

EDITORIAL

Clinical and Experimental Allergy

Hygiene hypothesis, allergy and BCG: a dirty mix?

C. C. Obihara* and P. G. Bardin†

*Department of Paediatrics, St Elisabeth Hospital, Tilburg, The Netherlands and †Department of Respiratory and Sleep Medicine, Monash University and Medical Centre, Melbourne, Australia

Allergic disease incidence has increased globally, not only in industrialized countries but also in developing countries [1]. One of the more plausible explanations for this increase is the 'hygiene hypothesis', which suggests that a decrease of or an altered exposure to microbes in the environment, due to improved sanitation and personal hygiene, smaller family sizes, shorter duration of breastfeeding, immunizations and lack of serious childhood infections results in alteration of immunoregulation [2]. There is considerable evidence from animal and experimental studies to implicate mycobacteria as potential microbial participants in the hygiene hypothesis, mainly through studies showing that infection with these organisms or exposure to their products leads to regulatory mechanisms that restore immune homeostasis [3, 4]. An inverse relationship between atopic disease and *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) immunization or wild-type *Mycobacterium tuberculosis* infection has been observed in some populations [5–8], but not in others [9, 10]. The discrepancy noted in epidemiological studies may be related to the fact that a relationship may be less direct than initially assumed. For example, it may be modified under the influence of factors such as genetic variability, time of exposure, amount and length of exposure, type of infection and burden of environmental allergens and other mycobacteria, which may differ between populations and settings [11, 12].

An inverse association between BCG immunization and atopic disease was observed in 10 of 23 published epidemiological studies (Table 1) [5, 6, 13–20]. Six of 16 published studies in Europe observed an inverse relationship between BCG immunization and atopy [13–18]. Two of four studies from Asia [5, 16], one of two studies from

Africa [6], one of three studies from the Americas [19] and the only study from Australia [20] observed an inverse relationship between BCG immunization and atopic disease.

Adding to the present body of evidence, Miyake et al. [21] report in this issue their observation in 5567 BCG-immunized Japanese children from Okinawa. The study had the following methodological strengths: subjects were homogenous in age and background, methods to measure the prevalence of atopic diseases were well validated and an adequate sample of the population under study was obtained. Shortcomings included a low number of non-BCG-immunized children for comparison and inability to control for confounding factors such as pollution and exposure to *M. tuberculosis* itself. The prevalence of allergic disorders in the current study was also different from reports in mainland Japan. The principal finding was that the group with a positive tuberculin skin test (TST) (≥ 10 mm) had a significantly lower prevalence of wheeze, asthma and atopic eczema than that with a negative TST [21]. When groups were divided according to a history of allergic disease in either parent, inverse associations became more pronounced in children with a negative parental history. Although there was no relationship between BCG immunization history and allergic disease (as expected, given the varying responses to vaccination), the findings based on more precise TST measurements again suggested an inverse relationship between BCG immunization and atopic disease.

The biological framework to explain the inverse relationship between infection and atopic disease is that the lack of microbial stimulation leads to either an inappropriate T helper (Th)-type immune response or an inappropriate Th cell regulatory mechanism, resulting in the development of atopic disease [22]. It was assumed previously that the critical period for the establishment of the Th cell balance is early childhood (when the Th2-skewed immune system gradually becomes less Th2-skewed in non-atopic individuals, but not in atopic individuals). However, recent evidence of genetically predisposed adult migrants developing atopic disease when

This editorial discusses the findings of the paper in this issue by Y. Miyake et al. [21] pp ...

Correspondence:

Dr Charles C. Obihara, Department of Paediatrics, St Elisabeth Hospital, P.O. Box 90151, 5000 LC Tilburg, The Netherlands.
E-mail: c.obihara@elisabeth.nl

they move from a developing country with a low prevalence to a westernized country with a high burden of atopic disease seems to refute this assumption [11]. Recent evidence also stresses the importance of reduced immune suppression rather than of erratic immune deviation to explain the increasing global incidence of atopy [22]. This means that low microbial exposure does not act by inducing a lower production of Th1-cytokines, but by decreasing the activity of regulatory T cells (Tregs). Immune suppression by Tregs is mediated through the production of inhibitory or anti-inflammatory cytokines, such as IL-10 and TGF- β [11, 22]. Although numerous fundamental scientific studies have supported aspects of the hygiene hypothesis, a translational connection remains to be established between the experimental scientific data supporting the hypothesis and epidemiological findings as reported by Miyake et al. [21] and others [5, 6, 13–20].

Important factors may explain differences in the outcomes of epidemiological studies; they include heterogeneity in study design, variability in BCG-induced cellular immunity, influence of other mycobacteria (including *M. tuberculosis*) and simultaneous *M. bovis* BCG and allergen exposure in the study population.

Bacillus Calmette–Guérin–induced cellular immunity

Only five studies that observed a significant inverse relationship between BCG immunization and atopic disease performed a TST in the study population [5, 13, 15, 16, 20]. The remaining studies either assumed a positive history or a record of BCG immunization or used the presence of a BCG scar as a proxy for BCG-induced immunity, although both are not specific for this vaccine effect. None of the studies have so far applied a more reliable method (such as IFN- γ release assays, IGRAs) of distinguishing TST reactivity induced by BCG from responses induced by other mycobacteria. In children, the specificity of the TST is limited by exposure to routine neonatal BCG immunization and non-tuberculous mycobacteria. The degree of BCG-induced-TST reactivity is dependent on several factors including BCG vaccine strain, age, nutritional status, frequency of skin testing and years since BCG immunization.

Influence of other mycobacteria

BCG immunization seems to evoke weaker and shorter immune stimulation and memory, and is therefore unable to mimic the persistent immune stimulation provided by frequent exposure to wild-type *M. tuberculosis* [11, 12]. This is supported by studies showing that *M. bovis*-BCG is a poorer inducer of IL-12 production than natural *M. tuberculosis* infection [11]. The effect of neonatal BCG vaccination disappeared in African children at the age of

5 years [11]. *M. tuberculosis* infection is associated with a larger TST size (usually ≥ 10 mm) than *M. bovis*-BCG or environmental mycobacteria (usually ≤ 10 mm) [5, 7, 8]. In the studies in Japanese children, 58% of the children in Shirakawa's study and 49% in Miyake's study had a strongly positive TST of ≥ 10 mm [5, 21]. Although the authors could not exclude the effects of other mycobacteria or natural *M. tuberculosis* infection on the TST sizes in the study population, the strong positive TST reactivity suggests an influence of *M. tuberculosis* [5]. This assumption is supported by recent studies in South African children from an area with endemic tuberculosis in which not only was a strongly positive TST observed in more than 50% of the children but also a group median TST size of ≥ 18 mm and no observed influence of other environmental mycobacteria was seen [7, 8]. There was a significant inverse relationship between a TST ≥ 10 mm and atopic disease symptoms [adjusted odds ratio (OR) 0.43; 95% confidence interval (CI) 0.24–0.79], and atopic rhinitis (adjusted OR 0.06; 95% CI 0.007–0.5) [7, 8]. Most of the studies from Europe, on the other hand, either did not perform a TST or observed a positive TST in a much smaller proportion of the study population. In addition, the fact that a stronger association was observed between BCG immunization and atopic disease in non-European migrant children suggests a different pattern of exposure to mycobacterial agents [13, 14]. These observations suggest that the proportion of strongly positive TST (a marker of the delayed-type hypersensitivity stimulation) rather than a history of BCG immunization may be important. Future studies adopting both the TST and more specific IGRAs are needed to exclude the possible influence of *M. tuberculosis* infection on the relationship between BCG immunization and atopic disease.

Infectious burden

Triggering of the innate immune system through endotoxin exposure and the derived effect (increased or decreased atopic inflammation) may not only depend on the exposure but also on the 'dose' of endotoxin [11]. A possible implication of this is that the protective effect of infections, such as that caused by mycobacteria, on atopic disease would logically be more evident in populations with a high and persistent burden of these infections (where exposure to these chronic infections is more likely to be intense and persistent) than in populations with a low burden (as in most affluent westernized countries, where exposure is more likely to be sporadic and intermittent). In line with this observation, it has been proposed that the difference in the 'dose' and persistence of infections that trigger the innate immune system may be important in explaining the lower prevalence of atopic disease in the developing world [11, 12].

Table 1. Overview, characteristics and the most important findings of studies that observed an inverse relation between *Mycobacterium bovis*-bacillus Calmette-Guérin and atopic disease

Individual study (year)	Inverse relationship	Study type, sample size and the most important findings	Comments
<i>Europe</i>			
Strannegård [13] (1998)	Yes	Cross-sectional, 6497 children (6312 Swedish born and 185 foreign born); 4–9 years. An inverse association between BCG vaccination and atopic disease was only found in foreign-born children.	TST performed. Among foreign-born children, those without BCG vaccination had a higher prevalence of atopy (28%) than BCG-vaccinated ones (13%).
Gruber [14] (2002)	Yes	Cross-sectional birth cohort, 38 808 German children (6102 foreign born); 6 years. A weak and transient inverse relation between BCG vaccination in infancy and atopic disease in pre-school children.	No TST performed. Although a weak protective effect of BCG vaccination against asthma was observed in all children, a strong protective effect against atopic diseases, eczema, asthma and hayfever was observed among foreign-born children.
Annus [15] (2004)	Yes	Cross-sectional, 717 Estonian children (309 BCG vaccinated); 10–11 years. Lower prevalence of allergic symptoms in BCG-vaccinated children with a positive TST compared with those with a negative TST.	TST performed. BCG vaccination or TST result obtained retrospectively from school records. Atopic children were more likely to have a positive TST after re-vaccination.
Townley [16] (2004)	Yes	Cross-sectional, 1704 children in 3 countries; 2 years. In Turkish children, an inverse association was observed between positive TST and allergic symptoms in BCG-vaccinated children but not in unvaccinated children.	TST performed. The study used the same protocol to study children in three international sites – Turkey, Thailand and Argentina. Neonatal BCG vaccination given at each site, TST performed at 9–12 months, and questionnaire on atopy at 2 years of age. Inverse relation found in Turkey and Thailand, but not in Argentina.
Martignon [17] (2005)	Yes	Cross-sectional, 717 French children; 12–15 years. A history of BCG vaccination was related to less atopic disease symptoms.	No TST performed. BCG vaccination based on records only. The study was originally not designed to test the relationship between BCG vaccination and atopy, but the effect of all childhood immunizations and atopy.
Garcia-Marcos [18] (2005)	Yes	Cross-sectional, 9590 Spanish schoolchildren; 6–7 years. A weak inverse association was observed between BCG vaccination and asthma and AR.	No TST performed. BCG vaccination based on records only.
<i>Asia</i>			
Shirakawa [5] (1997)	Yes	Cross-sectional, 867 Japanese children; 12–13 years. A lower prevalence of atopic symptoms, allergic sensitization, higher serum IFN- γ and lower serum IL-4 levels in BCG-vaccinated children with a positive TST than in BCG-vaccinated children with a negative TST.	TST performed. Th1 and Th2 cytokine profiles were also tested. TST ≥ 10 mm in 58% children. The lowest risk of atopic disease was observed in the group of children who remained TST positive at 6 and 12 years. Authors debated on the possible role of natural <i>M. tuberculosis</i> in the TST sizes.
Townley [16] (2004)	Yes	Cross-sectional, 1704 children in 3 countries; 2 years. In children from Thailand; an inverse association between positive TST and allergic symptoms in BCG-vaccinated children.	TST performed. The study used the same protocol to study children in three international sites – Turkey, Thailand and Argentina. Neonatal BCG vaccination given at each site, TST performed at 9–12 months, and questionnaire on atopy at 2 years of age. Inverse relation found in Turkey and Thailand, but not in Argentina.
<i>Africa</i>			
Aaby [6] (2000)	Yes	Cross-sectional, 400 children from Guinea-Bissau (BCG records in 271); 3–14 years. A lower frequency of positive SPT to HDM in those with BCG vaccination records than in those without (21% vs. 40%).	BCG vaccination was defined as a positive history of neonatal BCG immunization or the presence of BCG scar only. No TST performed, making it difficult to correct for the possible additive influence of other mycobacteria in the environment.

Table 1. continued

Individual study (year)	Inverse relationship	Study type, sample size and the most important findings	Comments
<i>America</i>			
Da Cunha [19] (2004)	Yes	Cross-sectional, 1621 Brazilian children; 12–16 years (BCG records in 1089). A lower risk of asthma only in those with a neonatal BCG scar and a past history of AR, but not in those without a history of AR.	No TST performed. BCG scar used as a proxy for neonatal BCG vaccination. Neonatal BCG vaccination was not associated with the overall prevalence of reported wheezing or asthma. However, in the sub-group reporting current allergy and sneezing, neonatal BCG was associated with a 37% reduction of prevalence of lifetime asthma.
<i>Australia</i>			
Marks [20] (2003)	Yes	Cross-sectional in birth cohort, 751 Australian children; 7–14 years (BCG records in 309). BCG vaccination was associated with less asthma symptoms only in children with a positive family history of atopic disease. BCG vaccination was also associated with lower levels of allergen-stimulated IL-10 production <i>in vitro</i> .	TST performed and cytokine profile measured. BCG-vaccinated subjects, who had TST ≥ 5 mm and those who demonstrated an <i>in vitro</i> IFN- γ response to <i>M. tuberculosis</i> -PPD did not have lower rates of allergic sensitization or a lower prevalence of allergic disease than TST or IFN- γ nonreactors.

BCG, bacillus Calmette-Guérin; TST, tuberculin skin test; Th, T helper; SPT, skin prick test; HDM, house dust mite; AR, allergic rhinitis; PPD, purified protein derivative.

Simultaneous exposure to infection and allergen

Priming of allergen-specific Th1 responses may be important in establishing a memory response capable of long-term protection against an allergen-specific Th2 response [23]. Recombinant mycobacteria mice challenged with an allergen underwent a switch from a Th2 to a Th1 profile, which was specific only for vaccines expressing the allergen peptide. In an experimental model, simultaneous mycobacterial infection and allergen challenge stimulated an immune response strong enough to suppress Th2 activity [24]. Stimulation of T cells with ovalbumin (OVA) only induced Th2-dominant responses, whereas concomitant stimulation with both OVA and *M. tuberculosis* caused induction of antigen-specific Th1 responses, with secretion of IFN- γ , mediated by IL-12. If this phenomenon, where both *M. tuberculosis* and allergens are simultaneously presented by antigen-presenting cells to the T cell, also applies to humans, then it is most likely to occur in a population where *M. tuberculosis* infection and allergen exposure are both highly prevalent.

Definition of atopy

The lack of uniformity in the definition of atopic disease makes it difficult to compare the results of studies and may account for some of the heterogeneity of the study results. While some studies defined atopy as questionnaire-reported symptoms, or positive SPT or elevated serum-specific IgE to common environmental allergens, others used both definitions [12]. The adoption of the World Allergy Organization definition of atopy [25] would make it easier to compare future findings of studies on this topic.

References

- Ait-Khaled N, Odhiambo J, Pearce N *et al*. Prevalence of symptoms of asthma, rhinitis and eczema in 13- to 14-year-old children in Africa: the international study of asthma and allergies in childhood phase III. *Allergy* 2007; **62**:247–58.
- Strachan D. Hay fever, hygiene and household size. *BMJ* 1989; **289**:1259–60.
- Wang CC, Rook GA. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. *Immunology* 1998; **93**:307–13.
- Erb K, Holloway J, Sobeck A, Moll H, Le Gros G. Infection of mice with *Mycobacterium bovis*-bacillus Calmette-Guérin (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med* 1998; **187**:561–9.
- Shirakawa T, Enomoto T, Shimazu S, Hopkin J. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**:77–9.
- Aaby P, Shaheen S, Heyes C *et al*. Early BCG vaccination and the reduction in atopy in Guinea-Bissau. *Clin Exp Allergy* 2000; **30**:644–50.

- 7 Obihara CC, Kimpen JLL, Gie RP *et al.* *Mycobacterium tuberculosis* may protect against allergy in a tuberculosis endemic area. *Clin Exp Allergy* 2006; **36**:70–6.
- 8 Obihara C, Beyers N, Gie R *et al.* Inverse association between *Mycobacterium tuberculosis* infection and atopic rhinitis in children. *Allergy* 2005; **60**:1121–5.
- 9 Ota M, van der Sande M, Walraven G *et al.* Absence of association between delayed type hypersensitivity to tuberculin and atopy in children in The Gambia. *Clin Exp Allergy* 2003; **33**:731–6.
- 10 Omenaas E, Jentoft H, Vollmer W, Buist A, Gulsvik A. Absence of relationship between tuberculin reactivity and atopy in BCG vaccinated adults. *Thorax* 2000; **5**:454–8.
- 11 Obihara CC, Kimpen JLL, Beyers N. The potential of *Mycobacterium* to protect against allergy and asthma. *Curr Allergy Asthma Rep* 2007; **7**:223–30.
- 12 Obihara CC, Bollen CW, Beyers N, Kimpen JL. Mycobacterial infection and atopy in childhood: a systematic review. *Pediatr Allergy Immunol* 2007; **18**:551–9.
- 13 Strannegård I, Larsson L, Wennergren G, Strannegård Ö. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. *Allergy* 1998; **53**:249–54.
- 14 Gruber C, Meinlschmidt G, Bergmann R *et al.* 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**:177–81.
- 15 Annus T, Montgomery S, Riikjäär M, Björkstén B. Atopic disorders among Estonian schoolchildren in relation to tuberculin reactivity and the age at BCG vaccination. *Allergy* 2004; **59**:1068–73.
- 16 Townley R, Barlan I, Patino C *et al.* The effect of BCG vaccine at birth on the development of atopy or allergic disease in young children. *Ann Allergy Asthma Immunol* 2004; **92**:350–5.
- 17 Martignon G, Oryszczyn M, Annesi-maesano I. Does childhood immunization against infectious diseases protect from the development of atopic disease? *Pediatr Allergy Immunol* 2005; **16**:193–200.
- 18 Garcias-Marcos L, Suárez-Varela MM, Canflanca IM *et al.* BCG immunization at birth and atopic diseases in a homogeneous population of Spanish schoolchildren. *Int Arch Allergy Immunol* 2005; **137**:303–9.
- 19 Da Cunha S, Cruz A, Dourado I *et al.* Lower prevalence of reported asthma in adolescents with symptoms of rhinitis that received neonatal BCG. *Allergy* 2004; **59**:857–62.
- 20 Marks G, Ng K, Zhou J *et al.* 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. *J Allergy Clin Immunol* 2003; **111**:541–9.
- 21 Miyake Y, Arakara M, Tanaka K, Sasaki S, Ohya Y. Tuberculin reactivity and allergic disorders in schoolchildren, Okinawa, Japan. *Clin Exp Allergy* 2008; doi: 10.1111/j.1365-2222.2007.02869.x.
- 22 Zuany-Amorim C, Sawicka E, Manlius C *et al.* Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002; **8**:625–9.
- 23 Janssen R, Kruisselbrink A, Hoogteijling L *et al.* Analysis of recombinant mycobacteria as T helper type 1 vaccines in an allergy challenge model. *Immunol* 2001; **102**:441–9.
- 24 Sano K, Haneda K, Tamura G, Shirato K. Ovalbumin (OVA) and *Mycobacterium tuberculosis* bacilli cooperatively polarize anti-OVA T-helper (Th) cells towards a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia. *Am J Respir Cell Mol Biol* 1999; **20**:1260–7.
- 25 Johansson SGO, Bieber T, Dahl R *et al.* Revised nomenclature for allergy for global use: report of the nomenclature review committee of the world allergy organization, October 2003. *J Allergy Clin Immunol* 2004; **113**:832–6.