

Allergic Sensitisation in Tuberculosis and Leprosy Patients

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Key Words

Allergy · Specific IgE · Mycobacterial infection · Leprosy · Tuberculosis

Abstract

Background: A negative association has been observed between infections and allergy in several studies. The aim of the present study was to examine whether tuberculosis and leprosy patients have more or fewer allergies than healthy individuals. **Method:** Sera from tuberculosis patients, leprosy patients and healthy controls were analysed by ELISA and Pharmacia Unicap[®] for serological markers for allergy and mycobacterial infection. The serological markers for allergy were total IgE, specific IgE using Phadiatop and specific IgE to the dust mite allergen *Dermatophagoides pteronyssinus* 1 (*Der p* 1). Serological markers for mycobacterial infections included specific IgG to a mixture of bacille Calmette-Guérin culture filtrate antigens, to purified mannose-capped lipoarabinomannan (manLAM) and to purified secreted antigen 85B. **Results:** Both tuberculosis and leprosy patients had significantly higher levels of total IgE than controls. Furthermore, a significantly higher level of specific IgE (Phadiatop) was also found in the tuberculosis patients compared with controls. A similar result, but not statisti-

cally significant, was observed for the leprosy group. Specific IgG to antigen 85B and to manLAM was found to be significantly higher in both tuberculosis and leprosy patients compared with controls. In addition, leprosy patients had significantly more IgG to the BCG culture filtrate antigen than controls. **Conclusions:** The results indicate that patients with mycobacterial infections have allergic sensitisation more frequently compared with healthy controls. This is seemingly in contrast with the notion that there is a negative association between allergy and infection ('hygiene hypothesis'). However, since only one in ten of those infected with *Mycobacterium tuberculosis* will develop the disease, patients with active mycobacterial disease represent a selected group. A similar relationship applies for leprosy. It is conceivable that those predisposed to allergy are less resistant to mycobacterial infections.

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Introduction

In the past decades there has been a significant increase in the prevalence of allergic diseases and asthma in western countries [1–3]. Although the predisposition to developing allergy clearly is inheritable, the rapidity of the in-

Table 1. Demographic characteristics of patients

Group	n	Female n (%)	Male n (%)	Un- known n (%)	Age mean $\pm$ SD years	Type of leprosy/TB	New cases n (%)	Reinfection n (%)
TB	102	46 (45)	55 (54)	1 (1)	28 $\pm$ 12	Pulmonary TB	91 (89)	11 (11)
Leprosy	19	3 (16)	16 (84)	0	29 $\pm$ 8	LL: n = 4 BL: n = 5 BT: n = 2 TT: n = 6 Unknown: n = 2	19 (100)	0 (0)
Controls	29	22 (76)	7 (24)	0	27 $\pm$ 11			

LL = Lepromatous leprosy; BL = borderline lepromatous leprosy; BT = borderline tuberculoid leprosy; TT = tuberculoid leprosy; TB = tuberculosis.

crease in allergic diseases indicates that the environment must be involved. However, it is still unknown which environmental factors are responsible. The Th1/Th2 balance has attracted a lot of attention and has been mechanistically linked to the increase in allergy. In general, the immunological T-helper cell 1 (Th1) response is associated with bacterial and viral infections, cancer and autoimmune diseases, and the Th2 response is associated with helminthic infections and allergy. It has been argued that Th1 cells are able to down-regulate Th2 cells in some systems, and therefore have a beneficial role in allergic disease. A well-known theory is the so-called 'hygiene hypothesis'. Supported by indirect evidence [4–12], it proposes that lack of infections in early childhood contributes to the marked increase in allergic diseases. In 1997, a Japanese study reported that responders to tuberculin had lower serum levels of total IgE and Th2 cytokines (IL-4, IL-10, IL-13), higher serum levels of the Th1 cytokine IFN- γ , and also lower prevalence of atopic diseases when compared with tuberculin non-responders [7]. The authors suggested that exposure and response to *Mycobacterium tuberculosis* may reduce allergic sensitisation. However, additional studies have tested whether immunisation with bacille Calmette-Guérin (BCG) can protect against atopic diseases, but have shown conflicting results [13–18]. The discrepancies may be ascribed to the differences in BCG vaccination regimes and bacterial strains used, the populations and age groups studied, the period during which the effects were evaluated, and the pattern of natural exposure to mycobacteria.

Further studies are needed to explore the relationship between mycobacteria and allergies. The aim of the pres-

ent investigation was to examine whether tuberculosis and leprosy patients have more or less allergic sensitisation than healthy individuals. Our hypothesis was that leprosy and tuberculosis patients would have less allergic sensitisation due to the anti-allergic effects of the mycobacteria-driven Th1 response.

Materials and Methods

Sera

Sera were collected from 102 tuberculosis patients, 19 leprosy patients and 29 healthy BCG-vaccinated controls from Mumbai, India, and stored at -20°C . Both the tuberculosis and leprosy patients were randomly selected among cases registered at the Maharashtra Lokahi Seva Mandal, a non-governmental organization involved in tuberculosis and leprosy programmes in western Mumbai. In addition, some leprosy patients were selected from The Foundation for Medical Research (FMR), a research and referral institution located in central Mumbai. The diagnosis of tuberculosis was based on positivity in direct sputum smear microscopy and chest X-ray. Blood samples were taken on the day the patients were diagnosed under medical supervision and with informed consent. The healthy controls used were hospital workers from FMR living in the same area as the patients, but probably from a higher socioeconomic class. Samples were collected in the years from 1996 to 1997 and are described in more detail in table 1. The sera were analysed for total IgE, specific IgE to a mixture of allergens (Phadiatop test) and to dust mite allergen *Dermatophagoides pteronyssinus* 1 (*Der p* 1), IgG to BCG culture filtrate (BCG c.f.), antigen 85B and mannose-capped lipoarabinomannan (manLAM). Not all parameters could be measured in a few samples due to a low volume.

Antigens

M. bovis, BCG Moreau was cultured as surface pellicles on the wholly synthetic Sauton medium for 3 weeks. The culture superna-

tant was used for coating. Antigen 85B [19] was purified from concentrated culture filtrate using a combination of ion exchange chromatography on Q-Sepharose Fast Flow and a Resource Q column, and by hydrophobic interaction chromatography on a Resource Phe column (Pharmacia, Uppsala, Sweden). The manLAM was kindly provided by Prof. J.T. Belisle at Colorado State University. These molecules represent highly immunogenic antigens that are either released from the mycobacteria (in BCG c.f.), or located within the mycobacterial cell wall (manLAM), or are both present within and released from the mycobacterial cell wall (85B) [20, 21].

Antibodies

The mouse anti-human IgE used was a mixture of clone 4.15 and 7.12 from A. Saxon (UCLA, Calif., USA). The labelled antibodies were alkaline phosphatase (ALP)-goat anti-human IgE (ϵ -chain-specific, Sigma A3525, St. Louis, Mo., USA) and ALP-rabbit anti-human IgG (Dako D336, Denmark).

ELISA, General Features

Polystyrene plates (Nunc-Immuno™ Plate, Maxisorp™ Surface, Nunc Brand Products, Roskilde, Denmark) were used. The coating step was performed with a 50-mM carbonate-bicarbonate buffer of pH 9.6. The plates were kept for 1 h at room temperature and stored overnight at 4°C. They were washed five times (Skan Washer 400, Model 12019, Skatron, Norway) between each incubation step with 0.01 M Tris-HCl buffer of pH 7.4 containing 0.05% Tween 20 (T/Tw). For all incubations, 1% of BSA/Tris-T20 (T/Tw/BSA) was used. The plates were blocked for 1 h at room temperature with T/Tw/BSA (100 μ l/well). For a second layer, 50 μ l/well of diluted serum was added in duplicate. The plates were incubated for 2 h at room temperature and stored overnight at 4°C. For a third layer, 100 μ l/well of ALP-labelled conjugate (diluted in T/Tw/BSA) was added and incubated for 3 h at room temperature. Next, the plates were developed at room temperature for 20–40 min with *p*-nitrophenyl phosphate (Sigma 104® phosphatase substrate, Sigma) diluted in 10% of diethanolamine buffer (pH 9.8, 37°C) to 1 mg/ml. Optical density was measured at 405 nm on a MRX Microplate Reader connected to PC using Revelation software (Dynatech Laboratories, Chantilly, Va., USA) for instrument operation and calculations.

Total IgE ELISA

Each well was coated with 100 μ l of mouse anti-human IgE (2 μ g/ml), blocked and then incubated with serum. Serial two-fold dilutions of human serum containing a known amount of total IgE (3.5 μ g/ml) were used in triplicate (50 μ l/well) for a standard curve. For a third layer, ALP-conjugated goat anti-human IgE was added (diluted 1:3,500) before development and reading of the plates.

IgG Anti-BCG c.f., Anti-Antigen 85B and Anti-manLAM ELISA

The plates were coated (100 μ l/well) with solutions of BCG c.f. (0.5 μ g/well), antigen 85B (0.25 μ g/well) or manLAM (0.25 μ g/well), blocked and then incubated with diluted serum. Serial four-fold dilutions of a human serum pool containing defined amounts of IgG anti-BCG c.f., IgG anti-85B or IgG anti-manLAM antibodies, were used in triplicate (50 μ l/well) for standard curves. For a third layer, ALP-conjugated rabbit anti-human IgG (diluted 1:400) was added. Finally, the plates were developed and read.

UniCAP 100

UniCAP 100 is an automated allergy-testing system from Pharmacia Diagnostics (Uppsala, Sweden), incorporating sample and reagent handling, washing, reading, calculations, integrated processing and printing. This system was used to measure both specific IgE to the common allergen *Der p* 1 (Art. No. 14-4107-07), and specific IgE to a combination of different inhalant allergens (Phadiatop, Art. No. 14-4405-35). The sera were diluted 1:2 when analysed with the Phadiatop. The test was performed according to the manufacturer's instructions and the results are expressed in both kU_A/l (quantitative measurements ≥ 0.35 kU_A/l) and percent positivity (qualitative measurements). A qualitatively positive result is defined as a specific IgE concentration ≥ 0.35 kU_A/l (UniCAP class ≥ 1).

Statistics

The non-parametric Kruskal-Wallis test was used to compare groups. Multiple comparisons versus a control group (Dunn's method) were then used to identify the differing groups. Results with the Phadiatop test and levels of specific IgE to *Der p* 1 were analysed both quantitatively and qualitatively. We used Dunn's method for quantitative measurements, and a χ^2 test to analyse qualitative data (atopy positivity or negativity). Spearman rank order correlations were performed to correlate the different parameters in both the leprosy and tuberculosis groups. The Mann-Whitney U test was used to compare gender differences in tuberculosis patients and controls. *p* values < 0.05 were considered significant.

Results

Allergy Markers

Sera from leprosy and tuberculosis patients and controls were analysed for allergy markers. These were total IgE, Phadiatop and specific IgE to the major dust mite allergen *Der p* 1. Comparison of the groups revealed significantly higher levels of total IgE in both the leprosy and tuberculosis patients compared with the controls (fig. 1a). The tuberculosis patients had significantly higher levels (quantitative measurements in kU_A/l) of specific IgE (Phadiatop) compared with controls (fig. 1b). A similar trend was observed for leprosy patients, although the results were not statistically significant. Phadiatop positivity (qualitative results) was 83, 72 and 52% among the tuberculosis patients, leprosy patients and controls, respectively. When a more strict definition of a positive test for specific IgE (> 0.7 kU_A/l) was used, similar differences were found. The statistical differences between the groups were similar regardless of using quantitative or qualitative data. However, there was no difference in levels of specific IgE to the particular allergen *Der p* 1 between tuberculosis/leprosy patients and controls (fig. 1c). *Der p* 1 positivity was 68, 58 and 62% in tuberculosis patients, leprosy patients and controls, respectively.

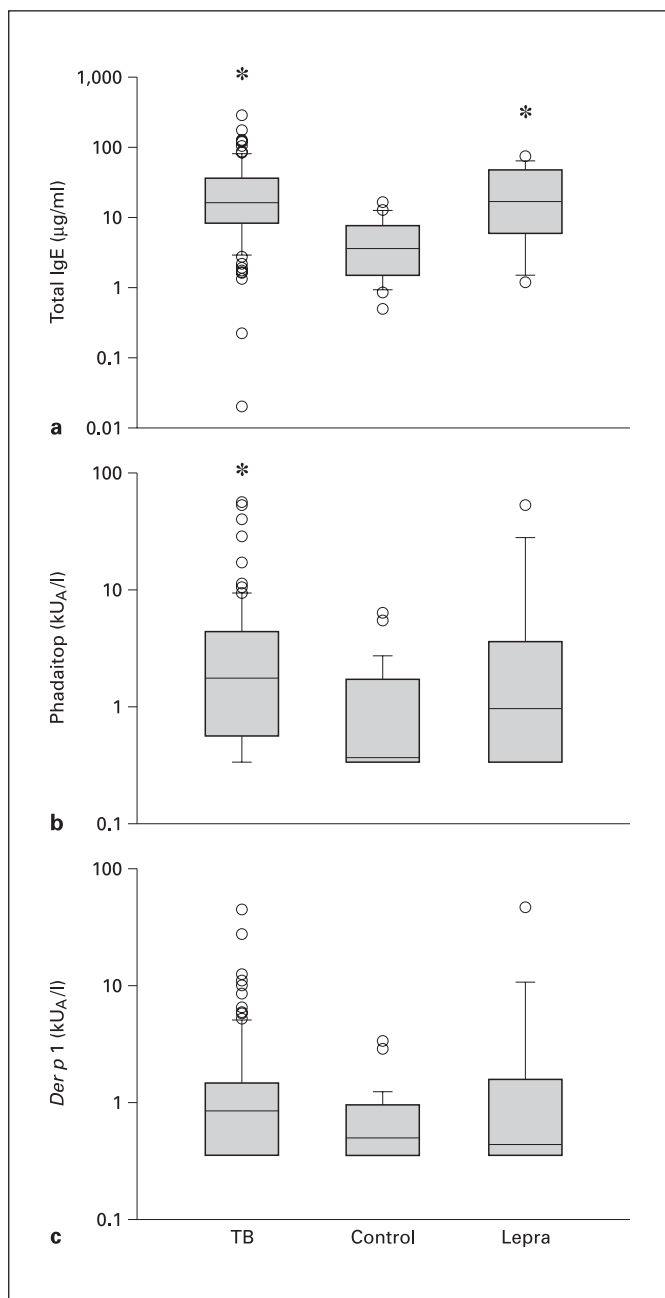


Fig. 1. The box plot indicates the 25th percentile, the median and the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles. **a** Higher levels of total IgE were measured in tuberculosis (TB, $n = 102$) and leprosy ($n = 19$) patients than in controls ($n = 29$). **b** Tuberculosis patients ($n = 93$) had significantly higher levels and of specific IgE (Phadiatop) than controls ($n = 29$). The same tendency was seen in the leprosy patients ($n = 18$). **c** No significant difference was observed in levels of specific IgE to *Der p 1* between the tuberculosis patients ($n = 101$) or leprosy ($n = 19$) patients and controls ($n = 29$) (* $p < 0.05$).

Markers for Mycobacterial Infection

The sera were also analysed for serological markers for mycobacterial infection. These were specific IgG to antigens in BCG c.f., to manLAM, and to the antigen 85B. Both tuberculosis and leprosy patients had higher levels of specific IgG to antigen 85B and manLAM compared with controls. There was no significant difference in IgG anti-BCG c.f. levels between tuberculosis patients and controls. However, the leprosy patients also had significantly higher levels of specific IgG antibodies to BCG c.f. antigens compared with controls. Table 2 shows an overview of the results for the allergy and mycobacterial infection markers among patients and controls.

Gender Differences

When tuberculosis patients and controls were separated by gender, similar significant differences were found between patients and controls for both allergy and mycobacterial markers (table 3). The only exception was for IgG anti-manLAM levels which were not statistically significantly different between male tuberculosis patients and male controls. However, this is probably due to the few male controls. Similar analyses were not performed in the leprosy group due to a small number of patients. Interestingly, the levels of allergy markers were higher in male than female tuberculosis patients ($p \leq 0.008$).

Correlations between Allergy and Mycobacterial Markers

Tables 4 and 5 show correlations between the allergy and mycobacterial markers among leprosy and tuberculosis patients, respectively. There was a positive correlation between IgG anti-85B and total IgE in the leprosy group ($\rho = 0.64$, $p = 0.004$) and in the TB group ($\rho = 0.23$, $p = 0.021$) (tables 4, 5). As expected there were positive intercorrelations between allergy markers and between mycobacterial markers (data not shown).

Discussion

We compared the presence of allergy and mycobacterial infection markers in tuberculosis and leprosy patients with that of healthy controls. Our main finding is that tuberculosis and leprosy patients seem to have higher levels of allergy markers compared with the controls. The particular finding of similar IgE anti-*Der p 1* levels in the groups does not exclude this fact. The results from the Phadiatop test were qualitatively and quantitatively analysed, both showing significant difference between tuber-

Table 2. Comparison of leprosy and tuberculosis patients versus controls

Marker	Group	Median	Range	Significance p < 0.05
Total IgE μg/ml	Control	3.60	0.50–16.15	
	TB	16.18	0.02–280.56	Yes
	Leprosy	16.64	1.18–73.00	Yes
Phadiatop kU _A /l	Control	0.38	0.35–6.50	
	TB	1.82	0.35–56.98	Yes
	Leprosy	1.00	0.35–51.39	No
IgE anti- <i>Der p 1</i> kU _A /l	Control	0.50	0.35–3.32	
	TB	0.85	0.35–44.30	No
	Leprosy	0.44	0.35–46.50	No
IgG anti-85B AU/ml	Control	2,328	170–6,616	
	TB	3,638	359–107,516	Yes
	Leprosy	3,441	1,600–204,795	Yes
IgG anti-manLAM AU/ml	Control	738	29–3,943	
	TB	1,086	34–21,846	Yes
	Leprosy	3,491	517–35,225	Yes
IgG a-BCG c.f. AU/ml	Control	340	28–1,778	
	TB	387	29–7,193	No
	Leprosy	1,004	291–13,909	Yes
Multiple comparisons versus a control group (Dunn's method).				

culosis patients and controls. Generally, the Phadiatop test is used as a yes-no test for diagnosing inhalant atopy. However, studies have shown that there is a correlation between results from quantification with the Phadiatop and the sum of specific IgE concentrations for individual allergens [22–24]. Furthermore, the rate of respiratory allergic disease has shown a linear increase with the increase in specific IgE (Phadiatop) [23]. Thus, the quantitative difference found in specific IgE level between tuberculosis and control groups may reflect a higher probability of allergic symptoms in the tuberculosis group. As expected, we observed significantly higher levels of specific IgG to 85B and manLAM in tuberculosis and leprosy patients compared with controls. Moreover, there was no significant difference in IgG anti-BCG c.f. levels between tuberculosis patients and controls, which probably is due to mycobacterial exposure of controls. However, the leprosy patients also had significantly higher levels of specific IgG antibodies to BCG c.f. antigens compared with controls. This difference might be due to a stronger humoral (Th2) response among the leprosy patients. An immunological spectrum presents itself in leprosy, as tuberculoid leprosy is characterised by a Th1 immune response, while lepromatous leprosy is characterised by a Th2 immune response. Patients with tuberculoid

leprosy have a strong cellular immunity and low antibody response, while patients with lepromatous leprosy have high levels of antibodies and strongly reduced cellular immunity [25].

The higher prevalence of allergic sensitisation in patients with mycobacterial infection compared with controls was the opposite of what one would expect according to the hygiene hypothesis and reports showing that mycobacteria elicit Th1-type immune responses. This study does not provide information of causal effect. However, one explanation could be that the patients are poor Th1 immune responders, and according to the Th1/Th2 paradigm, have a strong Th2 bias and thus increased susceptibility to allergy development as well as mycobacterial infection. Since only 10% of those infected with *M. tuberculosis* actually develop the disease, individuals with active tuberculosis represent a selected group. A similar relationship applies for leprosy. Previous studies have also shown significantly higher levels of total IgE in tuberculosis patients compared with healthy controls [26–28]. However, conflicting results have been reported when Th1 and Th2 markers in patients with active tuberculosis were compared to patients with a history of previous tuberculosis [28–30].

Table 3. Gender differences: comparison of male and female TB patients versus that of male and female controls, respectively

Marker	Group	Median	Range	Significance p
Total IgE μg/ml	Control male	3.22	0.92–16.65	0.002
	TB male	26.87	1.63–280.56	
	Control female	4.17	0.50–12.59	
	TB female	14.25	0.02–119.95	
Phadiatop kU _A /l	Control male	0.93	0.35–2.02	0.017
	TB male	3.02	0.35–53.64	
	Control female	0.35	0.35–6.50	
	TB female	0.92	0.35–56.98	
IgE anti- <i>Der p 1</i> kU _A /l	Control male	0.63	0.35–2.85	0.178
	TB male	1.03	0.35–44.30	
	Control female	0.47	0.35–3.32	
	TB female	0.49	0.35–27.4	
IgG anti-85B AU/ml	Control male	1,545	172–4,391	0.005
	TB male	4,590	644–73,803	
	Control female	2,359	170–6,616	
	TB female	3,209	359–107,516	
IgG anti-manLAM AU/ml	Control male	789	343–1,198	0.411
	TB male	969	40–11,423	
	Control female	675	29–3,943	
	TB female	1,320	34–21,846	
IgG anti-BCG c.f AU/ml	Control male	280	31–414	0.091
	TB male	410	29–7,193	
	Control female	347	28–1,778	
	TB female	351	47–3,032	
Mann-Whitney rank sum test.				

Table 4. Correlations (Spearman's ρ) between parameters in the tuberculosis group

	IgG anti-man LAM	IgG anti-85B	IgG anti-BCG c.f.
Total IgE	0.13	0.23*	0.15
Phadiatop	0.03	0.16	0.11
IgE anti- <i>Der p 1</i>	0.04	0.13	0.09

Spearman rank order correlation. * Correlation is significant at the 0.02 level.

Table 5. Correlations (Spearman's ρ) between parameters in the leprosy group

	IgG anti-man LAM	IgG anti-85B	IgG anti-BCG c.f.
Total IgE	0.14	0.64**	0.44
Phadiatop	–0.21	0.33	0.05
IgE anti- <i>Der p 1</i>	–0.06	0.25	0.22

Spearman rank order correlation. ** Correlation is significant at the 0.004 level.

Adams et al. [29] observed that successful treatment of tuberculosis infections decreased total IgE levels in the patients. However, no difference was observed in allergen-specific IgE levels [29]. Additionally, Lienhardt et al. [28] studied the Th2 activity (plasma levels of total IgE, sCD30, and macrophage-derived chemokine (MDC/

CCL22) and Th1 activity (plasma levels of a soluble form of the lymphocyte activation gene (LAG)-3 molecule) in tuberculosis patients before and after treatment. They observed that tuberculosis patients had higher Th2 activity and lower Th1 activity than patients with a history of tuberculosis, and that this balance was reversed after suc-

cessful treatment [28]. On the other hand, a Turkish study found that atopic markers (the rate of skin prick tests) and specific IgE (Phadiatop), apart from total IgE concentrations, were significantly lower in patients with active tuberculosis compared with patients with inactive tuberculosis [30]. This study did not compare the same patients before and after treatment. However, both these studies are compatible with the notion that allergic patients and individuals predisposed for allergy are more susceptible to developing tuberculosis or leprosy. In addition, the controls have probably been exposed to mycobacteria, and therefore one may expect an increased Th1 response and a reduced Th2 response in this group of individuals. Consequently, a shift in the Th1/Th2 balance among the controls may contribute to the observed difference in IgE levels between controls and patients. It is also possible that the increase in allergy markers may be temporary and not connected to allergy. Lienhardt et al. [28] proposed that the shift in Th1/Th2 balance could be due to mechanisms inducing either an expansion of the Th2 response or a decrease in the Th1 response.

There are some limitations to the material employed in the present study. The healthy controls were hospital workers living in the same area as the patients, but probably from a higher socio-economic class. People with a higher socio-economic status generally have more allergies [31, 32], but fewer parasitic infections [33], than people with a lower status. Total IgE reflects both parasitic infection and allergy. Since patients with mycobacterial infections probably come from lower socio-economic classes, their total IgE levels may partly be due to helminthiasis. In addition, smoking habits, of which information is not available, is also known to influence the total IgE level. Hence, total IgE, which often is included but usually not considered an ideal allergy marker, is even less so in the present study. On the other hand, the tuberculosis patients also had significantly increased levels of specific IgE, as revealed by the Phadiatop test, which indicate a higher degree of allergic sensitisation. As seen from table 1, the distribution of gender among controls was different from that of the tuberculosis and the leprosy patients, and is therefore a confounding variable. However, comparison of data from female and male tuberculosis patients with that of female and male controls still revealed significantly higher levels of markers for allergy in the tuberculosis patients (table 3). Due to the small number of leprosy cases, similar analyses were not performed for that group.

The finding of positive correlation only between total IgE and IgG anti-85B, which was strong ($p = 0.64$) for

lepra patients and weak ($p = 0.23$) for tuberculosis patients (tables 4, 5), may be explained by the fact that the mycobacterial markers used are not diagnostic for the mycobacterial diseases. However, the positive correlation observed could also be caused by high total IgE due to concomitant parasitic infections (unknown) or be secondary to a Th2-shift induced by the mycobacterial infection itself.

Further investigations are needed to study whether mycobacterial infection will reduce or increase allergy in a model which includes tuberculosis patients before and after treatment. Although the Th1/Th2 paradigm is a useful conceptual framework, it does not explain the whole story behind the observed rise in frequency of allergic diseases. Recent findings have led to increased attention towards regulatory T cells [12, 34]. Hence, a mechanistic study would also be of interest in which Th1, Th2, and T-regulatory responses should be measured.

Conclusions

We observed that tuberculosis and leprosy patients had higher levels of allergy markers than controls. However, this apparent contradiction to the hygiene hypothesis might be explained by the point of view that tuberculosis and leprosy patients represent selected groups of individuals who are predisposed to be weak Th1 and strong Th2 responders, and therefore are more susceptible to contracting both mycobacterial infection and developing allergy.

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References

- ▶ 1 Von Mutius E: The rising trends in asthma and allergic disease. *Clin Exp Allergy* 1998;28(suppl 5):45–49.
- ▶ 2 Beasley R, Crane J, Lai CK, Pearce N: Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000;105:S466–S472.
- ▶ 3 Kosunen TU, Hook-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T: Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002;32:373–378.
- ▶ 4 Strachan DP: Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–1260.
- ▶ 5 Von Mutius E, Martinez FD, Fritzsche C, Nicolai T, Reitmeir P, Thiemann HH: Skin test reactivity and number of siblings. *BMJ* 1994;308:692–695.
- ▶ 6 Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, Goudiaby A: Measles and atopy in Guinea-Bissau. *Lancet* 1996;347:1792–1796.
- ▶ 7 Shirakawa T, Enomoto T, Shimazu S, Hopkin JM: The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77–79.
- ▶ 8 Kramer U, Heinrich J, Wjst M, Wichmann HE: Age of entry to day nursery and allergy in later childhood. *Lancet* 1999;353:450–454.
- ▶ 9 Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL: Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–543.
- ▶ 10 Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S: Exposure to food-borne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;320:412–417.
- ▶ 11 Matricardi PM, Rosmini F, Panetta V, Ferrigno L, Bonini S: Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol* 2002;110:381–387.
- ▶ 12 Yazdanbakhsh M, Kreamsner PG, van Ree R: Immunology – allergy, parasites, and the hygiene hypothesis. *Science* 2002;296:490–494.
- ▶ 13 Alm JS, Lilja G, Pershagen G, Scheynius A: Early BCG vaccination and development of atopy. *Lancet* 1997;350:400–403.
- ▶ 14 Omenaas E, Jentoft HF, Vollmer WM, Buist AS, Gulsvik A: Absence of relationship between tuberculin reactivity and atopy in BCG vaccinated young adults. *Thorax* 2000;55:454–458.
- ▶ 15 Cavallo GP, Elia M, Giordano D, Baldi C, Cammarota R: Decrease of specific and total IgE levels in allergic patients after BCG vaccination: preliminary report. *Arch Otolaryngol Head Neck Surg* 2002;128:1058–1060.
- ▶ 16 Gruber C, Meinschmidt G, Bergmann R, Wahn U, Stark K: Is early BCG vaccination associated with less atopic disease? An epidemiological study in German preschool children with different ethnic backgrounds. *Pediatr Allergy Immunol* 2002;13:177–181.
- ▶ 17 Marks GB, Ng K, Zhou J, Toelle BG, Xuan W, Belousova EG, Britton WJ: The effect of neonatal BCG vaccination on atopy and asthma at age 7 to 14 years: a historical cohort study in a community with a very low prevalence of tuberculosis infection and a high prevalence of atopic disease. *J Allergy Clin Immunol* 2003;111:541–549.
- ▶ 18 Annus T, Montgomery SM, Riikjarv MA, Bjorksten B: Atopic disorders among Estonian schoolchildren in relation to tuberculin reactivity and the age at BCG vaccination. *Allergy* 2004;59:1068–1073.
- ▶ 19 Wiker HG, Harboe M, Lea TE: Purification and characterization of two protein antigens from the heterogeneous BCG85 complex in *Mycobacterium bovis* BCG. *Int Arch Allergy Appl Immunol* 1986;81:298–306.
- ▶ 20 Wiker HG, Harboe M: The antigen 85 complex: A major secretion product of *Mycobacterium tuberculosis*. *Microbiol Rev* 1992;56:648–661.
- ▶ 21 Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, Besra GS: Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science* 1997;276:1420–1422.
- ▶ 22 Williams PB, Siegel C, Portnoy J: Efficacy of a single diagnostic test for sensitization to common inhalant allergens. *Ann Allergy Asthma Immunol* 2001;86:196–202.
- ▶ 23 Matricardi PM, Nisini R, Biselli R, D'Amelio R: Evaluation of the overall degree of sensitization to airborne allergens by a single serologic test: implications for epidemiologic studies of allergy. *J Allergy Clin Immunol* 1994;93:68–79.
- ▶ 24 Wever AM, Wever-Hess J, van Schayck CP, van Weel C: Evaluation of the Phadiatop test in an epidemiological study. *Allergy* 1990;45:92–97.
- ▶ 25 Ridley DS, Jopling WH: Classification of leprosy according to immunity. A five-group system. *Int J Lepr Other Mycobact Dis* 1966;34:255–273.
- ▶ 26 Ohnishi T, Zayas K, Sato E, Matsui T, Sekizawa K, Sasaki H: Pulmonary tuberculosis and serum IgE. *Clin Exp Immunol* 2000;122:13–15.
- ▶ 27 Suzuki N, Kudo K, Sano Y, Ito K: Can *Mycobacterium tuberculosis* infection prevent asthma and other allergic disorders? *Int Arch Allergy Immunol* 2001;124:113–116.
- ▶ 28 Lienhardt C, Azzurri A, Amedei A, Fielding K, Sillah J, Sow OY, Bah B, Benagiano M, Diallo A, Manetti R, Manneh K, Gustafson P, Bennett S, D'Elia MM, McAdam K, Del Prete G: Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. *Eur J Immunol* 2002;32:1605–1613.
- ▶ 29 Adams JF, Scholvinck EH, Gie RP, Potter PC, Beyers N, Beyers AD: Decline in total serum IgE after treatment for tuberculosis. *Lancet* 1999;353:2030–2033.
- ▶ 30 Mungan D, Sin BA, Celik G, Gurkan OU, Acican T, Misirligil Z: Atopic status of an adult population with active and inactive tuberculosis. *Allergy Asthma Proc* 2001;22:87–91.
- ▶ 31 Matricardi PM, Franzinelli F, Franco A, Caprio G, Murru F, Cioffi D, Ferrigno L, Palermo A, Ciccarelli N, Rosmini F: Sibship size, birth order, and atopy in 11,371 Italian young men. *J Allergy Clin Immunol* 1998;101:439–444.
- ▶ 32 Schafer T, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, Wichmann HE, Ring J: School education and allergic sensitization in adults. *Allergy* 2001;56:1206–1210.
- ▶ 33 Traub RJ, Robertson ID, Irwin P, Mencke N, Andrew Thompson RC: The prevalence, intensities and risk factors associated with geohelminth infection in tea-growing communities of Assam, India. *Trop Med Int Health* 2004;9:688–701.
- ▶ 34 Akbari O, Stock P, Dekruyff RH, Umetsu DT: Role of regulatory T cells in allergy and asthma. *Curr Opin Immunol* 2003;15:627–633.