

Does the Atopic Phenotype Prevent Development of Active Tuberculosis Infection?

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This study investigated the relationship between the atopic phenotype and the development of active tuberculosis. A total of 82 human immunodeficiency virus-negative males with active pulmonary tuberculosis and 88 healthy controls were enrolled into this prospective study. Serum immunoglobulin E (IgE) levels were measured and skin prick tests performed before initiation of treatment. Skin prick tests were positive in 34.1% of the tuberculosis patients and 39.8% of the controls. Allergic respiratory symptoms

were significantly less frequent in skin prick test positive tuberculosis patients (21.4%) compared with skin prick test positive controls (62.9%). Median IgE levels in atopic tuberculosis patients were significantly higher than in atopic controls. The low rate of atopic respiratory complaints seen in the tuberculosis patients, despite having similar allergic skin prick test sensitivities to the controls, could be attributed to a weak T-helper (Th) 2 immune reaction and its effects on Th1–Th2 interaction.

KEY WORDS: *MYCOBACTERIUM TUBERCULOSIS*; ATOPY; SKIN PRICK TESTS; TOTAL IMMUNOGLOBULIN E

Introduction

The remarkable increase in the prevalence of asthma and atopy over recent years in Western societies cannot be explained solely by changes in genetic factors or improvements in diagnostic procedures.^{1,2} Currently the most plausible explanation for the rising frequency of allergic diseases is the hygiene hypothesis, as presented by Strachan in 1989.³ He suggested that some aspects of modern lifestyles in affluent nations, including smaller family size, improvements in indoor comfort and

increased standards of personal hygiene, have minimized exposure to infectious agents and their by-products such as endotoxins. These features of contemporary lifestyles have favoured the development of T-helper (Th) 2 immune responses to environmental allergens and the corresponding development of atopic diseases. Infections such as tuberculosis, measles and diphtheria may decrease the risk of developing atopic allergic disorders, according to the hygiene hypothesis. Interest has focused particularly on the role of

Mycobacterium tuberculosis, which is known to induce Th1-type immune responses, suppressing the development of Th2 type immune responses that are characteristic of atopic disorders.⁴ Both Th1 and Th2 cells have been reported to cross-regulate each other negatively *in vitro* and in experimental animals.⁵ Furthermore, in a study conducted among Japanese schoolchildren, there was a strong inverse association between delayed hypersensitivity to *Mycobacterium tuberculosis* and atopy: positive tuberculin responses predicted a lower incidence of asthma, lower serum immunoglobulin (Ig) E levels, and cytokine profiles biased toward the Th1 type.⁶ The inverse relationship between tuberculosis infection and allergic disorders has also been observed in other studies,^{7,8} although some contradict these findings.^{9,10} There are, however, no reports in the literature concerning the effect of atopic disorders on active tuberculosis infection.

The aim of the present study was to investigate the relationship between the atopic phenotype and the development of active tuberculosis.

Patients and methods

STUDY DESIGN AND PATIENTS

This prospective study was conducted in the Department of Pulmonology, Gulhane Military Medical Academy, Haydarpaşa Teaching Hospital, Istanbul, Turkey, between March 2005 and March 2006. Since recruitment for the study was undertaken in the military hospital where the majority of patients are males, those included in the study were, therefore, male. Patients hospitalized in our clinic for pulmonary tuberculosis who had no known history of tuberculosis, who had not been treated for tuberculosis in the past and who were negative for human immunodeficiency virus were included in the study. Active

pulmonary tuberculosis was determined by positive sputum culture and/or clinical and radiological findings after the exclusion of other possible diagnoses. Patients were treated with standard combination chemotherapy (isoniazid, rifampicin, ethambutol and pyrazinamide).

The control group consisted of randomly selected healthy male subjects, selected from male soldiers and male hospital employees, with no known personal or family history of active pulmonary tuberculosis. The control subjects were asymptomatic and had no history of chronic illnesses. None of the study participants in either group had helminthic infections.

Detailed clinical evaluation of allergy symptoms, exacerbating factors, a history of personal and/or familial atopy and management of symptoms was performed for both the tuberculosis patients and the healthy controls. The number of scars caused by Bacillus Calmette-Guérin (BCG) immunizations were noted in the pulmonary tuberculosis patients. This was not recorded in the control group because BCG immunization is routinely applied in Turkey, therefore it was assumed that all healthy controls would have been vaccinated.

All participants provided written informed consent before taking part in the study. The study was approved by Gulhane Military Medical Academy ethics committee.

SKIN PRICK TESTS

Allergy skin prick tests were performed using a lancet on the volar aspect of the forearm and antihistamines were withheld for 7 days beforehand. A standardized panel of antigens including house dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), mould, grass, tree, weed, feather and dander mix (ALK-Abelló, Madrid, Spain) was applied to the skin,

together with positive (0.1% histamine phosphate) and negative (phosphate-buffered physiological saline) controls. After 15 min the results were scored by an allergist as follows:¹¹ 0, no reaction; 1+, erythema $\leq$ 15 mm; 2+, erythema $>$ 15 mm or induration $<$ 3 mm; 3+, induration $>$ 3 mm and $<$ 6 mm; 4+, induration $\geq$ 6 mm or pseudopod formation. A positive test was regarded as a score of +1 or more to at least one of the test allergens.

Tuberculin skin prick tests were performed according to the Mantoux technique, applying 5 IU of purified tuberculin protein derivative intradermally into normal-looking skin on the forearm and then taking a reading between 48 and 72 h later. Indurations $>$ 15 mm were considered to be a positive result.

IGE AND EOSINOPHIL MEASUREMENT

Serum was taken for measurement of IgE from the controls and also from the tuberculosis patients before the initiation of treatment. Total IgE was determined with a microparticle enzyme immunoassay using an IMx[®] analyser (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions.

Peripheral blood eosinophil counts were estimated microscopically.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS[®] 11.00 for Windows[®] statistical software (SPSS Inc., Chicago, IL, USA). The rate of skin prick test positivity was compared between the two groups using the two-tailed *t*-test or χ^2 test as appropriate. Other statistical analyses were made using the Kruskal-Wallis and Mann-Whitney *U*-tests. A *P*-value $<$ 0.05 was considered to be statistically significant.

Results

A total of 82 tuberculosis patients and 88 healthy controls were enrolled into the study and their demographic and clinical characteristics are given in Table 1. The atopy rate and the rate of positive skin prick tests were not significantly different between patients and controls. Mite hypersensitivity was the leading allergen sensitivity in both groups (Table 1). There was, however, a significant difference in the frequency of allergic respiratory symptoms in tuberculosis patients with a positive skin prick test compared with controls with a positive skin prick test ($P = 0.001$) (Table 1, Fig. 1).

Overall, median IgE levels were not significantly different between the tuberculosis patients (72 IU/ml, range 0 – 1112 IU/ml) and the control group (62 IU/ml, range 8 – 2956 IU/ml). However, median IgE levels in atopic tuberculosis patients (212 IU/ml, range 16 – 1112 IU/ml) were significantly higher than in atopic controls (106 IU/ml, range 30 – 2956 IU/ml) ($P < 0.05$) (Fig. 2), whereas levels in non-atopic tuberculosis patients (46 IU/ml, range 0 – 542 IU/ml) and non-atopic controls were similar (43 IU/ml, range 8 – 1476 IU/ml).

Mean peripheral blood eosinophil counts for the tuberculosis and control groups, were 323.0 ± 273.3 and 202.9 ± 199.3 cells/mm³, respectively ($P < 0.005$).

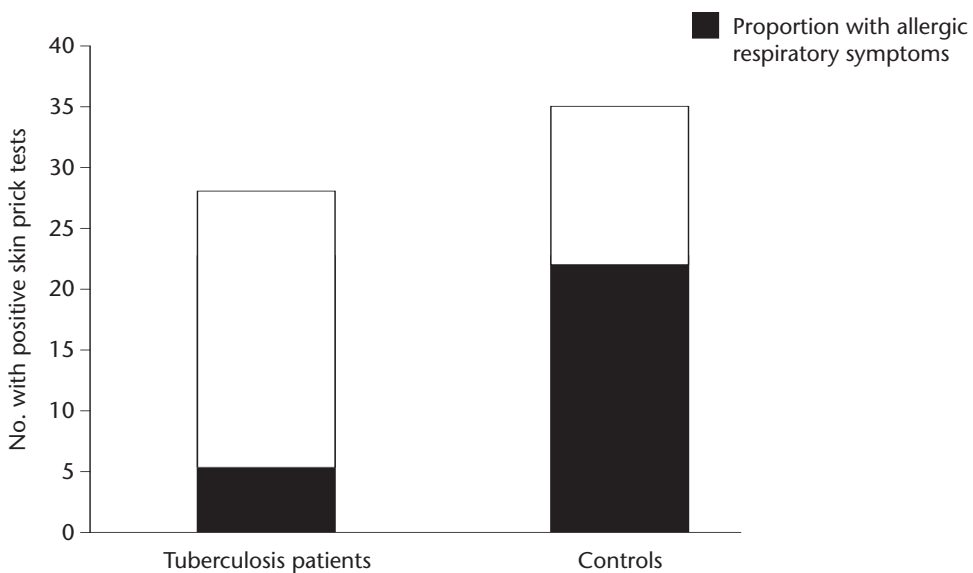
There were no correlations between the number of BCG scars and the tuberculin response, atopy and serum IgE levels in patients with pulmonary tuberculosis.

Discussion

Infection with *M. tuberculosis* is known to induce Th1-type immune responses. Initial levels of serum total IgE concentrations in tuberculosis patients have been shown to be significantly higher than in healthy controls, but these significantly decrease after curative

TABLE 1:
Demographic and clinical characteristics in patients with active tuberculosis and controls

	Tuberculosis patients (n = 82)	Controls (n = 88)
Age (years)		
Mean $\pm$ SD	22.9 $\pm$ 5.41	26.5 $\pm$ 7.69
Range	18 – 45	19 – 44
Family history of atopy	9 (11.0%)	13 (14.8%)
Positive skin prick test	28 (34.1%)	35 (39.8%)
Allergic respiratory symptoms	6/28 (21.4%) ^a	22/35 (62.9%)
Allergen sensitivity of atopic subjects		
Mite	22/28 (78.6%)	25/35 (71.4%)
Grass pollen	12/28 (42.9%)	18/35 (51.4%)
Mould	8/28 (28.6%)	8/35 (22.9%)
Feather	8/28 (28.6%)	3/35 (8.6%)
Tree pollen	6/28 (21.4%)	11/35 (31.4%)
Weed pollen	6/28 (21.4%)	10/35 (28.6%)
Dander	2/28 (7.1%)	4/35 (11.4%)

^aP = 0.001 versus control.**FIGURE 1:** Allergic respiratory symptoms in tuberculosis patients and controls with a positive skin prick test ($P = 0.001$ for the frequency of allergic respiratory symptoms between tuberculosis patients and controls)

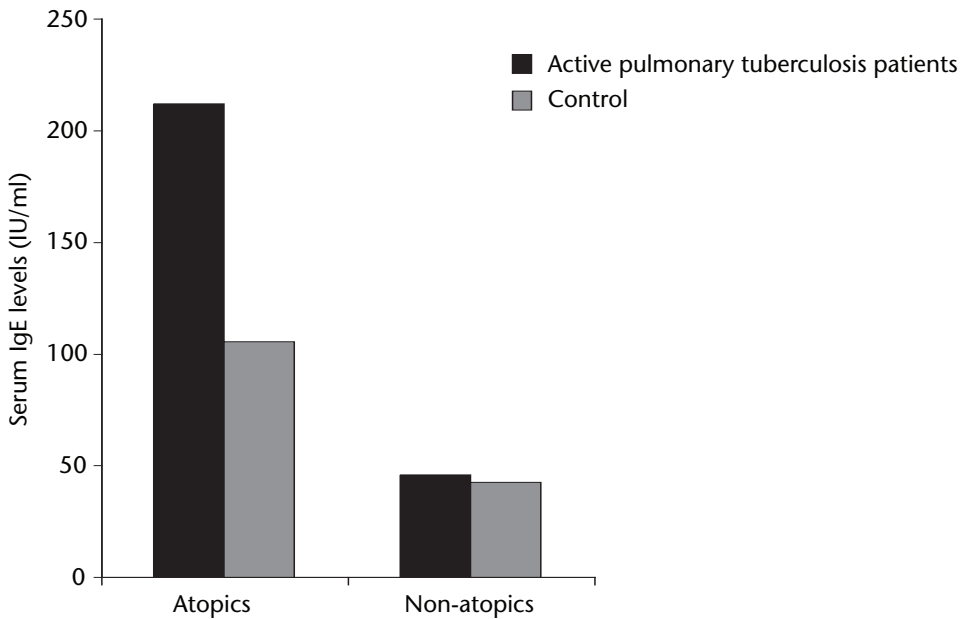


FIGURE 2: Serum immunoglobulin E (IgE) levels in tuberculosis patients and controls with and without atopy

treatment.¹² In some studies, a Th2-type cytokine response has been observed against mycobacterial antigens in the sera of tuberculosis patients.^{13,14} The higher total serum IgE levels, the predictive marker of the Th2 immune response, may indicate a limited Th2 response in the initial phase of tuberculosis infection. The subsequent decrease in serum IgE levels can be explained by a shift of immune responses towards the Th1 pathway.

In the present study, the median IgE levels were not significantly different between the patients with pulmonary tuberculosis and the control group. The median IgE levels in atopic tuberculosis patients were, however, significantly higher than in atopic controls, suggesting that patients with an atopic phenotype were able to exhibit a more pronounced Th2 response in tuberculosis. In addition, significantly fewer allergic

respiratory symptoms occurred (before the active infection) in atopic tuberculosis patients compared with the healthy controls, even though the skin prick test results were not significantly different between the two groups. These observations suggest that the patients with active pulmonary tuberculosis did not have the ability to generate an active allergic response without an atopic phenotype. The Th2 response is an unwanted response for limiting and controlling tuberculosis. Cellular immunity rather than humoral immunity has a predominant role against tuberculosis.¹⁵ The protective effect of antigen-specific antibodies produced by B-cells stimulated by interleukin (IL)-4 and IL-5 is known to be weak. The role of plasma cells has not been clearly defined, although they are frequently seen in tuberculosis lesions.¹⁶

Complement receptors (CRs) 1, 3 and 4 play

a major role in the phagocytosis of *M. tuberculosis* by human monocytes and macrophages. The macrophage mannose receptor (MR) is a prototypical pattern recognition receptor that binds with high affinity to mannose, and is frequently found on the surface of a variety of microbes, including *M. tuberculosis*.¹⁷ The expression of CRs and MRs by macrophages seems subject to the influence of a variety of mediators, including prostaglandin E₂ (PGE₂) and the cytokines interferon- γ (IFN- γ) and IL-4. PGE₂ and IL-4, a Th2-type cytokine, upregulate CR and MR expression in general, and IFN- γ has been demonstrated to decrease receptor expression and function, resulting in diminished ability of mycobacteria to adhere to macrophages.^{18,19} This could be seen as a positive effect of IL-4 in the early phase of host defences against the tuberculosis bacillus.¹⁹ Infected macrophages and dendritic cells produce IL-12, which is crucial in controlling early *M. tuberculosis* infection. IL-12 regulates the immune system towards a Th1 response, with production of IFN- γ and downmodulation of IL-10 and IL-4. IL-12 produced from phagocytic and antigen-presenting cells is the most important factor in driving Th precursor cells to become Th1 cells.^{20,21} IFN- γ and tumour necrosis factor- α are also important for macrophage activation. Patients with genetic defects in IL-12, the IL-12 receptor or IFN- γ are more susceptible to disseminated BCG and *M. avium* infections.²²

A study conducted among Japanese schoolchildren showed a strong inverse association between delayed type hypersensitivity to tuberculosis and atopy.⁶ Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels and cytokine profiles biased toward a Th1 type. In contrast, there were no significant associations between tuberculin response and atopy or serum IgE levels in our

present study.

It has been shown that the interaction between the CD40L (CD154) molecule on activated T-cells and its receptor, CD40, on macrophages and dendritic cells provides a strong signal for the production of IL-12, and the Th2 cytokines IL-4 and IL-13 also enhance IL-12 production.^{23–25} Th1 and Th2 cells have been reported to cross-regulate each other negatively *in vitro* and in experimental animals.⁵ The present study suggests that the inverse relationship between the Th1 and Th2 responses of the immune system is more complex than a simple see-saw effect.²⁶

A large study on the correlation between allergic diseases and tuberculosis, involving 235 477 children from 23 centres, suggested that exposure to *Mycobacterium* spp may reduce the risk of childhood asthma and allergic rhinitis.⁷ Although these results are generally regarded as supporting the 'hygiene hypothesis', they could also explain the positive effect of allergic diseases in preventing the development of active tuberculosis.

Primary tuberculosis is typically a disease of infancy and childhood however, in developed countries, it is also seen in increasing numbers of adult patients who have not been previously infected with *M. tuberculosis*. In 90% of cases it is a clinically asymptomatic, self-limiting disease with development of an appropriate immune response. In a small group of patients (10%), asymptomatic low-virulence dormant bacilli survive and remain in the fibrotic lesions. Subsequent immune deprivation or certain other conditions can lead to reactivation of the tuberculosis, causing break-out post-primary infection.²² In the present study, the vast majority of the patients probably had post-primary tuberculosis. Although these patients had similar allergic skin prick test

sensitivities to the control group, atopic respiratory complaints were rarely seen in the skin prick test positive patients before the diagnosis of tuberculosis. This could be attributed to the preventive effects of primary tuberculosis infection over the breakthrough of the atopic phenotype. In recent years, contradictory data have been published concerning the interpretation of the relationship between tuberculosis infection and the 'hygiene hypothesis'.^{9,10} In the initial phase of pulmonary tuberculosis, the ability to produce a strong Th2 response may help the formation of a powerful Th1 response and may facilitate limitation of the infection. We believe that pulmonary

tuberculosis patients with a similar allergic skin prick test sensitivity to healthy controls but who are unable to demonstrate a vigorous atopic allergic disease phenotype when healthy, might be indicative of a weak Th1–Th2 interaction.

In conclusion, the present study suggests that the relationship between atopy and tuberculosis is very complex and that large, detailed, prospective community-based studies are needed in order to clarify the link between cause and effect.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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