Delayed Hypersensitivity to Tuberculin, Total Immunoglobulin E, Specific Sensitization, and Atopic Manifestation in Longitudinally Followed Early Bacille Calmette-Guérin-Vaccinated and Nonvaccinated Children

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ABSTRACT. *Background.* Bacille Calmette-Guérin (BCG) is a strong T helper 1 incentive and, thus, may contribute to a decreased risk of T helper 2-dependent atopic disease.

Objective. To investigate the natural course of specific immunoglobulin E (IgE) responses and atopic disease in BCG-vaccinated and nonvaccinated children.

Participants. Seven hundred seventy-four children from a prospectively followed birth cohort.

Outcome Measures. Physical examination and case history were performed at 3, 6, 12, 18, 24, 36, 48, 60, 72, and 84 months of age. Total and specific serum IgE levels to 9 common inhalant and food allergens were determined (CAP; Pharmacia, Freiburg, Germany) at 12, 24, 36, 60, 72, and 84 months of age. Purified protein derivative (PPD) skin testing was performed at 84 months.

Results. Period and lifetime prevalences of atopic dermatitis and recurrent wheezing tended to be lower in the BCG-vaccinated group early in life, whereas no such trend was found after the second birthday or for allergic rhinitis. The proportion of children remaining free of clinical manifestations tended to be higher in the BCG-vaccinated group but differences decreased over time. No statistically significant differences were found for total IgE levels (median). Atopic sensitization tended to be lower among BCG-vaccinated children during the first 2 years of life. The diameter of the skin reaction to PPD did not correlate with total serum IgE. Clinical and serologic correlates of atopy were not significantly different between children with a skin test diameter of ≥5 mm and those with a smaller diameter.

Conclusion. These results do not support the hypothesis that BCG vaccination in early infancy is associated with a subsequently markedly decreased risk of atopic sensitization or allergy. In addition, PPD skin test reactivity was not impaired in atopic individuals. *Pediatrics* 2001;107(3). URL: http://www.pediatrics.org/cgi/content/full/107/3/e36; *Bacille Calmette-Guérin, atopy, total immunoglobulin E, allergic sensitization, allergy prevention, delayed type hypersensitivity.*

ABBREVIATIONS. Th, T helper cell; BCG, bacille Calmette-Guérin; PPD, purified protein derivative; IgE, immunoglobulin E; DTH, delayed-type hypersensitivity.

Exposure to mycobacteria results in preferential secretion of T helper cell (Th) 1 cytokines and subsequent activation of macrophages.[1] Balancing release of Th2 cytokines has been observed repeatedly later in the course of mycobacterial infection[2] but seems to play a less important role.[3,4] Hence, it seems to be possible that vaccination with bacille Calmette-Guérin (BCG) may skew the cytokine microenvironment toward preferential selection of Th1 cytokines and contribute to a decreased risk of Th2-dependent atopic disease. This hypothesis is supported by data from murine systems in which BCG-infected mice show a cytokine shift toward a Th1-like pattern and subsequent development of airway allergy is inhibited.[5,6]

In humans, an inverse correlation between purified protein derivative (PPD) skin test reactivity, total immunoglobulin E (IgE), and atopic disease in Japanese BCG-vaccinated schoolchildren was described, leaving open the degree of contribution of subclinical infection.[7] Evidence for a possible inhibiting effect of mycobacterial infection on atopy comes from 2 cross-sectional studies. In adolescents, national tuberculosis notification rates were found to be inversely associated with wheeze and asthma lifetime prevalences.[8] In a Finnish retrospective study, a protective effect on the prevalence of respiratory allergies was found for female adults if they were infected before 17 years of age.[9] In contrast to natural infection, cross-sectional studies in Scandinavian BCG-vaccinated children and nonvaccinated controls revealed no differences in atopic sensitization and disease.[10–12] However, in Guinea-Bissau, BCG-vaccinated children were less allergic to local indoor aeroallergens, and the skin-prick tests were particularly negative among those children who were vaccinated during their first week of life.[13]

This study was designed to investigate longitudinally the effect of exposure to BCG vaccination in early childhood on development of total IgE levels, specific sensitization, and atopic disease in BCG-vaccinated and nonvaccinated individuals of a prospectively followed birth cohort of 1314 children

from the former West Germany during their first 7 years of life. In addition, the size of delayed-type hypersensitivity (DTH) to tuberculin was investigated in relation to serum IgE and manifestations of atopic disease.

METHODS

Study Participants

In 1990, a cohort of 1314 neonates recruited in 5 German cities (Berlin, Düsseldorf, Freiburg, Mainz, and Munich) was selected for a prospective observational study (MAS-90). Of these, 499 neonates (38%) were selected as being at high risk for atopy (2 or more close atopic family members and/or cord-blood IgE values above 0.9 kU/L), and the remainder were at random risk.

The 1314 cohort infants and their parents were regularly seen for follow-up visits at ages 3, 6, 12, 18, 24, 36, 48, 60, 72, and 84 months. Parents filled in a questionnaire and gave a structured interview about their children's diseases and atopic symptoms. To keep the reporting bias low, parents kept a diary in which details of the children's diseases were recorded. Parents recorded information in the diary whenever the child became sick. The study coordinators monitored the diary at the indicated examination intervals. The children received a standardized physical examination by trained study physicians.

Definition of Atopic Diseases and Allergic Sensitization

A basic description of morphologic skin phenomena and their localization was used to construct a computer algorithm for the definition of atopic eczema according to the morphologic criteria given by Seymour et al.[14] A similar but more simple scheme was completed by the mothers regarding the case history of the preceding period.[15] Obvious recurrent wheezing bronchitis required at least 2 wheezing episodes with shortness of breath. Obvious atopic rhinitis was diagnosed in the case of stuffed and/or running nose without a cold lasting for 2 or more months during the preceding observation period, plus the diagnosis by a physician.[15] An infant was considered sensitized if the IgE antibody titer of 1 or more of the 9 allergens tested was ≥0.35 kU/L.

BCG Vaccination

In Germany, children believed to be at increased risk for tuberculous infection used to be vaccinated within their first weeks of life. Vaccination documents were available for all children of the cohort. BCG vaccinations were performed with an attenuated BCG strain (10^5 bacteria, Copenhagen strain 1331, BCG vaccine; Behring, Marburg, Germany).

PPD Skin Testing

Skin testing with mycobacterial antigen was performed at 7 years of age by intradermal injection of 10 IU PPD (Behring) at the volar aspect of the arm. The average diameter of the induration at the test site was evaluated 72 hours after administration.

Determination of Allergen Concentration in House Dust

The levels of major mite (Der p 1 and Der f 1) and cat (Fel d 1) allergens were determined from domestic carpet dust samples by sandwich enzyme-linked immunosorbent assay as described previously.[16]

Determination of IgE

Venous blood samples were obtained at birth (cord-blood), 12, 24, 36, 60, 72, and 84 months of age. Serum was separated by centrifugation at 3500 rpm for 13 minutes, and serum samples were stored at −20°C until analysis. Sera were analyzed for total IgE and specific IgE against 9 common inhalant outdoor (birch t3, grass g6), indoor (mite d1, cat e1, dog e2), or food allergens (egg f1, milk f2, wheat f4, soy f14). Analysis was performed in one laboratory by CAP-RAST FEIA (Pharmacia, Freiburg, Germany). At 5 years of age, CAP test results of 418 children were compared with skin prick test results for 5 respiratory allergens (cat, dog, mite, birch, and grass). Skin tests were considered positive if the maximum wheal diameter was >3 mm without reaction of negative control (saline) and the skin index was >0.6. The skin index was calculated as the ratio of the diameter of the allergen wheal to the histamine (histamine-dihydrochloride 10 mg/L) wheal. The overall efficiency, calculated as the proportion of concordant positive and negative results, is 92.2%. The sensitivity and specificity are 83.8% and 92.5%, respectively.

Statistical Methods

Statistical analysis was performed by using the *SPSS for Windows, Version 7.0* (SPSS, Chicago, IL). Pearson's χ^2 tests were applied for the assessment of association in 2-dimensional contingency tables.[17] Fisher's exact test was used when the expected frequency of any cell was <5. The Mann-Whitney U test was used for comparisons of continuous variables. For correlations, Spearman's ρ was calculated. For categorization of clinical diagnoses, only definite diagnosis was regarded as atopic disease. Specific IgE antibodies to food and inhalant allergens were grouped into values below detection limit (<0.35 kU/L) and detectable values (≥0.35 kU/L). Cord-blood IgE levels were categorized as not elevated (<0.9 kU/L) or elevated (≥0.9 kU/L). An infant with documented BCG vaccination was regarded as vaccinated; all other infants were regarded as nonvaccinated. Statistical significance was defined by a 2-sided α level of 0.05. Bonferroni's adjustments were used for multiple comparisons.

Ethics

The parents of all participating children in the study gave their informed consent. The research protocol was approved by the local ethics committee.

RESULTS

Of the 1314 cohort children, 169 were BCG-vaccinated (12.9%).

Nine hundred eighty-two of the cohort children were seen at a follow-up visit at 7 years of age (participation rate: 74.7%). Seven hundred eighty-seven children (59.9%) were examined at all scheduled examination dates up to their seventh birthday—100 of the BCG-vaccinated children (59.2%) and 687 of the nonvaccinated children (60.0%). Children were excluded from the analysis because of a history suggestive of tuberculosis infection ($n = 5$) or BCG vaccination after their first birthday ($n = 8$). The resulting population consists of 774 children, including 92 children (11.9%) who were BCG-vaccinated in their first year. The difference between this BCG vaccination rate and the rate of early BCG-vaccinated children in the whole cohort (12.1%) was not statistically significant ($P = .899$). The median age for BCG vaccination was 30 days (range: 1–343 days).

The 774 children did not differ significantly from the whole cohort in terms of gender, season of birth, atopic family history, cord-blood IgE levels, cat or mite allergen exposure, number of siblings at birth or at 5 years of life, and typical childhood infections (measles, mumps, rubella, and pertussis). However, the breastfeeding rate (>4 weeks) was significantly higher among the 774 continuously followed children compared with the whole cohort (78.0% vs 69.1%; $P < .001$). Also, vaccination rates were significantly higher in the 774 children than in the whole cohort (vaccinations against diphtheria/tetanus, 85.4% vs 78.5%; measles, 64.6% vs 51.9%; mumps, 65.6% vs 52.5%; rubella, 64.7% vs 51.8%; $P < .001$) except vaccination against pertussis (28.9% vs 25.2%; $P = .061$).

Among the 774 continuously followed children,

the BCG-vaccinated and nonvaccinated were similar in those characteristics. However, non-German children were overrepresented in the BCG-vaccinated group (9.8% vs 3.5%, P = .011) and a high proportion of the BCG-vaccinated children (39.1%) were born in Mainz, 1 of the 6 study centers (Table 1).

Period prevalences of atopic manifestations were not statistically different between the BCG-vaccinated and the nonvaccinated group and any time point (Fig 1). There was a tendency toward lower period prevalences of atopic dermatitis in the first year of life (Fig 1A). Period prevalence of recurrent wheezing followed a biphasic course in both groups and prevalences of the BCG-vaccinated group were nonsignificantly lower during the first phase (Fig 1B). Allergic rhinitis was not prevalent in the first 2 years of life and no significant differences between both groups were found thereafter (Fig 1C). Comparing the period prevalences of any of these clinical manifestations, no significant differences were found between groups (Fig 1D).

Lifetime prevalences of atopic dermatitis tended to be nonsignificantly lower in BCG-vaccinated children. Similarly, wheezing bronchitis tended to be lower among BCG-vaccinated children up to 5 years of age. Statistical significance of this difference was achieved at 12 months of life. No statistically significant differences were found for the lifetime prevalences of allergic rhinitis (Table 2). In the first 3 yea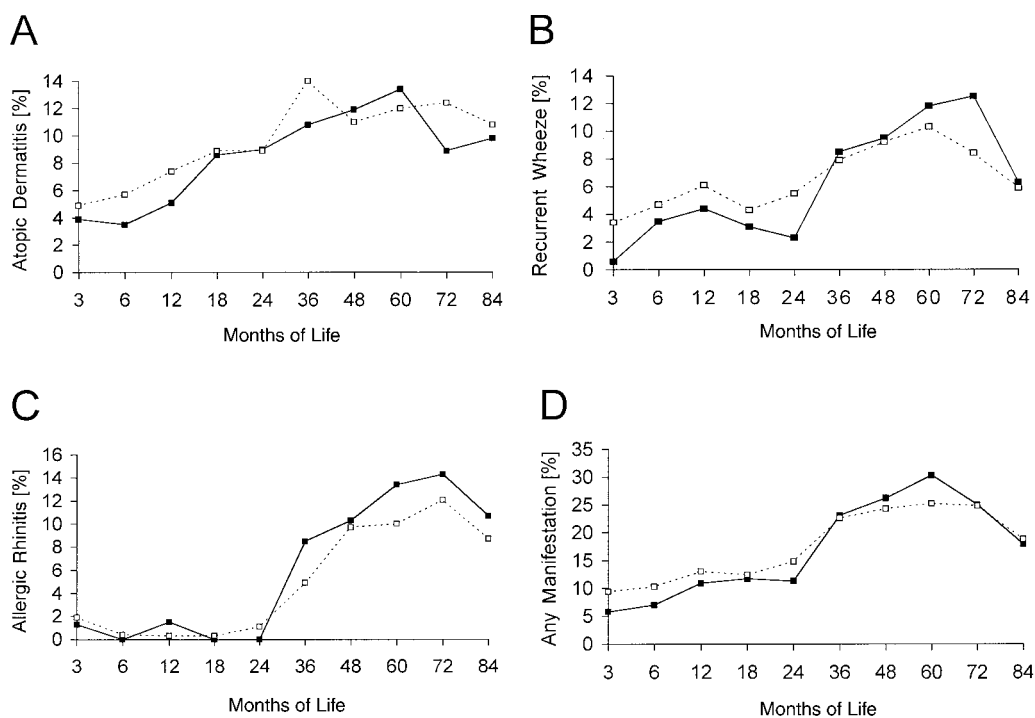rs of life, the proportion of infants remaining free of atopic manifestations over time tended to be nonsignificantly higher in the BCG-vaccinated group (P = .343; Fig 2).

Total serum IgE values (median) were not lower in the BCG-vaccinated group than in the nonvaccinated group at any age (Fig 3). Using 0.35 kU/L as a cutoff level for specific sensitization to 1 or more of 9 common inhalant and food allergens (birch, timothy grass, egg, milk, soy, wheat, mite, cat, and dog), sensitization rates tended to be lower among the BCG-vaccinated group than among the nonvaccinated group during the first 2 years. Statistical significance was achieved at 2 years (Fig 3). At this time point, the sensitization rate was 12.7% in the BCG-vaccinated group and 25.1% in the nonvaccinated group (P = .033). Calculating the percentage of positive specific IgE tests from the total number of tests performed at the indicated time points, a nonsignificantly lower proportion of tests were positive in the BCG-vaccinated group than in the nonvaccinated group during the first 3 years (at 12 months, 2.8% vs 3.0%; at 24 months, 4.1% vs 5.6%; at 36 months, 4.9% vs 6.3%). Later in life, this tendency was reversed and differences achieved statistical significance at ages 5 and 7 (at 60 months, 13.0% vs 9.6%, P = .006; at 72 months, 13.1% vs 11.8%; at 84 months, 16.4% vs 12.3%, P = .002).

A subpopulation of 492 children was tuberculin skin tested at 7 years of age. Fifty-eight of 492 children (11.8%) were BCG-vaccinated. BCG-vaccinated

TABLE 1. Patient Characteristics

| Variable	Nonvaccinated (n = 682)	BCG-Vaccinated (n = 92)	P Value
Male (%)	52.5	51.1	.800
Season of birth (%)			
March to August	45.2	46.7	
September to February	54.8	53.3	.775
Cord-blood IgE			
>0.9 kU/L (%)	18.9	10.8	.099
Nationality (%)			
German	96.5	90.2	
Other	3.5	9.8	.011
Family history (%)			
Mother atopic	35.2	35.9	.906
Father atopic	28.6	34.8	.219
Breastfeeding (%)			
0–4 wk	22.0	22.0	
1–6 mo	44.4	47.3	
>6 mo	33.6	30.8	.844
Median Fel d1 exposure (μg/g carpet dust, mean 6th/18th mo of life)	64	45	.106
Median Der p1 + Der f1 (μg/g carpet dust, mean 6th/18th mo of life)	255	202	.106
Siblings at birth (%)			
None	52.9	46.3	
1	35.0	36.6	
≥2	12.1	17.1	.355
Infections during the first 2 y of life (%)			
Measles	2.1	1.1	1.000
Mumps	0.3	.0	1.000
Rubella	1.6	2.2	.660
Pertussis	11.3	9.8	.779
Vaccinations during the first 2 y of life (%)			
Diphtheria/tetanus	85.5	84.8	.983
Measles	64.5	65.2	.895
Mumps	65.7	65.2	.929
Rubella	64.7	65.2	.917
Pertussis	28.4	32.6	.409

Fig 1. Period prevalences of atopic manifestations in 92 BCG-vaccinated (solid squares and solid line) and 682 nonvaccinated German children (open squares and dotted line) up to the age of 7 years.

TABLE 2. Lifetime Prevalences (%) of Atopic Dermatitis, Recurrent Wheezing, and Allergic Rhinitis in Early BCG-Vaccinated ($n = 92$) and Nonvaccinated Children ($n = 682$)*

Age (Months)	Atopic Dermatitis		Recurrent Wheezing		Allergic Rhinitis	
	BCG	Nonvaccinated	BCG	Nonvaccinated	BCG	Nonvaccinated
3	2.2	6.0	3.3	4.5	.0	.4
6	5.4	9.5	3.3	6.7	2.2	2.6
12	7.6	14.8	4.3	11.6	3.3	2.8
18	9.8	19.5	7.6	14.4	3.3	3.1
24	13.0	22.7	9.8	17.9	3.3	3.5
36	19.6	27.7	16.3	21.4	13.0	7.2
48	20.7	29.3	20.7	24.3	19.6	14.4
60	28.3	32.8	27.2	28.9	27.2	19.1
72	29.3	34.5	32.6	30.4	30.4	22.9
84	29.3	35.3	33.7	30.9	32.6	24.2

* No statistical significant differences were found after Bonferroni's correction.
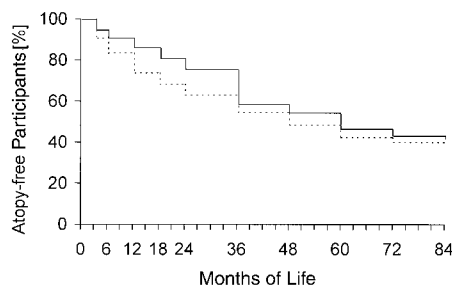

Fig 2. Cumulative proportion of 92 BCG-vaccinated (solid line) and 682 nonvaccinated German children (dotted line) remaining atopy-free up to the age of 7 years ($P = .343$).

and nonvaccinated children did not differ significantly with regard to gender, atopic manifestation of mother or father, duration of breastfeeding, mean indoor allergen exposure (cat and mite), number of siblings, common childhood infections, and vaccinations during early childhood (data not shown).

Three hundred sixty-seven of 492 children (74.6%) showed no DTH to tuberculin and 58 of 492 (11.8%) showed a reaction with an average diameter ≥ 5 mm. The diameter was significantly larger among BCG-vaccinated than nonvaccinated children ($4.1 \pm .8$ mm vs $1.1 \pm .2$ mm; $P < .001$). However, among those who showed a reaction with an average diameter ≥ 5 mm, only 18 were BCG-vaccinated.

At 7 years of age, DTH to tuberculin did not significantly differ between 48 children with and 442 children without atopic dermatitis ($1.8 \pm .6$ mm vs $1.5 \pm .2$ mm; $P = .977$), between 29 children with and 461 children without recurrent wheeze ($.9 \pm .4$ mm vs $1.5 \pm .2$ mm; $P = .312$), between 36 children with and 454 children without allergic rhinitis ($1.5 \pm .5$ mm vs $1.5 \pm .2$ mm; $P = .793$), or between 84 children with 1 or more of these conditions and 406 children without any of these conditions (1.5 ± 0.4 mm vs 1.5 ± 0.2 mm; $P = .607$). No significant differences were found in the BCG-vaccinated and nonvacci-
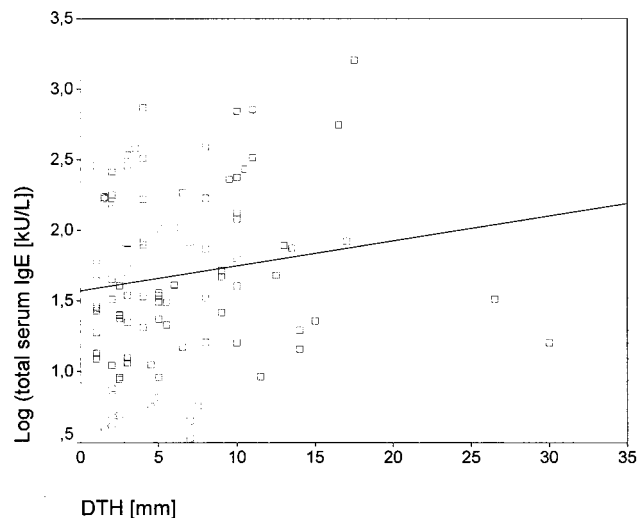
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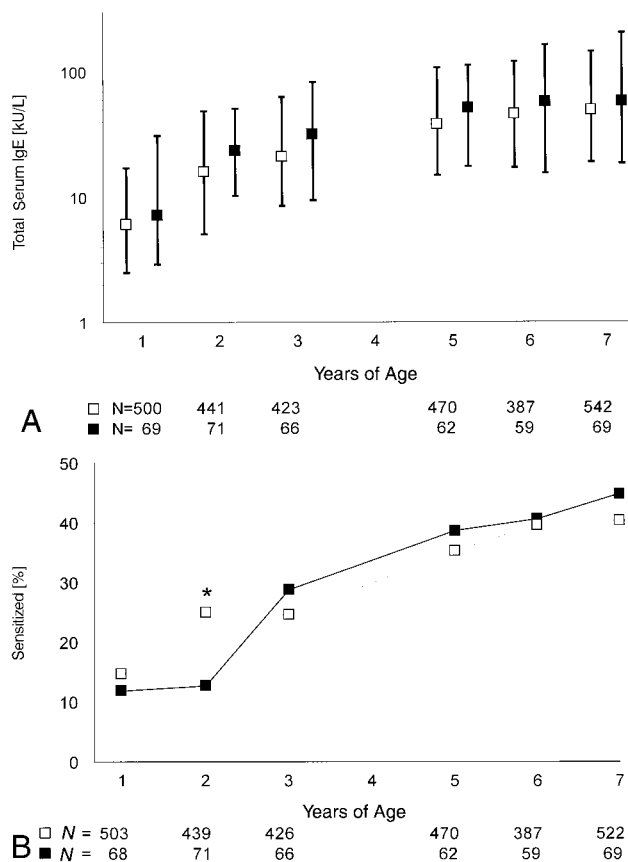
Fig 3. Total serum IgE levels (top panel) and allergic sensitization to common airborne and nutritive allergens (bottom panel) in BCG-vaccinated (closed squares) and nonvaccinated German children (open squares) up to age 7 years. Bars represent median, 25th, and 75th percentile (*$P < .05$).

Fig 4. Size of delayed type hypersensitivity to tuberculin (DTH) in relation to total serum IgE levels at 7 years of age (all 125 detectable test results, $r = 0.022$).

nated subpopulations (data not shown). Compared with children with a DHT ≥5 mm, children with a smaller DTH had similar rates of atopic dermatitis (10.3% vs 9.7%; $P = 1.000$), recurrent wheeze (3.4% vs 6.3%; $P = .559$), allergic rhinitis (8.6% vs 7.2%; $P = .600$), or any of these manifestations (15.5% vs 17.4%; $P = .869$). Children with a PPD ≥10 mm did not differ statistically significant from those with a diameter below regarding atopic sensitization or clinical manifestations of atopy (data not shown).

Total IgE was measured in 454 of the PPD skin-tested children. The DTH diameter correlates very poorly with total IgE at 7 years of age ($r_s = 0.022$; Fig 4), similarly in the 55 BCG-vaccinated ($r_s = 0.021$) and the 399 nonvaccinated children ($r_s = 0.015$). Children with a DTH ≥5 mm were found to have similar total serum IgE values in comparison with children who showed a lower DTH (153.4 ± 39.9 kU/L vs 141.9 ± 19.3 kU/L; $P = .966$).

DTH does not differ significantly between 184 children sensitized to common airborne or nutritive allergens at their seventh birthday and 278 nonsensitized children (1.4 ± .3 mm vs 1.5 ± 0.2 mm; $P = .177$). Also, DTH was found not to be significantly different between sensitized and nonsensitized children of the BCG-vaccinated or nonvaccinated subpopulation (data not shown). Among 55 children with a DTH ≥5 mm, the rate of sensitization to

common airborne or nutritive allergens was nonsignificantly lower than among those 407 children with a smaller diameter (36.4% vs 40.3%; $P = .680$).

DISCUSSION

Our results do not support the hypothesis that BCG vaccination in early infancy is associated with a subsequently decreased risk of atopic sensitization or of clinical manifestations of atopic disease in general. Given the good comparability of the BCG-vaccinated group and the nonvaccinated group, some transient tendencies in favor of BCG-vaccinated children that were observed early in life may actually be related to the difference in the BCG vaccination status. It could be speculated that a BCG-induced shift of the cytokine environment toward a Th1-type pattern contributed to those transient differences and that later in life additional environmental influences override an initial BCG effect. However, in this study the observed differences mostly failed to be significant. They may be stronger in even larger patient samples.

Several factors may have contributed to what could be regarded as conflicting evidence between the Japanese and the European BCG studies. First, the vaccine strains and doses used differ. The BCG Tokyo strain 172 used in the Japanese study differs biochemically from the Copenhagen strain used in the Swedish and our study.[18,19] Differences in the immunogenicity of different strains have been described,[20] but to our knowledge no direct comparison of the Danish and Japanese strain is presently available. The Japanese vaccine is administered in a 10-fold higher dose to achieve comparable indurations evoked by the Copenhagen strain 1331.[7] Therefore, vaccine and dose differences could have been contributed to a stronger influence of BCG vaccination on development of elevated IgE levels and atopic disease.

Second, genetic and environmental differences between Japanese and European children cannot be ruled out as a possible cause for a greater susceptibility to BCG immunization.

Third, an immunity skewed toward the Th2-type cytokine pattern at the cost of Th1-type cytokines is a characteristic of being atopic. The cutaneous induration expected in children infected with mycobacteria on testing with tuberculin is a Th1-dependent type I allergic reaction. Thus, the decreased tuberculin skin reactions in atopics documented in the Japanese study may represent a marker of atopy rather than pointing toward BCG as a cause of being nonatopic. Reactivity to mycobacterial antigens has been shown to be reduced in Finnish allergic children.[21] A similar phenomenon has been demonstrated in atopic pertussis-vaccinated children who respond with a smaller induration than do nonatopic children.[22] Similarly, an inverse relation of total serum IgE levels and in vitro lymphoproliferative responses to tetanus toxoid was found in diphtheria–pertussis–tetanus-vaccinated children.[23] In our study, however, clinical and serologic indicators of atopy were not significantly different between children with positive and negative PPD skin tests and the correlation of total serum IgE and DTH was close to zero (Fig 4). In accordance with these results, a lack of correlation between tuberculin skin test reactivity and atopy has been found among adult Norwegians who had been BCG-vaccinated at the age of 14 years.[12]

Finally, although Shirakawa et al[24] claimed that a fixed determination of atopy and diminished tuberculin responses by genetic factors is unlikely because of intraindividually changed tuberculin responses from positive to negative and vice versa over time, environmental mycobacterial infections known to be more common at lower latitudes may have coinfluenced tuberculin responses and development of atopy by continuously stimulating Th1-like clones. It would be interesting to see how these children shifting from positive to negative tuberculin response behave toward IgE and atopy over time. Moreover, a high proportion of children with DTH responses ≥40 mm would also be consistent with a *Mycobacterium tuberculosis*-infected subpopulation within the Japanese sample. Epidemiologically compared, populations of countries with high tuberculosis notification rates tend to have lower prevalences of wheeze and asthma.[8] Thus, mycobacterial infection by respiration route may be more effective in inhibiting the development of allergic airway disease.

There is a theoretical chance that other vaccines than BCG or infections stimulated a Th1-type immune response in the children of our cohort that could have buffered any additional differences derived from the BCG vaccination that were seen in the Japanese study. However, in this study, other common childhood vaccines and infections were not a major confounder (Table 1).

Th2-skewed cytokine milieu seems to be part of the regular prenatal development of the immune system,[25] but it can be exaggerated and prolonged postnatally in genetically predisposed individuals.[26–29] It has been suggested that the immune system of individuals at risk be skewed more toward a Th1 response to prevent atopy. This seems to be possible with mycobacteria in murine models.[5,6,30]

However, based on available data in children a possible preventive effect of a single early BCG vaccination against atopy in later life is at best controversial and no imperative to reintroduce BCG vaccination in regions with low prevalence of tuberculosis. In contrast, prokaryotic DNA fragments are a promising Th1-stimulating agent.[31] Because autoimmune diseases such as insulin-dependent diabetes mellitus are Th1-associated and because a shift toward Th2-type protects the fetus in pregnancy,[32,33] caution should prevail when immunization strategies are being considered to shift the cytokine balance from atopy.

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