

Atopic allergy and delayed type hypersensitivity in Estonian children

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Summary

Background A shift in the balance of T helper (Th) cell subsets towards a polarized Th2 population is generally accepted to occur in atopic disease, however, both Th1 and Th2 disorders have increased over the past decades in Western communities.

Objective The aim of our study was to investigate delayed type hypersensitivity (DTH) response in atopic and non-atopic children in a population with a low prevalence of allergic disorders.

Methods Skin prick tests (SPT) were performed with fresh egg white and extracts of five inhalant allergens, i.e. cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), birch and timothy, and DTH response was evaluated by Multitest® CMI in 72 Estonian 4- to 6-year-old children.

Results The frequency of response to diphtheria was significantly increased in SPT-positive children (55% vs. 26%, $\chi^2 = 5.5$; $P = 0.038$). The induration to diphtheria (2.4 ± 0.5 vs. 0.9 ± 0.2 mm; $P = 0.004$), and tetanus (3.5 ± 0.6 vs. 2.1 ± 0.3 mm; $P = 0.025$) was significantly greater in the SPT-positive children. The cumulative size of induration in the positive DTH tests was significantly greater in the SPT-positive children (9.0 ± 1.2 vs. 5.2 ± 0.6 mm, $P = 0.01$).

Conclusion In this group of children our findings do not support the hypothesis of an immune deviation with decreased Th1 and increased Th2 responses leading to atopic disease, but rather a process of immune modulation whereby both Th1 and Th2 responses are increased in atopic subjects.

Keywords atopic sensitization, children, delayed type hypersensitivity, Multitest® CMI, skin prick tests, Th1/Th2

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Introduction

A shift in the balance of T helper (Th) cell subsets towards a polarized Th2 population is generally accepted to occur in atopic disease. However, both Th1 and Th2 disorders have increased markedly over the past decades in Western communities, suggesting that common environmental factors may contribute to both Th1- and Th2-associated disease. The prevalence of type 1 diabetes (a Th1 disorder) and allergic asthma (a Th2 disorder) is associated in studies at both a population [1] and individual [2] level. These observations are not well explained by a reciprocal balance between Th1 and Th2 responses.

Studies on delayed hypersensitivity responses in atopic subjects have provided conflicting results. An inverse relationship between atopic sensitization and delayed type hypersensitivity (DTH) to mycobacteria was observed in a Japanese study [3]. However Alm et al. found that the size of the tuberculin reaction was unrelated to both IgE levels and skin prick positivity to common allergens [4, 5]. In a Swedish study, atopic children developed stronger DTH responses to environmental

mycobacteria than non-atopic children [6]. As the prevalence of atopy varies in different countries it is of interest to examine the relationship between DTH responses and atopy in different populations to determine if atopic subjects show evidence of a decreased Th1 immunity, as might be expected to result from a process of immune deviation. In this study we compare DTH responses in atopic and non-atopic Estonian children.

Materials and methods

The study group comprised 72 Estonian 4- to 6-year-old children. Fifty-six of them participated in a prospective study of the development of allergic disease [7]. Six were skin prick test (SPT)-positive and 50 SPT-negative. Another 16 children with asthma or allergic rhinitis and positive SPT were recruited at the Allergy Centre at the Children's Clinic of Tartu University Clinics. Immunization history was obtained from the immunization records. All children received bacille Calmette–Guérin (BCG) vaccine (Statens Serum Institute, Copenhagen, Denmark) on the 3–5th day of life. All had a vaccine-induced scar (mean diameter 5.7 ± 3 mm). All children received diphtheria–tetanus and pertussis (whole-cell vaccine) from different European manufacturers (National Public Health Institute, Finland;

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Behring, Germany) at 3, 4.5 and 6 months during the first year of life and at 2 years of age.

Skin testing

None of the children had been using oral steroids or long-acting antihistamines during the last 3 months, or short-acting antihistamines during the last week before testing. SPTs were performed with fresh egg white and extracts of five inhalant allergens, i.e. cat, dog, house dust mite (*Dermatophagoides pteronyssinus*), birch and timothy (Solu-Prick SQ, ALK-Abelló, Hørsholm, Denmark) according to the instructions of the European Academy of Allergology and Clinical Immunology [8] on the volar aspects of the forearms. The potency of the inhalant allergens was 10 histamine equivalent prick units (HEP). Histamine dihydrochloride, 10 mg/mL, was used as positive control. In 15 min the weal size was marked with ballpoint pen and transferred with transparent tape to paper. The child was considered to be SPT-positive if the weal reaction to any allergen was ≥ 3 mm, calculated as the mean of the longest and right angle diameters.

DTH responses were evaluated by Multitest[®] CMI (Pasteur Mérieux, Lyon, France). A multipuncture applicator has eight heads loaded with seven antigens, i.e. diphtheria, candida (*albicans*), proteus, streptococcus (group C), tetanus, trichophyton, tuberculin and glycerin as negative control. The tests were performed according to the instructions of the manufacturer on the volar aspect of the forearm. The test results were read after 48 h and the diameter of the induration was calculated as for SPT. The reaction was considered to be positive if the induration was at least 2 mm according to the suggestions of the manufacturer. Anergy was defined as negative reaction to all antigens.

Ethical considerations

The parents of all participant children were informed and gave their consent. The Human Research Ethics Committee of Tartu University approved the study.

Statistical analysis

Calculations were performed with the statistical package Stat-View (SAS[®] Institute Inc., Cary, NC, USA). The association between SPT and DTH reactivity was compared by the chi-square (χ^2) test. The Mann-Whitney *U*-test was used for comparison of the size of DTH responses in the groups. Wilcoxon signed rank correlation was used for comparing DTH reaction and histamine-induced weal size. A *P*-value of < 0.05 was considered significant.

Results

Twenty-two children reacted to one or more SPT. Fourteen of the 22 (64%) were sensitized to house dust mite, nine (41%) to cat, four (18%) to dog and six (27%) to birch pollen. Nine children were sensitized against two or more allergens. SPT-positive children had larger histamine-induced weals (5.0 ± 0.4 vs. 4.0 ± 0.2 ; $P = 0.005$).

Figure 1 shows the size and percentage of DTH responses in the SPT-positive and -negative children. Twelve children (17%) were anergic. No children reacted to candida, streptococcus or

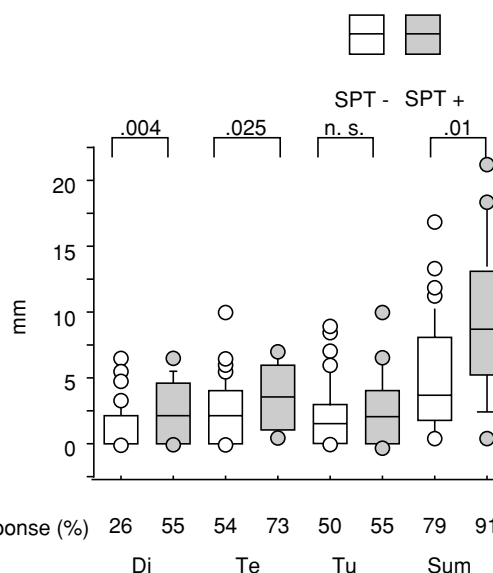


Fig. 1. Delayed type responses in atopic and non-atopic children. Di, diphtheria; Te, tetanus; Tu, tuberculin; Sum, sum of antigens.

trichophyton, and five children reacted to proteus antigen. SPT-positive children had a positive DTH response to one or more antigens more often than SPT-negative children (91% vs. 76%; $P = 0.25$); however, the difference was not significant. Comparison of the responses to individual antigens showed that the frequency of response to diphtheria was significantly increased in SPT-positive children (55% vs. 26%, $\chi^2 = 5.5$; $P = 0.038$).

The induration to diphtheria (2.4 ± 0.5 vs. 0.9 ± 0.2 mm; $P = 0.004$), and tetanus (3.45 ± 0.6 vs. 2.1 ± 0.3 mm; $P = 0.025$) was significantly greater in the atopic children. In addition, when the size of induration of all positive tests were combined the total was significantly greater in the atopic children (9.0 ± 1.2 vs. 5.2 ± 0.6 mm; $P = 0.01$; Fig. 1). The response to each antigen individually is presented in Fig. 2.

Discussion

It has been postulated that a reduction in Th1 immune deviation due to an altered microbial environment in early life has contributed to the marked increase of allergic diseases (Th2-type diseases) in recent decades in developed societies [9]. However, over the same time frame, the incidence of Th1 diseases such as type 1 diabetes and coeliac disease has also increased in the same populations [1, 2, 10, 11]. Estonia has a lower level of both atopy [7, 12, 13] and Th1 disorders, e.g. coeliac disease [14] and type 1 diabetes [15], as compared to the neighbouring Scandinavian countries Finland and Sweden [10, 16, 17]. The concept of immune deviation has led to the thinking that, in allergic subjects with increased Th2 responses, Th1 responses will be reduced and vice versa. However, epidemiological findings raise the possibility that environmental factors might enhance or decrease both Th1 and Th2 in the same direction [1]. To investigate this question further we have examined DTH responses in atopic and non-atopic children.

The incidence of anergy was 17% among Estonian children. This is twice as high as in an Italian study incorporating children

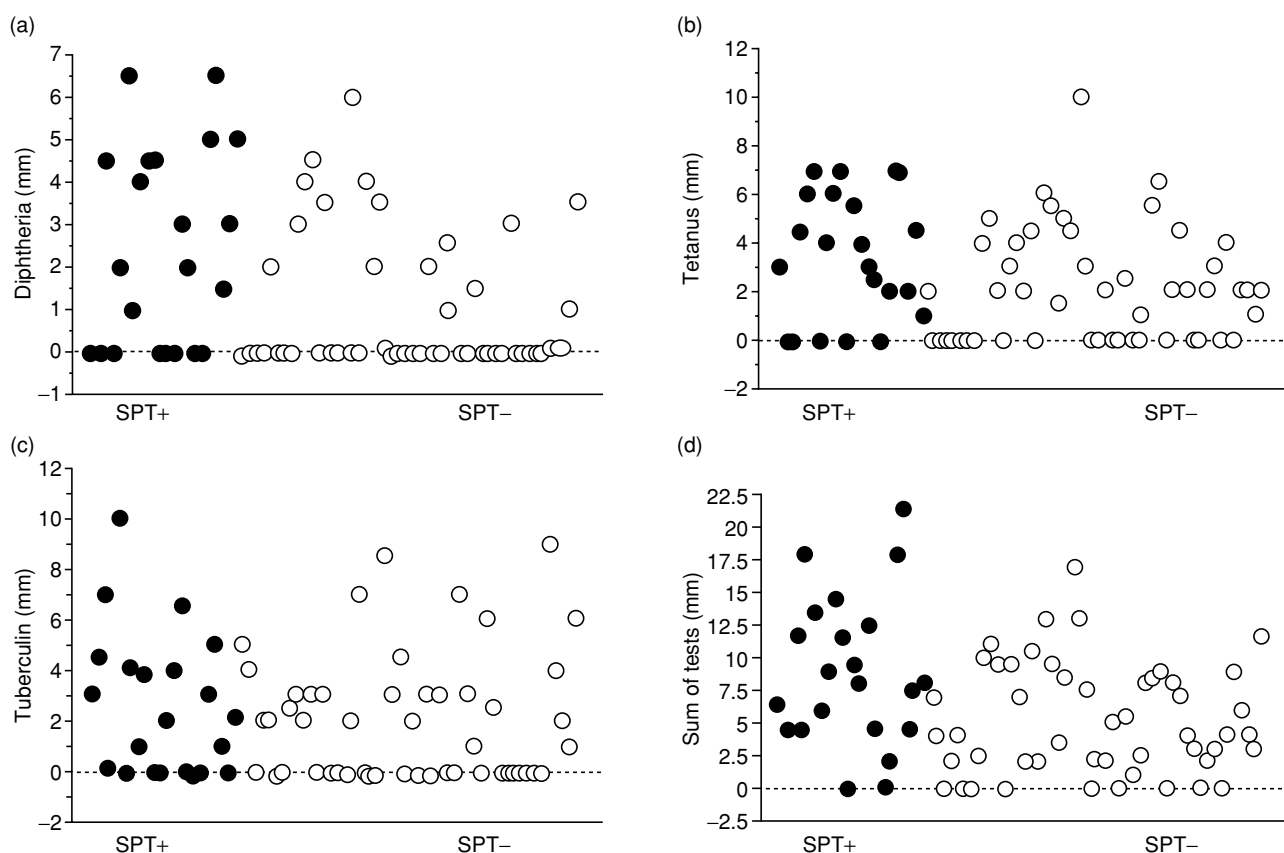


Fig. 2. Delayed type responses against various antigens presented individually in atopic (●) and non-atopic (○) children. (a) Diphtheria antigen, (b) tetanus antigen, (c) tuberculin, (d) sum of tests.

from 4 months to 16 years [18] and in an American study incorporating children from 6 months to 7 years of age [19], both of whom have a higher incidence of childhood atopic disease [20, 21]. In the present study we demonstrate in Estonian children that DTH responses are increased, rather than decreased, in atopic subjects. This finding extends the previous observations of Strannegård who demonstrated increased DTH reactivity to atypical mycobacteria in Swedish atopic children [6]. A number of other epidemiological and immunological observations, such as the concurrent increase of Th1 and Th2 diseases in the same populations [1], the decreased incidence of both Th1 and Th2 disorders in some developing countries as compared to developed countries [22] and decreased Th1 and Th2 immune responses to environmental antigens in parasite-infected children [23] are not well explained by the concept of immune deviation. The findings of this study do not support a process of immune deviation, which results in decreased DTH responses in atopic subjects, but rather immune modulation whereby both Th1 and Th2 responses are increased in atopic subjects. Our results agree with recent data demonstrating differences in the Th1/Th2 response in two regions of Germany. The atopic children from western Germany showed Th2 polarization; in eastern Germany, as in Estonia, atopy was associated with Th0 responsiveness [24]. They are also consistent with recent observations that Th1 as well as Th2 responses to allergens are enhanced in atopic children [25]. Although the mechanisms are unclear, a process of immune modulation may be effected at the level of antigen presenting or regulatory T cells.

This concept of immune modulation is more consistent with our present findings and previous observations on the epidemiology of Th1 and Th2 disorders, and may also be indicative of the reported regional differences in the Th1/Th2 response pattern.

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