

Original article

Atopic disorders among Estonian schoolchildren in relation to tuberculin reactivity and the age at BCG vaccination

Background: Published data about a relationship of atopic diseases to *Bacillus Calmette-Guérin* (BCG) vaccination and tuberculin responses are inconsistent. Our aim was to determine this association in a country with a low prevalence of allergies.

Methods: A random sample of 10–11-year-old schoolchildren in Tallinn was studied by a parental questionnaire ($n = 979$) and skin-prick tests ($n = 643$), according to the International Study of Asthma and Allergies in Childhood. Data about BCG vaccinations and tuberculin tests were obtained from school records ($n = 723$).

Results: The prevalence of allergic symptoms and atopy was similar in children vaccinated during the first month of life and later. Positive tuberculin responses (≥ 5 mm) were inversely related to symptoms of asthma [odds ratio (OR) 0.10 (95% confidence interval 0.00–0.68) for exercise-induced wheezing; OR 0.37 (0.12–0.99) for night cough], and eczema [OR 0.53 (0.28–0.98)] but not to atopy. However, among BCG-revaccinated children, atopy tended to be more common in tuberculin responders, and the atopic children were significantly more likely to have a positive tuberculin response after the revaccination than would be predicted by their first test.

Conclusions: We found no protective effect of early BCG vaccination against atopy in school age, although tuberculin responses and allergic symptoms were inversely related.

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The increasing prevalence of atopic diseases in developed countries during the last decades has been attributed to the lack of induction of immune response caused by less exposure to certain infectious agents, including mycobacteria (1). In animal models, *Bacillus Calmette-Gu  rin* (BCG) vaccination protects against subsequent development of IgE antibody formation and allergen-induced airway inflammation (2, 3), and even against established allergy (4–6).

In human beings, the relationship between exposure to mycobacteria and atopic diseases is inconsistent. In ecological studies, based on the data from the International Study of Asthma and Allergies in Childhood (ISAAC), the prevalence of asthma symptoms in 13–14-year-old children was weakly inversely associated with national tuberculosis notification rates (7), but not with immunization rates for tuberculosis (8). A history of tuberculosis was related to a lower prevalence of allergic diseases in Finnish women (9). Early BCG vaccination protected children against atopy in Guinea-Bissau (10) and in Germany (11, 12), while in Sweden (13, 14) a general protective effect was not observed, and was restricted to children with a foreign background. There are also

reports suggesting a therapeutic effect of mycobacteria on pre-existing allergic disease (15–17).

Delayed-type hypersensitivity to tuberculin is a marker of Th1-mediated immunity to mycobacteria. Positive tuberculin responses in Japanese schoolchildren were reported to be related to lower prevalence of atopic sensitization and allergic diseases (18). The findings have been interpreted either as the result of an inhibiting effect of different mycobacteria on atopy or as a reflection of an impaired Th1 immunity among atopic individuals (14, 19). Nevertheless, several subsequent studies in different populations have failed to demonstrate any association between atopic disorders and tuberculin responsiveness (20–23), and some studies even revealed stronger responses to mycobacteria among allergic children (14, 24). Similarly, in a recent Estonian study, delayed-type hypersensitivity responses to diphtheria and tetanus antigen were stronger in atopic children (25).

In Estonia, all newborn babies are vaccinated with BCG at birth as the incidence of tuberculosis is relatively high [incidence rate 51/100 000 in 1997; (26)]. At the same time, the prevalence of allergic diseases and atopic sensitization is significantly lower than in Western

countries (27–29). Therefore, the aim of our study was to determine whether tuberculin reactivity and the age at BCG vaccination were associated with atopic disorders in Estonian schoolchildren.

Methods

Study population

A total of 1158 children between 10 and 11 years of age from 20 schools randomly selected among the Estonian language schools in Tallinn were studied between December 1996 and April 1997 as a part of the ISAAC Phase II study. The methods employed to assess the prevalence of allergic diseases and atopic sensitization were described in detail elsewhere (29). Briefly, the parents of 85% (979/1158) of the children answered to the standard ISAAC core questionnaire, enquiring about symptoms of wheezing, rhinoconjunctivitis and eczema. Of these children, 66% (643/979) were skin-prick tested (SPT) with seven prevalent airborne allergens, i.e. *Dermatophagoides pteronyssinus*, *D. farinae*, cat and dog dander, birch pollen, timothy pollen, and *Alternaria tenuis* (Soluprick SQ, ALK, Hørsholm, Denmark). The test was considered positive if the sum of the orthogonal diameters of the wheal was ≥ 3 mm and atopy was defined as at least one positive reaction.

BCG vaccination and tuberculin skin testing

The data concerning BCG vaccinations and tuberculin testing were obtained retrospectively from the children's medical records at school, which were available for 723 individuals (74% of the participating children) from 17 of 20 participating schools. Of the 723 children with medical records, 717 (99%) had received BCG vaccination. At the time of their first BCG vaccination, 638 (89%) of the children were less than 1 month old (usually 3–4 days), 42 (6%) were 1–11 months, and 37 (5%) at least 1 year old. A Soviet BCG strain (Minmedbioprom, USSR) containing 500 000–1 500 000 live microbes in one dose of 0.05 mg was used for vaccination. Altogether 282 (39%) children with a negative tuberculin response (no induration, nor hyperaemia) at 7 years of age were vaccinated a second time.

The children were periodically tested for tuberculin conversion in polyclinics and in school medical offices. As testing had become optional by the end of 1990s, the frequency and the age of testing varied between the medical offices. Of the 717 BCG-vaccinated children, 715 were tested at least once. The number of recorded tuberculin tests for one child varied from 1 to 8 (mean 3.5). The most common age for tuberculin testing was 7 years. In our analysis we used the results of the tuberculin tests performed at the age of 2–3, 6–7 and 10–11 years. If a child was tested at another age, the result of the test was included into the closest of the ranges. Altogether 471 children had been tested at least once at 1–4 years, 599 at 5–8 years, and 343 at 9–12 years of age (65, 83 and 47% of the children with medical records, respectively). The children were tested using intradermal Mantoux test with 2 tuberculin units of purified protein derivate (Minmedbioprom, USSR/Russia) on the volar aspect of the forearm. The results were recorded after 72 h as the diameter of the induration. The test was considered positive if the diameter of the wheal was ≥ 5 mm.

Statistical analysis

Statistical analyses were performed using statistical software programme SAS. To compare the differences in prevalence rates

between groups, odds ratios (OR) with 95% confidence intervals (CI) and chi-squared tests, or Fisher's exact tests if appropriate, were employed. Multivariate logistic regression analyses were used to calculate OR with adjustments for different covariables.

Ethical considerations

The study was approved by the institutional review board of Tallinn Institute of Experimental and Clinical Medicine. The parents of the participating children gave their informed consent.

Results

The prevalence of questionnaire-reported symptoms of asthma, allergic rhinitis, eczema, and positive SPT were similar in children vaccinated during the first month of life and later (Table 1). Adjustment for sex, age and the number of BCG vaccinations did not appreciably influence the results (data not shown).

The proportion of positive tuberculin responses (induration ≥ 5 mm) increased from 18% at 2–3 years to 45% at 10–11 years of age ($P < 0.001$) and large responses (≥ 10 mm) increased from 6 to 22%, respectively ($P < 0.001$) (Table 2). Of the 343 children who were tuberculin tested at 10–11 years of age, 101 (29%) had been revaccinated with BCG at 7–8 years. The prevalence of positive tuberculin responses was similar in children who were and were not revaccinated (50% vs 42%; $P = 0.16$).

The relationship of tuberculin responses at different ages to clinical symptoms and sensitization is shown in Table 3. The prevalence of lifetime and current symptoms at 10–11 years of age was similar in children with and without a positive tuberculin response at 2–3 and

Table 1. Prevalence rates, crude odd ratios (OR), and 95% confidence intervals (CI) of allergic symptoms and positive skin-prick tests at 10–11 years of age in relation to the age at BCG vaccination

	Age at BCG vaccination		
	≤ 1 month ($n = 638$) (%)	> 1 month ($n = 79$) (%)	OR (95% CI)
Lifetime prevalence			
Wheezing	125/627 (20)	17/76 (22)	0.86 (0.48–1.54)
Diagnosed asthma	15/618 (2)	3/74 (4)	0.59 (0.16–2.14)
Runny nose	127/627 (20)	16/77 (21)	0.97 (0.54–1.72)
Itchy rash	155/625 (25)	25/78 (32)	0.70 (0.42–1.16)
12-month prevalence			
Wheezing	49/625 (8)	8/77 (10)	0.74 (0.33–1.61)
Exercise-induced wheezing	31/623 (5)	7/78 (9)	0.53 (0.23–1.25)
Night cough	54/625 (8)	10/78 (13)	0.65 (0.31–1.32)
Runny nose	103/626 (16)	13/77 (17)	0.97 (0.34–1.82)
Rhinoconjunctivitis	32/625 (5)	4/77 (5)	0.98 (0.34–2.86)
Itchy rash	118/625 (19)	19/78 (24)	0.72 (0.41–1.27)
Flexural dermatitis	93/624 (15)	13/78 (17)	0.88 (0.47–1.64)
≥ 1 positive skin-prick test	61/417 (15)	7/54 (13)	1.15 (0.49–2.63)

Table 2. The distribution of tuberculin responses at different ages and in relation to BCG revaccination

Tuberculin test	2–3 years (<i>n</i> = 471) (%)	6–7 years (<i>n</i> = 599) (%)	10–11 years (%)		
			All tested (<i>n</i> = 343)	BCG-revaccinated at 7–8 years (<i>n</i> = 101)	No revaccination (<i>n</i> = 242)
Tuberculin response					
0–4 mm	384 (82)	452 (75)	190 (55)	50 (50)	140 (58)
5–9 mm	60 (13)	80 (13)	78 (23)	23 (23)	55 (23)
10–14 mm	23 (5)	57 (10)	59 (17)	22 (22)	37 (15)
≥15 mm	4 (1)	10 (2)	16 (5)	6 (6)	10 (4)

Table 3. Prevalence rates, crude odd ratios (OR), and 95% confidence intervals (CI) of allergic symptoms and positive skin-prick tests at 10–11 years of age in relation to tuberculin response at 2–3, 6–7, and 10–11 years of age

	Tuberculin response at 2–3 years (<i>n</i> = 471)			Tuberculin response at 6–7 years (<i>n</i> = 599)			Tuberculin response at 10–11 years (<i>n</i> = 343)		
	≥5 mm (%)	<5 mm (%)	OR (95% CI)	≥5 mm (%)	<5 mm (%)	OR (95% CI)	≥5 mm (%)	<5 mm (%)	OR (95% CI)
Lifetime prevalence									
Wheezing	15/86 (17)	78/377 (21)	0.81 (0.44–1.49)	31/145 (21)	92/443 (21)	1.04 (0.66–1.64)	24/150 (16)	41/185 (22)	0.67 (0.38–1.17)
Diagnosed asthma	1/86 (1)	10/376 (3)	0.43 (0.05–3.55)	3/146 (2)	10/438 (2)	0.90 (0.24–3.39)	1/148 (1)	5/185 (3)	0.24 (0.03–2.17)
Runny nose	20/85 (24)	74/379 (20)	1.26 (0.72–2.22)	33/145 (23)	87/445 (20)	1.21 (0.77–1.91)	23/151 (15)	42/186 (23)	0.61 (0.35–1.08)
Itchy rash	20/84 (24)	103/378 (27)	0.83 (0.48–1.45)	32/143 (22)	118/446 (26)	0.80 (0.51–1.25)	34/150 (23)	52/185 (28)	0.75 (0.46–1.23)
12-month prevalence									
Wheezing	6/86 (7)	30/376 (8)	0.86 (0.35–2.15)	9/144 (6)	37/442 (8)	0.73 (0.34–1.55)	5/150 (3)	16/184 (9)	0.36 (0.10–1.07)
Exercise-induced wheezing	5/84 (6)	21/377 (6)	1.07 (0.39–2.93)	9/143 (6)	20/444 (5)	1.42 (0.63–3.20)	1/150 (1)	12/186 (6)	0.10 (0.00–0.68)*
Night cough	4/86 (5)	42/378 (11)	0.39 (0.14–1.12)	7/145 (5)	45/445 (10)	0.45 (0.20–1.02)	6/150 (4)	19/186 (10)	0.37 (0.12–0.99)**
Runny nose	18/85 (21)	60/380 (16)	1.43 (0.80–2.58)	30/145 (21)	69/445 (16)	1.42 (0.88–2.29)	17/150 (11)	30/185 (16)	0.66 (0.35–1.25)
Rhinoconjunctivitis	5/85 (6)	19/379 (5)	1.18 (0.43–3.27)	11/144 (8)	22/445 (5)	1.59 (0.75–3.36)	5/150 (3)	12/185 (6)	0.50 (0.17–1.44)
Itchy rash	17/84 (20)	72/378 (19)	1.08 (0.60–1.95)	22/144 (15)	93/445 (21)	0.68 (0.41–1.13)	25/150 (17)	41/184 (22)	0.70 (0.40–1.21)
Flexural dermatitis	15/84 (18)	55/378 (15)	1.28 (0.68–2.39)	19/144 (13)	72/444 (16)	0.79 (0.46–1.35)	17/150 (11)	36/184 (20)	0.53 (0.28–0.98)***
≥1 positive skin-prick test	6/59 (10)	39/262 (15)	0.65 (0.26–1.61)	12/98 (12)	49/303 (16)	0.72 (0.37–1.42)	15/106 (14)	19/127 (15)	0.94 (0.45–1.95)

* $P = 0.008$; ** $P = 0.036$; *** $P = 0.041$.

6–7 years. However, at 10–11 years, the tuberculin responses and symptoms were inversely related. Thus, among the children with tuberculin response ≥ 5 mm at this age, the prevalence of current exercise-induced wheezing, night cough, and flexural dermatitis was lower than among the children with smaller response. Adjustment for sex and age at the tuberculin test did not appreciably influence the results (data not shown). No such association was found between tuberculin responses at any age and the prevalence of positive SPT at 10–11 years.

When the children who were and were not revaccinated with BCG at 7–8 years, were analysed separately, the association between allergic symptoms and tuberculin response at 10–11 years was similar and in the same direction in both groups, although in the group of revaccinated children, because of the smaller sample size, it did not achieve statistical significance (data not shown). However, sensitization tended to be inversely associated with tuberculin responsiveness (10% vs 17%, OR 0.54, 95% CI 0.20–1.38) only among children who

were not revaccinated. Conversely, among the revaccinated children, positive SPT tended to be more common in tuberculin responders than in non-responders (24% vs 11%, OR 2.54, 95% CI 0.73–10.45). Furthermore, the atopic children were significantly more likely to have a positive tuberculin response after the BCG revaccination than would be predicted by their first test. Among the 141 non-atopic subjects who were revaccinated, 28 (20%) had a subsequent positive tuberculin response, while 105 (53%) of 200 non-atopic subjects were positive after the first BCG vaccination. In contrast, among the 21 revaccinated atopic subjects eight (38%) showed a positive tuberculin response after revaccination, while 15 (42%) of 36 showed a positive tuberculin response after the first BCG vaccination. There was a significant association of the interaction of BCG revaccination and atopy with a positive tuberculin response, OR = 4.02 (95% CI 1.13–14.38), $P = 0.032$ after adjustment for the main effects (BCG revaccination and atopy) as well as sex, age at the last tuberculin test and size of the first test induration.

Discussion

We found no association between the age at BCG vaccination and the prevalence of an atopic disorder. Thus, the prevalence of atopic symptoms and sensitization were similar in schoolchildren who were vaccinated during the first month of life (mostly at the age of 3–4 days) and in children immunized later.

Stimulation of Th1-type immunity during the first months of life has been suggested to enhance protection against allergy later in life (30, 31). In a German study, however, the influence of BCG vaccination on allergic diseases decreased over time (12). In our study, the children were 10–11 years old when their allergic status was assessed. By this age, any effect of early vaccination could have been overshadowed by more recent exposure with natural mycobacteria in a region of relatively high incidence of tuberculosis.

In Guinea-Bissau, BCG vaccination was associated with decreased risk of atopic sensitization and the protective effect of BCG against atopy was greater if the vaccination was received at an early age, especially during the first week of life (10). In a Swedish study, the protective effect of BCG vaccination was restricted to children with a foreign background (14). Thus, the effect of BCG on the development of allergic diseases may be different for populations with different degree of allergy risk and different lifestyle. This suggestion is supported by the comparison of neonates from Eastern and Western Germany, showing differences in T cell reactivity already at birth, i.e. decreased interferon-gamma and tumor necrosis factor-alpha and increased interleukin-4 production by neonates in Western Germany (32). Divergent reports on the effects of BCG vaccination on the development of allergy between studies may also possibly be explained by differences in BCG strains and doses.

A positive tuberculin response was inversely related to current symptoms of asthma and atopic eczema, but only for tuberculin tests performed at 10–11 years, i.e. at the same time as symptoms were assessed. The lack of any association with earlier tuberculin tests indicates that if there is any causal relationship between exposure to mycobacteria and allergy, the effect is likely not longlasting. In addition, we found no relationship between tuberculin responses at any age and the prevalence of positive SPT.

We used retrospective data for tuberculin tests which were performed and read by several different persons. This could lead to some misclassification of the tuberculin responses. However, this misclassification would be random with respect to atopic disorders. Although the data about tuberculin responsiveness was not available at every age period for all study groups, it is unlikely to bias

the results substantially, because the prevalence of allergic diseases and atopic sensitization was similar among children with and without the data.

While positive tuberculin responses in Japanese schoolchildren correlated inversely with atopic sensitization and allergic diseases (18), several more recent studies have failed to demonstrate any association between atopic disorders and tuberculin responsiveness (20–23). Possible explanations for this inconsistency include differences in study design (especially the time of the testing), tuberculin preparations, doses, assessment of the test results, concurrent environmental factors, and immune responsiveness of different populations.

Besides a possible protective effect of mycobacterial infection against atopy, an inverse relationship between tuberculin responsiveness and atopic disorders could also be explained by altered immune responsiveness in the atopic individuals (14), as atopic diseases have been associated with deviation towards Th2-type immune response and decreased capacity to produce Th1 cytokines (33). However, there is now increasing evidence questioning this possibility. For example, the prevalence of both atopic and autoimmune diseases have increased in parallel during the last decades, and the disorders are significantly associated and share common risk factors (34, 35). Furthermore, concomitant Th1 and Th2 cytokine production to allergens was recently demonstrated in atopic children (36).

In the subgroup of BCG-revaccinated children, SPT positivity tended to be associated with a positive tuberculin response. Furthermore, among atopic children, a positive tuberculin response was more common after the BCG revaccination than would be predicted by their first test. Increased delayed-type hypersensitivity to mycobacteria among allergic children has also been reported in some other studies (14, 24). Similarly, in a recent study, Estonian children, if atopic, showed stronger delayed-type hypersensitivity responses to diphtheria and tetanus antigens than non-atopic children (25). The observations are not easily accommodated within the Th1/Th2 concept as the cause of allergic disease, but could rather indicate differences in immune regulation between allergic and non-allergic children.

In conclusion, our study revealed no protective effect of early BCG vaccination against atopy in school age, although positive tuberculin responses were inversely related to current symptoms of asthma and eczema.

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