

Is the Mycobacteria-Derived Purified Protein Response in Atopic Asthmatic Children Different?

A Case Study from Istanbul, Turkey

Hüseyin Serdar Kale^a Yücel Taştan^b Osman Pinçe^c Emel Altuncu^a
Ethem Erginöz^d

^aDepartment of Paediatrics, Bakirkoy Maternity and Infant Hospital, ^bDepartment of Paediatrics, Cerrahpaşa Medical School, Istanbul University, ^cDepartment of Paediatrics, Division of Immunoloji-Allerji, Bakirkoy Maternity and Infant Hospital, and ^dDepartment of Public Health, Cerrahpaşa Medical School, Istanbul University, Istanbul, Turkey

Key Words

Atopic asthmatic children · Asthma, children · Bacillus Calmette-Guérin · Mycobacteria-derived purified protein

Abstract

Background: The response to mycobacteria-derived purified protein (PPD) is mediated primarily by T-helper-1 response and is expected to be inhibited in atopic diseases. The aim of this study was to investigate whether the PPD response is different in atopic asthmatic children. **Methods:** 40 atopic asthmatic children (mean age 8.3 ± 4.9 years) and 40 healthy age- and sex-matched children who had received bacillus Calmette-Guérin (BCG) vaccination were included in the study. Five PPD units were administered intradermally to all children and were interpreted after 48 h. **Results:** There was no correlation between serum total IgE level and PPD induration ($p = 0.054$). The PPD induration was not statistically different between the children who used inhaled corticosteroid and those who did not. Although the PPD positivity (induration ≥ 5 mm) rate was higher in atopic asthmatic children (50%) than in healthy children (32.5%), the difference was not found to be statistically significant.

The PPD induration in atopic asthmatic children (7.41 ± 5.58 mm) was found to be greater than the one in healthy children (5.21 ± 3.39) ($p < 0.039$). The induration in atopic asthmatic children (5.21 ± 3.77) and healthy children (4.43 ± 2.32) did not show a difference in children who were vaccinated only once with BCG, but it was found to be statistically significantly greater in atopic asthmatic children (12.50 ± 5.90) than healthy children (7.08 ± 4.70) who were vaccinated with BCG twice ($p < 0.012$). The proportion of having a PPD induration of ≥ 10 mm was found to be higher in atopic asthmatic children than in the healthy ones (32.5 vs. 12.5%) ($p < 0.032$). **Conclusion:** Our data showed that the PPD response was stronger in BCG-vaccinated atopic asthmatic children than in healthy BCG-vaccinated ones.

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Introduction

In recent years, a rising trend of allergic diseases has been observed in many developed countries [1]. The reasons for this are not fully understood. The lack of exposure during early childhood to infections such as tuberculosis and measles may possibly increase the risk of

developing atopic diseases in these countries [2, 3]. Recently, interest has focused particularly on the role of mycobacterial infections. In the relationship between atopic and mycobacterial diseases, T-helper (Th) lymphocytes have a central role. Th lymphocytes are classified into two subgroups, Th1 and Th2, according to their production of the cytokines [4]. Th1 and Th2 responses cross-regulate each other negatively [5]. Th1 responses are characterized by production of interferon (IFN)- γ , and interleukin (IL)-2, that mediate T-cell cytotoxic activity and delayed-type hypersensitivity (DTH) reaction [6]. It is known that mycobacterial infections and *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine induce intense Th1 responses and suppress the development of Th2 responses that are characteristic of atopic diseases [7–11]. Th2 responses are characterized by production of cytokines IL-3, IL-4, IL-5 and IL-13 which lead to immunoglobulin E (IgE) production [4]. According to the Th2 hypothesis for asthma, atopic diseases are caused by a relative increase in Th2 response in combination with a decrease in Th1 response [4].

The first epidemiological evidence for the relationship between atopic disorders and mycobacterial diseases came from the study by Shirakawa et al. [12] who reported that there was an inverse association between mycobacteria-derived purified protein (PPD) responses and atopic disorders in Japanese schoolchildren. They suggested that mycobacterial exposure may influence the immune response to allergens. To date, these findings have been widely used in support of the hygiene hypothesis for allergic diseases. Subsequently, there has been an inability to confirm these findings in epidemiologic studies in other communities [13–18]. Studies from countries with a low incidence (e.g. Sweden [14], Norway [16] and Germany [17]) and a high incidence of tuberculosis (e.g. Turkey [13], China [15] and The Gambia [18]) showed that there were no differences between allergic and healthy children with respect to the PPD response. However, some epidemiologic studies showed that exposure to *M. tuberculosis* reduced the risk of developing asthma [19–20].

The PPD skin test is a standard and simple test, and is still widely used in the screening of tuberculosis. It is believed that a strong PPD response is a strong indicator of active tuberculosis. The PPD response is a DTH and mediated primarily by Th1 response, and it is expected to be suppressed in atopic diseases. However, an animal study showed that the DTH reaction in skin was not always mediated by Th1 response, and Th2 response may also mediate DTH responses. Thus, it is possible that

PPD responses in the asthmatic and normal control are different [21]. Özen et al. [22] recently reported that PPD induration in allergic children was greater than in healthy children.

The purpose of the present study was to evaluate whether the PPD response in atopic asthmatic children is different.

Material and Methods

This study was carried out prospectively from January to December of 2001 in the Allergy Unit and General Polyclinics of Bakirkoy Maternity and Infant Hospital in Istanbul. Both the study group and control group consisted of children who had received BCG vaccination within the first 2 months of age. In accordance with the program of the Turkish Ministry of Health, two doses of BCG vaccine are given to all children in Turkey routinely at the ages of 2 months and 7 years.

Forty randomly selected children (18 females and 22 males) who had had atopic asthma for more than 6 months were included in the study; their mean age was 8 ± 3.49 (range 3–14) years. Forty healthy sex- and age-matched children served as the control group and their mean age was 7.6 ± 2.99 (range 3–14) years.

Atopic asthmatic disease was defined by a positive skin prick test (Stallergenes, Kamp Lintfort, Germany) to house dust mite allergen (wheal diameter >3 mm than the negative control), a history of recurrent dyspnea, wheezing episodes and a high serum total IgE level. Positive reactions to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinea* mites were most commonly recorded (80%). The severity of asthma was graded from I (intermittent asthma) to IV (severe persistent asthma) according to the classification of the Global Initiative for Asthma [23]. Twenty-six (65%) children were found to be mildly asthmatic (grade II) and 14 (35%) were found to be moderately persistent asthmatic (grade III).

Age, sex, number of BCG vaccinations and BCG scars, age at the time of vaccinations, atopy history and history of atopy and tuberculosis in the family were recorded at the study entry. The times of BCG vaccinations were also monitored from their own vaccination schedules. The hospital follow-up time of asthmatic children, their atopic status and the duration of treatment were determined from their follow-up records. Children under acute asthmatic attack, under therapy/prophylaxis of tuberculosis or with a positive family history of active tuberculosis or with malnutrition, immunocompromised patients, children revaccinated within the last 6 months and patients with a history of taking corticosteroids except by inhalation within the last 15 days, and those who had viral infections, were excluded from the study. Stools of all children were examined for parasites three times and those with parasitosis were also excluded. The direct smear examination for parasites was performed in their stool three times in both groups and children with parasitosis were also excluded.

Five units of PPD (Statens Serum Institute, Copenhagen, Denmark) were administered intradermally into the volar site of the left forearm using a 27-gauge needle and a PPD syringe to all children by the same skilled person throughout the study. Injection of 0.1 ml physiological saline intradermally into the volar site of the right forearm was used as negative control. The diameter of the

Table 1. Characteristics of atopic asthmatic and healthy children

	Atopic asthmatic children n = 40	Healthy children n = 40	p
Age, years (mean $\pm$ SD)	8.3 $\pm$ 4.9	7.6 $\pm$ 2.9	NS
PPD induration diameter, mm (mean $\pm$ SD)	7.41 $\pm$ 5.58	5.21 $\pm$ 3.39	0.039
PPD induration $\geq$ 5 mm, n (%)	20 (50)	13 (32.5)	NS
$\geq$ 10 mm, n (%)	13 (32.5)	5 (12.5)	0.032
PPD induration (mean $\pm$ SD), mm			
1 = BCG scar (n = 28)	5.21 $\pm$ 3.77	4.43 $\pm$ 2.32	NS
2 $\geq$ BCG scars (n = 12)	12.50 $\pm$ 5.90	7.08 $\pm$ 4.70	0.012
Total serum IgE level			
Geometric mean, UI/ml	618.80	34.73	0.0001
NS = Not significant.			

induration was measured transversely to the long axis of the forearm by using ballpen method after 48 h [24]. If an induration in the negative control occurred, it was subtracted from the PPD induration diameter. The erythema was not taken into account. An induration of $\geq$ 5 mm was accepted as a positive result. Children with an induration of $\geq$ 10 mm were examined for the presence of tuberculosis by chest X-rays, cultures of three gastric aspirates and through family screening.

Before PPD testing, a 2-ml venous blood sample was taken from each child and the quantitative total serum IgE levels were assayed using the BN ProSpec System (reagent; N Latex IgE mono, Dade Behring, Germany) [25]. The study was conducted in accordance to the standards of the institutional ethic committee. The aim of the study and the procedures were explained to the children and their parents and informed consent was obtained.

The geometric means of serum antibody levels were calculated by logarithmic transformation. The Mann-Whitney U test was used for comparison of the means between the groups, χ^2 was used to compare the proportions, and the Pearson correlation method was used to determine any association. Significance was set at $p < 0.05$.

Results

The characteristics of the study and control groups are presented in table 1. By strictly adhering to the protocol of the study, the distribution of ages and sexes of children in both groups were similar. In the study group, the median time of hospital follow-up was 2.7 ± 2 years. The rate of positive family history of atopic diseases (e.g., asthma, eczema, allergic rhinoconjunctivitis) in other members of the family (e.g., parents, brothers and sisters) in the study group (75%) was higher than in the control

group (5%). The median intervals between study time and last BCG vaccination in the study group (5 ± 3.37 years) and control group (5.1 ± 3.13 years) were not different. In each of the groups, 28 (70%) children had received one BCG vaccine and 12 children (30%) had received two BCG vaccines. The number of BCG scars was equal to the number of vaccinations in both groups. The geometric mean of serum total IgE in the study group (618.80 IU/ml) was significantly higher than in the control group (34.73 IU/ml) ($p = 0.0001$).

The mean PPD induration was significantly greater in atopic asthmatic children (7.41 ± 5.58 mm) than in healthy children (5.21 ± 3.33 mm) ($p = 0.039$). In evaluating the PPD induration diameter according to BCG vaccination status, in one-time BCG-vaccinated children, the diameter of PPD induration in atopic asthmatic children (5.21 ± 3.77 mm) did not show a significant difference from the one in healthy children (4.43 ± 2.32 mm) ($p > 0.05$), on the other hand, it was found to be significantly greater in atopic asthmatic children (12.50 ± 5.90 mm) than the healthy ones (7.08 ± 4.70 mm) in twice BCG-vaccinated children ($p < 0.012$). There was no correlation between serum total IgE level and diameter of PPD induration ($p = 0.054$).

In the study group, 26 children (65%) had been using inhaled corticosteroids (400 μ g/day of beclomethasone dipropionate) for 10 ± 6 months on average. The PPD induration of the children who used inhaled corticosteroids was not found statistically different from the ones who did not use them. Although the rate of PPD positivity (induration $\geq$ 5 mm) in atopic asthmatic children

(50%) was higher than in the healthy children (32.5%), no statistically significant difference was found. But 13 (32.5%) of the children in the study group and 5 (12.5%) in the control group had a PPD induration of ≥ 10 mm and the difference was statistically significant ($p = 0.032$). Active tuberculosis was not observed in any children with an induration of ≥ 10 mm.

Discussion

The Th2 hypothesis for asthma states that asthma is caused by a relative increase in Th2 response in combination with a decrease in Th1 response. Shirakawa et al. [12] reported that an inverse association existed between the PPD response and atopic diseases. They showed that a strongly positive PPD response was associated with a lower incidence of allergic disease, lower levels of serum IgE, and higher levels of Th1-type cytokines. Subsequently, there has been an inability to confirm these findings in similar studies [13–18].

In contrast to previous studies, our study showed that PPD induration in atopic asthmatic children was statistically significantly greater than in healthy children ($p = 0.039$). This difference was especially observed in twice BCG-vaccinated children ($p = 0.012$). The PPD positivity (induration ≥ 5) did not show a significant difference, but the proportion of the children who had PPD induration of ≥ 10 mm was significantly higher among atopic asthmatic children (32.5%) than healthy children (12.5%) ($p = 0.032$). In our study, supporting the results of previous studies, no correlation was also found between serum, the total IgE level and PPD induration. Our findings support the earlier ones reported by Özen et al. [22]. They reported that the rate of PPD induration of ≥ 10 mm was found to be more significant in allergic children (27%) than the nonallergic ones (6%), and the diameter of PPD induration was found to be greater in allergic children who were vaccinated once or more with BCG than the nonallergic ones. The results obtained from these two studies show that some allergic children have an increase of DTH response to PPD. Both of these studies are reported from a country with a high incidence of tuberculosis.

There are a lot of epidemiological studies from countries with a low incidence (e.g. Sweden [14], Norway [16] and Germany [17]) and high incidence of tuberculosis (e.g. Turkey [13], China [15] and The Gambia [18]) and they show that there are no differences between allergic and healthy children in respect to PPD induration and

PPD positivity rate. These results suggest that the Th1 response is not completely suppressed in atopic children.

The PPD response is variable and depends on the vaccine strain and the dosage used, the age at vaccination, the time elapsed since vaccination and the number of vaccinations [26–28]. Although active infections were not detected in children with PPD induration ≥ 10 mm in this study, active tuberculosis, BCG vaccination and the cross-reaction with mycobacteria other than tuberculosis (MOTT) may cause an increase in the PPD response. On the other hand, because of long-term hospital follow-up (median 2 years) of atopic asthmatic children, it is probable that they may be faced with MOTT any time, which may result in the increase of the PPD response. But there is no report in Turkey about the prevalence of MOTT, which is believed to be widespread. Accordingly, higher frequencies of skin reactions of MOTT have been reported in an allergic group by Stannegard et al. [16].

The majority (65%) of atopic asthmatic children were on long-term inhaled steroid therapy in this study. It is known that steroids generally suppress the Th1 response [29]. Therefore, the PPD response was expected to be suppressed in our patients. On the other hand, increased a PPD response might be due to activation of tuberculosis as a result of steroid use. Our study did not support these hypotheses because the PPD induration was not statistically different between the children who used inhaled corticosteroid or not. Accordingly, it is reported that long-term use of inhaled steroids does not cause tuberculosis activation [30]. Nevertheless, how the PPD response is affected by inhaled steroid is not stated in this study.

In the assumption of a dominant Th2-type response in atopic asthmatic children, the suppression of the PPD response was expected to occur, as the PPD response, a DTH reaction, was mediated by Th1-type response. Several hypotheses can be suggested to explain the increase of the PPD response in atopic asthmatic children. Firstly, there is an increased reaction of dermis to irritant agents in atopic children, so a PPD reaction may also have increased as well. Accordingly, Nasif et al. [31] showed that allergic individuals presented an excessive response against irritant agents. In our study, excessive reactions were not seen in the study group to such an irritant agent like physiological saline. Three cases in each group developed a dermal induration of 2 mm as a reaction to the physiological saline applied, and it was subtracted from the diameter of PPD induration while the calculations were done. For that reason, we think that irritation was

not responsible for the increase of PPD reactivity as expected. During asthmatic attacks, the response to PPD and irritant agents may be different. Our study group was chosen from asthmatic children without an attack. Secondly, although the PPD reaction is mediated by primary Th1 response, Th2 cells may also have a role in increasing PPD reaction in atopic asthmatic children. It is known that transforming growth factor- β (TGF- β) produced by Th2 cells suppresses the cellular immunity. It is postulated that the suppression of TGF- β by other over-reactive Th2 cells in atopic people may have caused a reactivation of cellular immune responses and an increase of PPD reaction. This hypothesis was supported by an experimental study [32].

Finally, the PPD response is mediated by primary Th1 response which is expected to be suppressed in atopic asthmatic children. In contrast to this fact, there may be no suppression of Th1 immune response in some atopic asthmatic children, so the PPD reaction may also have increased as well. The increase of IFN- γ may be considered responsible for the abnormal response in atopic asthmatic children who have increased PPD reactions of ≥ 10 mm, because PPD induration shows a positive correlation with serum IFN- γ level in healthy people [33]. On the other hand, an experimental study showed that there was a strong DTH response to chlamydial infection in IFN- γ knockout mice with a Th2-dominant cytokine production pattern [21]. These results suggest that IFN- γ is not essential for DTH and that Th2 cells may also me-

diates DTH responses in animal models. Unfortunately, we did not evaluate the cytokine profile in our cases.

IFN- γ is a Th1-mediated cytokine produced mainly from CD4⁺ T cells, CD8⁺ T cells and natural killer cells, but according to the Th2 hypothesis, it must be suppressed. However, there are some controversial reports about IFN- γ being released in atopic asthmatic children. In a previous study it was reported that the amount of IFN- γ produced by PPD-specific lymphocytes in atopic patients was less than the amount produced in a healthy control group [34]. However, Corrigan and Kay [35] reported that increased serum levels of IFN- γ were found in patients with acute severe asthma during exacerbations, and that levels were reduced after treatment. It has recently been reported that there is an increased frequency of IFN- γ producing CD4⁺ and CD8⁺ T cells in asthmatics compared with normal subjects [36]. In contrast to the Th2 hypothesis, these results suggest that Th1 responses may have a role in asthma pathogenesis.

In conclusion, when tuberculosis is screened in tuberculosis-prevalent countries, it should be taken into consideration that the PPD response can be stronger and overemphasized as an active infection, in part due to certain BCG-vaccinated atopic asthmatic children. Some previous studies and our findings suggest that the 'Th2 hypothesis' for asthma is too simplistic and should be re-evaluated. Accordingly, we need further comprehensive studies.

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