, more specifically for evaluating (suspected) food allergy in children younger than 4 years [17]. At the Paediatric Dermatology Unit of the Sophia Children's Hospital, the SAFT is usually performed in AD children younger than 3 years to evaluate food allergy. In this cohort, both skin tests were performed in all children, independent of

a history of AD. In the SAFT, the food was applied, in the same condition as usually consumed, on the back of the child, using large Finn chambers. The test was read after 10, 20, and 30 min. The results were scored as follows: 0 = no reaction; 1+ = erythema; 2+ = erythema and oedema within the area of the Finn chamber; 3+ = erythema and oedema up to the outer area of the Finn chamber. Reactions with a score of 2+ or 3+ were regarded as positive.

A microlancet with a 1-mm needle with shoulders (ALK, Benelux) was used in the prick-prick test. The needle was pricked into the food preparation and subsequently vertically into the skin of the lower arm. A drop of histamine dihydrochloride solution (10 mg/mL, ALK, Benelux) was used as positive control and a drop of saline as negative control. The test was read after 15 and 30 min. The reaction to the food was evaluated by comparing it with the reaction to histamine. The reaction to food was considered positive if the average weal diameter was at least 3 mm larger than the negative control.

The oral provocation test was performed in a standardized way at the day-care unit, under appropriate safety conditions. After examination and registration of the blood pressure and pulse rate, a small amount of the food was given to the child. All observed reactions were noted. Positive reactions consisted of an urticarial rash, perioral redness, flare-up of eczema, itching, increased pulse rate, depression of blood pressure, and respiratory or gastrointestinal complaints. If no reactions were observed after 1 h, a double amount of the food was given; after 2 h, the amount was doubled again. After 3 h, the food was given without restrictions. The total amount given correlated to age-related average 'daily intake'. If a positive reaction was observed, the test was stopped. If no reaction was observed, the child was dismissed after 8 h. The parents were urged to contact the dermatologist if any late reaction was observed, in order to evaluate this reaction.

Statistics

Statistical analyses were performed using SPSS for Windows, version 8.0. Parameters were evaluated by computing the relative risk factor (RR) with Cornfield's 95% confidence interval (CI) as a measure of association or parameters were tested using an independent sample *t*-test. The used *t*-test anticipated with a partial skewness of the values. *P*-values less than 0.05 were considered significant.

Results

Prevalence of atopic disease

In this group of high-risk newborns, the prevalence of atopic disease was 16% (18/110 children) at 6 months of age, 25%

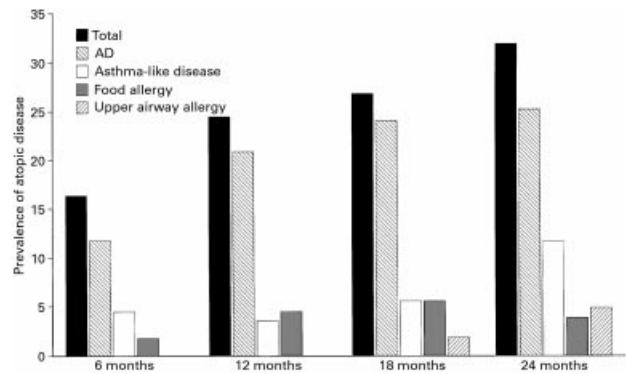


Fig. 1. Prevalence of atopic disease at the consecutive time points tested. Children were assessed for atopic symptoms at 6, 12, 18 and 24 months of age. Results were expressed as the total percentage atopic children at the individual time points or the percentage of the children with atopic dermatitis, asthma-like disease, food allergy and upper airway allergy.

at 12 months of age and increased gradually to 32% (33/103 children) at 24 months of age (Fig. 1). The prevalence of AD increased markedly after birth to 21% at 12 months and 25% at 24 months. The severity of the eczema in these children was in most children classified as mild (objective SCORAD < 15). The prevalence of asthma-like disease started to increase mainly after the first year of life from 4% at 12 months to 12% at 24 months of age. Five children (5%) had a food allergy at 6 months of age, all in combination with AD. The prevalence of food allergy did not show marked changes during the first 24 months of life, whereas the prevalence of upper-airway allergy in the children started to develop after 12 months of life to 4% at 24 months of age.

Of the five children with a food allergy in combination with AD at 12 months, at 24 months two children revealed to have asthma-like disease (40%) and all five children still had AD (100%).

Time course of total IgE levels

Figure 2(a) shows the development of mean total IgE levels between birth and 24 months of age in children whether or not they had developed AD or asthma-like disease at 24 months of age. The mean total IgE levels measured at the consecutive time points increased gradually over time, whereas this increase was more prominent in children with atopic features at 24 months, especially AD. At all time points tested, the difference in total IgE levels between AD or asthma-like disease and nonatopic children just failed to reach significance.

Time course of specific IgE

Specific IgE in plasma of children at 12 months of age was

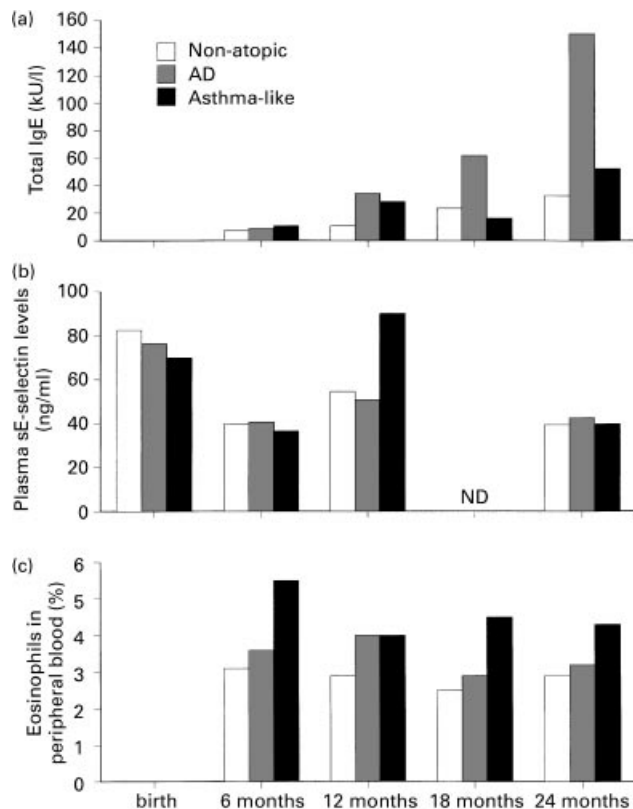


Fig. 2. Total plasma IgE levels (a), sE-selectin levels in plasma (b), and the percentage of eosinophils in peripheral blood (c) at consecutive time points. The results are expressed as mean total plasma IgE levels in kU/l, mean plasma sE-selectin levels in ng/mL, or mean percentage eosinophils in nonatopic children, children with atopic dermatitis, and children with asthma-like disease as defined at 24 months of age. No significant differences were observed between the patient groups and these markers at the different time points.

only found against food allergens, whereas in children at 24 months of age specific IgE against both food and inhalant allergens was observed (Tables 1 and 2). Three out of 12 children (25%) with food-specific IgE revealed

to have clinical symptoms to the corresponding food at the age the specific-IgE was found (Table 1). Out of the five children with HDM-specific IgE, four (80%) had clinical symptoms of an upper-airway allergy. Food and upper-airway allergy was significantly more frequently observed in children with specific IgE to food or inhalant allergens, respectively, compared to children without specific IgE (Table 1).

Food-specific IgE in children before 24 months of age was significantly associated with atopic disease (RR = 2.86), AD (RR = 2.39), food allergy (RR = 1.32) and upper-airway allergy (RR = 1.20) at 24 months of age. Inhalant-specific IgE in children was significantly associated with upper-airway allergy (RR = 5.00) at 24 months of age. We could not assess in our cohort a causal relationship between specific IgE measured before 24 months of age and the development of atopic disease at 24 months.

Time course of plasma sE-selectin levels and blood eosinophil count

At the consecutive time points, sE-selectin levels were not significantly different between children diagnosed as having no atopic disease, AD or asthma-like disease (Fig. 2b). In all patient groups the sE-selectin levels were not significantly higher at birth than at later time points. Between the sE-selectin levels and the development of atopic disease at 24 months of age no associations were observed.

The percentage eosinophils in peripheral blood did not increase with consecutive time points and no significant differences were found between the different patient groups at the consecutive time points (Fig. 2c).

Time course of proliferative responses

In vitro proliferative responses of PBMC to cow's milk, egg-white (Fig. 3) and HDM were not significantly different for the various allergens at any time point tested between children who had or had not developed atopic disease. Atopic

Table 1. Overall results of age-related plasma levels of allergen-specific IgE

	Food† or upper airway allergy	Atopic disease at 24 months‡			
		AD	FA	A	UA
Specific IgE to food allergens (n = 12)	3*	8*	3	3	2
Specific IgE to inhalant allergens (n = 5)	4*	3	1	2	4
No specific IgE§ (n = 84)	2	17	1	8	0

AD, atopic dermatitis; A, asthma-like disease; FA, food allergy; UA, upper-airway allergy. * $P < 0.05$ relative to children without specific IgE. †Number of children with a food or upper-airway allergy corresponding with the specificity of IgE. ‡Number of children with atopic disease at 24 months of age. §At all tested time points no specific-IgE was detectable in these children.

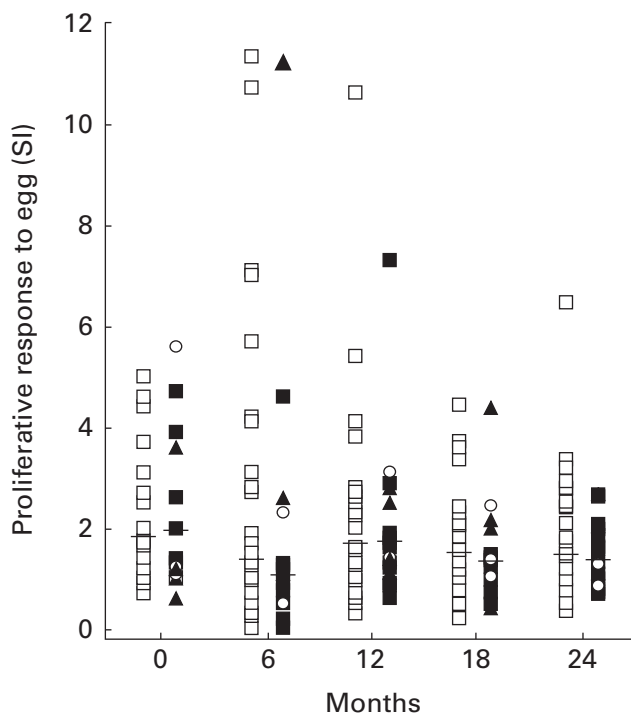


Fig. 3. Proliferative responses of PBMC of children who had developed atopic disease (■, ○ and ▲) and nonatopic children (□). Proliferation of PBMC at the different time points was determined by measuring [^{3} H]-thymidine incorporation on day 7 of culture after stimulation with 50 μ g/mL egg-white extract. Results are expressed as SI and means of the groups are indicated. Each point represents the mean of four from an individual PBMC sample. Atopic children were subdivided in children who had developed clinical symptoms to egg (○), children with specific-IgE against egg-white (▲) and children without clinical symptoms or specific IgE to egg-white (■). No significant differences exist between the patient groups and the different time points.

children who had developed a food or upper-airway allergy or sensitization at any of the different time points, did not differ in their proliferative responses from the other atopic children without a food or upper-airway allergy or sensitization (children with clinical symptoms to egg or with specific IgE to egg-white are marked in Fig. 3). Allergen-specific proliferative responses were not related to the development of atopic disease at 24 months of age.

Skin tests and oral provocation test

A positive skin test for food was observed in 11 out of 97 children tested (11%) (Table 3). Significantly more children with a positive skin test had AD at 12 months (8/11) compared to skin test negative children (15/86). Also significantly more children with a positive skin test had food allergy (4/11) compared to children with a negative skin test (1/86). A positive skin test at 12 months was significantly

associated with atopic disease (RR = 2.86), AD (RR = 2.90) and food allergy (RR = 1.36) at 24 months.

A positive skin test for egg-white or cow's milk could be found in 5% of the children. Out of the 14 positive skin test children, only 5 (36%) were related to the diagnosis of food allergy (Table 4). This discrepancy between a positive skin test and food allergy was mainly due to false positive peanut prick-prick tests. Eight positive prick-prick tests for peanut were observed, while no symptoms induced by this food product were registered.

Oral provocation tests were performed in seven children to evaluate the clinical impact of the skin tests. These provocation tests were negative in all cases related to peanut, whereas in one child with a history of symptoms to egg, peanut and banana, the egg provocation test was positive. Provocation to banana was not performed in this child, because of a strong flare-up of the eczema during the skin test with banana.

Discussion

Detection of children at risk for atopy is important for initiating preventive measures. In 133 newborns at high risk for the development of atopic disease, we studied the development of several markers for allergic sensitization and inflammation at consecutive time points up to 2 years of age. An important difference in the conduct between this and most other studies is that we did not select patient groups retrospectively. This study underscored the relevance of sensitization, but not of inflammatory markers in relation to the clinical expression of atopic disease and whether they preceded atopic manifestations up to 24 months of age.

The prevalence of atopic manifestations (AD, asthma-like disease, food and upper-airway allergy) rose from 16% at 6 months to 25% and 12 months and to 32% at 24 months, which is in agreement with the expected genetic predisposition of 30% in the case of an atopic mother [32]. The prevalence for AD at 1 (21%) and 2 years (25%) of age is in agreement with other studies [33,34]. Most children, who had AD at 24 months of age, had their initial symptoms within their first 12 months. The prevalence for asthma-like disease in our cohort at 1 (4%) and 2 (12%) years was lower than that in the 'Isle of Man study', being 19% and 27%, respectively [33]. This could be due to differences in the selection criteria for high-risk children or the severity of the AD.

AD within the first months of life, mostly the initial expression of atopic disease, is considered to be a risk factor for the development of asthma at later ages [35]. In accordance with Sporik *et al.* [36] we could not confirm AD as a risk factor for asthma. However, our results suggest that the combination of AD and food allergy at 12 months of

Table 2. Individual results of age-related plasma levels of allergen-specific IgE

study number	Allergic* sens.	Specific-IgE (kU/L)				Clinical symptoms†
		6 months	12 months	18 months	24 months	
28	peanut	0.36	ND	–	–	no
101	peanut	1.1	–	–	–	no
61	EW	–	0.58	–	–	no
70	EW	0.56	0.37	0.55	–	yes
91	EW	1.08	0.40	–	ND	no
126	EW	–	–	0.78	0.83	yes
128	EW	2.31	0.47	0.43	–	no
109	EW	0.36	3.39	4.66	16.6	yes
	wheat	0.42	2.81	2.75	8.96	?
	peanut	–	–	0.69	1.54	?
	Soy	–	–	–	0.88	?
40	CM	–	1.19	ND	3.38	no
57	CM	–	–	0.45	1.42	no
113	CM	–	0.79	0.54	–	no
63	CM	–	–	0.46	1.72	no
39	HDM	–	–	–	4.9	yes
40	HDM	–	–	–	15.16	no
57	HDM	–	–	1.7	25.8	yes
109	HDM	–	–	22.7	93.3	yes
119	HDM	–	–	–	1.04	yes

CM, cow's milk; EW, egg-white; HDM, house-dust mite. (–) < 0.35 kU/l; ND, RAST not done; ?, not clear. *Inhalant and food allergens to which IgE was directed. †Clinical symptoms to the food or inhalant allergen corresponding with the specificity of IgE.

Table 3. Overall results of skin test results and atopic symptoms at 12 and 24 months of age

Skin test†	Food allergy‡ corresponding to SPT	Atopic disease at 12 months§				Atopic disease at 24 months§			
		AD	FA	A	UA	AD	FA	A	UA
Positive (<i>n</i> = 11)	4	8*	4*	0	0	8*	3	2	0
Negative (<i>n</i> = 86)	1	15	1	4	0	18	1	10	4

AD, atopic dermatitis; A, asthma-like disease; FA, food allergy; UA, upper-airway allergy; **P* < 0.05. †Skin tests for cow's milk, egg and peanut were performed at the age between 9 and 12 months in all children, independent of having a history of AD. ‡Children with a food allergy as defined in Materials and Methods section, for the corresponding food as positive in the skin test. §Number of children with atopic disease at 12 or 24 months of age.

age was a risk factor for asthma-like disease at 24 months, although the sample size of children who had a food allergy was too small for definite recommendations.

Several studies have demonstrated that early and long-lasting sensitization to food and inhalant allergens must be regarded as a risk factor for the development of atopic disease [20,23,35]. Allergic sensitization is frequently asymptomatic, but sensitization is generally considered the first step in developing atopic disease [6,37].

Between 12 and 24 months the IgE levels increased markedly in AD and gradually in the nonatopic children, resulting in an increasing difference between the total IgE levels. Surprisingly, the increase of the total IgE levels is much stronger in AD children than in asthma-like disease children. This could be explained by clinical heterogeneity in the group of children with asthma-like disease or may be related to disease progression in the affected target organs.

Table 4. Individual results of skin test results and atopic symptoms at 12 and 24 months of age

Skin test†	Positive test‡	Food allergy§
Pr-Pr	EW 3 Peanut 8	3/3 0/8
SAFT	CM 1	1/1
Pr-Pr plus SAFT	EW 1 Banana 1	0/1 1/1

Pr-Pr, prick-prick test; SAFT, skin application food test; CM, cow's milk; EW = egg-white. †Skin tests for cow's milk, egg and peanut were performed at the age between 9 and 12 months in all children, independent of having a history of AD. ‡Number of food positive skin tests. Two children had more than one positive test: one had a positive prick-prick tests for egg and peanut and another had a positive prick-prick tests for egg, peanut and banana in combination with a positive SAFT for banana. §Children with a food allergy as defined in Materials and Methods section, for the corresponding food as positive in the skin test.

The clinical heterogeneity in both groups of children, atopic and nonatopic, may influence the impact of these total IgE levels, as well as of the other markers. The nonatopic group of children can potentially be divided in children who will not develop atopic disease and in children who at 2 years have not yet developed atopic disease. The heterogeneity in the atopic group of children is related to the observation that before the age of 2 years it is difficult to distinguish between temporary and persistent atopic symptoms, especially for asthmatic features [38]. This implies that in the wheezing group a part of the children will appear to be free of such symptoms and mostly nonatopic some years later. Clinical implications of total IgE levels and the other markers as risk factors for the development of atopic disease will become more obvious after longer follow-up.

In agreement with the MAS-90 study, specific IgE antibodies in children before the age of 2 years were predominantly observed against food allergens [39]. Out of the children with food-specific IgE antibodies, however, only 25% had a clinically relevant food allergy. This percentage is in agreement with previous studies, showing that only 30% to 40% of patients with food-specific IgE experienced clinical symptoms after ingestion of the food-at-risk [10,16].

Food-specific IgE antibodies were significantly associated with the clinical expression of AD, food and upper-airway allergy, and HDM-specific IgE antibodies with upper-airway allergy. However, we could not detect that specific IgE preceded the clinical expressions of atopic disease in our cohort, as most children with atopy at 24 months had that atopic manifestation already before

24 months. This is in contrast with the findings of Lilja *et al.* [16] showing that three out of four children with detectable specific IgE to food at 6 months of age, developed clinical symptoms of atopic disease before 18 months of age.

In 5% of the children a positive skin test was observed for egg or milk, as is in line with other studies [32,40]. A positive skin test was related to the clinical expression of AD and food allergy. With regard to specific IgE, we could not detect that a positive skin test preceded the clinical expressions of atopic disease. Skin sensitivity and IgE antibodies to egg in the first year of life have been reported to be associated with upper-airway allergy at 7 years of age [19,40]. This predictive effect over prolonged periods is important and underscores the further follow up of our cohort.

We found that different markers related to inflammation, plasma sE-selectin levels and blood eosinophil counts, were not related to or would precede the clinical expression of atopic disease in children before 2 years of age. This could imply that the development of the inflammatory process has not (yet) resulted in the presence of these markers in the circulation. This may be a consequence of level of symptoms, since the determinations were performed in children without exacerbations of their atopic disease at the moment of examination.

In conclusion, in these genetically predisposed children the total prevalence of atopic disease at 2 years of age was 32%. The prevalence of AD increased markedly during the first 12 months, while asthma-like disease started to increase mainly after 12 months of age. Markers for atopic disease were a positive skin test in relation to the clinical expression of AD and food allergy. Food-specific IgE antibodies were associated with AD, food and upper-airway allergy, and HDM-specific IgE antibodies with upper-airway allergy. Moreover, the combination of AD and food allergy at 12 months is the strongest risk factor in our cohort for the development of asthma-like disease at 24 months of age.

None of the markers for allergic sensitization or inflammation could be shown to precede the clinical expression of atopic disease at 24 months. This may be the case in follow-up after 2 years of age as in most other studies where markers such as skin tests or specific IgE were claimed to be useful in predicting atopic disease, the subjects studied were older than 2 years [5,6,37,40].

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References

- Nakagomi T, Itaya H, Tominaga T, Yamaki M, Hisamatsu S, Nagagomi O. Is atopy increasing? *Lancet* 1994; 343:121–2.
- Sampson HA. Pathogenesis of eczema. *Clin Exp Allergy* 1990; 20:459–67.
- Sampson HA. The immunopathogenic role of food hypersensitivity in atopic dermatitis. *Acta Derm Venereol* 1992; 176:S34–7.
- Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. *J Pediatr* 1988; 113:447–51.
- Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. *J Allergy Clin Immunol* 1995; 95:1179–90.
- Sigurs N, Hattevig G, Kjellman N-IM, Nilsson L, Björkstén B. Appearance of atopic disease in relation to serum IgE antibodies in children followed from birth for 4–15 years. *J Allergy Clin Immunol* 1994; 94:757–63.
- Hide DW, Arshad SH, Twiselton R, Stevens M. Cord serum IgE: an insensitive method for the prediction of atopy. *Clin Exp Allergy* 1991; 21:739–43.
- Ruiz RGG, Richards D, Kemeny DM, Price JF. Neonatal IgE: a poor screen for atopic disease. *Clin Exp Allergy* 1991; 21:467–72.
- Johnson EE, Irons IS, Patterson R, Roberts M. Serum IgE concentration in atopic dermatitis. Relationship to severity of disease and presence of atopic respiratory disease. *J Allergy Clin Immunol* 1974; 54:94–9.
- Sampson HA. The role of food allergy and mediator release in atopic dermatitis. *J Allergy Clin Immunol* 1988; 81:635–45.
- Niggeman B, Sielaff B, Beyer K, Binder C, Wahn U. Outcome of double-blind, placebo-controlled food challenge tests in 107 children with atopic dermatitis. *Clin Exp Allergy* 1999; 29:91–6.
- Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *J Pediatr* 1990; 117:561–7.
- Hide DW. Food allergy in children. *Clin Exp Allergy* 1994; 24:1–2.
- Rancé F, Juchet A, Brémont F, Dutau G. Correlations between skin prick tests using commercial extracts and fresh foods, specific IgE, and food challenges. *Allergy* 1997; 52:1031–5.
- Kjellman N-IM, Björkstén B, Hattevig G, Falth-Magnusson K. Natural history of food allergy. *Ann Allergy* 1988; 61:83–7.
- Lilja G, Oman H. Prediction of atopic disease in infancy by determination of immunological parameters: IgE, IgE- and IgG-antibodies to food allergens, skin prick tests and T lymphocyte subsets. *Pediatr Allergy Immunol* 1991; 2:6–13.
- De Waard-van der Spek FB, Elst EF, Mulder PGH, Munte K, Devillers ACA, Oranje AP. Diagnostic tests in children with atopic dermatitis and food allergy. *Allergy* 1998; 53:1–5.
- Sampson HA, Albergo R. Comparison of results of skin tests, RAST, and placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984; 74:26–33.
- Hattevig G, Kjellman B, Björkstén B. Clinical symptoms and IgE responses to common food proteins and inhalants in the first 7 years of life. *Clin Allergy* 1987; 17:571–8.
- Nickel R, Kulig M, Forster J, Bergmann R, Bauer CP, Lau *et al.* Sensitisation to hen's egg at the age of twelve months is predictive for allergic sensitisation to common indoor and outdoor allergens at the age of three years. *J Allergy Clin Immunol* 1997; 99:613–7.
- Kondo N, Kobayashi Y, Shinoda S, Kasahara K, Kameyama T, Iwasa S, Orii T. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67:1003–7.
- Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO. Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996; 26:780–8.
- Kondo N, Fukutomi O, Agata H, Motoyoshi F, Shinoda S, Kobayashi Y, *et al.* The role of T lymphocytes in patients with food-sensitive atopic dermatitis. *J Allergy Clin Immunol* 1993; 91:658–68.
- Laan MP, Koning H, Baert MRM, Oranje AP, Buurman WA, *et al.* Levels of soluble intercellular adhesion molecule-1, soluble E-selectin, tumor necrosis factor- α , and soluble tumor necrosis factor receptor p55 and p75 in atopic children. *Allergy* 1998; 53:51–8.
- Wolkerstorfer A, Laan MP, Savelkoul HFJ, Neijens HJ, Mulder PGH, Oudesluys-Murphy AM, *et al.* Soluble E-selectin, other markers of inflammation and disease severity in children with atopic dermatitis. *Br J Dermatol* 1998; 138:431–5.
- Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, *et al.* Eosinophilic inflammation in asthma. *N Eng J Med* 1990; 323:1033–9.
- Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD-index: Consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997; 195:10–9.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
- Laan MP, Baert MRM, Vredendaal AECM, Savelkoul HFJ. Differential mRNA expression and production of IL-4 and IFN- γ in stimulated PBMC of house-dust mite allergic patients. *Eur Cyt Network* 1998; 9:75–84.
- Leeuwenberg JFM, Smeets EF, Neefjes JJ, Ahern TJ, Jeunhomme TMAA, Cinek T, Buurman WA. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology* 1992; 77:543–9.

- 31 Oranje AP, Van Gysel D, Mulder PGH, Dieges PH. Food-induced contact urticaria syndrome (CUS) in atopic dermatitis: reproducibility of repeated and duplicated testing with a skin provocation test, the skin application food test (SAFT). *Cont Derm* 1994; 31:314–8.
- 32 Kjellman N-IM, Nilsson L. Genetic factors in the development of allergy. In: Schatz M, Zeiger R. eds. *Asthma and Allergy in Pregnancy and Early Infancy* 1993: 371–93.
- 33 Hide DW, Matthews S, Tariq S, Arshad SH. Allergen avoidance in infancy and allergy at 4 years of age. *Allergy* 1996; 51:89–93.
- 34 Halken S, Host A, Hansen LG, Osterballe O. Effect of an allergy prevention programme on incidence of atopic symptoms in infancy. A prospective study of 159 high-risk infants. *Allergy* 1992; 47:545–53.
- 35 Bergmann RL, Edenharter G, Bergmann KE, Forster J, Bauer CP, Wahn V, *et al.* Atopic dermatitis in early infancy predicts allergic airway disease at 5 years. *Clin Exp Allergy* 1998; 28:965–70.
- 36 Sporik R, Holgate ST, Cogswell JJ. Natural history of asthma in childhood – birth cohort study. *Arch Dis Child* 1991; 66:1050–3.
- 37 Kulig M, Bergmann R, Niggemann B, Burow G, Wahn U, The Mas Study Group. Prediction of sensitisation to inhalant allergens in childhood: evaluating family history, atopic dermatitis and sensitisation to food allergens. *Clin Exp Allergy* 1998; 28:1397–403.
- 38 Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. *J Allergy Clin Immunol* 1998; 102:915–20.
- 39 Bergmann RL, Bergmann KE, Lau-Schadensdorf S, Luck W, Dannemann A, Bauer CP, *et al.* Atopic diseases in infancy. The German multicenter atopy study (MAS-90). *Pediatr Allergy Immunol* 1994; 5 (Suppl. 1):19–25.
- 40 Burr ML, Merrett TG, Dunstan FDJ, Maguire MJ. The development of allergy in high-risk children. *Clin Exp Allergy* 1997; 27:1247–53.