

The effect of neonatal BCG vaccination on atopy and asthma at age 7 to 14 years: An historical cohort study in a community with a very low prevalence of tuberculosis infection and a high prevalence of atopic disease

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Background: There are conflicting reports on the effect of BCG vaccination on the subsequent development of atopy and asthma. There are no data on the effects of neonatal BCG vaccination on cytokine responses of lymphocytes that are exposed *in vitro* to allergens.

Objectives: We sought to test the hypothesis that neonatal BCG vaccination or, alternatively, evidence of an immunologic memory of this vaccination is associated with a reduced prevalence of allergic sensitization, asthma, eczema, and hay fever during childhood.

Methods: An historical cohort study was conducted among 7- to 14-year-old children who were born in 2 districts in Sydney, Australia, and whose mothers were born in southeast Asia. One district had routinely administered BCG vaccination to infants born to overseas-born mothers and the other had not. Eligible subjects were identified from birth registers. Consenting subjects completed questionnaires, performed spirometric and airway hyperresponsiveness testing, and had allergen skin prick testing and tuberculin skin testing. Blood was collected to measure total serum IgE levels and for *in vitro* lymphocyte culture in the presence of an extract of house dust mite, the dominant allergen in this region, and purified protein derivative of *Mycobacterium tuberculosis* (tuberculin). IL-4, IL-5, IL-10, and IFN- γ were measured in the culture supernatant.

Results: The cohort included 309 BCG-vaccinated subjects and 442 non-BCG-vaccinated subjects. BCG-vaccinated sub-

jects did not have a lower rate of allergic sensitization than nonvaccinated subjects. However, among the subgroup of subjects with a family history of rhinitis or eczema, BCG vaccination was associated with a lower prevalence of current asthma (defined as recent wheezing plus airway hyperresponsiveness; relative risk, 0.46; 95% CI, 0.22-0.95). BCG vaccination was also associated with lower levels of allergen-stimulated IL-10 production *in vitro*. Among the BCG-vaccinated subjects, the 44 (14.3%) who had tuberculin skin test reaction sizes of 5 mm or greater and the 31 (18.3%) who demonstrated an *in vitro* IFN- γ response to purified protein derivative of *M. tuberculosis* did not have lower rates of allergic sensitization and, overall, did not have a lower prevalence of allergic disease than tuberculin skin test or IFN- γ nonreactors.

Conclusion: We conclude that neonatal BCG vaccination has an effect on T-cell allergen responsiveness 7 to 14 years after vaccination and that among a subgroup of subjects with an inherited predisposition to allergic disease, this is associated with clinically relevant beneficial effects. The findings of this study encourage the view that external influences on the immune system in the neonatal period have consequences that extend into later childhood and influence the expression of asthma. Genetic factors are likely to modify the effect of those external factors. (J Allergy Clin Immunol 2003;111:541-9.)

Key words: Atopy, immune deviation, cytokines, bacille Calmette-Guerin

There is evidence to suggest that respiratory and non-respiratory viral infections and other systemic infections in early life might protect against the development of atopy.¹⁻³ The observation that children who are born into large sibships⁴ or who attend childcare centers from an early age⁵ are relatively protected against the development of atopy is consistent with this hypothesis. It might also be consistent with the finding that children who live on farms with livestock in early life are less likely to have atopy.⁶ The proposed mechanism for this protective effect of early-life infections is immune deviation: infectious agents induce the establishment of clones of antigen-specific memory T lymphocytes, which secrete antiallergic T_H1 cytokines.⁷

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Abbreviations used

AHR: Airway hyperresponsiveness
 HDM: House dust mite
 NSW: New South Wales
 PPD: Purified protein derivative of *Mycobacterium tuberculosis*
 SPT: Skin prick test
 TST: Tuberculin skin test

Mycobacterial infections are a potent stimulus to T_H1-like lymphocyte responses. Shirakawa et al⁸ studied 867 children aged 12 and 13 years in Japan and found that those who had evidence of a delayed-type hypersensitivity reaction to the mycobacterial antigen tuberculin (58% of the cohort) had lower levels of serum IgE than those who did not. It is likely that in most cases the initiating antigen exposure was vaccination with BCG, an attenuated strain of *Mycobacterium bovis* that was administered routinely in infancy and again at age 6 years in this community. The implication is that this early-life mycobacterial infection promoted the T_H2 to T_H1 switch and hence inhibited the expression of atopy.

Immunization with BCG is a useful model to test the role of early-life stimulation of T_H1 cells in subsequent immune maturation. In common with other mycobacterial infections, BCG is a potent T_H1 stimulus.⁹ In many communities it is administered during the neonatal period. Follow-up of these vaccinated infants presents an opportunity to examine the effect of this T_H1 stimulus administered during this critical period of immune system maturation. Previous studies addressing this question have produced conflicting results.

In Swedish children with a strong family history for allergic disease, the administration of BCG vaccine in the first 6 months of life was not followed by a reduced risk of having positive allergen skin prick test (SPT) responses at age 3 to 7 years.¹⁰ However, the effect of BCG immunization in those without a family history of atopy is not known. Furthermore, some of the children in this study might have been evaluated too early in life to clearly define their atopic status.¹¹ A further study from Sweden found that among older children who were migrants to Sweden, those with BCG vaccination had lower rates of symptoms of allergic disease.¹² No such difference was apparent among Swedish-born children. The interpretation of this study is limited by the lack of objective measures of atopic status. In a study of 3- to 14-year-old children in Guinea-Bissau who were being followed after an episode of measles or measles vaccination, the absence of evidence of BCG vaccination was associated with a substantially increased risk of atopy: 40% versus 21% had SPT wheal responses of 2 mm or greater to one or more allergens.¹³ In all these studies, there is a potential for selection bias associated with the decision to offer and accept BCG vaccination.

Existing data do not allow any conclusions to be drawn on the effect or lack of effect of neonatal BCG vaccination on the risk of acquiring atopy or atopic disease. Furthermore, they do not adequately test the alternative explana-

tion for the observation of Shirakawa et al⁸ that the response to BCG immunization, rather than the vaccination itself, is linked to the risk of acquiring atopy. A large cross-sectional study among BCG-vaccinated children from Hong Kong did not find any association between tuberculin skin test (TST) reaction size and the prevalence of atopy, asthma, or other allergic disease.¹⁴ However, it was not possible to exclude the potential confounding effect of actual tuberculosis infection in this study setting.

The principal aim of this study was to test the hypothesis that neonatal BCG vaccination reduces the subsequent incidence of allergic sensitization and the prevalence of asthma, eczema, and hay fever during childhood. The secondary hypothesis was that among children who have received neonatal BCG vaccination, the intensity of delayed-type hypersensitivity to tuberculin is inversely related to the prevalence of allergic sensitization and disease. The study reported here overcomes the problems of selection bias inherent in previous studies by taking advantage of a serendipitous difference in BCG vaccination policy between 2 otherwise similar localities in Sydney. It allows an examination of the effect of BCG vaccination in an environment with a very low prevalence of tuberculosis infection and a high level of exposure to house dust mite (HDM) allergen.

METHODS

An historical cohort study was conducted among subjects who were born in Sydney, Australia, during the period from 1985 to 1994. Outcomes were measured during the period from April 1999 to January 2001, when subjects were aged 7 to 14 years.

The study protocol was approved by the Human Research Ethics Committees of South Western Sydney Area Health Service, Central Sydney Area Health Service (RPAH Zone), the University of Sydney, and the New South Wales (NSW) Department of Health.

Study population

The target population comprised children who were born in one of 2 localities in southwestern Sydney during the period from 1985 to 1994 and whose mothers were born in China, Hong Kong, Taiwan, the Philippines, or Vietnam. In one locality (locality A) neonatal BCG vaccination was not routinely administered, except to children traveling overseas for an extended period. In the other locality (locality B) children whose parents originated in countries with a high prevalence of tuberculosis were routinely offered BCG vaccination within the first 8 weeks of life. The strain used was BCG (CSL) (CSL Bioscience, Melbourne, Australia), which was originally derived from BCG (Glaxo).

Eligible members of the cohort were identified in locality A by reference to the local hospital's computerized hospital information system. Details of all discharges during the study period of women whose country of birth was among those listed above and whose discharge diagnosis was confinement with live delivery were entered into a database. In locality B the principal hospital did not have a computerized hospital information system before 1994. For that hospital during the years 1988 and 1990 to 1994, data equivalent to those described above were obtained from the Midwives Data Collection, which is maintained by the NSW Department of Health. Because some children from this locality were born at another nearby hospital, their records were also obtained from the Midwives Data Collection. Finally, the birth register of the principal hospital in this locality was inspected for the years 1985, 1986, 1987, and 1989 to

identify mothers with Vietnamese and Chinese names. The hospital records of these mothers were then retrieved to establish whether they were born in one of the countries listed above.

Study procedures

An attempt was made to identify the most recent addresses and telephone numbers of all identified mothers by using each hospital's computerized information system, the NSW electoral roll, and an online version of the telephone directory. Eligible subjects were initially contacted by telephone to confirm their address details and to establish their willingness to receive written information about the study. Contact was made in English, Chinese, or Vietnamese, as appropriate. Only those subjects with whom initial telephone contact could be made were considered part of the cohort.

An information sheet about the research, a consent form, and a study questionnaire to be completed by the parents were sent to all parents who agreed to receive it. They were then contacted again by telephone, and if they agreed, an appointment for testing the child was made.

At the testing center, the nature of the study protocol was explained to all subjects and their parents, and written informed consent was obtained from those who agreed to participate. All study materials were published in English, Chinese, and Vietnamese and Chinese- and Vietnamese-speaking research assistants were available to explain the study and assist with completing the questionnaire.

After consent was obtained and the questionnaire was completed, subjects underwent allergen SPTs, spirometry, a methacholine inhalation challenge test, a venous blood collection for IgE assay and lymphocyte studies, and a TST. The child's personal health record was reviewed for evidence of immunizations (including BCG). Two observers independently examined the child for evidence of a BCG vaccination scar. Subjects returned 3 days later for TST reading. A movie pass or book voucher was offered as a recompense for participating in the research.

Questionnaire

The questionnaire included items measuring the following: wheezing ever or in the last 12 months; hay fever or eczema ever; a physician's diagnosis of asthma or ever having treatment for asthma or wheezing; parental history of ever having asthma, hay fever, or eczema; parental smoking during the first 2 years of the child's life; attendance at a childcare center or family daycare before the age of 2 years¹⁵; and consumption of fresh fish at least once per week. The questionnaire used for this study was closely based on a validated and repeatable questionnaire that has been extensively used in studies of asthma and allergy in children (eg, the study by Peat et al¹⁶).

Skin prick tests

SPTs were performed with extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (both HDMs), cockroach, cat, rye grass, *Cladosporium* species, *Alternaria alternata*, and *Aspergillus fumigatus* (Hollister-Stier, Miles Inc, Elkart, Ind) by using a standardized method.¹⁷ Glycerine and histamine (10 mg/mL) were used as negative and positive controls, respectively. Mean allergen wheal diameters of 3 mm or greater and greater than those caused by the glycerine control were regarded as positive. Subjects with any positive allergen wheal responses were classified as atopic. If all allergen wheal responses were negative and the histamine wheal response was less than 3 mm, the test was regarded as void.

Spirometry and methacholine inhalation challenge test

Spirometric function was measured with a dry rolling seal spirometer linked to a portable computer (S&M Spirometers, Mijn-

hardt BV, Bunnik, Netherlands), according to the American Thoracic Society's specifications.¹⁸

The presence and degree of nonspecific airway hyperresponsiveness (AHR) was assessed by using the rapid methacholine inhalation challenge test¹⁹ in subjects whose initial FEV₁ was greater than 70% of the predicted value.²⁰ A maximum cumulative dose of 6.1 μ mol was administered. Subjects with a greater than 20% decrease in FEV₁ during the test were defined as having AHR.

Subjects with both wheezing in the preceding 12 months and AHR were classified as having current asthma.²¹

Tuberculin skin test

Ten units of tuberculin (CSL, Melbourne, Australia) was injected intradermally on the volar surface of the left forearm. The diameter of the indurated area was measured 72 hours later by using the ballpoint method.²² The TST was performed after blood collection.

Lymphocyte responses

Venous blood was drawn into heparinized containers and transferred to the laboratory, where PBMCs were separated by means of centrifugation over Ficoll-Hypaque. PBMCs were then resuspended at 10⁶/mL in AIM-V medium (without serum) and cultured in a 5% CO₂ atmosphere at 37°C. For each sample, PBMCs were cultured separately with the mitogen PHA (Sigma, St Louis, Mo) at 10 μ g/mL, purified protein derivative of *Mycobacterium tuberculosis* (PPD; Serumstatens Institut, Copenhagen, Denmark) at 10 μ g/mL, an aqueous ultrasonicated extract of HDM (CSL Biosciences, Melbourne, Australia) at 50 μ g/mL, and medium alone for 48 hours. Supernatants were then harvested and stored at -70°C before analysis.

For cytokine analysis, the supernatants were thawed, and 2-site capture immunoassays with OptEIA mAb sets (BD Pharmingen, Los Angeles, Calif) streptavidin-horseradish peroxidase conjugate, and recombinant cytokines as standards were used to measure the cytokines IL-4, IL-5, IL-10, and IFN- γ . The limits of detection of these assays are 3.1 pg/mL for IL-4 and IL-5, 6.2 pg/mL for IL-10, and 31 pg/mL for IFN- γ .

In cases in which the subject's cytokine response to polyclonal (PHA) stimulation was less than 3 times the control (medium only) level, that subject's results for that cytokine from the specific stimulation (ie, PPD and HDM) cultures were considered void. Assay results of less than the detection limit were assigned a value equal to the detection limit for the purposes of analysis. For each subject and each cytokine, a stimulation index was calculated as the ratio of the cytokine response to HDM to the control (medium only) cytokine response.

Nonresponder survey

Parents of children who refused to take part in the survey were asked if they would agree to be interviewed over the telephone. Those who agreed answered questions about wheezing, asthma, eczema, and hay fever.

Data analysis

The agreement in BCG status assignment between the personal health record and the examination for a BCG vaccination scar was quantified as the Cohen κ value, the chance corrected measure of agreement.²³

The distributions of serum IgE and dose-response ratio were skewed and were log-transformed for analysis.

Data for normally distributed continuous variables were summarized as means and differences between groups with 95% CIs. Data for log-normally distributed continuous variables were summarized as geometric means and relative percentage differences between one group and another with 95% CIs. These were transformed val-

TABLE I. Characteristics of the 2 cohorts

	BCG vaccinated (n = 309)	Non-BCG vaccinated (n = 442)	P value for difference
Age, y (mean $\pm$ SD)	9.5 $\pm$ 2.4	9.7 $\pm$ 2.0	.2
Male sex, n (%)	170 (55.0%)	258 (58.5%)	.3
Mother's country of birth, n (%)			.0001
China	62 (20.1%)	90 (20.4%)	
Hong Kong	32 (10.4%)	10 (2.3%)	
Philippines	22 (7.1%)	66 (14.9%)	
Vietnam	193 (62.5%)	276 (62.4%)	
Parental history of asthma (either parent)	38 (12.3%)	62 (14.1%)	.5
Parental history of hay fever or eczema (either parent)	145 (47.1%)	233 (52.8%)	.12
Maternal smoking before age 2 y	2 (0.7%)	7 (1.6%)	.2
Paternal smoking before age 2 y	126 (40.8%)	225 (50.9%)	.006
Fish consumption			.18
Not at all	38 (12.3%)	69 (15.6%)	
Once per week	156 (50.7%)	235 (53.2%)	
More than once per week	114 (37.0%)	138 (31.2%)	
Attending child care before age 2 y	91 (29.6%)	67 (20.3%)	.007

ues of ratios, which were calculated as the antilog of the difference between the group means of the logged values. Data for binary categorical variables were summarized as percentage prevalences and relative risks with 95% CIs.

Differences and relative risks are all adjusted for confounders. Confounders were identified in multiple linear or logistic regression models that included a main effect term identifying BCG vaccination status and a range of potential confounders as covariates. For the models with IgE and SPTs as outcomes, the potential confounders tested were sex, mother's country of birth, parental asthma, parental hay fever or eczema, parental smoking when the child was less than 2 years of age, child's consumption of fish at least once per week, and attendance at childcare or family daycare before the age of 2 years. For the clinical, lung function, and airway responsiveness models, the potential confounders tested included the presence of positive SPT responses in addition to those listed above.

Before selection of actual confounders, the model was used to test for effect modification. All of the potential confounders, except dietary fish intake, attendance at childcare, and parental smoking, were also tested as potential modifiers of the effect of BCG vaccination status. This was achieved by including interaction terms in the logistic regression model. When effect modification was identified, further analysis was undertaken separately in the 2 levels of the effect modifier.

Actual confounders were identified by means of a stepwise elimination procedure whereby covariates were retained in the model if they were significant predictors of outcome ($P < .05$). Differences adjusted for the identified confounders were estimated directly from the final linear regression model. Relative risks adjusted for the identified confounders were estimated by means of log-linear regression. The log-linear regression analysis was undertaken by using Proc Genmod software (SAS, SAS Institute, NC) with binomial error distribution and log link.

Cytokine levels and stimulation indices were not normally distributed and included many values of less than the assay detection limit. Hence nonparametric methods were used for analysis. Distributions were summarized as percentages with stimulation indices of greater than 2. Differences between groups were tested by using the Wilcoxon 2-sample test.

RESULTS

Agreement between BCG record and BCG scar

Among 820 subjects who had an examination of their personal health record and an examination for a BCG vaccination scar, 510 (62.2%) had neither a record of BCG vaccination nor a BCG vaccination scar, 280 (34.2%) had both a record and a scar, 10 (1.2%) had a record but no visible scar, and 20 (2.4%) had a scar but no record. The κ statistic was 0.92 (95% CI, 0.89-0.95), indicating very good agreement between the 2 detection methods. For the remainder of the analysis, subjects were considered to have received a BCG vaccination if they had either a record of BCG vaccination or a visible BCG vaccination scar.

Study population

In locality A there were 2003 records for women who met the study criteria. Telephone contact was established with 638 of these subjects, of whom 444 (69.6%) agreed to participate. Two of these had evidence of BCG vaccination. The remaining 442 (99.6%) formed the non-BCG-vaccinated cohort.

In locality B there were 1229 records for women who met the study criteria. Telephone contact was established with 523 of these subjects, of whom 380 (72.7%) agreed to participate. Examination revealed that 71 of these subjects did not have a record or scar evidence of BCG vaccination. The remaining 309 (81.3%) formed the BCG-vaccinated cohort.

The 2 cohorts had a similar distribution of age, sex, and family history of asthma and hay fever (Table I). Most mothers, including a similar proportion in both cohorts, were born in Vietnam or China. However, the non-BCG-

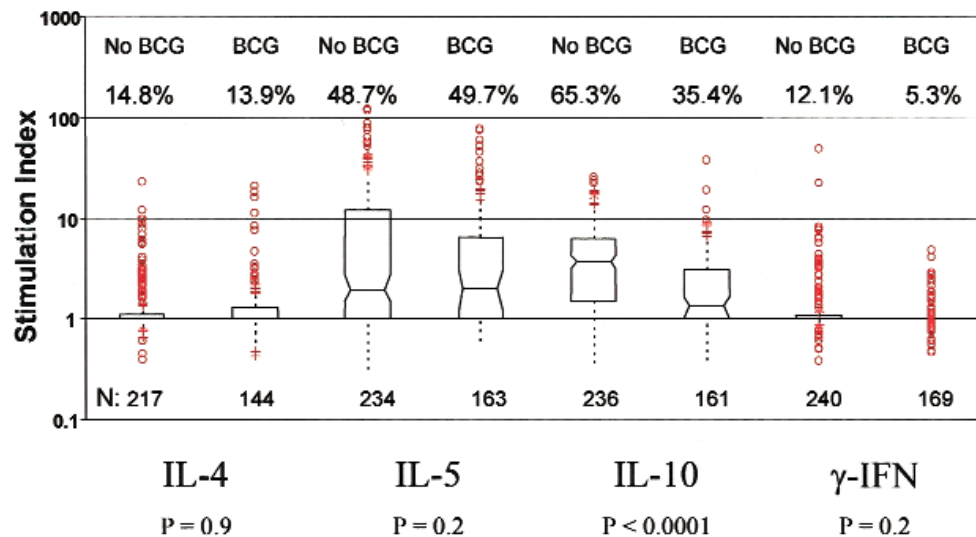


FIG 1. Box and whiskers plot of stimulation index values for the cytokines IL-4, IL-5, IL-10, and IFN- γ secreted in vitro in response to HDM stimulation of PBMCs in culture. Subjects are classified according to BCG immunization status. *P* values are shown for the Wilcoxon 2-sample test for the difference between BCG-vaccinated and non-BCG-vaccinated subjects. Percentages of subjects with stimulation indexes of greater than 2 are shown for each cytokine and group. *N*, Number of valid observations for each cytokine and group.

vaccinated cohort included a higher proportion of subjects whose mothers were born in the Philippines, and the BCG-vaccinated cohort included a higher proportion of Hong Kong-born mothers. Very few of the mothers had smoked when their children were infants, but a relatively high proportion of fathers had been smokers during this time, including a slightly greater proportion among the non-BCG-vaccinated cohort. There was a trend toward more regular fresh fish consumption in the BCG-vaccinated cohort, but the size of the difference was small. A higher proportion of the BCG-vaccinated cohort had attended childcare or family daycare in early life.

Among the 824 respondents, 192 (23.3%) were assessed at home visits because they refused to attend the hospital testing center. These subjects completed the questionnaire, had their BCG status assessed, and had allergen SPTs performed but did not complete other aspects of the protocol. A further 150 (18.2%) subjects did not have a methacholine inhalation challenge test, usually because they refused consent or were unable to complete the test. Subjects who did not have challenge tests had a similar prevalence of atopy (75% vs 73%, $P = .4$) and recent wheezing (21% vs 24%, $P = .4$) compared with subjects who did have this test.

Nonresponders survey

Of 337 subjects who refused to take part in the survey, 203 (60%) agreed to respond to questions about the presence of wheezing, hay fever symptoms, and eczema symptoms. Responses to these questions indicated that compared with those who did participate in the main study, those who refused to take part had a lower prevalence of recent wheezing (5.4% vs 22.6%, $P < .001$), recent hay fever symptoms (3.0% vs 38.6%, $P < .001$),

and recent eczema symptoms (6.9% vs 18.9%, $P < .001$). These differences between responders and nonresponders did not differ between subjects in locality A and those in locality B ($P > .05$).

Effect of BCG vaccination status of allergy, clinical, and immunologic outcomes

Atopy was highly prevalent in both cohorts (Table II), but there was no significant difference in the prevalence of positive SPT responses to any allergen and specifically to HDM allergen, between the BCG-vaccinated and the non-BCG-vaccinated cohorts. Among children whose parents had a history of hay fever or eczema, serum IgE levels did not differ between the BCG-vaccinated and the nonvaccinated cohort. In those with a negative family history for hay fever and eczema, IgE levels were actually higher in the BCG-vaccinated cohort.

In keeping with the high prevalence of atopy, the prevalence of hay fever and eczema was also high, and these did not differ between the cohorts (Table II). The prevalence of wheezing, AHR, and asthma was also high and, among those without a family history of allergic disease, did not differ between the BCG-vaccinated and nonvaccinated cohorts. However, in children who had one or both parents with hay fever or eczema, the BCG-vaccinated cohort manifest a trend toward a lower prevalence of frequent wheezing and had a significantly lower prevalence of current asthma than the nonvaccinated cohort (Table II). The mean FEV₁/forced vital capacity ratio was 0.92 in both cohorts and did not differ between the cohorts.

Fig 1 shows the stimulation indices for cytokines secreted in vitro in response to HDM stimulation of PBMCs in culture. Fifteen percent of IL-4 assays, 6%

TABLE II. Effect of BCG vaccination status on allergy and clinical outcomes

		BCG vaccinated (n = 309)
		Prevalence
Any positive SPT response		221/308 (71.8%)
Positive SPT response to HDM		191/308 (62.0%)
Wheezing ever ($P = .14$) [†]	Neither parent has hay fever or eczema	57/163 (35.0%)
	One or both parents has hay fever or eczema	57/145 (39.3%)
Wheezing in the last 12 mo ($P = .06$) [†]	Neither parent has hay fever or eczema	38/163 (23.3%)
	One or both parents has hay fever or eczema	35/145 (24.1%)
Wheezing ≥ 4 times in the last 12 mo ($P = .037$) [†]	Neither parent has hay fever or eczema	16/163 (9.8%)
	One or both parents has hay fever or eczema	11/145 (7.6%)
Diagnosed asthma or ever having medicines for asthma or wheezing		130/306 (42.5%)
AHR ($P = .03$) [†]	Neither parent has asthma	40/128 (31.3%)
	One or both parents has asthma	8/23 (34.8%)
Current asthma ($P = .019$) [†]	Neither parent has hay fever or eczema	13/88 (14.8%)
	One or both parents has hay fever or eczema	6/62 (9.7%)
Eczema ever		69/307 (22.5%)
Hay fever ever		134/308 (43.5%)
		Geometric mean
Serum total IgE ($P = .003$) [†]	Neither parent has hay fever or eczema	227.1 kIU/L (n = 95)
	One or both parents has hay fever or eczema	192.1 kIU/L (n = 83)

*Relative risk, adjusted for confounders by means of log-linear regression, is shown (see the "Methods" section). The actual confounders for which the parameter estimates are adjusted are included in the last column.

[†]Significance test for difference between 2 levels of the effect modifier.

[‡]Relative percentage difference.

TABLE III. Effect of TST status on allergy and clinical outcomes among BCG-vaccinated cohort

		TST ≥ 5 mm (n = 44)
		Prevalence
Any positive SPT response		31/44 (70.5%)
Positive SPT response to HDM		26/44 (59.1%)
Wheezing ever		11/44 (25.0%)
Wheezing in the last 12 mo ($P = .0005$) [†]	Neither parent has hay fever or eczema	5/20 (25.0%)
	One or both parents has hay fever or eczema	2/25 (8.0%)
Wheezing ≥ 4 times in the last 12 mo		3/44 (6.8%)
Diagnosed asthma or ever having medicines for asthma or wheezing ($P = .03$) [†]	Neither parent has hay fever or eczema	8/18 (44.4%)
	One or both parents has hay fever or eczema	6/25 (24.0%)
AHR		13/31 (41.9%)
Current asthma		3/31 (9.7%)
Eczema ever		5/43 (11.6%)
Hay fever ever		21/44 (47.7%)
		Geometric mean
Serum total IgE	Boys	184.7 kIU/L (n = 21)
	Girls	650.4 kIU/L (n = 10)

*Relative risk, adjusted for confounders by means of log-linear regression, is shown.

[†]Significance test for difference between 2 levels of the effect modifier.

[‡]Relative percentage difference.

of IL-5 assays, 7% of IL-10 assays, and 4% of IFN- γ assays were regarded as void because of low levels of production of these cytokines in response to polyclonal activation with the mitogen (see the "Methods" section). These were excluded from the analyses shown here. In the remaining subjects, stimulation indices were greater than 2.0 in 14% of subjects for IL-4, 49%

of subjects for IL-5, 53% of subjects for IL-10, and 9% of subjects for IFN- γ . The stimulation indices for IL-10 were higher in the non-BCG-vaccinated cohort (median, 3.69) than in the BCG-vaccinated cohort (median, 1.34) and did not differ between BCG-vaccinated and non-BCG-vaccinated subjects for IL-4, IL-5, and IFN- γ .

Non-BCG vaccinated (n = 442)		
Prevalence	Relative risk* (95% CI)	Adjusted for:
337/441 (76.4%)	0.97 (0.89-1.05)	Sex and family history of asthma
290/441 (65.8%)	0.95 (0.86-1.10)	Sex, family history of asthma, age group, and childcare attendance
62/208 (29.8%)	1.23 (0.95-1.59)	Any positive SPT response, family history of asthma
104/233 (44.6%)	0.97 (0.79-1.20)	Any positive SPT response, family history of asthma, fish intake
34/208 (16.4%)	1.47 (0.99-2.19)	Any positive SPT response, family history of asthma, mother's country of birth
66/233 (28.3%)	0.88 (0.65-1.19)	Any positive SPT response, family history of asthma, age group
14/208 (6.7%)	1.38 (0.71-2.72)	Family history of asthma
33/233 (14.2%)	0.58 (0.31-1.09)	Any positive SPT response, family history of asthma
191/442 (43.2%)	1.06 (0.93-1.23)	Any positive SPT response, family history of asthma
65/258 (25.2%)	1.26 (0.92-1.73)	Any positive SPT response
24/42 (57.1%)	0.61 (0.32-1.13)	None
14/153 (9.2%)	1.99 (0.95-4.14)	Any positive SPT response
31/148 (21.0%)	0.46 (0.22-0.95)	Any positive SPT response, family history of asthma
112/441 (25.4%)	0.92 (0.71-1.19)	Any positive SPT response, family history of allergy
213/435 (49.0%)	0.99 (0.85-1.15)	Any positive SPT response, family history of allergy, and mother's country of birth
Geometric mean	Relative percentage difference‡ (95% CI)	
147.1 kIU/L (n = 121)	54% (2% to 133%)	None
253.1 kIU/L (n = 121)	-25% (-49% to 9%)	Fish in diet

TST <5 mm (n = 263)		
Prevalence	Relative risk* (95% CI)	Adjusted for:
184/255 (72.2%)	0.91 (0.75-1.09)	Sex, family history of asthma
164/258 (63.6%)	0.87 (0.68-1.10)	Sex, family history of asthma
100/263 (38.0%)	0.80 (0.46-1.39)	Family history of asthma
33/143 (23.1%)	1.33 (0.60-2.97)	Any positive SPT response, family history of asthma, father smoking
33/120 (27.5%)	0.35 (0.09-1.36)	Any positive SPT response, family history of asthma, age group
22/263 (8.4%)	0.73 (0.24-2.27)	Sex, family history of asthma
54/142 (38.0%)	1.07 (0.62-1.05)	Any positive SPT response, family history of asthma, father smoking
61/120 (50.8%)	0.51 (0.25-1.06)	Sex, family history of asthma
36/122 (29.5%)	1.37 (0.84-2.21)	Any positive SPT response
18/122 (14.8%)	0.66 (0.21-2.09)	None
61/262 (23.3%)	0.50 (0.21-1.17)	None
114/262 (43.5%)	1.03 (0.77-1.39)	Any positive SPT response
Geometric mean	Percentage difference‡ (95% CI)	
278.4 kIU/L (n = 86)	-37.4% (-65.8% to +14.7%)	Age group
129.5 kIU/L (n = 63)	+402% (+96% to 1190%)	None

Effect of TST reaction size and IFN- γ response to PPD on immunologic and clinical outcomes

Among 169 subjects from the BCG-vaccinated cohort who had data for both TST and in vitro IFN- γ response to PPD, 121 (71.6%) had TST results of less than 5 mm and stimulation indexes for IFN- γ response to PPD of less

than 2.0, 17 (10.1%) had TST results of 5 mm or greater and stimulation indexes of less than 2.0, 19 (11.2%) had TST results of less than 5 mm and stimulation indexes of greater than 2.0, and 12 (7.1%) had TST results of 5 mm or greater and stimulation indexes of greater than 2.0. The κ statistic for the agreement between these 2 tests was 0.27 (95% CI, 0.09-0.45), indicating poor agreement.

Forty-four (14.3%) of the BCG-vaccinated cohort had TST reaction sizes of 5 mm or greater. Table III shows that the prevalence of atopy, as measured on the basis of positive SPT responses, was not lower in subjects with positive TST results. Geometric mean IgE levels were actually higher in girls with positive TST results than in girls with negative TST results. Overall, the prevalence of allergic disease was not lower in subjects with positive TST results compared with in subjects with negative TST results. However, among the subgroup with a family history of eczema or rhinitis, there was a strong trend toward a reduced prevalence of recent wheezing and diagnosed asthma and eczema in subjects with positive TST results compared with in subjects with negative TST results. There were no differences between the subjects with positive and negative TST results in the level of cytokine responses to HDM stimulation of PBMCs in vitro (all $P > .2$, data not shown).

The 31 BCG-vaccinated subjects who had a stimulation index of greater than 2 for the IFN- γ response to PPD in vitro did not differ from those with a PPD stimulation index of less than 2 with respect to any of the allergy or clinical outcomes (data not shown), except that they had a significantly higher prevalence of hay fever (68% vs 44%; relative risk, 1.26; 95% CI, 1.03-1.55), and the girls among them had a significantly higher prevalence of atopy, as measured on the basis of positive SPT responses (86% vs 56%; relative risk, 1.52; 95% CI, 1.12-2.09). Subjects with a PPD stimulation index of greater than 2 had higher levels of response to HDM stimulation in vitro (data not shown).

DISCUSSION

This historical cohort study has demonstrated that neonatal BCG vaccination does not reduce the subsequent cumulative incidence of allergic sensitization, as measured on the basis of SPT responses or the total serum IgE concentration during childhood. There was some in vitro evidence of immune deviation in the vaccinated cohort manifesting as a reduced prevalence and level of IL-10 secretion by T cells stimulated with specific allergen. In the population as a whole, there was no effect of BCG vaccination on the prevalence of asthma, eczema, or hay fever in childhood. However, in a substantial subset of subjects who had a family history of allergic disease, neonatal BCG vaccination was associated with a reduced prevalence of asthma. Our secondary hypothesis, that the intensity of delayed-type hypersensitivity to tuberculin is inversely related to the prevalence of allergic sensitization and disease in subjects who have received BCG vaccination, is not supported by these data.

The serendipitous difference in BCG vaccination policy between these 2 nearby localities has meant that the 2 cohorts compared in this study are similar in most respects. This, together with our finding that 80% of the subjects identified from locality B and virtually none in locality A had received vaccination, gives us confidence

that the selection of subjects for BCG vaccination was not influenced by any extraneous factors that could conceivably bias this investigation.

Response rates from those who could be traced were around 70%. Our telephone interviews with nonresponders indicate that in both localities they probably had a lower prevalence of allergic disease than those who responded. Although this is relevant to the interpretation of overall prevalence rates, it is unlikely to lead to an important bias in the assessment of the study hypotheses.

The sample size was sufficient to adequately test the principal hypothesis. However, the smaller size of the BCG-vaccinated cohort and the low proportion who had positive TST results meant that the power to test the secondary hypotheses concerning the relationship between sustained cell-mediated immune response to BCG vaccination and allergic outcomes was low, and the CIs around the estimates were consequently broad.

A strength of this investigation is the low prevalence of tuberculosis²⁴ and tuberculosis infection²⁵ among Australian-born individuals living in Australia. This has meant that the interpretation of the effect of neonatal BCG vaccination on TST and in vitro PPD IFN- γ responses is not confounded by subsequent actual tuberculosis infection. An additional feature of this study was our capacity to detect immunologic recall of BCG vaccination by 2 means: intradermal tests of delayed hypersensitivity and in vitro tests of IFN- γ response to PPD. This latter response was detected in 31 of 169 subjects, and 61% of these responses were not detected by the TST.

Our findings contrast with those of the most similar previous study,¹⁰ conducted in Sweden, which found that children with a family history of allergic disease did not gain protection from allergic disease as a result of neonatal BCG vaccination. However, in the Swedish study children were assessed between the ages of 3 and 7 years, a time at which the clinical features of allergic disease might not yet be manifest. Overall, the prevalence of atopy and allergic disease was much lower in the Swedish study (10% in the BCG-vaccinated group and 12% in the nonvaccinated group) than in the current study, in which over 70% of subjects showed evidence of sensitization. It is possible that other unidentified factors driving this high prevalence are susceptible to the protective effects of BCG vaccination.

In addition to determining total IgE levels and specific skin test responses as a measure of atopy, allergen-specific and polyclonal T-cell responses were measured. There were no significant differences in allergen-stimulated IL-4, IL-5, or IFN- γ levels between the BCG-vaccinated and the nonvaccinated cohorts. However, neonatal BCG vaccination appears to be associated with reduced IL-10 responses to HDM allergen. The control and consequences of IL-10 secretion by lymphocytes appears to be complex, but others have shown that enhanced IL-10 secretion is associated with atopy.²⁶

Our failure to detect differences between BCG-vaccinated and nonvaccinated subjects in the prevalence of SPT reactivity, total serum IgE levels, and other allergic

diseases (eczema and hay fever) is surprising and calls into question our hypothesized causal pathway: immune deviation causing reduced atopy and, consequently, reduced asthma. These findings imply that immune deviation might have effects on clinical airway disease without a measurable effect on immediate hypersensitivity. Furthermore, the effects of BCG vaccination might not be generalizable to all forms of allergic disease.

We conclude that neonatal BCG vaccination does have a measurable effect on T-cell allergen responsiveness 7 to 14 years after vaccination and that among a subgroup of subjects with an inherited predisposition to allergic disease, this is associated with clinically relevant beneficial effects. This interaction between genetic predisposition and protective environmental exposures has recently been observed in relation to early-life exposure to pets.²⁷

The findings of this study encourage the view that external influences on the immune system in the neonatal period have consequences that extend into later childhood and influence the expression of asthma. Genetic factors are likely to modify the effect of those external factors.

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