

Patterns of sensitization in infants and its relation to atopic dermatitis

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Longitudinal studies in infant populations using validated diagnostic criteria of atopic dermatitis and sensitization are rarely reported, and disease definitions, testing procedures, age of study population and evaluation of objective markers vary between countries and studies. The objectives of this prospective birth cohort study were to investigate: (i) the prevalence, the cumulative incidence and the pattern of transient and persistent sensitization to common food- and aeroallergens in unselected infants, (ii) the association between sensitization and the development of atopic dermatitis (AD) and (iii) the association between selected perinatal risk factors with respect to AD and post-natal sensitization. During a one-year period a cohort of 562 unselected newborns was established and followed up at the age of 3, 6, 9, 12 and 18 months of age. At all time points infants were examined clinically and by histamine release (HR), total- and specific immunoglobulin E (IgE) and skin prick test (SPT). Sensitization ever to ≥ 1 allergen at 18 months of age was 59%, 50% and 6% using HR, IgE and SPT, respectively. A transient sensitization to ≥ 1 allergen was found in 47%, 42% and 4% and a persistent sensitization in 17%, 10% and 3%, respectively. Sensitization to environmental allergens was frequently observed in infancy when testing with HR and IgE. Results of SPT gave much lower frequencies. Reactivity to foods was more frequent than to aeroallergens. The dominant pattern was low-level transient sensitization. This is important to know when sensitization tests are used in the course of examination of infants with eczematous skin diseases. Specific definitions of sensitization like persistent reactivity, high-level sensitization and poly sensitization were clearly associated with AD. A maternal history of AD was a valuable tool in predicting AD in early infancy; a similar finding was less obvious with regard to post-natal sensitization. Cord-specific IgE and cord-HR positive reactivity did not prove better tools than cord-total IgE in predicting AD within the first 18 living months.

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The 'atopic syndrome' is defined as a personal or familial tendency to produce immunoglobulin E (IgE) antibodies in response to low doses of allergens, and to develop typical symptoms of asthma, rhinoconjunctivitis and atopic dermatitis (AD) (1). Environmental allergens play a role in facilitating the development of atopic diseases and atopic sensitization. A complex interplay between the environment and a genetically

determined atopic propensity is most likely determinant for development and progression of atopic diseases (2).

As atopic diseases have increased two-three fold since the 1960s, especially in the Western world countries, several studies concentrate on specific markers for early identification of individuals at risk. Elevated cord IgE and family history have been proposed as important

markers of atopic diseases (3–6), especially essential for primary as well as secondary allergy prevention strategies.

Sensitizations to food allergens, particularly to hen's egg in early childhood have been suggested to be strongly associated with atopic diseases such as childhood asthma. Children with persistent sensitization were at an increased risk of being asthmatic, whereas an early and transient reaction conferred no significant risk (7).

A division of dermatitis into an allergic and non-allergic type is now suggested (1, 8). According to the new nomenclature dermatitis is divided into eczema, contact eczema or other dermatitis. Eczema can be divided into atopic eczema (IgE-associated) or non-atopic eczema (non-IgE-associated). In this report the term atopic dermatitis is selected due to the application of the Hanifin and Rajka criteria (9, 10).

Longitudinal studies in infant populations using validated diagnostic criteria of atopic dermatitis and sensitization are rarely reported, and disease definitions, testing procedures, age of study population and evaluation of objective markers vary between countries and studies.

The aims of the present study were (i) to describe the pattern of sensitization to common food- and aeroallergens in a group of unselected infants followed prospectively from birth to the age of 18 months, (ii) to identify atopic infants using different methods to detect sensitization, (iii) to investigate the associations between high level-, transient- and persistent sensitization and the development of AD and (iv) to evaluate the association between some perinatal risk factors and selected atopic phenotypes.

Methods

Study population

The Danish Allergy Research Centre cohort ('DARC birth cohort') (11), a prospective birth cohort study, recruited 562 mature unselected newborns from November 1998 to November 1999 at Odense University Hospital, Denmark. The infants were followed up at the age of 3, 6, 9, 12 and 18 months of age. At recruitment, parents were interviewed and information was obtained regarding self-reported family history of atopic diseases (atopic dermatitis, asthma and/or hay fever) ever diagnosed by a doctor. Parents were offered a skin prick test (SPT) to 10 common aeroallergens. Cord blood for the determination of total IgE, specific IgE and histamine release (HR) was collected routinely. At each follow-up a physical examination,

blood-sampling and SPT was performed. All infants were offered the same test procedures. Blood samples obtained varied in dependence of the method due to problems in obtaining a blood sample in some infants.

Skin prick test

SPT was performed as a skin 'prick-prick' on the volar aspect of the forearms with a 1 mm lancet (ALK- ABELLÓ, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/ml) and diluents were used as positive and negative controls, respectively. The wheal reactions were read after 10 min, outlined with a marker and transferred to paper with translucent tape. The largest diameter (D) and its perpendicular (d) of each wheal were measured in millimetre. The results were given as mean diameters using the formula: $(D + d)/2$. SPT was performed with cow's milk, hen's egg, dog, cat, house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides Farinae*). From the 12 months follow-up birch, grass (timothy), mugwort, *Alternaria alternata*, *Cladosporium herbarum* and horse dander was tested. Fresh cow's milk (3.4 g protein/100 ml) was tested at each time point. Due to a high number of small-sized wheals, only mean wheal diameter ≥ 2 mm bigger than the negative control after 10 min were used in the later analysis.

IgE-antibody measurement

Total IgE levels and specific IgE to cow's milk, egg white, codfish, wheat, peanut, grass (timothy), birch, mugwort, cat, dog, horse, house dust mite (*D. Pteronyssinus*, *D. Farinae*), *Alternaria alternata*, *Cladosporium herbarum* were determined. Specimens were analysed at the Department of Clinical Chemistry, University Hospital of Odense, Denmark. Blood samples were frozen and stored at -86°C until analysed by Magic Lite (ALK ABELLÓ, Denmark). Total IgE levels in cord blood were analysed by categorizing in < 0.3 , ≥ 0.3 IU/ml. Measurable specific IgE was determined as $\geq$ class 1 (≥ 1.43 SU/ml) and $\geq$ class 2 (≥ 4.00 SU/ml).

Histamine release from basophiles

Histamine release from basophile leukocytes was performed by a glass-fibre-based histamine assay (12). Cow's milk, egg, grass (phleum pratense), birch (*betula verrucosa*) and house dust mite (*D. pteronyssinus*) were analysed. The following definitions were used to describe histamine release: (i) Basophil releasability: the maximum

histamine (ng/ml) released at any concentration and (ii) Basophil sensitivity: the lowest concentration of allergen or anti-IgE giving > 15 ng/ml of HR.

Definitions

Atopic dermatitis was defined as suggested by Hanifin-Rajka (9, 10) and was the number of infants ever diagnosed AD the first 18 months of life. A parental history of atopy was defined as a self-reported history of asthma, hay fever and/or atopic dermatitis ever diagnosed by a doctor. A parental positive SPT was defined as positive, if mean wheal diameter to ≥ 1 of 10 common aeroallergens was ≥ 3 mm bigger than the negative control after 10 min. The *prevalence* of sensitization was defined as the proportion of infants sensitized at a certain time point, and the *cumulative incidence* as the proportion of infant's sensitized $\geq$ once during the first 18 months of life.

An infant was defined as (i) *never sensitized*, if reactivity within each method was never found to any of the investigated allergens, as (ii) *transiently sensitized*, if all allergens showed maximally a singular reactivity ever, as (iii) *persistently sensitized*, if at least for one allergen reactivity was never followed by a negative test result in the following obtainable blood tests, as (iv) *mono-sensitized*, if reactivity occurred to one allergen only, as (v) *poly sensitized*, if reactivity occurred to two or more allergens, and as (vi) *high-level-sensitized*, if measurable specific IgE was determined as $\geq$ class 2 and HR as $\geq$ class 2.

Statistical analysis and data banking

All data were entered twice in two separate Microsoft Access databases (Office 2000, Microsoft Corporation, Washington, DC, USA). A comparison of raw data was performed. Discrepancies were corrected manually. Statistical analysis was performed with STATA 7.0 for windows (STATA Corporation, TX, USA). The associations between criteria of AD and investigated factors of atopic history and sensitization were expressed as odds ratios with 95% confidence interval. A significance level of 0.05 was used.

Ethical aspects

Written and informed consent was obtained from all parents. The study was approved by the Human Research Ethics Committee for Funen and Vejle County (#19980102).

Results

Test material

AD ever was diagnosed at least once in 61/553 (11%) infants examined ≥ 1 by SPT and IgE. 553 infants were SPT tested $\geq 1\times$ (girls 274, boys 279), 527 (girls 261, boys 266) $\geq 2\times$, 504 (girls 252, boys 252) $\geq 3\times$, 456 children (girls 228, boys 228) $\geq 4\times$. Mean wheal diameter ($D + d/2$) at 3, 6, 12 and 18 months of all positive reactions ≥ 2 mm were 2.9, 3.2, 3.8 and 3.6 mm; of all positive reactions ≥ 3 mm 3.8, 3.7, 4.5 and 4.3 mm, respectively. The mean wheal diameter and lower and upper 5% percentiles of the positive histamine control increased steadily during study period (Table 1).

Excluding results from cord blood, specific IgE measurements were performed in 501 infants (girls 247, boys 254) $\geq 1\times$, 445 (girls 223, boys 222) $\geq 2\times$, 365 (girls 183, boys 182) $\geq 3\times$, 232 (girls 119, boys 113) $\geq 4\times$ and using HR 418 infants were tested at $\geq 1\times$ (girls 205, boys 213), 289 $\geq 2\times$ (girls 134, boys 155); 150 $\geq 3\times$ (girls 78, boys 72); 38 $\geq 4\times$ (girls 19, boys 19).

Prevalence of IgE, HR and Total IgE in cord blood

Specific IgE to ≥ 1 allergen was found in 52 of 221 girls (23.5%), in 59 of 227 boys (26.0%) $\geq$ class 1; in 11 girls (5.0%) and in 16 boys (7.0%) $\geq$ class 2. HR to ≥ 1 allergen was found in 64 of 181 girls (35.4%) and in 66 of 170 boys (38.8%) $\geq$ class 1; in six girls (3.3%) and in 9 boys (5.3%) $\geq$ class 2. Total IgE ≥ 0.1 IU/ml was found in 40.4% of the girls and in 55.2% of the boys, and ≥ 0.3 IU/ml was detected in 34 of 223 girls (15.2%); in 62 of 230 boys (27.0%).

Prevalence at 3, 6, 12 and 18 months of age

The frequency of positive reactivity to any allergen, to two or more allergens, to any food allergens or aeroallergens is presented in Table 1. Within each method mono-sensitization was the most frequent pattern of sensitization at each time point. Sensitization to two or more allergens was detectable at 3 months of age. In a few infants reactivity to three or more allergens was observed starting at the 6 months follow-up with a detection limit of $\geq$ class 2 with respect to IgE or a cut-off limit of ≥ 3 mm using the SPT.

At each time point reactivity to ≥ 1 food allergen was consistently much more frequent than reactivity to ≥ 1 aeroallergen. The frequencies of sensitization at the lowest detection limit were distinctly higher than frequencies at a higher cut limit, but the difference was smallest for SPT.

Table 1. The prevalence (%) of positive reactivity using skin prick test (SPT), specific immunoglobulin E (IgE) and histamine release (HR) at 3, 6, 12 and 18 months of age

Age	SPT							
	3 months		6 months		12 months		18 months	
	502		482		467		430	
Wheal size	≥2 mm	≥3 mm	≥2 mm	≥3 mm	≥2 mm	≥3 mm	≥2 mm	≥3 mm
1 Allergen	1.4	0.4	3.1	2.3	5.6	3.9	2.6	2.1
≥1 Allergen	2.0	0.8	5.0	3.1	7.7	5.4	5.6	3.7
≥2 Allergen	0.6	0.4	1.9	0.8	2.1	1.5	3.0	1.6
≥3 Allergen	0.0	0.0	0.4	0.4	1.1	0.9	1.9	0.9
≥1 Food	1.2	0.8	4.8	3.1	6.6	4.3	4.2	3.2
≥1 Aero	0.8	0.0	0.8	0.2	3.0	1.9	3.7	1.6
Hen's egg	0.6	0.4	3.1	1.9	6.0	3.6	3.5	2.6
Milk	0.6	0.4	1.7	0.8	1.2	1.2	1.0	0.7
Fresh milk	0.6	0.4	2.1	1.6	1.5	1.3	1.2	1.2
Mite pterony	0.0	0.0	0.0	0.0	0.2	0.2	0.7	0.5
Mean SPT	2.9	3.8	3.2	3.7	3.8	4.5	3.6	4.3
Mean of Histamine	2.8 (1.5–4.5)		3.0 (1.5–4.5)		3.4 (2.0–6.7)		3.6 (1.9–6.7)	

Tested infants	IgE and HR															
	IgE: 443		HR: 154		IgE: 381		HR: 176		IgE: 389		HR: 297		IgE: 331		HR: 269	
	≥class 1		≥class 2		≥class 1		≥class 2		≥class 1		≥class 2		≥class 1		≥class 2	
Class	IgE	HR	IgE	HR	IgE	HR	IgE	HR	IgE	HR	IgE	HR	IgE	HR	IgE	HR
1 Allergen	17.6	22.1	5.4	2.6	18.9	31.3	7.4	4.5	14.7	29.3	4.9	3.7	17.8	30.9	6.0	4.1
≥1 Allergen	22.6	37.0	5.9	4.5	25.2	41.5	8.9	5.7	21.3	39.4	7.2	7.4	24.7	37.5	8.5	6.7
≥2 Allergen	5.0	14.9	0.5	1.9	6.3	10.2	1.6	1.1	6.7	10.1	2.3	3.7	6.7	6.7	2.4	2.6
≥3 Allergen	1.1	1.9	0.0	0.6	1.6	1.1	0.5	0.6	2.9	2.7	1.0	1.3	3.3	2.2	0.9	1.9
≥1 Food	15.8	35.7	2.3	4.5	19.4	39.2	6.0	5.1	20.3	36.0	6.9	7.4	21.5	36.1	7.6	6.3
≥1 Aero	9.3	5.2	3.8	0.6	8.7	4.5	3.1	1.1	3.1	8.1	0.8	1.7	6.3	4.1	2.4	2.2
Hen's egg	2.7	33.8	1.1	3.9	7.9	34.7	3.4	3.4	9.8	29.6	3.6	5.7	10.0	32.7	6.1	5.2
Milk	1.8	14.9	0.7	2.6	2.9	13.6	1.6	2.8	3.6	14.5	1.3	5.1	3.9	9.3	0.9	3.7
Mite pterony	2.9	0.6	1.4	0.0	1.6	1.1	0.5	0.6	0.5	2.7	0.5	0.7	0.3	1.5	0.6	1.1
Peanut	0.9	NT	0.2	NT	3.3	NT	1.6	NT	2.6	NT	1.6	NT	2.2	NT	1.2	NT

Prevalence of allergens at 3, 6, 12 and 18 month of age not demonstrated in Table 1.

SPT (≥2 mm/≥3 mm), mite farinae, cat, dog, horse, birch, grass (≤ 0.9/ ≤ 0.9), alternaria, cladosporium, mugwort (0); IgE (≥class 1/≥ class 2), wheat (≤ 11.5/ ≤ 0.6), cod fish (≤ 1.6/ ≤ 0.5), mite farinae, dog, cat, birch, grass, alternaria, cladosporium (≤ 2.0/ ≤ 1.3); HR (≥class 1/≥class 2), birch, grass (≤ 5.7/ ≤ 5.1); Mean SPT, mean of positive SPT reactivity (mm); Mean histamine, mean wheal of positive SPT histamine controls (mm) and lower and upper 5% percentiles (mm); NT, not tested.

Comparing results of sensitization to ≥1 allergen with results of ≥1 food allergen gave almost the same frequencies, suggesting that sensitization to ≥1 allergen almost always involved reactivity to at least one food allergen.

Results of the cumulative incidence at 18 months

During the study new cases of sensitization were found at any time point, regardless of allergens tested and method used. Cumulative incidence data at 18 months demonstrated that within each method at least 80% of all those sensitized were sensitized to ≥1 allergen within the first 12 living months.

Sensitization to foods, and especially to hen's egg, was more frequent than sensitization to any

of the investigated inhalant allergens (Table 2). Unexpectedly, IgE reactivity to wheat ≥class 1 was found in 149 infants, whereas sensitization ≥class 2 was observed in four infants only. 20 infants had reacted to peanut at 18 months of age, and of these nine were sensitized ≥class 2 at least once.

Reactivity to fresh cow's milk was similar to reactivity to standardized cow milk's extract.

Low-level sensitization was distinctly more frequent for all allergens than sensitization ≥class 2 using the HR method and results of specific IgE. The frequency difference between SPT reactivity ≥2 mm and reactivity ≥3 mm was less pronounced. The majority of infants had or had had a reaction to only one allergen.

Table 2. The cumulative incidence (CI) and persistency (P) of selected allergens at 18 months of age

		n ¹	≥1 ¹	CI (%)	≥1	≥2	Non-sing (%)	N ²	P	P (%)
SPT	Wheal (mm)									
Egg	≥2	553	37	6.7	37	18	48.6	32	18	56
	≥3		25	4.5	25	13	52.0	22	10	46
Milk	≥2	553	13	2.4	12	7	58.3	12	6	50
	≥3		11	2.0	11	3	27.3	8	3	38
Milk fresh	≥2	553	12	2.2	12	7	58.3	11	7	64
	≥3		11	2.1	11	5	45.5	10	5	50
Mite Pterony	≥2	553	5	0.9	5	1	20.0	3	1	33
	≥3		2	0.4	2	0	0	2	1	50
IgE	Class									
Egg	≥1	501	62	12.4	57	29	50.9	48	29	60
	≥2		33	6.6	32	18	56.3	27	18	67
Milk	≥1	500	44	8.8	28	9	32.1	22	9	41
	≥2		17	3.4	10	4	40.0	9	4	44
Peanut	≥1	497	20	4.0	20	7	35.0	15	7	47
	≥2		9	1.8	9	5	55.6	7	5	71
Mite Pterony	≥1	500	25	5.0	22	1	4.5	19	1	5
	≥2		10	2.0	8	1	12.5	7	1	14
HR	Class									
Egg	≥1	418	213	50.9	172	58	33.7	125	45	36
	≥2		30	7.2	20	9	45.0	16	9	56
Milk	≥1	418	94	22.5	79	15	19.0	59	13	22
	≥2		22	5.3	16	9	56.3	13	9	69
Mite Pterony	≥1	418	13	3.1	9	1	11.1	5	1	20
	≥2		4	1.0	3	1	33.3	1	1	100

Cumulative incidence not demonstrated in Table 2.

n¹, Number of infants with at least one test performed; ≥1¹, Number of infants with at least one positive reaction; CI, Cumulative incidence of at least one positive reaction among infants tested at least once; ≥1, Number of infants with at least one positive reaction and at least 2 tests performed; ≥2, Number of infants with at least two positive tests and at least 2 tests performed; Non-sing, Percentage of infants with at least 2 positive reactions among infants with at least one positive reaction and tested at least twice; n², Number of infants with at least one positive reaction and at least one subsequent blood test; P, Number of infants in this subpopulation with persistency, i.e. a positive reaction never followed by a negative; SPT, skin prick test; SPT ≥ 2 mm/≥3 mm, Dog (2.0/0.7), cat (1.8/0.5), horse, birch, grass, mite farinae (≤ 0.6), cladosporium, alternaria, mugwort (0); IgE ≥ class 1/≥class 2, Wheat (29.9/0.8), dog (4.4/0.6), cod fish (4.2/1.0), mite farinae (3.2/1.2), alternaria (3.2/1.0) birch and grass (2.8/1.4), cladosporium (1.8/0.6), cat (1.6/0.6); HR ≥ class 1/≥class 2, birch (6.7/2.2), grass (4.1/1.2).

Using HR sensitization ever to ≥1 allergen at 18 months of age was observed in 248 (122 girls, 126 boys). The cumulative incidence was 59.3% (95% CI 54.4–64.1%) and 9.6% (95% CI 6.9–12.8%) using a cut limit of ≥class 1 and ≥class 2, respectively. Using specific IgE sensitization occurred ≥1× in 253 (127 girls, 126 boys), corresponding to an incidence rate of 50.4% (95% CI 46.0–54.9%); sensitization ≥class 2 occurred in 14.5% (95% CI 11.6–18.0%), whereas SPT revealed an incidence rate of 10.7% (95% CI 8.2–13.5%) of ≥2 mm, sensitization occurring in 31 girls and in 28 boys, and in 6.3% (95% CI 4.4–8.7%) at a cut limit of at least 3 mm's.

Transient and persistent reactivity

Within each test method the percentage of infants with ≥2 positive test results of ≥class 1 varied between 5% and 50% demonstrating that a *transient* (or a singular) reactivity to any of the tested allergens was the most characteristic pat-

tern of sensitization in infants (Table 2). Considering ≥class 2 the fractions of non-singular reactivity were higher, but still for most allergens transient reactivity was the dominant pattern. In contrast, no distinct differences were found with respect to the frequency of non-singular reactivity comparing ≥2 and ≥3 mm in the SPT procedure.

For all allergens and methods the percentages of *persistence* were close to the percentages of non-singular reactivity (Table 2).

Considering infants tested ≥2×, a *transient* reaction to ≥1 allergen ≥class 1 was observed in 136 (girls 65, boys 71) using the HR; in 189 (girls 96, boys 93) using the IgE measurements, and in 35 (girls 22, boys 13) using SPT ≥2 mm's, and in 21 (girls 9, boys 12) with SPT reactivity of ≥3 mm's.

A *persistent reaction* ≥class 1 to ≥1 allergen was found in 49 (girls 23, boys 26) and in 12 (girls 5, boys 7) ≥class 2 using HR; in 43 (22 girls and 21 boys) and in 23 (girls 11, boys 12) using RAST ≥class 1 and ≥class 2, respectively. The SPT

demonstrated persistent reactivity in 25 (girls 12, boys 13) ≥ 2 mm reactivity, and in 17 (girls 9, boys 8) of ≥ 3 mm's.

Association between sensitization and AD

An AD diagnosis was less frequent in infants never sensitized in comparison to infants sensitized to ≥ 1 allergen. The association was more pronounced at higher detection levels and for the SPT, compared to the other two methods (Table 3).

The frequency of an AD diagnosis increased with increasing number of allergens, as shown in Fig. 1. However, the association was not pronounced for HR and low-level specific IgE.

No significant association was found between transient reactivity and AD analysed by the HR and the IgE methods, although the associations were better at a higher detection limit (Table 4). A significant association, though, was found between transient reactivity and AD using SPT procedure, in which the association was strongest with whealing of at least 3 mm's.

Table 3. The association between sensitization to ≥ 1 allergen ever (3–18 months) and cumulative atopic dermatitis (AD) cases ever at 18 months of age

	Cumulative incidence of AD ever			
	$\geq$ class 1 (≥ 2 mm, SPT)		$\geq$ class 2 (≥ 3 mm, SPT)	
	AD (%)	OR (95% CI)	AD (%)	OR (95% CI)
HR				
None	10.6	1.00	10.9	1.00
≥ 1 Allergen ever	13.3	1.30 (0.68–2.54)	25.0	2.74 (1.11–6.27)
Specific-IgE				
None	9.3	1.00	9.6	1.00
≥ 1 Allergen ever	15.0	1.73 (0.97–3.14)	27.4	3.56 (1.83–6.75)
Skin prick test				
None	8.0	1.00	9.0	1.00
≥ 1 Allergen ever	37.3	6.83 (3.45–13.25)	42.9	7.57 (3.33–16.71)

Number of AD cases ever (HR: 51, IgE: 61, SPT: 61).

SPT, skin prick test; HR, histamine release; IgE, immunoglobulin E.

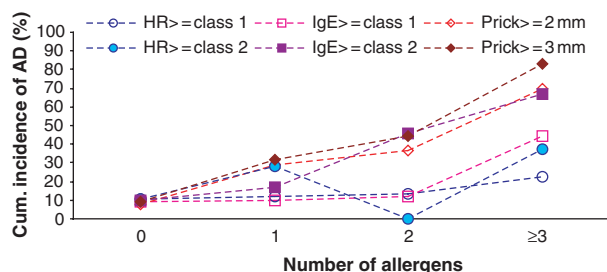


Fig. 1. The association between sensitization (number of allergens) and atopic dermatitis cases ever at 18 months of age.

The sensitivity of positive SPT ≥ 3 mm to any food or inhalant allergens was 24/61 among the patients with AD. Sensitization to milk and/or egg allergens occurred in 16/61 (26%) of children with AD (12 positive to egg and six positive to milk). The positive predictive value of SPT ≥ 3 mm to egg was 12/25 (48%) as regards the diagnosis of AD. The positive predictive value SPT ≥ 3 mm to milk was 6/11 (55%) as regards the diagnosis of AD. A total of 35/553 (6%) has SPT ≥ 3 mm and the positive predictive value of SPT as regards the diagnosis of AD in the total population was 24/35 (69%).

Food allergy was proved by controlled elimination/challenge procedures in 7/61 (11.5%) of children with AD (six were allergic to egg and two were allergic to milk and one to peanut). Within the children with AD the positive predictive value of a positive SPT to egg as regards the diagnosis of food allergy was 6/12 (50%) and for milk 2/6 (33%).

A persistent reactivity to at least one allergen, even at the lowest detection level, was associated with AD, especially for the IgE and SPT procedure.

In Fig. 2 different definitions of sensitization are compared with respect to the percentage of infants sensitized and the frequency of AD among sensitized infants. It seems that definitions based on SPT or IgE are more powerful tools in diagnosing AD cases than HR based definitions.

Association between selected predictors, AD and persistent reactivity

At the age of 18 months more boys than girls had or had had atopic dermatitis (Table 5). An association was found between elevated cord blood IgE (≥ 0.3 IU/ml) and AD. Cord-blood reactivity to ≥ 1 allergen using the specific IgE measurements demonstrated a significant association with AD, however, it decreased at a higher detection limit. The association was not present using HR-reactivity in cord blood, but visible at a higher detection level of $\geq$ class 2. A significant association was found between a maternal history of atopic diseases, in which a history of AD gave a better association than a history of asthma (A) and/or hay fever (HF). A significant association was found between both maternal and paternal histories of atopy plus a simultaneous positive SPT to ≥ 1 allergen.

There was no significant difference between gender and the pattern of sensitization (HR: OR = 1.01 ($\geq$ class 1), OR = 1.30 ($\geq$ class 2); IgE: OR = 1.08 ($\geq$ class 1), OR = 1.07 ($\geq$ class 2);

Table 4. The association between never sensitized, transient and persistent reactivity and atopic dermatitis (AD) ever at 18 months of age

	Cumulative incidence of AD ever in infants tested at least twice			
	≥class 1 (≥2 mm, SPT)		≥class 2 (≥3 mm, SPT)	
	AD (%)	OR (95% CI)	AD (%)	OR (95% CI)
HR				
Never sensitized	14.4	1.00	14.0	1.00
Transiently sensitized	10.3	0.68 (0.31–1.47)	25.0	1.98 (0.43–7.32)
Persistently sensitized	22.4	1.72 (0.69–4.10)	25.0	1.98 (0.32–8.76)
Specific-IgE				
Never sensitized	11.0	1.00	11.0	1.00
Transiently sensitized	10.6	0.96 (0.48–1.90)	18.2	1.81 (0.70–4.48)
Persistently sensitized	34.9	4.36 (1.86–9.89)	43.5	6.25 (2.17–17.33)
Skin prick test				
Never sensitized	8.2	1.00	8.2	1.00
Transiently sensitized	20.0	2.82 (0.97–7.16)	38.1	6.93 (2.32–19.28)
Persistently sensitized	56.0	14.33 (5.55–37.16)	52.9	12.67 (4.03–39.72)

Number of AD cases ever (HR: 51, IgE: 61, SPT: 61).

SPT, skin prick test; HR, histamine release; IgE, immunoglobulin E.

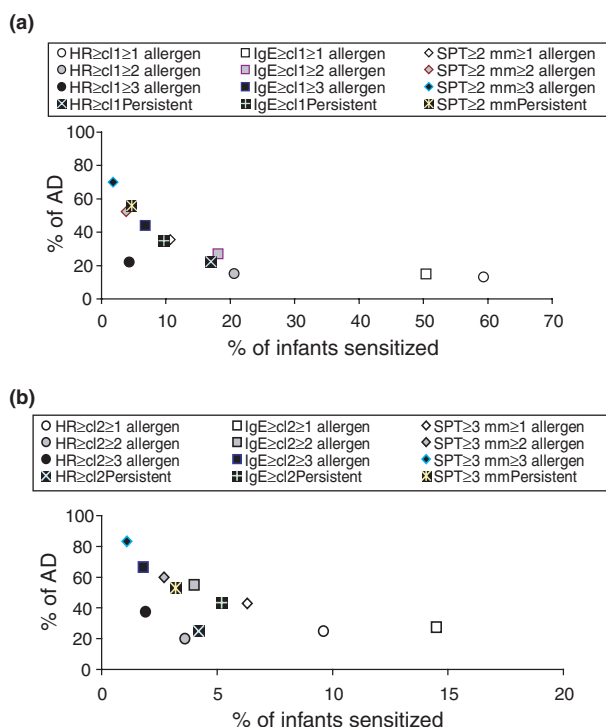


Fig. 2. The association between sensitization and atopic dermatitis ever at 18 month, using different definitions of sensitization: ≥1 allergen, ≥2 allergens, ≥3 allergens (a) [≥class 1 (HR, IgE) or ≥2 mm (SPT) or Persistent sensitization (≥class 1 (HR, IgE) or ≥2 mm(SPT))] (b) [≥class 2 (HR, IgE) or ≥3 mm (SPT) or Persistent sensitization (≥class 2 (HR, IgE) or ≥3 mm(SPT))] SPT, skin prick test; HR, histamine release; IgE, immunoglobulin E.

SPT: OR = 1.14 (≥2 mm), OR = 1.22 (≥3 mm)). As only weak associations were found between family history and family atopy verified by SPT and the development of any/transient post-natal

sensitization to ≥1 allergen ≥class 1, associations between the previous factors and the development of a *persistent* sensitization to ≥1 allergen were analysed. Results demonstrated a significant association between elevated cord IgE and persistent SPT ≥2 mm (OR = 2.78), whereas only weak associations were found with respect to specific IgE measurements and HR persistency.

Discussion

Prospective studies in infant cohorts followed regularly since birth and combining clinical examinations with different test methods to determine level of sensitization are rare. This study demonstrated that a marked response to different food- and aeroallergens was detectable in infants by IgE and HR as young as 18 months. Reactivity status up to 18 months of age was predominantly low-level sensitization to one allergen using different methods to detect responsiveness. Reactivity to two or more allergens and high-level sensitization of ≥class 2 was detectable in some infants as early as 3 months of age. Regardless of method, reactivity to foods was considerable more frequent than reactivity to aeroallergens. This finding was in agreement with another recent study (13).

Comparing results of sensitization to ≥1 allergen with results of ≥1 food allergen gave almost the same frequencies, suggesting that sensitization to ≥1 allergen almost always involved reactivity to ≥1 food allergen. Prospective analysis of serum IgE titres to ubiquitous environmental allergens in children have been detected in both atopic and normal individuals, the

Table 5. The association between selected perinatal risk factors and atopic dermatitis (AD) cases ever at 18 months of age

N (AD): 51 (HR), 61 (IgE, SPT)	AD cases ever at 18 months	
	AD (%)	OR (95% CI)
Gender		
Girls	9.1	1.00
Boys	13.7	1.59 (0.89–2.86)
Cord-total IgE		
<0.3 IU/ml	9.7	1.00
≥0.3 IU/ml	16.7	1.87 (0.90–3.77)
Cord-specific IgE, ≥1 allergen		
None	8.8	1.00
≥class 1	18.5	2.37 (1.20–4.60)
≥class 2	14.8	1.41 (0.33–4.40)
Cord-HR, ≥1 allergen		
None	11.1	1.00
≥class 1	8.1	0.70 (0.28–1.61)
≥class 2	13.3	1.42 (0.15–6.72)
Maternal history of atopy (AD/A/HF)		
None	8.3	1.00
Asthma and/or hay-fever	18.2	2.46 (1.15–5.04)
Atopic dermatitis	24.1	3.53 (1.61–7.39)
Maternal history of atopy + cord-IgE ≥ 0.3		
No	10.3	1.00
Yes	21.9	2.45 (0.84–6.24)
Maternal history of atopy + prick ≥ 1 allergen		
No	9.6	1.00
Yes	23.8	2.94 (1.41–5.86)
Paternal history of atopy (AD/A/HF)		
None	10.5	1.00
Asthma and/or hay-fever	15.9	1.62 (0.83–3.06)
Atopic dermatitis	16.1	1.64 (0.47–4.71)
Paternal history of atopy + cord-IgE ≥ 0.3		
No	10.5	1.00
Yes	33.3	4.27 (1.24–13.03)
Paternal history of atopy + prick ≥ 1 allergen		
No	10.5	1.00
Yes	19.4	2.05 (1.06–3.85)

A, Asthma; HF, hay fever; SPT, skin prick test; HR, histamine release; IgE, immunoglobulin E.

magnitude and the duration of these responses vary with IgE-responder phenotype. The initial food-specific IgE responses may terminate spontaneously by the age of 2–3 yr (14, 15), leaving IgG responses to the same antigens into adulthood (16). Differences in the immunologic responsiveness of the gastrointestinal tract and of the airway mucosa, or differences in allergenicity between foods and inhalants may explain different patterns of sensitization to foods and inhalants (17, 18).

In the present study the prevalence of a positive SPT to ≥1 allergen was found in 5.4% and 3.7% (≥3 mm) at 12 and 18 months, respectively. The result was significantly lower than figures of 8.5% and 12% reported in a previous Danish study conducted in the same area using RAST (19), however closer to results of 5% in a British study using SPT (3).

Incidence data showed that responsiveness to ≥1 allergen was reached within the first year of life in the majority of infants ever sensitized, revealing results of a high-level response of ≥1 allergen in 80% using HR, in 89% using IgE measurements and in 91% and 97% using the SPT at ≥2 and ≥3 mm, respectively. In the majority of cases persistent reactivity to both foods and aeroallergens occurred around or after the 6 months follow-up with the exception of peanut.

IgE mediated persistent reactivity to peanut was observed in seven children (1.6%) within the first 18 living months; in five sensitization ≥class 2 occurred before 6 months of age. In another cohort study skin prick test positivity to peanut was observed in 1.2% by 4 yr of age (20). Transient reactivity occurred in 13 of 20 infants ever sensitized to peanut. Another study found that allergy to nuts persists even in those who develop early allergy (21).

Sensitization to peanut in infancy may occur through breast milk (22). Information on peanut ingestion by mothers during pregnancy and lactation was not obtained in this study, but in Denmark no recommendation on avoidance of peanuts or other highly allergenic foods are given, neither during pregnancy nor lactation.

Fresh foods are generally considered superior as test allergens (23). In this study, both SPT with fresh cow's milk and standardized allergen extract was performed simultaneously. Testing with fresh milk did not give more positive reactions than the standardized allergen extract. However, using fresh cow's milk the frequency of persistent reactivity was higher. The cumulative incidence of skin prick test positive reactivity to cow's milk at 18 months of age was 2.2%.

SPT is considered the preferred *in vivo* diagnostic method for immediate type allergy, and it is also a useful tool in small children (24). A tendency towards lower skin reactivity to histamine in infants below 2 yr of age has been reported (25, 26), and in this study whealing of less than 3 mm in diameter was frequently observed. A positive SPT of ≥1 mm was found ≥1× in 91 infants (16.5%), of ≥2 mm in 59 (10.7%) and of ≥3 mm in 35 (6.3%). Results of persistence to each of the tested allergens revealed, that persistence was improved with a cut-off limit of ≥2 mm. Due to this result SPT reactivity of ≥2 mm was included in the analysis. Results of mean wheal diameters of the positive histamine control substantiated this finding, as mean wheal diameters during study increased from 2.8 mm at 3 months of age to 3.6 mm at 18 months of age.

The mechanisms behind the maturation of the immune system are not fully known. The initial priming of the immune system seems to occur in early post-natal life, or possibly already *in utero*. Neonates in cord blood possess responsiveness to both dietary and inhalant antigens (2) and some studies have identified skewing of allergen-specific response at birth towards a Th₂-predominant profile. It has been proposed that the production of post-natal high IgE levels is due to a genetically determined immunologic deficiency in the capacity to generate Th₁ responses, which may function as 'protection' in early infancy against potentially pathogenic Th₂ responses increasing the risk of developing allergic diseases (27, 28).

In this study a large percentage of infants developed transient (a singular) reactivity to one or more allergens. Transient reactivity was not associated with AD, although transient SPT reactivity ≥ 3 mm's constituted an exception. However, the frequent finding of transient reactivity, and especially a transient low-level reactivity to foods and aeroallergens without clinical symptoms of AD, could substantiate earlier findings, that transient reactivity represents the normal immune response to ubiquitous environmental allergens in early childhood (29). The relevance of a transient reaction in infancy with respect to the development of AD later in childhood can only be evaluated at later follow-up. Another group have found transient skin test reactivity to foods to be common in early infancy (29).

Persistent reactivity was clearly associated with AD and support earlier findings that 'when atopic individuals are exposed to even minute amounts of allergens, they respond with a persistent production of IgE reactivity' (30). Furthermore, our results demonstrated, that in an atopic infant, the number of allergens, which the child had reacted to over time, was clearly related to AD. The association was less obvious using the HR method and RAST $\geq$ class 1.

The HR method has been shown to be a valuable tool in diagnosing sensitization in atopic children compared to conventional methods such as SPT (31). In this study the HR method was less discriminatory in detecting AD cases. A potential decreased capability in infants to release histamine from basophiles was considered. Altered histamine releasability in infants could not explain a lowered ability to distinguish between AD and non-AD cases. Maximum histamine releasability induced by anti-IgE in infants who were negative to any of the tested allergens (releasability less than 15 ng/ml) was less than 15 ng/ml in only a few percentages. In

contrast, in the majority of cases releasability induced by anti-IgE was good and scored as at least anti-IgE class 3. A tendency towards higher capability to release histamine was found in children sensitized to two or more allergens.

A clear distinction of dermatitis into immunologic subgroups of extrinsic and intrinsic eczema was impossible within the first 18 months of life, as HR, IgE and SPT gave different patterns of sensitization, even at higher detection levels. Preliminary results of agreement within each method between three definitions of high-level sensitization: (i) persistent sensitized, (ii) high class sensitized ($\geq$ class 2) and (iii) poly sensitized gave low values of agreement, except for the SPT.

Cord blood IgE as a risk factor and its use as a predictor of allergic diseases in infancy has been questioned (32, 33). In this study elevated cord-IgE was found to be associated with AD. Reactivity in cord blood to ≥ 1 allergen detected by either HR or specific IgE did not prove better tools although associations improved at a higher detection level. A significant association between family members with a positive SPT supported the importance of an atopic family trait and the result was in line with other studies (34, 35). A maternal report of AD was more significant than a paternal report (6, 17).

Elevated cord-IgE, positive parental history and positive parental SPT were used for the prediction of sensitization in infancy. Results of persistent reactivity suggested that the majority of infants ever sensitized at 18 months of age were without any marked demonstrable risks at birth. A significant association was found between cord-IgE and persistent SPT ≥ 2 mm, however. No demonstrable difference with respect to post-natal sensitization was found between girls and boys.

In summary this study showed that sensitization to different food and aeroallergens was frequently observed in infancy as determined by histamine release and specific IgE. Reactivity to foods was more frequent than to aeroallergens. The dominant pattern was low-level transient sensitization, most likely reflecting the normal immune response. Specific definitions of persistent reactivity, high-level sensitization and poly sensitization were clearly associated with AD in early childhood. A maternal history of AD was a valuable tool in predicting AD in early infancy; a similar finding was less obvious with regard to post-natal sensitization. Cord-specific IgE and cord-HR positive reactivity did not prove better tools than cord-total IgE in predicting AD within the first 18 living months.

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