

Short communication

The association of airway hyperresponsiveness and tuberculin responses

Background: The balance between the two subsets of T cell is pivotal for allergic sensitization.

Objective: We conducted a cross-sectional study of 486 children vaccinated with bacillus Calmette-Guérin (BCG), aged 10–13 years, to evaluate whether tuberculin responses may contribute to airway hyperresponsiveness (AHR).

Methods: Tuberculin skin test, allergic skin test, and methacholine challenge test were done. The methacholine concentration causing a 20% fall (PC_{20}) in forced expiratory volume in 1 second (FEV_1) was used as a threshold of AHR. Atopy was defined as a reaction showing a mean wheal size of ≥ 3 mm to one or more allergens on skin prick test (SPT). Two tuberculin units of polysorbate-stabilized purified protein derivatives (PPD) were injected intradermally into the volar surface of the forearm. Reactions were read at 48–72 h as the transverse diameter in millimeters of induration.

Results: Of the children in the study, 12.3% (60/486) had PPD induration; 7.8% (38/486) of children had PPD induration of greater than 10 mm. The PPD induration size was 10.5 ± 1.03 mm (confidence interval (CI) 7.19–12.33) in atopic children and 11.2 ± 0.76 mm (CI 7.89–13.1) in nonatopic children. The differences of PPD induration diameter between the two groups were not significant. There was no difference of log PC_{20} between PPD induration ≥ 10 mm and < 10 mm (0.13 ± 0.18 vs. 0.42 ± 0.05). The difference of log PC_{20} between positive and negative tuberculin response was not significant. Children with atopy had lower log PC_{20} than those without atopy (0.16 ± 0.07 vs. 0.51 ± 0.05 , $P = 0.001$). After adjusting for sex, age, height, weight, tuberculin response, atopy was associated with AHR in multivariate analyses (odds ratio = 1.895, CI 1.285–2.505, $P = 0.002$).

Conclusion: These data suggested that a tuberculin response due to mycobacterial infection status have no effect on AHR in schoolchildren.

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A decline in infection is probably a partial explanation for rising trends of allergy worldwide and mycobacterial infection is amongst the most promising candidates (1, 2).

Heterogeneity among $CD4^+$ T cells has become widely accepted because it explained immune deviation, that is, the inverse relationship between humoral and cell-mediated immunity, which has been observed clinically and experimentally for decades. The balance between the two subsets of T cells is important for allergic sensitization. T-helper 1 (TH1) cells activate mononuclear phagocytes that are involved in cell-mediated immunity, while T-helper 2 (TH2) cells dominate parasitic infection and type I hypersensitivity (3).

Bronchial asthma is defined as a chronic inflammatory disorder of the airways in which many cells such as

mast cells, eosinophils, T-lymphocytes, neutrophils, and epithelial cells, and cellular elements play a role (4). There is accumulating evidence that $CD4^+$ lymphocytes with a Th2 cytokine pattern play an important role in the pathogenesis of asthma. $CD4^+$ T cells secrete a characteristic pattern of lymphokines, including interleukin (IL)-2, a growth factor for T cells that stimulates them to expand clonally, and interferon (IFN)- γ , a major mediator of macrophage activation in infection with *Mycobacterium tuberculosis* (1). Shirakawa et al. (2) reported that a positive tuberculin response in bacillus Calmette-Guérin (BCG)-vaccinated school children in Japan correlated with a lower incidence of atopic disorders. But Yilmaz et al. (5) did not find any relationship between tuberculin response and atopy status later in life in BCG-immunized subjects.

To our best knowledge, there has been no published

data investigating association of airway hyperresponsiveness (AHR) and tuberculin response. Therefore, we observed tuberculin response and AHR to investigate the relationship between tuberculin positivity and AHR in schoolchildren vaccinated with BCG after birth in epidemiological survey.

Materials and methods

Study group

All children were 10–13 years of age. The BCG vaccine strains used in Korea are generally French Pasteur strain 1173P₂ and they contain 1–6 million colony forming units/ml. All children were vaccinated with 0.1 ml BCG at the ages of 4 weeks after birth. Questions on respiratory and allergic disorders included those developed for the International Study of Asthma and Allergies in Childhood (ISAAC). The focus of this survey is on responses to the ISAAC questions on 'wheeze in the last 12 months', 'number of wheezing attacks in the last 12 months', and on 'sleep-disturbing' and 'speech-limiting' wheezing in the last 12 months. Symptoms of allergic rhinoconjunctivitis ('sneezing or runny, or blocked nose without a cold' and 'itchy-watery eyes') were assessed (6). In addition, a question on morning cough as a symptom of nonspecific airway irritation was asked: 'Did you frequently cough in the morning right after waking up in the last 12 months?'. A total of 486 patients were enrolled in this study (Table 1). No subjects took any drugs, such as antihistamines, cromolyn, theophylline or sympathomimetics, which might interfere with the results of skin tests within 72 h of the tests being performed. All the children in the study had neither received BCG nor been tuberculin skin tested in the previous 6 months, nor did they have any condition known to cause anergy (immunosuppression by drugs or disease, viral infections, malnutrition, administration of live-virus vaccines) at the time of the study. The study was approved by the Research Committee of Seonam University and written informed consent was obtained from guardians before the study.

Allergy skin test

Allergy skin prick test with commercially available inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Aspergillus* spp., alder, birch, hazel, rye, timothy, mugwort, ragweed, cockroach; Allergopharma Co, Reinbek, Germany) and histamine (10 mg/ml, Allergopharma Co, Germany) were performed on the volar side of both forearms. After 15 min, the mean diameter of the wheal formed by the allergen was compared with that formed by histamine. Atopy was defined as a mean wheal size of ≥ 3 mm to at least one of the inhalant allergens (7).

Tuberculin skin test

An area of skin on the volar surface of the forearm was cleaned with acetone or ether. Two tuberculin units of polysorbate-stabilized purified protein derivatives (PPD RT23, Copenhagen, Denmark) were injected intradermally into the volar surface of the forearm. Reactions were read at 48–72 h as the transverse diameter in millimeters of induration, which was palpated using a finger. The diameter of erythema was not considered. The diameter (in millimeters) of the induration was measured transversely to the long axis of forearm. A positive reaction was defined as one showing PPD induration ≥ 10 mm.

Spirometry

Spirometry was performed according to American Thoracic Society standards (8) using SensorMedics 2200 spirometer (Cardiopulmonary Care Company, Yorba Linda, CA). The repre-

sentative values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were selected according to International Thoracic Society criteria (9) and the reference values were taken from the reports by Choi et al. (10) and Kim et al. (11)

Airway hyperresponsiveness

Methacholine challenge tests were carried out by a modified method described by Chai et al. (12) Concentrations of 0.075, 1.25, 2.5, 5, 10, and 25 mg/ml methacholine were prepared by dilution with buffered saline. A Micro-dosimeter (S & M Instrument Co, Doylestown, PA) was used to deliver the aerosol generated by a De Vilbiss 646 nebulizer (Sunrise Medical HHG Inc., Pittsburgh, PA). Subjects inhaled five breaths of increasing concentrations of methacholine until FEV₁ fell by more than 20% of its basal value or the highest concentration was reached. The largest value of triplicate FEV₁ at 30 or 90 or 180 s after each inhalation was adopted for analysis. The reduction in FEV₁ after inhalation of increasing concentrations of methacholine was compared to the post saline values. If PC₂₀ was less than 16 mg/ml, a subject was considered to have AHR to methacholine.

Statistical analyses

All data were analysed using the spss version 7.5 for windows. Data are expressed as mean $\pm$ standard error of mean (SEM). Statistical analysis was performed by Mann-Whitney *U*-test and chi-square test. Spearman's correlations were used. In multivariate analysis, logistic regression was performed using a stepwise selection method with $P < 0.05$ for entry into the model. Atopy, tuberculin response, and AHR were treated as binary outcome variables in logistic regression analysis. A *P*-value of < 0.05 was considered significant.

Results

Of the children in this study 12.3% (60/486) had a tuberculin response. 7.8% (38/486) of the children had PPD induration greater than 10 mm. The PPD induration size was 10.5 ± 1.03 mm (CI 7.19–12.33) in atopic children and 11.2 ± 0.76 mm (CI 7.89–13.1) in nonatopic children. The difference of PPD induration diameter between the two groups was not significant. 47.3% (18/38) of the children with positive PPD responses and 46.8% (210/448) of the children with negative tuberculin responses had PC₂₀ less than 16 mg; 13.9% (68/486) of children had PC₂₀ less than 4 mg/ml. The difference of PC₂₀ between positive and negative PPD responses was not significant (Fig. 1). PPD induration diameter is not different between positive AHR and negative AHR. There was no difference between PPD responses of children with lung symptoms and of those without lung symptoms [10.62 ± 0.84 mm (CI 7.28–12.18) vs. 11.52 ± 0.84 mm (CI 8.24–13.0)]. There was no difference between PPD responses of children with nose symptoms and of those without nose symptoms [10.73 ± 0.69 mm (CI 7.22–12.5) vs. 11.61 ± 1.19 mm (CI 7.91–13.57)]. There was no difference of log PC₂₀ between PPD induration ≥ 10 mm and < 10 mm (0.13 ± 0.18 vs. 0.42 ± 0.05). The difference of log PC₂₀ between positive and negative tuberculin response was not significant. Children with atopy had lower log PC₂₀ than those without atopy (0.16 ± 0.07 vs. 0.51 ± 0.05 ,

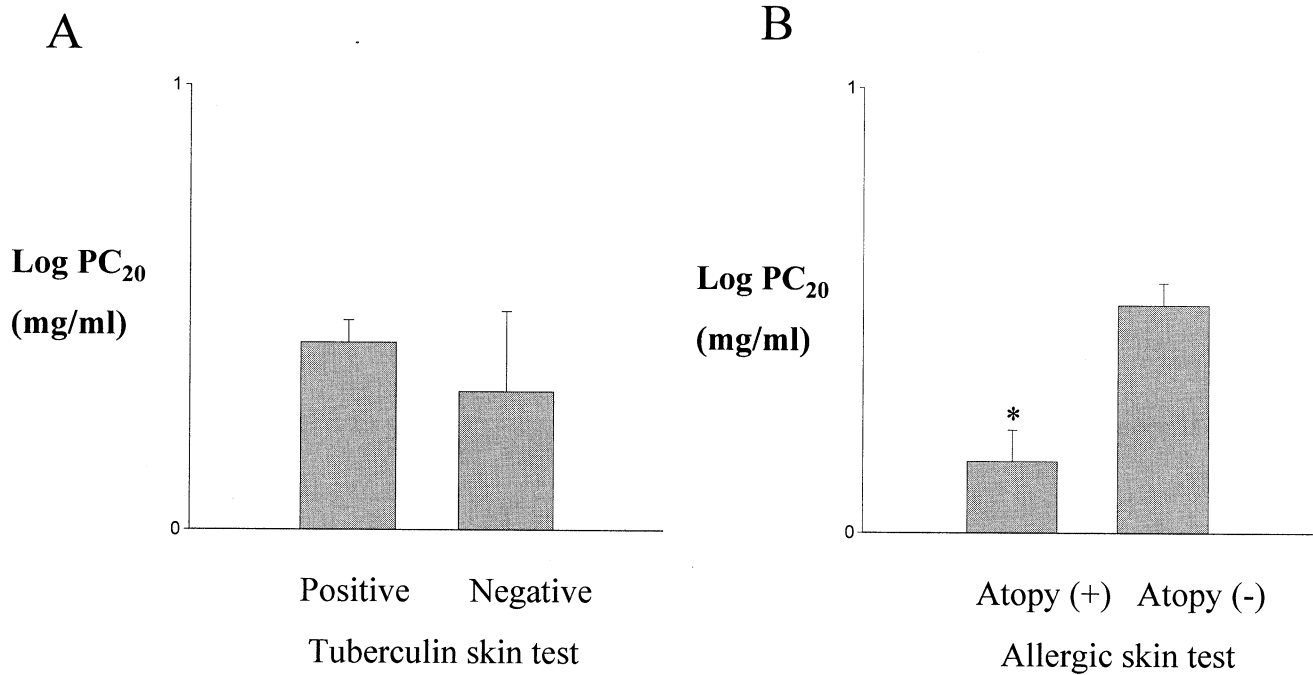


Figure 1. A) Log PC₂₀ of children with positive and negative tuberculin skin test. B) Log PC₂₀ of children with regard to atopy. * $P < 0.05$ compared to those without atopy.

$P = 0.001$, Fig. 1]. After adjusting for sex, age, height, weight, tuberculin skin test result, atopy was associated with AHR in multivariate analyses (odds ratio = 1.895, CI 1.285–2.505, $P = 0.002$, Table 2).

Discussion

In this study, we did not find any relationship between tuberculin responses and AHR in children vaccinated with BCG. But children with atopy had lower PC₂₀ than those without atopy, suggesting that atopy is a more important factor for AHR than tuberculin responses in BCG vaccinated children.

Polarization of T-helper cells from T-helper 0 cells is regulated, among other things, by dendritic cells, the strength of antigen stimulation and the cytokines present during priming. T-helper subsets can be distinguished by their cytokine profiles. TH1 cells produce mainly IL-2 and IFN- γ and TH2 cells produce mainly IL-4, IL-5 and IL-10 (13–15). The same

cytokines may also induce a shift in the balance between TH1 and TH2 responses. Via the release of particular cytokines the subsets may mutually modulate the response.

Asthma develops in early life (16). Infections seem to afford protection against the development of asthma and allergy (17) and children with eczema occasionally undergo temporarily spontaneous remission after severe bacterial and viral infection (18). Young adults who had experienced measles in childhood were significantly less likely to be atopic than those who had been vaccinated and/or had no measles (19). The BCG vaccine is a potent immunoadjuvant for induction of Th1-type responses and increases production of IFN- γ (20). In this study because all children were BCG vaccinated, we attempted to prove the hypothesis that a pre-existing Th1-type immune response elicited by BCG immunization could suppress allergic sensitization and AHR. Based on the concept of the TH1/TH2 balance comprising the

Table 1. Characteristics of subjects

	Tuberculin response	
	≥ 10 mm	< 10 mm
Age (years)	11.02 $\pm$ 0.12	10.98 $\pm$ 0.03
Sex (male/female)	18/20	212/236
Atopy (yes/no)	17/21	135/313
FEV ₁ (%pred.)	104.4 $\pm$ 1.07	103.8 $\pm$ 0.41
FVC (%pred.)	103.5 $\pm$ 1.05	102.9 $\pm$ 0.43
FEV ₁ /FVC	101.2 $\pm$ 0.53	99.7 $\pm$ 0.13

Table 2. Multivariate logistic regression on AHR

Variables	Odds ratio	95% confidence interval	P-value
Atopy*	1.895	1.285–2.505	0.002
Tuberculin response	1.264	0.641–2.496	
Sex (male/female)	0.770	0.534–1.110	
Age (years)	1.114	0.843–1.472	
Height (cm)	1.002	0.958–1.047	
Weight (kg)	1.114	0.843–1.472	

*Stepwise logistic regression was used to select factors significantly associated AHR risk ($P < 0.05$). All variables in the table were included simultaneously in the logistic regression model.

dichotomy in T-helper cell populations with a mutual inhibition of TH1 and TH2 cell subsets, it was recently hypothesized that infections involving a TH1 response suppress the TH2 response. Infections of the TH1 type like *Bordetella pertussis* (21), *Listeria monocytogenes* (22), *Mycobacterium bovis* (23), *Leishmania* (24), and many other bacteria go along with increased production of IFN- γ which impairs the TH2 response and thus reduces the progression of allergy/asthma. Other infections, like respiratory syncytial virus infection, which was held responsible for pediatric asthma (25) for a long time, brucellosis, rhinovirus and *Aspergillus fumigatus* infection (26) induce TH2 responses and may therefore increase the prevalence of allergic disorders.

Shirakawa et al. (2) reported that positive tuberculin responses among Japanese schoolchildren aged 12–13 years correlated with a lower incidence of atopic disorders and low serum IgE levels, and there was an inverse correlation between tuberculin response and total IgE levels. Recently any relationship between tuberculin response and atopy status in later life in BCG-immunized subjects in Turkey was not detected (5). In a young adult population, who had been BCG vaccinated at the age of 14, no relationship between a positive tuberculin reaction and atopy was observed (27). In this study, we checked AHR using a methachol-

ine challenge test in schoolchildren vaccinated with BCG after birth. In contrast to the results of Shirakawa et al. (2) we found that there was no relationship between tuberculin responses and atopy. We evaluated the hypothesis that a pre-existing Th1-type immune response elicited by BCG immunization could suppress allergic sensitization and AHR. Herz et al. (20) reported inhibition of AHR by BCG. However, in our study there was no relationship between tuberculin response and AHR, suggesting that a tuberculin response due to mycobacterial infection status has no effect on AHR in schoolchildren. But children with atopy had lower PC₂₀ than those without atopy, suggesting that atopy is a more important factor for AHR than tuberculin response.

In conclusion, our results indicate that tuberculin response was not correlated with AHR in schoolchildren vaccinated with BCG. Further studies are necessary to elucidate the balance between the two subsets of T cells for allergic sensitization.

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