

Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria

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By influence on the Th1/Th2 cell balance, infectious agents may affect the development of atopic allergy. In this study, we investigated whether previous BCG vaccination or infection with atypical mycobacteria might be related to the development of atopic disease. The study, which involved skin testing with mycobacteria and answers to a questionnaire for more than 6000 children in Sweden, revealed a low prevalence of allergy among BCG-vaccinated children who were immigrants or adopted from other countries. Vaccinated children born in Sweden, however, did not have significantly lower allergy prevalence than age-matched, unvaccinated children. Furthermore, the overall frequencies of skin-test reactivity to the atypical mycobacteria *M. avium* and *M. scrofulaceum* were higher rather than lower in allergic than in nonallergic children. By contrast, there was a tendency toward a lower frequency of more strongly positive skin reactions (≥ 10 mm) to mycobacteria in allergic than in nonallergic children. These findings do not support the hypothesis that early mycobacterial infections have a suppressive effect on the development of atopic disease. Earlier findings of an apparent association between atopy and lack of previous mycobacterial infection may possibly be explained by a relatively decreased ability of atopic patients to mount strong Th1 cell-mediated immune responses.

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The prevalence of atopic diseases has been increasing in many parts of the world over the last 20–30 years (1–4). Generally, this has been found to be associated with a shift toward a “westernized” lifestyle. For instance, comparative studies have shown the prevalence of atopic diseases to be higher in West than in East Germany, although the gap between the different prevalences has gradually been narrowing since the unification of the two parts of the country (5). Likewise, the prevalence of atopy has been found to be lower in the Baltic countries than in Sweden (6).

The reasons for the increasing prevalence of atopy are not known. Suggested causes include increased allergen load (e.g., mites) (7), changed indoor climate (8, 9), exposure to tobacco smoke (10), and air pollution by automobile exhaust (11).

A recently advanced hypothesis suggests that changes in the general range of infections may

account for the increased prevalence of atopic disease (12). Support for this hypothesis has been obtained from findings of an inverse relationship between occurrence of atopy and number of siblings (13, 14) and from findings of a very high prevalence of asthma in certain islands with low prevalence of respiratory infections (reviewed by Martinez [15]). Further and stronger support for the role of infections in determining atopy prevalences was obtained in a study from Guinea-Bissau which found that subjects who had had measles during early childhood were about half as likely to develop sensitization and atopy as those who had not had measles (16). Similarly, a low prevalence of atopic allergy was recently found to be associated with serologic evidence of past hepatitis A infection (17).

The reason for the suggested inhibitory effect of infections on the development of atopy could be

that the infections, by inducing Th-1 cytokines, direct the immune response to this branch of the T-helper cell immune response (18). Interferon (IFN)-gamma, which is an important part of the Th-1 response, can suppress the Th-2 responses characteristic of atopy. Therefore, induction of IFN- γ , which especially occurs during infections by viruses, intracellular bacteria, fungi, and certain protozoa, might lead to a general suppression of the development of atopy (19). Mycobacteria, especially *M. tuberculosis*, are known to be very potent inducers of Th-1 immune responses (20). Recently, Shirakawa et al. (21) reported a strong inverse association between delayed hypersensitivity to *M. tuberculosis* and atopy. Therefore, the authors speculated that exposure and response to *M. tuberculosis* may, by modification of immune profiles, inhibit atopic disorders.

In countries where the prevalence of *M. tuberculosis* is low, infections with atypical mycobacteria or vaccination with BCG, which is often performed during the very first days of life, might have an effect similar to that of *M. tuberculosis*; i.e., a preventive effect against atopy. In this study, we investigated whether previous BCG vaccination or infection with atypical mycobacteria might be related to the development of atopic disease.

Material and methods

The study comprised 802 pre-school children (group I) and 3557 schoolchildren (group III) in Göteborg, the second largest city in Sweden, located in the western, coastal part of the country. The children were 4–5 years and 7–8 years of age, respectively. They were recruited from 72 different day-care centers and from altogether 66 different schools, covering various socioeconomic groups, as well as different geographic locations within the city. Another study group included 2138 8–9-year-old schoolchildren (group II) in Jämtland, an inland rural area further north in Sweden.

The investigations included detailed questionnaires as well as skin tests with purified protein derivative (PPD) and sensitins derived from the atypical mycobacteria *M. avium* and *M. scrofulaceum*. None of the children had earlier been tested with either tuberculin or any of the sensitins.

Skin tests

Injections and readings of skin tests, as well as checking for scars caused by previous BCG vaccinations, were performed at the day-care centers and schools, respectively. The testing teams at the day-care centers and schools in Göteborg comprised two specially trained registered nurses. The

same nurses were employed for the work throughout the study. Likewise, in Jämtland, the testing team comprised two specially trained nurses. The tests were performed in 1986–90. The injections were given simultaneously on the middle part of the dorsal surface of the right and left arms, respectively. The standard Mantoux test was applied. One injection consisted of 2 TU PPD RT 23 and one of a sensitin, either 0.1 μ g *M. avium* sensitin RS 10, or 0.1 μ g *M. scrofulaceum* sensitin RS 95. The volume of injected tuberculin or sensitin was 0.1 ml, and it was given intracutaneously. The *M. avium* sensitin was prepared from the strain "Maren Cecilie", derived from a Danish patient and being of serotype 2. The *M. scrofulaceum* sensitin was prepared from a culture originating in Canada (22).

The tuberculin and the sensitins were obtained from the Statens Seruminstitut, Copenhagen, Denmark. The reactions were read after 3 days by measuring the transverse diameter of the induration (mm). A minimum diameter of 3 mm was considered a positive reaction and a diameter of ≥ 10 mm was labeled "strongly positive".

Questionnaire

The parents were asked to answer a questionnaire on several aspects of their children's health: whether the children had any allergic diseases and, if so, what type; whether they had been BCG-vaccinated or tuberculin-tested; whether they had had any contact with patients with tuberculosis; and whether they kept any pets at home. The children were considered BCG-vaccinated only if the questionnaires contained information about the year of vaccination.

From the information in the questionnaires, it was possible to determine prevalences of various types of atopic as well nonatopic allergy in the various groups. However, to avoid possible errors due to biased interpretation of the answers in the questionnaires, all calculations described below were made with respect to allergy in general.

The children and their parents were provided with oral as well as written information, and the investigations were approved by the scientific ethical committees of the Universities of Göteborg and Umeå, respectively.

Statistical methods

Group comparisons were performed with Fisher's exact two-sided test, or, when groups included a very large number of subjects, the chi-square test. The significance of pair differences was determined by Wilcoxon's test.

Table 1. Prevalence of allergy in BCG-immunized and nonimmunized children

Place of birth	BCG vaccination	Prevalence of allergy			
		Group I (4–5 years) (%)	Group II (8–9 years, rural) (%)	Group III (8–9 years, city) (%)	Total (%)
Sweden	No	167/732 (22.8)	470/2059 (22.8)	687/3289 (20.9)	1324/6080 (21.8)
Sweden	Yes	7/34 (20.6)	13/38 (34.2)	29/160 (18.1)	49/232 (21.1)
Abroad	No	8/23 (34.8)	9/28 (32.1)	17/72 (23.6)	34/123 (27.6)
Abroad	Yes	2/13 (15.4)	2/13 (15.4)	4/36 (11.1)	8/62 (12.9)

Results

Prevalence of allergy in relation to BCG vaccination in infancy

According to the questionnaires, 340 children in the total material had been BCG-immunized. From this group of children, 46 were excluded, as the immunization had been performed at a later stage than during the first year of life or information about allergy was lacking. As Table 1 shows, no statistically significant differences in allergy prevalence were observed between BCG-immunized and nonimmunized children born in Sweden. In contrast, prevalences in immigrant children tended to be lower in BCG-vaccinated than in unvaccinated children in all groups, although none of the differences observed were statistically significant. However, when previous BCG vaccination was defined by occurrence of demonstrable scar, one statistically significant difference was noted. Thus, group III then included only one allergic child among 25 immunized children, as opposed to 17/72 unvaccinated children ($P=0.04$).

Since the difference in allergy prevalence between BCG-vaccinated and unvaccinated children born abroad could depend on the children's country of origin, further comparison on this aspect was made. It was then found that a considerably higher proportion of the unvaccinated than of the BCG-vaccinated children born abroad originated from "Western countries". Still, when only children born outside such countries (mainly Asian and South American countries) were considered, there was a higher prevalence of allergy in unvaccinated (19/75; 25.3%) than in BCG-vaccinated children (8/57; 14.0%). This difference, however, was not statistically significant.

Calculations of actual prevalences of atopic dermatitis, allergic rhinoconjunctivitis, or asthma were made after a careful review of the questionnaires. Even after exclusion of cases of doubtful atopic allergy, the results were similar to those described above regarding prevalence of defined atopy in the BCG-immunized compared with non-BCG-immunized children.

PPD reactivity in BCG-immunized and in non-BCG-immunized children

As expected, there was generally a much higher frequency of PPD reactivity in BCG-vaccinated than in nonvaccinated children (Table 2). The frequency of positive reactions (≥ 3 mm) was higher in allergic than in nonallergic subjects in five of the six group pairs ($0.05 < P < 0.10$). On the other hand, the frequencies of strongly positive reactions (≥ 10 mm) showed a reverse pattern in the BCG-vaccinated children. Thus, in these children, strongly positive reactions were observed almost twice as frequently in nonallergic as in allergic children. This difference, however, was not statistically significant.

*Reactivity to *M. avium* and *M. scrofulaceum**

All children in the study were tested for PPD reactivity and, simultaneously, for reactivity to either *M. avium* or *M. scrofulaceum*. Table 3 shows the results of the latter tests in allergic and non-allergic children that had not been BCG-vaccinated. As Table 3 shows, there was a consistently higher frequency of reactivity in allergic than in nonallergic children, regardless of the type of sensitin used in the test. Although the differences were rather small (3–5 percentage units), they were found in all six group pairs (Wilcoxon's test for pair differences; $P < 0.05$); and when the results obtained in the various groups were compared, it was again found that the differences were statistically significant.

A higher level of significance ($P=0.01$) was found for comparisons between sensitin reactivities in urban children and children living in the rural area of Jämtland. Thus, urban children had clearly higher sensitin skin reactivity to *M. avium*, as well as to *M. scrofulaceum*, than the rural children.

Occurrence of strongly positive skin test reactions in allergic and in nonallergic children

Although the frequency of skin reactivity to PPD and the two sensitins used in the study generally was higher in allergic than in nonallergic children, the reverse was true when only strongly positive

Table 2. PPD reactivity in BCG-immunized and nonimmunized children in relation to occurrence of allergy

BCG vacc.	Group	Allergy	n	PPD reactivity	
				≥3 mm (%)	≥10 mm (%)
+	I	-	25	20 (80)	9 (36.0)
+	I	+	7	7 (100)	1 (14.3)
+	II	-	41	12 (29.2)	5 (12.2)
+	II	+	10	5 (50.0)	1 (10.0)
+	III	-	115	58 (50.4)	22 (37.9)
+	III	+	17	10 (58.8)	2 (11.8)
-	I	-	567	46 (8.1)	7 (1.2)
-	I	+	180	20 (11.1)	1 (0.6)
-	II	-	1291	174 (13.5)	6 (0.5)
-	II	+	439	59 (13.4)	5 (1.1)
-	III	-	2307	330 (14.3)	11 (0.5)
-	III	+	729	107 (14.7)	6 (0.8)

Table 3. Skin test reactivity to *M. avium* and *M. scrofulaceum* sensitins in allergic and nonallergic children

Group	Allergy	Positive reaction (≥3 mm)	
		<i>M. avium</i> (%)	<i>M. scrofulaceum</i> (%)
I	-	49/281 (17.4)	51/286 (17.8)
I	+	25/110 (22.7)	16/70 (22.9)
II	-	156/655 (23.8)	172/636 (27.0)
II	+	53/199 (26.6)**	78/240 (32.5)
III	-	470/1208 (38.9)	492/1100 (44.7)
III	+	174/382 (45.5)**	161/347 (46.4)
All	-	675/2144 (31.5)*	715/2022 (35.4)
	+	252/691 (36.5)*	255/657 (38.8)

*Difference between allergic and nonallergic children was statistically significant ($P=0.047$). Note that allergic children had higher prevalence rates than nonallergic children in all group pairs (Wilcoxon's test for paired differences, $P<0.05$).

**Difference in reactivity to *M. avium* between groups II and III (rural and city) was statistically significant ($P=0.01$).

reactions were considered. This phenomenon was particularly evident for PPD reactivity in BCG-vaccinated children (Table 1), but it was also seen for sensin reactivity. Table 4 shows findings in the 4–5-year-old children. Although the relative differences between the allergic and nonallergic children were considerable, they did not attain statistical significance. This was true also when the frequency of strongly positive reactions was expressed in relation to overall numbers of positive reactions.

Discussion

The hypothesis that infections may inhibit development of atopy postulates that infectious agents, such as viruses and intracellular bacteria, preferentially stimulate the development of Th-1-type immunity (12, 15). Therefore, a relative lack of infections caused

Table 4. Frequencies of strongly positive reactions (≥10 mm) in allergic and nonallergic 4–5-year-old children

Group	PPD ^a (%)	<i>M. avium</i> ^a (%)	<i>M. scrofulaceum</i> ^a (%)
Allergic	2/187 (1.1)	1/117 (0.9)	2/77 (2.6)
Nonallergic	16/592 (2.7)	9/306 (2.9)	11/311 (3.5)

^a BCG-vaccinated children included in all groups. Difference between allergic and nonallergic children was not significant in any group.

by such agents during early childhood might lead to a shift of the Th-1/Th-2 balance to the Th-2 side, thereby predisposing for allergic sensitization and development of atopy. In this context, mycobacteria, which are very strong inducers of a Th-1-type immune response, are particularly relevant (20).

In Sweden, infection with *M. tuberculosis* is uncommon; presently, the annual incidence of tuberculosis is about 6/100 000 inhabitants. General vaccination against tuberculosis was stopped in 1975, but BCG vaccination of neonates or older children is still practiced occasionally when an increased risk of tuberculosis is anticipated, as, for instance, in immigrant children from highly endemic areas. Although tuberculosis is rare in Sweden, infection with atypical mycobacteria seems to be common, as judged by the results of our previous study of skin test reactivity to the mycobacteria *M. avium* and *M. scrofulaceum* in more than 7000 children from two regions in Sweden (23–25). These studies used questionnaires on allergy. It was considered that retrospective analysis of these data might yield information about any possible relationship between past mycobacterial infection and development of atopic allergy.

This study found that BCG-vaccinated children had a tendency toward a lower frequency of allergy than their age-matched, unvaccinated controls. This finding apparently agrees with the hypothesis of a protective effect of mycobacterial infection against the development of atopy. However, the low prevalence was not statistically significant, and it was observed only in immigrant children, not in children born in Sweden. Thus, the findings suggest that some factors, other than BCG vaccination, attributable to the environment in the children's country of origin, could have protected against development of allergy. This possibility would be in accordance with the well-known results of Morrison-Smith et al. (26) suggesting that environmental influences occurring very early in life are of decisive importance for later development of atopic disease. In agreement with our results, Alm et al. found no correlation between BCG vaccination and absence of atopy in a case-control study of Swedish children (27).

The present findings showed that the frequency of positive skin reactions to BCG and the atypical mycobacteria *M. avium* and *M. scrofulaceum* were increased rather than decreased in the allergic children. This might be taken as evidence against a possible protective role of mycobacteria in the development of atopy. However, it is possible that such a hypothetical protective effect is evoked only if the infection takes place very early in life. As judged by the about twice as high prevalence of skin test reactivity in 8–9-year-old children as in 3–4-year-old children in Göteborg, most of the infections by atypical mycobacteria occur after the first year of life.

The most plausible explanation of the increased frequency of positive skin tests in atopic children is that they have a decreased cell-mediated immunity. This is manifested by an increased tendency to develop severe infection by certain viruses such as Epstein-Barr virus, herpes simplex, and varicella-zoster virus (28, 29). Therefore, the present study may suggest that the decreased cell-mediated immunity which is associated with atopic disease (30) results in a slightly decreased ability to avert infection by atypical mycobacteria.

Although we found an increased rather than the expected decreased frequency of positive skin tests to mycobacteria, it should be noted that strongly positive reactions (≥ 10 mm), such as those that were exclusively considered in the study of Shirakawa et al. (21), were somewhat lower in atopic than in nonatopic children. This finding should not be surprising in view of the early findings of decreased delayed hypersensitivity and other T-cell-mediated reactions in patients with atopic dermatitis and other atopic diseases (31–33). Thus, the previous findings of Shirakawa et al. (21) of a relationship between a lack of strong PPD reactivity and atopy are not surprising.

The observation that infection by atypical mycobacteria appears to be much more common in the city of Göteborg than in the more northern, rural area of Jämtland has been made before (25). In the present context, it is interesting to note that it has been claimed that there is a latitude-dependent difference in the prevalence of allergy in Sweden, and the disorder has been reported to be more common in the northern than in the southern part of the country (4). In the present study, however, we did not find any significant difference in allergy prevalence between the two regions.

In conclusion, atopic children show evidence of past mycobacterial infections at least as often as nonatopic children but seem to have a decreased ability to mount a strong Th-1 cell-mediated delayed-hypersensitivity response. It has been speculated that an early mycobacterial infection in an infant

who is genetically predisposed to atopic disease may affect the Th-1/Th-2 cell balance, resulting in a decreased tendency to develop atopic disease. The present results do not support this hypothesis.

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