

Short communication

Tuberculin responses in children with allergic diseases

Background: The prevalence of allergic disorders has been increasing over the last 30 years, especially in developed countries. One factor associated with this rise may be the decline of many childhood infections. We investigated tuberculin responses in allergic children in order to see the development of delayed-type hypersensitivity reactions to tubercule bacillus infection.

Methods: The study sample was composed of 106 allergic and 100 nonallergic children vaccinated with bacille Calmette-Guerin (BCG). The standard Mantoux test was applied to all children. The reactions were read after 72 h by measuring the diameter of the wheal.

Results: The wheal size was 6.29 ± 5.09 mm (mean $\pm$ SD) in allergic children, and 2.79 ± 2.96 mm in nonallergic children. The difference between the two groups was significant ($P < 0.001$). In children with a single BCG scar, the mean purified protein derivative (PPD) wheal size for allergic children was 4.77 ± 4.79 mm, and for nonallergic children it was 2.48 ± 3.19 mm. The mean PPD wheal sizes in allergic and nonallergic children who had been vaccinated twice were 8.35 ± 4.80 mm and 3.33 ± 2.44 mm, respectively. This difference was statistically significant ($P < 0.05$). In 27.35% of the allergic children and 6% of the nonallergic children, the positive tuberculin responses (PPD ≥ 10 mm) were recorded. The difference was significant ($P < 0.05$).

Conclusions: Our results showed that response to tuberculin in BCG-immunized allergic children is higher than in BCG-immunized nonallergic children.

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Key words: BCG vaccination; childhood allergy; tuberculin response.

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Accepted for publication 13 May 2002

The prevalence of allergic diseases has been rising in many countries (1,2). Although the precise causes of this rise are not clearly known, the decline in the rate of childhood infectious diseases is considered to be one of them (3–5). Some viral, bacterial, fungal, and protozoal infections lead to secretion of IFN- α from Th 1 cells; thus inhibiting IgE synthesis through B lymphocytes. Therefore, these infections induce the secretion of Th 1 cytokines, which cause the immune response to shift into Th 1 type (6,7). Mycobacteria are specifically known for strongly stimulating the Th 1 response (8). Attenuated *Mycobacterium tuberculosis* vaccination may suppress the development of atopy by secreting IFN- α and interleukin(IL)-2 (9). Not having tuberculosis and other infectious diseases may lead to absence of an inhibitory effect on Th 2 immune mechanisms; thus an increase in the rate of allergic diseases in developed countries (10).

Unlike infections, in atopic diseases Th 2 cells are stimulated through allergen stimulation and synthesize IL-4, which leads to IgE secretion from B lymphocytes. In such people, the immune response has shifted to Th 2 type and the Th 1 response has been suppressed (11). According to this hypothesis, atopic response and delayed-type hypersensitivity to tuberculin are two different series of reactions, antagonist to each other.

The tuberculin response is therefore expected to decrease in allergic diseases. In this study, tuberculin response in allergic childhood diseases has been investigated.

Material and methods

The study involved 206 cases from the Pediatric Allergy Unit and General Pediatrics Policlinic of Dr Sami Ulus Children's Hospital between April and December 1999. The age of the children ranged between 1 and 14 years and each child had at least one BCG scar. In Turkey, all children are vaccinated with BCG after birth, at ages of 2 months and 6 years. Thus, all children in the study group were vaccinated twice with BCG—at least once—before they were 14 years old.

One hundred and six children of those followed were diagnosed with allergic diseases; they were selected as the study group. The other 100 healthy children who applied to the General Pediatrics Policlinic were assigned to the control group. These children had no systemic complaints or atopic symptoms in their own or their familial histories. All parents gave informed consent. Detailed histories of the children in the study group were received.

Following physical examination, we studied posteroanterior chest roentgenograms, serum total IgE levels, and direct fecal smear examination for parasites. Those with allergic diseases, who were also suspected of having tuberculosis in their history, or from chest roentgenograms and/or parasites in their feces, were excluded from

the study. The terminology for the definition of allergic diseases was adapted to the EAACI Position Paper (12).

Of the allergic children, 80 (75.5%) had allergic asthma, 17 (16%) had allergic rhinitis, 5 (4.7%) had allergic atopic eczema/dermatitis syndrome, and 4 (3.8%) had allergic urticaria. The clinical diagnosis of allergic disease was based on information from the child's family and from clinical and laboratory assessment. The following criteria were used: allergic asthma was described as wheezing and/or chronic cough and/or dyspnea associated with definite environmental exposure, and exclusion of other diseases for at least 3 months (13). A child was considered to have allergic rhinitis if there were symptoms of runny nose, itchy nose, sneezing, and stuffed nose after exposure to a particular allergen, and if all of these were unrelated to infection. The diagnosis of allergic atopic eczema/dermatitis syndrome (AEDS) was based on the description by Hanifin and Rajka (14).

Skin prick tests (SPT) (Soluprick ALK, Denmark) were done on the forearm of all allergic children. The tested allergens were *Dermatophagoides pteronyssinus*, *Dermatophagoides farinea*, sp. grass mix, sp. tree mix, wool, cow's milk, hen's egg, cacao, dog epithelium, cat pelt, and mixed feathers. SPT results were considered positive if the wheal was ≥ 3 mm, compared to the negative control. Serum total IgE was determined by enzyme linked immunoassay (ELISA).

None of the children in our study had a subsequent tuberculin test, or revaccination within the last 6 months. The children with viral infections (such as measles, mumps, varicella, and influenza), with malnutrition, metabolic disease, immunosuppression by malign disorders or corticosteroids, or administration of live-virus vaccines at the last 3 months, were excluded from the study because of possible effects on their response to the tuberculin test.

The standard Mantoux test was applied to allergic and nonallergic children. One injection consisted of 5 TU (tuberculin units) PPD (0.1 ml). The reactions were read after 72 h by measuring the diameter of the wheal.

In statistical analysis, group comparisons were performed with Student's *t*-test. *P* values of less than 0.05 were considered significant.

Results

There were 106 allergic children, consisting of 65 boys (61.3%) and 41 girls (38.7%). The nonallergic control group consisted of 56 boys (56%) and 44 girls (44%). The mean age of the study group was 7.00 ± 3.18 years and that of the control group was 6.91 ± 3.22 years. There were no differences between the groups in age, sex, weight, height, or BCG vaccine status ($P > 0.05$) (Table 1).

In the allergic group 54.7% of children had at least one positive SPT reaction, so they were also atopic. A positive reaction to mites *D. pteronyssinus* and *D. farinea* was the most common (70.1%).

The purified protein derivative (PPD) wheal size was 6.29 ± 5.09 mm (mean $\pm$ SD) in allergic children and 2.79 ± 2.96 mm in nonallergic children. The difference between two groups was significant ($P < 0.001$).

In 27.35% of the allergic children and 6% of the nonallergic children, the positive tuberculin responses (PPD ≥ 10 mm) were recorded. The difference was significant ($P < 0.05$).

The allergic and nonallergic children were divided into four groups: Group 1 (aged 1–3 years), Group 2 (4–6 years), Group 3 (7–10 years) and Group 4 (11–14 years) and the mean PPD values were calculated for each group.

The mean PPD wheal sizes (mean $\pm$ SD) in allergic and nonallergic children were, respectively, for Group 1 (1–3 years old) 4.50 ± 4.64 mm and 1.84 ± 3.53 mm; Group 2 (4–6 years) 5.17 ± 4.84 mm and 2.50 ± 3.09 mm; Group 3 (7–10 years) 6.69 ± 4.97 mm and 3.23 ± 2.33 mm; Group 4 (11–14 years) 9.37 ± 5.21 mm and 3.23 ± 3.34 mm. In Group 1 the difference was not significant ($P > 0.05$), but it was for the other groups ($P < 0.05$; $P < 0.001$) (Table 2).

Comparison of BCG scar numbers showed that 125 (60.7%) of the total (206 allergic and nonallergic children) had only one BCG scar and 81 (39.3%) had two BCG scars. The mean PPD value for those with one scar was 3.60 ± 4.19 mm, and 6.12 ± 4.65 mm for those with two scars. The difference was statistically significant ($P < 0.001$).

In evaluating PPD wheal size according to BCG vaccine status, in children with single BCG scar, the mean PPD wheal in allergic children was 4.77 ± 4.79 mm, and in nonallergic children it was 2.48 ± 3.19 mm ($P < 0.05$) (Table 3). The mean PPD wheal size in allergic and nonallergic children who had been vaccinated twice was 8.35 ± 4.80 mm and 3.33 ± 2.44 mm, respectively. This difference was statistically significant ($P < 0.05$) (Table 3).

Discussion

The tuberculin response among children with allergic diseases was investigated; a stronger response was revealed in this group compared to that of the healthy population (mean PPD value 6.29 mm in allergic group, 2.79 mm in control group; $P < 0.001$).

There is a significant rise in prevalence of atopic diseases in developed countries. The reasons for this increase may include the decrease of some viral and mycobacterial infections which inhibit Th 2 responses

Table 1. Characteristics of the study population

	Allergic group		Nonallergic group	
	(n)	(%)	(n)	(%)
Boys	65	61.3	56	56
Girls	41	38.7	44	44
Age range	1–14 years of age		1–14 years of age	
Mean age (years $\pm$ SD)	7.00 $\pm$ 3.18		6.91 $\pm$ 3.22	
BCG vaccine status				
one BCG scar	61	57.5	64	64
two BCG scars	45	42.5	36	36

Table 2. Mean purified protein derivative (PPD) wheal values of allergic and nonallergic children according to age group

Group (age range)	Allergic group			Nonallergic group		
	PPD (mm)	n	%	PPD (mm)	n	%
Group 1: 1–3 years*	4.50 ± 4.64	12	3.11	1.84 ± 3.53	13	13
Group 2: 4–6 years**	5.17 ± 4.84	39	36.8	2.50 ± 3.09	36	36
Group 3: 7–10 years***	6.69 ± 4.98	39	36.8	3.23 ± 2.33	34	34
Group 4: 11–14 years***	9.37 ± 5.21	16	1.15	3.23 ± 3.34	17	17

* $P > 0.05$.** $P < 0.05$.*** $P < 0.001$.

Table 3. Mean purified protein derivative (PPD) wheal size with respect to BCG vaccine status in allergic and nonallergic groups

BCG status ($P < 0.05$)	Allergic group			Nonallergic group		
	PPD (mm)	n	%	PPD (mm)	n	%
One scar	4.77 ± 4.79	61	57.5	2.48 ± 3.19	64	64
Two scars	8.35 ± 4.80	45	42.5	3.33 ± 2.44	36	36

through induction of a Th 1 immune response (3–5, 11). This hypothesis is supported by the study of Shirakawa et al. (15) in which asthma incidence, serum IgE, and Th 2 type cytokines such as IL-4, IL-10, and IL-13 were lower in children with positive tuberculin tests compared with those of children with negative results. It has been stated that Th 1 immune mechanism activated through BCG immunization may limit atopic response due to the increase in cytokines such as IFN- α .

However; Alm et al. (16) found no difference between the prevalence of allergic diseases of the children with BCG immunization and those without. In the same study about prevention of allergic disease by BCG immunization, the probability of a difference between the immunities provided by BCG immunization and by natural contact with *M. tuberculosis* has been emphasized. The immunity following BCG immunization may not stimulate an effective Th 1 immune response as does natural infection. Furthermore, it was concluded that results might be affected by certain factors, such as the differences among strains of BCG vaccines, and in immune responses of societies with different genetic constitutions (16, 17).

When the PPD responses of allergic patients in BCG vaccinated groups were examined, there was no relation between the tuberculin response and allergic disease (18–20). However, Stannegard et al. (18) have shown higher frequencies of skin reactions of atypical mycobacteria such as *M. avium* and *M. scrofulaceum* in allergic group. In the present study, however, the tuberculin response of those with allergic diseases was higher than that of the control group. As the mean PPD wheal size was 6.29 mm for the allergic group, it might have developed against BCG vaccination or atypical mycobacteria.

In atopic individuals due to decreased cellular immunity, atypical mycobacterial infection may not be prevented;

as a result of this the development of delayed type hypersensitivity reaction could account for the increased (PPD) reactivity levels in our patients (18). The difference in PPD response in children aged ≥ 3 years could involve the effect of contact with environmental atypical mycobacteria. However, PPD positivity in our patient group (≥ 10 mm) was significantly higher than in the control group. This condition leads to a general consideration of an increase in delayed hypersensitivity to mycobacteria in allergic people.

Two hypotheses could be stated concerning increased PPD response mechanism in atopic patients. First in allergic individuals, when the reaction of the dermis against irritants increased, PPD reaction may have increased as well. Similarly, Nassif et al. (21) showed that even in those with respiratory allergies, through increased cytokines and mediators in the serum, mucosa and skin, the dermis has presented excessive response due to the abnormal intrinsic inflammatory cell hyperactivity against irritants. Second the increased tuberculin response in allergic people may be attributed to the effect of Th 2 cells in PPD reaction. As already known, tuberculin reaction is a delayed-type hypersensitivity reaction, developing through T lymphocytes and activated macrophages, to intradermal injection of *M. tuberculosis* protein antigen. Basically, tuberculin response is a clinical example of a delayed type of hypersensitivity reaction, constituted by cellular immunity, in which Th 1 cells are involved. However, Th 2 cells play a role in the cellular immune response, as well. Classical Th 2 cells suppress the cellular immune response by secreting IL-4, IL-13, IL-10 cytokines. In addition to this, there is a group of cells different from classical Th 2 lymphocytes whose relation with Th 2 cells has not been clearly established (22). Secreted transforming growth factor- β (TGF- β) from this subgroup of Th 2 cells inhibits T lymphocyte proliferation

and function, and macrophages. In studies conducted on knockout mice lacking TGF- β , an increased cellular response to microorganisms and chemical irritants has been observed. In allergic people if there is a suppression of T cells secreting TGF- β , when there is an increased activation of classical Th 2 cells, the cellular response cannot be restricted, and there may be increased PPD response. This hypothesis may also explain the exaggerated clinical response to viral infections in allergic children.

Contradictory results found in the literature concerning the relevance of PPD measurement and allergic diseases confirm the existence of various factors affecting PPD response (15, 18, 19). von Hertzen et al. (23) have

investigated the effects of tuberculosis infection in adolescence or childhood on the development of asthma and other allergic diseases without an evaluation of PDD response.

To conclude, we find an investigation of atopy prevalence among people with a history of tuberculosis to be more beneficial than the measurement of PDD reaction, due to its limitations in investigations of the allergy-preventive effects of *M. tuberculosis* infection. We recommend further studies on the reasons for strong positive PDD reactions in allergic patients, as was presented in our study. While PPD results are evaluated, the probability of an allergic disease should also be taken into consideration.

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