

# Development of allergy and IgE antibodies during the first five years of life in Estonian children

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## Summary

**Background** Epidemiological studies have demonstrated a low prevalence of allergic diseases and atopic sensitization among schoolchildren and young adults in the formerly socialist countries of Central and Eastern Europe as compared to Western Europe.

**Objective** The aim of our study was to prospectively investigate IgE responses to food and inhalant allergens and the development of allergy during early childhood in a population with a low prevalence of atopic disorders.

**Methods** In a population-based prospective study, 273 children were followed from birth through the first 5 years of life, recording manifestations of allergy by questionnaires and clinical examinations at 0.5, 1, 2 and 5 years ( $n = 213$ ). Skin prick tests (SPT) were performed using natural foods (cow's milk, egg white) and commercial extracts of inhaled allergens (cat, dog, *D. pteronyssinus*, birch, timothy). In addition, serum IgE levels and circulating IgE antibodies against the seven allergens were determined.

**Results** The prevalence of allergic diseases at 5 years of life was 19%. Atopic dermatitis was the most common allergic disease at all ages. The point prevalence of positive skin prick tests was 7% at 0.5, 1 and 2 years of age, and 3% at 5 years. Circulating IgE antibodies against food allergens were common at all ages, i.e. 13, 23, 36 and 36%, respectively, at 0.5, 1, 2 and 5 years. The prevalence of circulating IgE antibodies to inhalant allergens increased from 1.5% at 0.5 years to 11% at 1, 19% at 2 and 47% at 5 years. The antibody levels were generally low, however. The value of positive SPT and the presence of IgE antibodies in the diagnosis of clinical allergy were low.

**Conclusion** The results of this prospective study carried out in a previously socialist country with a low allergy prevalence among schoolchildren and young adults indicate that transient sensitization in early childhood is followed by a down-regulation of skin reactivity.

**Keywords:** allergic diseases, atopic sensitization, SPT, IgE antibodies, children, down-regulation

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## Introduction

The prevalence of allergic diseases has increased world-wide, particularly during the last few decades and most strikingly so in industrialized countries with a market economy [1,2]. Furthermore, epidemiological studies of

atopic disorders have shown large world-wide differences in the prevalence among children [3]. The prevalence of allergy and atopic sensitization is low in the formerly socialist countries of Central and Eastern Europe [4–7]. It has been suggested that the increasing prevalence in industrialized countries with market economy is in part caused by major improvements in public health and personal hygiene practice, dietary changes, reduction of

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average family size and improvements of general living standards, i.e. a changed overall exposure to microbial stimulation [8].

Experimental, epidemiological and clinical observations strongly suggest that the conditions under which the primary encounter with an allergen takes place influence the immune response, i.e. whether tolerance or sensitization is triggered [8–12]. Previous studies from Scandinavia have shown that temporary low-level IgE responses to food and inhalant allergens are common during the first years of life, but are then down-regulated in non-atopic individuals, while they continue to increase in children who develop allergic manifestations [13].

We recently reported [14,15] that manifestations of allergic diseases and incidence of sensitization to foods and inhalants during the first two years of life were only slightly lower in Estonian children as compared to Scandinavian children [13,16], despite a much lower prevalence among older children [4] and adults in Estonia [17]. There was also a rather poor relationship between positive skin prick tests and the presence of circulating IgE antibodies [14,15], while there was a good correlation between these two tests among Swedish children [18], as well as in other industrialized countries with a market economy [19,20].

These findings could support the hypothesis that the high prevalence in Western countries is due to a faulty down-regulation of IgE antibody responses, i.e. to defective immune deviation [21,22]. Alternatively, the high prevalence of sensitization among the Estonian infants could indicate an increasing incidence as a consequence of changes in lifestyle after the country regained its independence.

In this 5-year prospective study we tested the hypothesis that the low prevalence of allergic diseases and atopic sensitization in the Estonian children is due to a more rapid immune deviation.

## Materials and methods

### Study population

Mothers who delivered at term at Tartu Women's Clinic during November 1993 – February 1994 and were living in Tartu County were invited to participate in the study. In all, 298 of 455 (65%) agreed to participate. Atopic status and socio-economic level of mothers who declined participation did not differ from those who participated in the study. The children were then followed up at 6 ( $n = 173$ ) and 12 months ( $n = 220$ ), and at 2 ( $n = 229$ ) and 5 years ( $n = 213$ ). The low participation rate at 6 months was due to the fact that many families could either not be contacted as they lacked telephones in their summer dwellings, or they had transport problems. The general living conditions

and the educational level of the parents were similar in those who did and did not attend the 6-month follow-up. There were 117 children who participated in all the four investigations. The incidence of clinical allergy and atopic sensitization during the first two years of life has been reported previously [14,15]. All clinical work of this study was carried out by KJ and MV.

The diagnosis of allergic disease at five years of age was based on clinical examination of the children (carried out during February – April 1999) and on data obtained from questionnaires, combined with an interview of the parents regarding allergy symptoms.

Asthma was defined as at least three episodes of wheezing, at least once verified by a doctor. Allergic rhinitis/conjunctivitis was defined as runny nose, blocked nose or itchy eyes appearing at least twice after exposure to a particular allergen and unrelated to infection. The diagnosis of allergic urticaria was based on generalized urticaria, appearing at least twice within one hour after exposure to a particular allergen. Atopic dermatitis was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution as suggested by Hanifin and Rajka [23]. Vomiting and/or diarrhoea appearing within two hours on at least two separate occasions after the intake of a certain food was considered as diagnostic for gastrointestinal allergy/intolerance. The term 'probable allergy' was used when most, but not all, the criteria for diagnosis were fulfilled.

### Skin prick tests

Skin prick tests (SPT) were done with natural egg white (EW) and extracts of 5 inhalant allergens, i.e. cat, dog, house dust mite (*D. pteronyssinus*), birch and timothy (Solu-Prick SQ, ALK-Abelló, Hørsholm, Denmark). All tests were performed by KJ and MV or a trained nurse according to the instructions of the European Academy of Allergy and Clinical Immunology [24] on the volar aspects of the forearms, using lancets from ALK. The potency of the inhalant allergens was 10 histamine equivalent prick units (HEP). Histamine dihydrochloride, 10 mg/mL, was used as positive control. The SPT was regarded as positive when the weal size, measured after 15 min, was 3 mm or more.

### Serum IgE and IgE antibodies

Venous blood samples were obtained from 207 children at 5 years of age using vacutainers. After clotting at room temperature, the samples were centrifuged and sera were frozen and stored at  $-20^{\circ}\text{C}$  until analysed.

Serum IgE (S-IgE) and circulating IgE antibodies against allergens were determined by a chemiluminescence

method, according to the recommendations of the manufacturer (Magic Lite SQ, ALK-Abelló). For determination of S-IgE, a normal sensitivity (NS, detection level 2–3000 kU/L) was employed. The blood samples were analysed for IgE antibodies against cow's milk, egg white, cat, dog, *D. pteronyssinus*, birch and timothy, employing the normal sensitivity (NS) method. The detection level for the test procedure was 0.5 standardized units per ml (SU/mL), corresponding to approximately 0.09 Paper Rast Units (PRU) in the test system manufactured by Pharmacia Upjohn (Uppsala, Sweden). The level of IgE antibodies was expressed as low (class 1; 1.43–4 SU/L ml), moderate (class 2; 4–20 SU/mL), high (class 3; 20–100 SU/mL), very high (class 4; 100–300 SU/mL), and extremely high (class 5; > 300 SU/L). In addition, IgE antibody measurements against *Ascaris* were done on samples with S-IgE level exceeding 300 kU/L.

#### Ethical considerations

The parents of all participant children gave their informed consent. The Human Research Ethics Committee of Tartu university approved the studies.

#### Statistical analysis

Calculations were performed with the statistical package StatView 4.5 for Macintosh (Abacus Concepts Inc, Berkeley, CA, USA).

Logarithmic transformation was used for IgE values and the values were presented as geometric means. Wilcoxon signed rank test was used for comparing IgE values and histamine-induced weal size at different ages. Mann–Whitney *U*-test was explored for comparing IgE values in subgroups. Chi-square test or when appropriate, Fisher's exact test was used to test differences in allergy symptoms, SPT results and circulating IgE antibody levels.

Statistical significance was set at the 5% level.

## Results

The prevalence of at least one allergic disease was 19% (41/213) at 5 years, as compared to 15% and 17% at one and two years (Table 1). Atopic dermatitis was the most common manifestation. The cumulative prevalence of allergic disease was 32%. In the group 'probable allergy', urticaria of uncertain aetiology was the most common manifestation.

The mean serum IgE level was 55 ( $\pm$  SD, 12–269) kU/L at 5 years, as compared to 5, 8 and 19 kU/L, respectively, at 6, 12 and 24 months of age ( $P < 0.01$ ). Fifty percent of the children had an IgE level below 50 kU/L and 26% below 20 kU/L. Thirty-one children (15%) had a serum IgE

**Table 1.** Point prevalence (%) and cumulative incidence (%) of obvious atopic diseases over the first 5 years of life in Estonian children

| Diagnosis                            | 0.5 year<br><i>n</i> = 173 | 1 year<br><i>n</i> = 220 | 2 years<br><i>n</i> = 223 | 5 years<br><i>n</i> = 213 | Cumulative<br><i>n</i> = 273 |
|--------------------------------------|----------------------------|--------------------------|---------------------------|---------------------------|------------------------------|
| Atopic dermatitis                    | 4<br>(2.5)                 | 10.5<br>(5)              | 15<br>(13.5)              | 10<br>(13)                | 19<br>(21.5)                 |
| Bronchial asthma                     | 0<br>(0)                   | 0.5<br>(1)               | 2<br>(2.5)                | 6.5<br>(5)                | 6<br>(5)                     |
| Allergic rhinitis/<br>conjunctivitis | 0<br>(0)                   | 0<br>(0)                 | 1<br>(1)                  | 5.5<br>(2.5)              | 5<br>(2.5)                   |
| Allergic urticaria                   | 2<br>(3.5)                 | 3<br>(1.5)               | 0<br>(0)                  | 1<br>(0)                  | 4.5<br>(5)                   |
| Gastrointestinal<br>allergy          | 2<br>(1)                   | 1<br>(1)                 | 1.5<br>(1.5)              | 0<br>(0)                  | 2<br>(1.5)                   |
| Any                                  | 8<br>(7)                   | 15<br>(11)               | 17<br>(16)                | 19<br>(19)                | 31<br>(32)                   |

The figures in brackets refer to the 117 children who participated in all the four follow-up investigations

level above 300 kU/L. As only 10 of the latter children had any allergic symptoms, and only three had a positive SPT, their sera were tested for the presence of IgE antibodies against *Ascaris*. Nineteen of the 31 sera (61%) contained IgE antibodies against *Ascaris* and in 7 samples the level of antibodies was class two or higher. The mean serum IgE levels were similar in allergic and non-allergic children (57 ( $\pm$  SD 13–260) kU/L and 54 ( $\pm$  SD 12–251) kU/L), respectively.

Only six of 208 (2.9%) children had at least one positive SPT, i.e. weal size  $\geq 3$  mm at 5 years of age (Table 2). An additional 2% (4/208) had a weak reaction with a weal size less than 3 mm but at least the size of the positive control. Three children were sensitized to cat and house dust mite, two against dog, two against birch and none against egg. Three children were sensitized to more than one allergen.

The mean size of the histamine-induced weal was significantly smaller at 0.5 and 5 years of age as compared to 1 and 2 years (Fig. 1).

Circulating IgE antibodies against one or more allergens were detected in 63% (131/207) of the children at 5 years and in 21% of the children (43/207) the level was class 2 or higher (Table 2). Circulating IgE antibodies were detected against at least one inhalant allergen in 47% (97/207) and against ovalbumin (OA) and/or cow's milk (CM) in 36% (75/207). Circulating IgE antibodies against food allergens were common at all ages, i.e. 13, 23 and 36%, respectively, at 0.5, 1 and 2 years, while the prevalence of circulating IgE antibodies to inhalant allergens increased from 1.5% at 0.5–11% at 1 and 19% at 2 years of age. The levels were

**Table 2.** Presence of circulating IgE antibodies (%)  $\geq$ class 1 ( $\geq 1$ ) and  $\geq$ class 2 ( $\geq 2$ ) and positive SPT (weal  $\geq 3$  mm). (%) over the first 5 years of life in Estonian children who were prospectively followed from birth

| Allergen                   |          | 0.5 year<br>% | n   | 1 year<br>% | n   | 2 years<br>% | n   | 5 years<br>% | n   |
|----------------------------|----------|---------------|-----|-------------|-----|--------------|-----|--------------|-----|
| $\beta$ -LG/<br>Cow's milk | $\geq 1$ | 12.0/         | 92  | 20.7/       | 116 | 25.8/        | 120 | 23.2/        | 207 |
|                            | $\geq 2$ | 0             |     | 3.4         |     | 0.8          |     | 3.4          |     |
|                            | SPT      | 1.7           | 172 | 0.9         | 220 | 0            | 222 |              | nd  |
| OVA/<br>Egg white          | $\geq 1$ | 4.2/          | 118 | 5.6/        | 126 | 20.6/        | 141 | 22.7/        | 207 |
|                            | $\geq 2$ | 0.8           |     | 4.0         |     | 9.2          |     | 2.4          |     |
|                            | SPT      | 5.2           | 172 | 4.1         | 220 | 1.8          | 222 | 0            | 208 |
| Cat                        | $\geq 1$ | 1.6/          | 128 | 4.2/        | 144 | 11.8/        | 144 | 13.6/        | 206 |
|                            | $\geq 2$ | 0             |     | 2.1         |     | 3.5          |     | 5.8          |     |
|                            | SPT      |               | nd  | 1.4         | 220 | 4.1          | 222 | 1.4          | 208 |
| Dog                        | $\geq 1$ | 0             | 65  | 3.9/        | 102 | 4.5/         | 67  | 17.0/        | 206 |
|                            | $\geq 2$ |               |     | 0           |     | 3.0          |     | 3.9          |     |
|                            | SPT      |               | nd  | 0.5         | 220 | 2.7          | 222 | 0.5          | 208 |
| <i>D. pter.</i>            | $\geq 1$ | 0             | 127 | 2.1/        | 143 | 7.6/         | 144 | 19.4/        | 206 |
|                            | $\geq 2$ |               |     | 0.7         |     | 2.8          |     | 6.3          |     |
|                            | SPT      |               | nd  | 1.8         | 220 | 2.3          | 222 | 1.4          | 208 |
| Birch                      | $\geq 1$ | 0             | 125 | 3.5/        | 142 | 3.5/         | 143 | 13.5/        | 207 |
|                            | $\geq 2$ |               |     | 1.4         |     | 0.7          |     | 3.9          |     |
|                            | SPT      |               | nd  |             | nd  | 0.5          | 222 | 1.0          | 208 |
| Timothy                    | $\geq 1$ | 0             | 68  | 1.3/        | 78  | 6.3/         | 95  | 17.9/        | 207 |
|                            | $\geq 2$ |               |     | 0           |     | 0            |     | 2.9          |     |
|                            | SPT      |               | nd  |             | nd  | 0            | 222 | 0            | 208 |

generally low, i.e. class 1. High levels, i.e.  $\geq$  class 3 were detected only in 5 samples against *D. pteronyssinus* and in two samples each against OVA, cat, dog and birch. At 6 months, none had such high levels, and at 1 and 2 years, each one had against *D. pteronyssinus* and cat, and 2 against OVA. The results for the subgroup of children who participated in all the four follow-up investigations are shown in Fig. 2. Out of 21 children with atopic dermatitis, only two had both a positive SPT and IgE antibodies  $\geq$  class 2, while five children had IgE antibody levels  $\geq$  class 2, but were SPT negative.

There was a poor correlation between the presence of circulating IgE antibodies and positive SPT and this tended to increase with age. The discrepancy between the two diagnostic methods was mainly due to the presence of circulating IgE antibodies class 1 or 2 in SPT negative children. This is illustrated in Table 3 for one food (egg white) and two inhalants (cat and *D. pteronyssinus*). One child had a positive SPT against birch, but lacked circulating IgE antibodies against birch.

Among the children with a diagnosis of allergic disease or probable allergy, positive SPT was 10 times more common than in the non-allergic children (5/71, 7% vs. 1/137, 0.7%, respectively) ( $P = 0.02$ ). Similarly, the percentage of children with circulating IgE antibodies against at least

one allergen was higher among allergic and probably allergic, as compared to non-allergic children (52/71, 73% vs. 79/136, 58%, respectively) ( $P = 0.03$ ). Levels corresponding to class 2 or higher were three times more common among allergic and probably allergic children than among non-allergic children (29/79, 37% vs. 15/135, 11%, respectively) ( $P < 0.001$ ). The sensitivity for clinical allergy of a positive IgE antibody test was 73% and the specificity was 42%.

## Discussion

The cumulative incidence of allergic diseases at 5 years of age was similar to recent Scandinavian findings [13,25–27]. Although the studies were not identically designed and the diagnostic criteria were slightly different, some comparisons can nevertheless be made. The 32% cumulative incidence in our study would somewhat overestimate the problem, since most children got the diagnosis only on one occasion. In the Scandinavian studies the prevalence was much higher in the children with, than without an atopic family history [27]. Such comparisons are not meaningful in Estonia, since the phenotypic expression of an atopic genotype is usually not expressed in adults [17]. The 7% prevalence of positive skin prick tests at 1 and

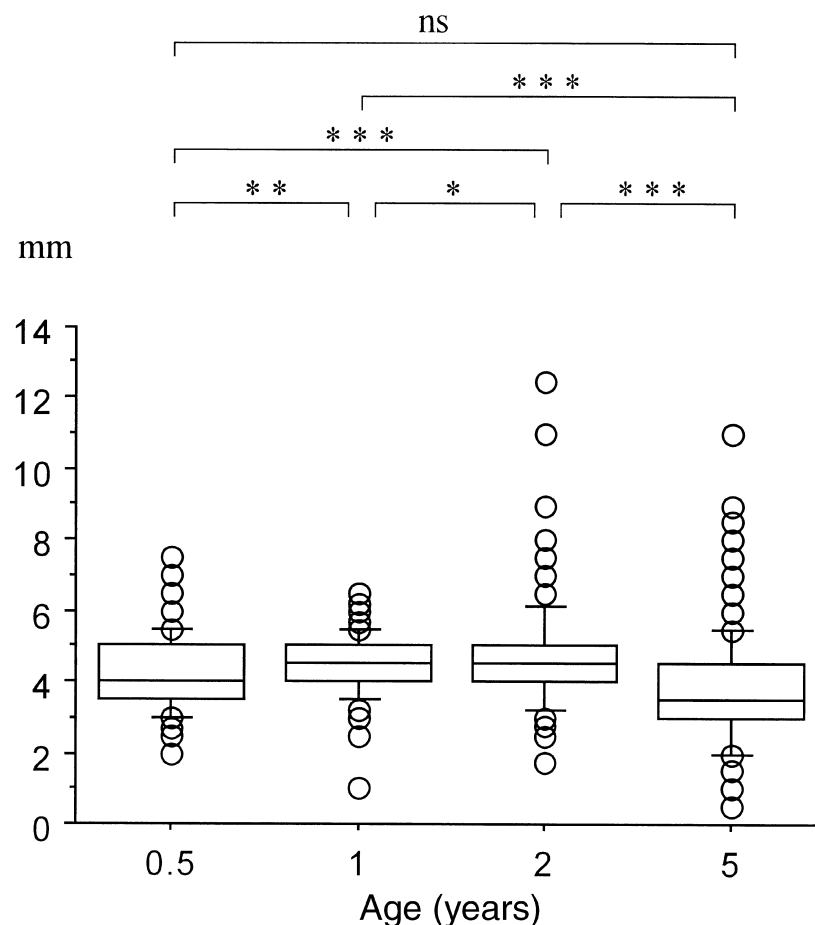


**Fig. 1.** The mean diameter of skin weal reactions to histamine (10 mg/mL) at different ages (mm, mean  $\pm$  SEM). \*\*\* $P$  < 0.0001; \*\* $P$  < 0.001; \* $P$  < 0.01.

2 years was lower than the 13% reported in a recent Swedish study of 7-month-old children who were unselected with regard to a family history of atopy [16]. The proportion of SPT-positive children was even lower at 5 years (2.9%) and the mean diameter of the histamine control had also diminished. The socio-economic status of the study group children did not differ from the socio-economic status of Estonians in general. Atopy problems were not related to family size, nor parental education (data not shown).

As reported previously [14], only 58% of children attended the follow-up at 6 months. To exclude this would have affected the results so we analysed the 117 children who participated in all the follow-ups separately. The prevalence of clinically manifest allergy, as well as sensitization, was similar among the children who participated all four times and in the entire study group at all ages.

In contrast to the SPT results, circulating IgE antibodies were commonly detected and the prevalence of IgE antibodies to inhalants increased with age. The prevalence of IgE antibodies to foods was also high. These results differ from a previous Swedish study [13] where IgE

antibodies to egg white and cow's milk reached a peak prevalence during the first year of life with high concentrations almost exclusively in atopics and then disappeared successively. In the Swedish study group, no child developed IgE antibodies to food allergens after the first year of life. In a recent German study, annual incidence rates of sensitization to food allergens decreased from 10% at 1 years of age to 3% at 6 years [28]. In our study a significant number of the children who were tested all four times developed IgE antibodies to foods after infancy, i.e. 30% to  $\beta$ -lactoglobulin and 20% to ovalbumin. The levels were, however, mostly low or moderate.

Several studies have shown a close correlation between SPT results and the presence of circulating IgE antibodies [18–20,29]. This was not the case in the Estonian children. The decreasing prevalence of a positive SPT from 2 to 5 years would support the hypothesis of a down-regulation of Th2 immunity [30] and would thus explain the low prevalence of sensitization in the Estonian schoolchildren [4].

The discrepancy between SPT results and presence of circulating IgE antibodies cannot be explained by technical

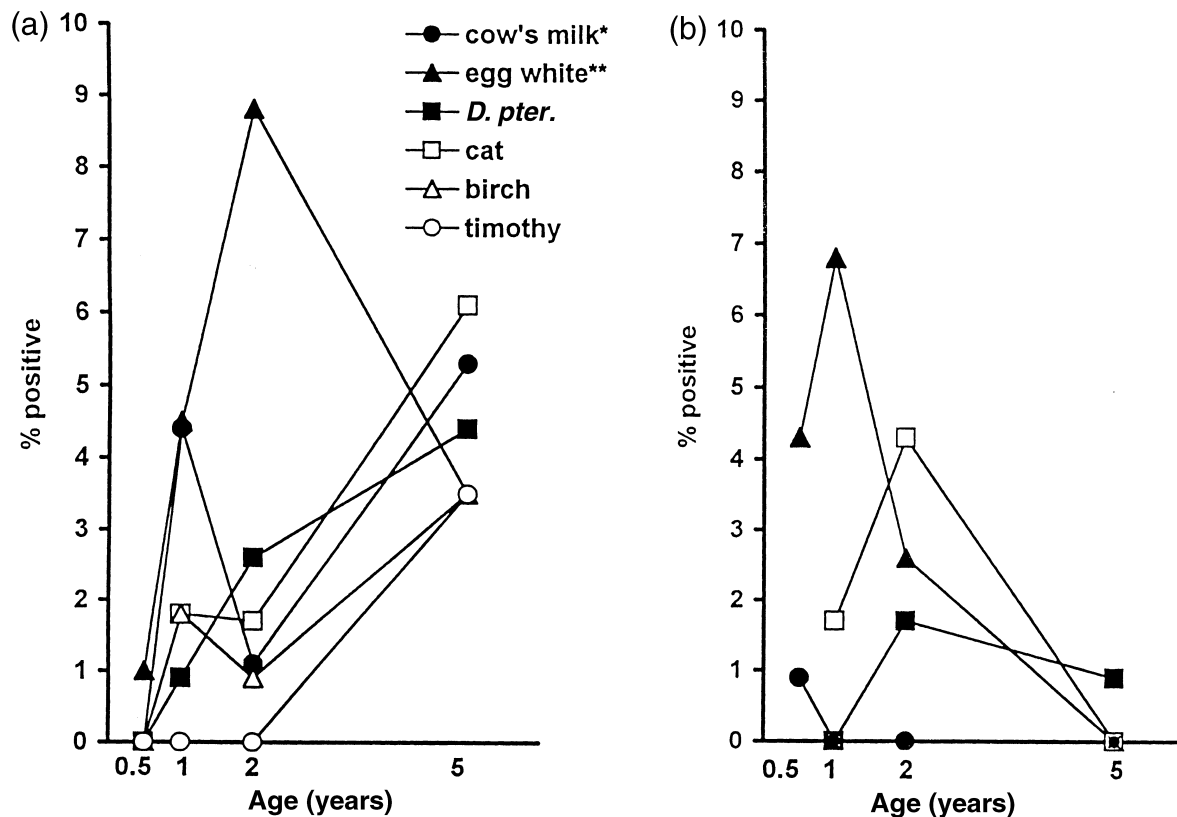


**Fig. 2.** (a) Circulating IgE antibodies and (b) skin prick test results against foods (\* $\beta$ -lactoglobulin was used for detecting circulating IgE antibodies and natural cow's milk for skin-prick tests; \*\*ovalbumin was used for detecting circulating IgE antibodies and natural egg white for skin prick tests) and inhalant allergens (*D. pteronyssinus*, birch, timothy).

factors. The tests were done by KJ and MV at 0.5, 1 and 5 years and by a trained nurse at 2 years. To reduce the individual variability the three persons performing the tests did 20 SPTs with histamine on the lower arms of the volunteers. The individual weal reactions and variations were similar for them. Furthermore, the prevalence of sensitization remained low (5%), even when weakly positive reactions were included (weal size < 3 mm but at least the size of positive control). Our findings could explain some discrepancy reported in epidemiological studies comparing Eastern and Western Germany after the unification [31–33]. It could also explain numerous reports from developing countries where the levels of circulating IgE antibodies are high, while the prevalence of positive SPTs and clinical allergy are low [34,35]. The assumption that there is a good agreement between the results of SPT and the determination of circulating IgE antibodies is based on numerous studies that were performed in industrialized countries with a market economy [18–20]. There is less agreement in developing countries, however, between the two methods to detect IgE antibodies. Thus, in tropical countries with high morbidity

from parasitic infections in children the IgE levels are high, yet the prevalence of positive SPTs is low [34,35]. This has been interpreted as a blocking effect of circulating IgE antibodies [36]. Three types of helminths are common in Estonian children as indicated by the Statistical Bureau of Ministry of Social Affairs in 1999, i.e. *Ascaris* 159, *Diphyllobothris* 8.7 and *Enterobius* 1068 cases per 100 000 children. An alternative explanation suggested by our observations in this prospective study could be that there is a down-regulation of skin reactivity in countries with a more traditional lifestyle and a low prevalence of allergy.

Our findings agree with reports from Gabon, showing a high frequency (32%) of circulating IgE antibodies to mites, while the prevalence of positive SPTs were only present in one third of the children [37]. Macaubas *et al.* [38] reported an inverse relationship between the weal diameter of SPTs and the levels of IL-10 in children with circulating IgE antibodies to house dust mite and negative SPT to the same allergen. There is evidence suggesting that IL-10 can also inhibit both antigen-specific and anti-CD3 antibody-mediated proliferation and cytokine production by Th2 cells [39,40]. Furthermore, Groux *et al.* [41]

**Table 3.** Number of children with positive skin prick tests (weal  $\geq 3$  mm) to egg white, cat and *D. pteronyssinus* in relation to the presence of circulating IgE antibodies ( $\geq 1$  class) to the allergens

| Allergen        | Age (years) | Circulating IgE | Skin prick test |      |
|-----------------|-------------|-----------------|-----------------|------|
|                 |             |                 | pos.            | neg. |
| Egg white       | 0.5         | yes             | 2               | 3    |
|                 |             | no              | 2               | 111  |
|                 | 1           | yes             | 2               | 5    |
|                 |             | no              | 5               | 114  |
|                 | 2           | yes             | 4               | 25   |
|                 |             | no              | 0               | 112  |
| Cat             | 5           | yes             | 0               | 46   |
|                 |             | no              | 0               | 158  |
|                 | 1           | yes             | 1               | 5    |
|                 |             | no              | 2               | 136  |
|                 | 2           | yes             | 4               | 13   |
|                 |             | no              | 4               | 123  |
| <i>D. pter.</i> | 5           | yes             | 2               | 26   |
|                 |             | no              | 0               | 176  |
|                 | 1           | yes             | 0               | 3    |
|                 |             | no              | 3               | 137  |
|                 | 2           | yes             | 1               | 10   |
|                 |             | no              | 3               | 130  |
|                 | 5           | yes             | 3               | 37   |
|                 |             | no              | 0               | 164  |

reported that IL-10 might enhance T cell unresponsiveness. Comparisons of IL-10 secretion levels in SPT-positive and SPT-negative children in our prospective study would therefore be of interest.

In conclusion, this prospective study from a country with a low prevalence of atopic disease shows that the incidence of atopic manifestations is high during the first two years of life and is then down-regulated, resulting in a low prevalence of positive skin prick tests at 5 years of age. These findings differ markedly from the results of prospective studies in Western Europe [13,28,42] and USA [11,43]. Thus, the progress of allergic disease from early childhood, i.e. the 'atopic march' is not so apparent in Estonian children as it is in Western Europe.

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Agency for the Protection of the Environment and Glaxo Wellcome Ltd, UK.

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