

Review Article

# Mycobacterial infection and atopy in childhood: A systematic review

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The epidemiological relation between mycobacterial infection and the prevalence of atopic disease in humans is still unclear. This is in contrast to studies in murine models in which a clear suppression of atopic symptoms was observed after exposure to mycobacteria or mycobacterial products. We therefore wanted to provide a systematic overview of the published literature on the relationship between mycobacterial infection and atopic disease and to evaluate the causal relationship in a meta-analysis. The EMBASE and MEDLINE databases were searched systematically for papers published in the English literature (1966–2005) on the relation between mycobacterial infection and atopic disease. Original observational or interventional studies involving the paediatric population were included. Two authors independently reviewed articles for data on mycobacterial exposure and atopic disease outcome. Any differences were resolved by discussion. Of a total of 1201 hits, 23 studies (19 cross-sectionals, three case-controls and one prospective cohort) met the inclusion criteria. Only a minority of studies (40%) observed an association between mycobacterial infection and the prevalence of atopic disease outcome. In the meta-analysis, only studies containing data on mycobacterial exposure and atopic disease outcome variables were included. Only cross-sectional studies, in which the relation between a positive tuberculin skin test and allergic symptoms was studied, observed statistically significant negative correlation (odds ratio 0.63; 95% confidence interval: 0.51–0.79). The results of this review show that the evidence of the relationship of mycobacterial infection and atopic disease is based on observations of cross-sectional studies. In a meta-analysis, calculations showed a high level of heterogeneity ( $I^2$ ) within studies with similar design making it difficult to pool effects. This may partly be explained by differences in the type and definition of mycobacterial infection and lack of uniformity in the definition of atopy. The results show that only a minority of studies in the literature shows any evidence of inverse relationship between mycobacterial exposure and atopic disease outcome. The fact that the present epidemiological evidence on the relationship between mycobacterial infection and the development of atopic disease is based mainly on cross-sectional observational studies indicates the need for population-based prospective studies to address this issue. This issue needs to be addressed in view of recent suggestions to developing mycobacterial-based vaccines against atopic disease in the future.

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During the last few decades, the prevalence of atopic disease in children has increased in countries with a Western lifestyle (1). The 'hygiene hypothesis' suggests that a relationship exists between improved hygiene and increase in atopic disease prevalence (2). An underlying mechanism for this hypothesis is that the lack of microbial stimulation leads to either an inappropriate T-helper (Th) type response or inappropriate Th cell-regulatory mechanisms (3, 4).

A specific infectious agent responsible for the hygiene hypothesis has not yet been identified, although many candidates have been suggested, such as measles, hepatitis A and respiratory infections (5–7). Animal models have identified mycobacteria as important potential candidates in the hygiene hypothesis by demonstrating that exposure to mycobacteria or mycobacterial proteins led to regulatory mechanisms that restored the immune homeostasis (4, 8, 9). This has led to the suggestion that absence of infections, such as that caused by mycobacteria, may be an underlying factor in the rising prevalence of atopic diseases in developed countries. In contrast, the epidemiological relation between mycobacterial infection and atopic disease in humans is still unclear and controversial (10–13). We reviewed systematically the evidence for this relation.

## Methods

### Literature sources and search terms

The EMBASE and MEDLINE databases were searched systematically to identify all papers published in the English literature on the relation between mycobacterial exposure or infection and atopic disease, including the period January 1966 and September 2005. For the search the following keywords were utilized both individually and in different combinations: '*Mycobacterium tuberculosis* (*M. tuberculosis*) infection, tuberculin skin test (TST) response, tuberculosis (TB), mycobacteria other than tuberculosis (MOTT) infection and/or *M. bovis* bacille Calmette-Guérin (BCG) immunization', and 'atopy, atopic disease, allergic disease or symptom and/or allergy'. Cross-referencing of important articles was also applied. All identified papers on the relation between mycobacteria and atopic disease were published after 1997, when Shirakawa et al. reported on this relation for the first time (13).

### Study selection

Original observational or interventional population or hospital-based studies involving

persons  $\leq 18$  yr of age in which the relation between mycobacterial exposure or infection and an atopic disease outcome was studied were included. The age range was chosen because it is assumed that immune deviation caused by infectious agents and the protective effect on the development of atopy mainly occurs in childhood (14). In addition, only studies with well-defined participants' characteristics and number of subjects with and without mycobacterial exposure or infection and number of subjects with and without atopic outcome measure(s) were included in the meta-analysis.

### Data abstraction

One author extracted and screened potentially relevant articles to determine whether they met the inclusion criteria. Two authors then independently abstracted data from each included article and reviewed cross-referencing for additional potentially relevant articles. Abstract discrepancies were resolved by repeated review and discussion.

We extracted the following types of data from each study identified as specified by our protocol: study design, country in which the study was conducted, sample size, age of participants, type and definition of mycobacterial exposure and atopic outcome variables.

The quality of each study was assessed using the following criteria: methods of selection of subjects, definition of exposure and outcome variables, the number of subjects with and without mycobacterial exposure or infection, and the number of subjects with and without atopic disease outcome.

### Statistical analysis

All analyses were performed using EXCEL software (Office version 2003; Microsoft Cooperation, Silicon Valley, CA, USA) and SPSS for windows version 11. First, odds ratios (ORs) were calculated with 95% confidence intervals (CI) for each study using the original data containing the number of subjects infected (exposed group) or not infected (non-exposed group) with mycobacteria, and number of subjects with and without atopic disease outcome. Secondly, studies were stratified according to study design (cross-sectional, retrospective, case-control or prospective), type of mycobacterial infection or exposure (BCG, MOTT or *M. tuberculosis*) and type of atopic disease outcome [questionnaire-reported allergic disease symptoms, elevated serum

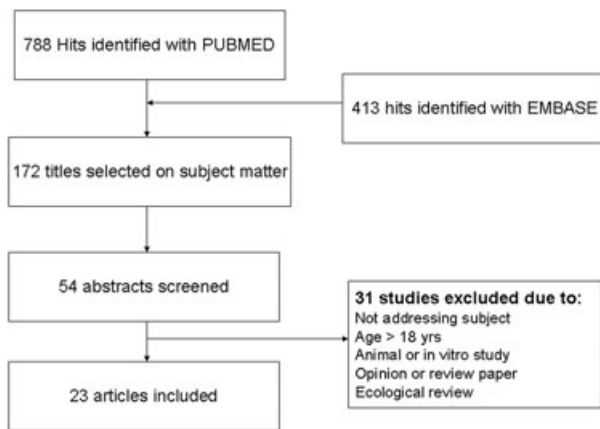


Fig. 1. Flowchart identification of articles.

allergen-specific immunoglobulin (Ig) E antibody or positive skin prick test (SPT) for environmental aeroallergens].

Pooled ORs were calculated using Mantel-Haenszel equations. We explored heterogeneity by calculating  $I^2$  within each stratum (study design type). The quantity,  $I^2$ , describes the percentage of total variation across studies within each stratum, which is due to heterogeneity rather than chance (15). Publication bias was assessed by visual examination of funnel plots of ORs as function of the inverse of standard errors.

## Results

### Description of studies

The selection procedure of relevant literature is illustrated in the flow diagram in Fig. 1. Of 788 hits identified with the MEDLINE search and 413 hits identified with the EMBASE search 172 titles on the subject matter were screened. Of these, 54 abstracts which met the selection criteria were screened and 23 which met the inclusion criteria were included in this review. All the studies which were identified with cross-referencing were also available on MEDLINE or EMBASE.

Table 1 summarizes the characteristics of studies. Of the 23 studies 19 were cross-sectional, three case-control and one prospective cohort (13, 16–37). Table 2 shows the type of *Mycobacterium* infection studied and methods used to define mycobacterial exposure or infection and atopic disease outcome. Two studies reported on the relation between MOTTs and atopic disease (17, 36), 21 on the relation between BCG immunization and atopic disease (13, 16–34, 37) and two on the relation between natural *M. tuberculosis* infection and atopic disease (22, 35).

### Definition of mycobacterial infection and atopic disease

**MOTT and atopic disease.** The two studies which studied the relation between MOTT and atopic disease used purified protein derivatives (PPDs)

Table 1. Characteristics of studies that evaluated the relation between mycobacterial infection and atopy

| Individual study reference  | Country of origin         | Year of publication | Study type      | Sample size  | Age group (yr) |
|-----------------------------|---------------------------|---------------------|-----------------|--------------|----------------|
| (1) Shirakawa et al. (13)   | Japan                     | 1997                | Cross-sectional | 867          | 12–13          |
| (2) Alm et al. (16)         | Sweden                    | 1997                | Case-control    | 574          | 4–7            |
| (3) Strannegård et al. (17) | Sweden                    | 1998                | Cross-sectional | 6497         | 4–9            |
| (4) Yilmaz et al. (18)      | Turkey                    | 2000                | Cross-sectional | 736          | 2.5–16         |
| (5) Nuhoglu et al. (19)     | Turkey                    | 2000                | Cross-sectional | 252          | 6–7            |
| (6) Aaby et al. (20)        | Guinea-Bissau             | 2000                | Cross-sectional | 400 (271)*   | 3–14           |
| (7) Wong et al. (21)        | China                     | 2001                | Cross-sectional | 980          | 10             |
| (8) Pahari et al. (22)      | UK                        | 2001                | Cross-sectional | 329          | 11–18          |
| (9) Gruber et al. (23)      | Germany                   | 2001                | Prospective     | 492 (92)*    | 7              |
| (10) Krause et al. (24)     | Greenland                 | 2001                | Cross-sectional | 1686         | 8–16           |
| (11) Jang and Son (25)      | Korea                     | 2002                | Cross-sectional | 486          | 10–13          |
| (12) Ozmen et al. (26)      | Turkey                    | 2002                | Case-control    | 206          | 1–14           |
| (13) Gruber et al. (27)     | Germany                   | 2002                | Cross-sectional | 38,808       | 6              |
| (14) Ota et al. (28)        | The Gambia                | 2003                | Cross-sectional | 507          | 8–12           |
| (15) Marks et al. (29)      | Australia                 | 2003                | Cross-sectional | 751 (309)*   | 7–14           |
| (16) Annus et al. (30)      | Estonia                   | 2004                | Cross-sectional | 717 (343)*   | 10–11          |
| (17) Mommers et al. (31)    | The Netherlands/Germany   | 2004                | Case-control    | 572 (75)*    | 7–8            |
| (18) Cooper et al. (32)     | Ecuador                   | 2004                | Cross-sectional | 1002 (959)*  | 7–17           |
| (19) Townley et al. (33)    | Turkey/Thailand/Argentina | 2004                | Cross-sectional | 1704 (655)*  | 2              |
| (20) Da Cunha et al. (34)   | Brazil                    | 2004                | Cross-sectional | 1612 (1089)* | 12–16          |
| (21) Obihara et al. (35)    | South Africa              | 2005                | Cross-sectional | 337          | 6–14           |
| (22) Bibakis et al. (36)    | Greece                    | 2005                | Cross-sectional | 806          | 8–18           |
| (23) Martignon et al. (37)  | France                    | 2005                | Cross-sectional | 718 (647)*   | 12–15          |

\*Number of children in the study who were actually tested for mycobacterial infection is shown in bracket.

Table 2. Test methods used to define mycobacterial infection and atopy, together with authors conclusion of findings

| Study reference                       | Measure used to indicate <i>Mycobacterium</i> infection |                                |             |                                | Measure used to indicate atopy |                                          |                                | Author's conclusion of findings |
|---------------------------------------|---------------------------------------------------------|--------------------------------|-------------|--------------------------------|--------------------------------|------------------------------------------|--------------------------------|---------------------------------|
|                                       | TST<br>(cut-off size<br>in mm)                          | BCG<br>immunization<br>history | BCG<br>scar | Elevated Th1<br>cytokine level | Allergic<br>symptoms           | SPT or<br>allergen-specific<br>IgE level | Elevated Th2<br>cytokine level | Inverse relation<br>(yes or no) |
| <b>MOTT</b>                           |                                                         |                                |             |                                |                                |                                          |                                |                                 |
| Strannegård et al. (17)               | +                                                       | +                              | +           | —                              | +                              | —                                        | —                              | No                              |
| Babakis et al. (36)                   | +                                                       | —                              | +           | —                              | +                              | +                                        | —                              | No                              |
| <b>BCG</b>                            |                                                         |                                |             |                                |                                |                                          |                                |                                 |
| Shirakawa et al. (13)                 | +                                                       | +                              | +           | +                              |                                | +                                        | +                              | Yes                             |
| Alm et al. (16)                       | —                                                       | +                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Strannegård et al. (17)               | +                                                       | +                              | +           | —                              | +                              | —                                        | —                              | No                              |
| Yilmaz et al. (18)                    | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Nuhoglu et al. (19)                   | +                                                       | +                              | —           | —                              | —                              | +                                        | —                              | No                              |
| Aaby et al. (20)                      | —                                                       | +                              | +           | —                              | —                              | +                                        | —                              | Yes                             |
| Wong et al. (21)                      | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Pahari et al. (22)                    | +                                                       | +                              | +           | —                              | +                              | —                                        | —                              | No                              |
| Gruber et al. (23)                    | +                                                       | +                              | —           | —                              | +                              | +                                        | —                              | No                              |
| Krause et al. (24)                    | —                                                       | +                              | —           | —                              | +                              | —                                        | —                              | No                              |
| Jang and Son (25)                     | +                                                       | +                              | —           | —                              | +                              | +                                        | —                              | No                              |
| Ozmen et al. (26)                     | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Gruber et al. (27)                    | —                                                       | +                              | —           | —                              | +                              | —                                        | —                              | Yes                             |
| Ota et al. (28)                       | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Marks et al. (29)                     | +                                                       | +                              | +           | +                              | +                              | +                                        | +                              | Yes                             |
| Annus et al. (30)                     | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | Yes                             |
| Mommers et al. (31)                   | —                                                       | +                              | —           | —                              | +                              | +                                        | —                              | No                              |
| Cooper et al. (32)                    | +                                                       | —                              | +           | —                              | +                              | +                                        | —                              | No                              |
| Townley et al. (33)                   | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | Yes                             |
| Da Cunha et al. (34)                  | —                                                       | +                              | +           | —                              | +                              | —                                        | —                              | Yes                             |
| Martignon et al. (37)                 | —                                                       | +                              | —           | —                              | +                              | —                                        | —                              | yes                             |
| <b>Natural <i>M. tuberculosis</i></b> |                                                         |                                |             |                                |                                |                                          |                                |                                 |
| Pahari et al. (22)                    | +                                                       | +                              | +           | —                              | +                              | —                                        | —                              | No                              |
| Obihara et al. (35)                   | +                                                       | +                              | +           | —                              | +                              | +                                        | —                              | Yes                             |

+, present; —, absent; TST, tuberculin skin test; BCG, bacille Calmette-Guérin; Th, T helper cell; SPT, allergy skin prick test; IgE, immunoglobulin E.

skin test for *M. avium*, *M. intracellulare* and *M. scrofulaceum* to define immune reaction to MOTT infection (17, 36). For the definition of atopic disease, Strannegård et al. (17) used questionnaire-reported allergic disease symptoms only, while Bibakis et al. (36) used both questionnaire-reported symptoms and positive SPT.

**BCG and atopic disease.** The relation between BCG immunization and atopic disease was studied in 21 studies (13, 16–34, 37; Table 2). Exposure to *M. bovis*-BCG was defined by positive parental recall of BCG immunization or by documented record of BCG immunization in 20 studies, by positive TST reactivity in 14 and by the presence of BCG scar on inspection in 14. Only two studies measured serum Th1 cytokine [interferon (IFN)- $\gamma$ ] levels as surrogate of mycobacterial infection (13, 29). In 19 studies, atopy was defined as positive questionnaire-reported allergic symptoms, in 15 as positive SPT or elevated serum-specific IgE to environmental aeroallergens and in two as elevated serum Th2 cytokine level [interleukin (IL)-4, IL-5 or IL-13; Table 2].

**Natural *M. tuberculosis* infection and atopic disease.** In two studies the relation between natural *M. tuberculosis* infection and atopic disease outcome was studied (Table 2; 22, 35). In both studies *M. tuberculosis* infection was defined as positive TST reactivity to *M. tuberculosis* PPD. Pahari et al. studied the relation between TST reactivity and reported symptoms of asthma in schoolchildren from England, as part of a contact-tracing exercise after a teacher at their school was diagnosed with sputum-positive pulmonary TB (22). In a study in South African children from a high TB incidence area, we studied the relation between TST reactivity and atopic rhinitis (35).

Relation between mycobacterial infection and atopic disease

**MOTT and atopic disease.** Strannegård et al. found a higher (instead of lower) prevalence of positive PPD to MOTT in allergic than in non-allergic Swedish children (17). Bibakis et al. did not find a significant relation between PPD reactivity to MOTT and atopy in Greek children (36).

**BCG and atopic disease.** In eight of the 21 studies which analysed the relation between BCG immunization and atopic disease, a significant inverse relation was observed between *M. bovis*-BCG exposure and one or more atopic disease outcome variables (13, 20, 27, 29, 30, 33, 34, 37; Table 2). All of these studies had a cross-sectional design. Shirakawa et al. observed lower frequency of allergic symptoms, positive SPT to environmental aeroallergens and lower serum Th2 cytokine level in BCG-immunized Japanese schoolchildren with positive TST than in BCG-immunized children with negative TST (13). Aaby et al. observed a lower frequency of positive SPT to house dust mite in children from Guinea Bissau with documented BCG immunization records than in those without (20). In a German birth cohort study, only a transient and weak inverse relation was observed between BCG immunization in infancy and the prevalence of atopic disease in children (27). In Australian children, Marks et al. observed in a cross-sectional study, in a birth cohort that in those with a positive family history of allergy, BCG immunization was associated with less symptoms of asthma (29). A similar effect was not observed in BCG-immunized children without a positive family history. Annus et al. reported lower prevalence of allergic symptoms in BCG-immunized Estonian children with positive TST compared to those with negative TST (30). In a study conducted simultaneously in three international sites, Townley et al. observed a significant inverse relation between TST reactivity and allergic history in BCG-immunized children from Turkey and Thailand, but not in BCG-immunized children from Argentina (33). In Brazilian children, Da Cunha et al. observed lower risk of asthma only in children with neonatal BCG scar and a past history of allergic

rhinitis, but not in those without a history of allergic rhinitis (34). In another study, French children with a positive BCG immunization history had less allergic disease symptoms than those without BCG immunization (37).

**Natural *M. tuberculosis* infection and atopic disease.** One of the two studies which evaluated the relation between positive TST (as a surrogate of *M. tuberculosis* infection) and atopic disease observed an inverse relation (Table 2). In schoolchildren from England, Pahari et al. did not observe an inverse relation between positive TST reactivity and asthmatic symptoms (22). In South African children, we observed a significant inverse relation between positive TST reactivity and atopic rhinitis. In addition, we observed a significant inverse relation between positive TST reactivity and SPT to aeroallergens in allergic children (35).

#### Meta-analysis of studies

Studies which did not report the original data containing the number of exposed and non-exposed subjects to mycobacteria, or the number of subjects with and without the atopic disease outcome were excluded from the meta-analysis. For the meta-analyses, studies were stratified according to design, type of mycobacterial exposure and atopic disease outcome. The results of these stratified pooled analyses are shown in Table 3. We assessed publication bias of studies by visual inspection of the relation between the inverse of precision ( $1/p$ ) and strength of the reported relation. The result was not suggestive of publication bias for the different strata. As shown in Table 3 cross-sectional studies that investigated the relation between BCG immunization or TST reactivity and atopic disease

Table 3. Results of the meta-analysis pooled according to study design

| Study design    | Number of studies | Number of patients | Exposure | Outcome | Test     | OR   | 95% CI |       | $I^2$ (%) |
|-----------------|-------------------|--------------------|----------|---------|----------|------|--------|-------|-----------|
|                 |                   |                    |          |         |          |      | Lower  | Upper |           |
| Cross-sectional | 4                 | 40,453             | BCG      | Asthma  | Clinical | 0.96 | 0.88   | 1.05  | 0         |
| Cross-sectional | 1                 | 451                | BCG      | Asthma  | MCIT     | 1.10 | 0.72   | 1.69  | NA        |
| Cross-sectional | 1                 | 6497               | BCG      | Atopy   | Clinical | 0.86 | 0.64   | 1.15  | NA        |
| Cross-sectional | 5                 | 4122               | BCG      | Atopy   | SPT      | 1.01 | 0.83   | 1.23  | 61        |
| Cross-sectional | 2                 | 482                | TST      | Asthma  | Clinical | 0.82 | 0.46   | 1.46  | 0         |
| Cross-sectional | 1                 | 153                | TST      | Asthma  | MCIT     | 1.73 | 0.77   | 3.89  | NA        |
| Cross-sectional | 3                 | 3554               | TST      | Atopy   | Clinical | 0.63 | 0.51   | 0.79  | 90        |
| Cross-sectional | 7                 | 3567               | TST      | Atopy   | SPT      | 0.92 | 0.76   | 1.12  | 12        |
| Case-control    | 1                 | 574                | BCG      | Atopy   | Clinical | 0.81 | 0.57   | 1.15  | NA        |
| Case-control    | 2                 | 1145               | BCG      | Atopy   | SPT      | 0.91 | 0.68   | 1.21  | 13        |

$I^2$ , coefficient of heterogeneity; CI, confidence interval; TST, tuberculin skin test; BCG, bacille Calmette-Guérin; SPT, allergy skin prick test; NA, not applicable due to too few studies for further analysis.

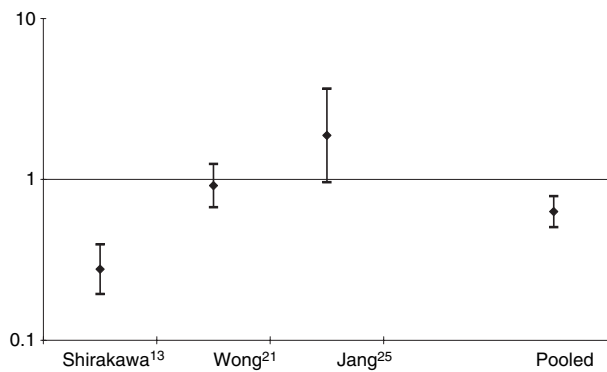


Fig. 2. Calculated and pooled odds ratio of cross-sectional studies which studied the relation between tuberculin skin test and symptoms of atopic disease as outcome variable.

showed a great degree of unexplained heterogeneity ( $I^2$ ). Therefore, these pooled results were not readily interpretable and could not be analysed further. Only the relation between positive TST and allergic symptoms was significant (OR 0.63; 95% CI: 0.51–0.79; Table 3). The individual and pooled ORs of these studies are illustrated in Fig. 2. The first study by Shirakawa et al. reported a significant association that was not reproduced by the other two analogous studies (13, 21, 25). Three case-control studies, one investigating the relation between BCG immunization and allergic disease symptoms and two defining atopy by SPT did not observe a significant relationship (16, 26, 31). The only prospective study also did not observe a significant relationship (23).

## Discussion

The results of this systematic review of the literature on the relation between mycobacterial infection and atopic disease show that only a minority of studies found evidence to support a protective effect of mycobacterial infection against the development of atopic disease. This contrasts to studies in murine models showing a suppression of atopic symptoms after exposure to mycobacteria or mycobacterial products (4, 8, 9). Moreover, this review reveals a high level of heterogeneity of published studies which made it difficult to pool effects of published data in a meta-analysis. Among the most important factors which could explain this heterogeneity, next to differences in the study population, design and methodology, are differences in type, definition, intensity and persistence (chronicity) of mycobacterial exposure or infection in the populations studied, and the lack of uniformity in the definition of atopy.

Immune activation after mycobacterial infection is dependent on the mycobacterial species (38). This is supported by the observation that MOTTs are poorer inducers of IL-12, the major cytokine in the induction of the Th1 response, than *M. tuberculosis* (39). Protective immunity in reaction to mycobacterial infection is mediated primarily by Th1 cells directed against mycobacterial proteins (38). An important marker of Th1-mediated immunity is the development of delayed type hypersensitivity (DTH), which can be evaluated by TST. TST reactivity becomes apparent in 3–6 wk following *M. tuberculosis* infection and may remain positive for the lifetime of the individual (40, 41). The size of TST indurations has been directly correlated with serum levels of IFN- $\gamma$ , implying that a large TST size may reflect strong Th1 immunity (42). A very strong TST response is more frequently associated with *M. tuberculosis* infection than with *M. bovis*-BCG or MOTT infection (13, 35, 40, 41). Although BCG immunization may cause a transiently reactive TST, most children who received BCG as infants have a weak or non-reactive TST at 5 yr of age (43). Moreover, BCG immunization had little impact on the interpretation of TST in children during a TB contact tracing investigation (44). It has been observed that BCG immunization (even in early life) cannot possibly mimic qualitatively or quantitatively the persistent immune stimulation provided by natural infection with *M. tuberculosis* (45). Although differences in the species of *Mycobacterium* may contribute in explaining some of the heterogeneity in findings between studies, they cannot logically explain the differences in results of studies which used the same *Mycobacterium* type.

Recent explanations of the hygiene hypothesis suggest that the intensity, frequency and persistence of infection with pathogens may influence the level of immune protection (45, 46). Pathogens that cause persistent and/or chronic infections may have evolved strategies to subvert the host's immune responses, by stimulating an increased production of anti-inflammatory or immunosuppressive mediators, which normally function to control or terminate the protective immune effector-responses of the host (47). This is either achieved through the direct induction of host immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)- $\beta$ , which are produced by innate immune cells in response to pathogen-derived antigens or through the production of regulatory T cells (47). Antigen-specific regulatory T cells have been described in several chronic infections including those by *M. tuberculosis* or *M. vaccae* (4, 48, 49). These

regulatory cells might be beneficial to the host by maintaining a balance between efficient effectors and memory responses, but with a low level of inflammatory response that causes minimal damage to the host. The Japanese children investigated by Shirakawa et al. received BCG immunization three times (at birth, at the age of 6 and 12 yr; 13). This is reflected by the high prevalence of positive TST reactivity (58%) and by the fact that the strongest effect was observed in the group of children which remained TST-positive at 6 and 12 yr. Moreover, the possible influence of natural *M. tuberculosis* infection was suggested. This contrasts to the majority of other studies in which the effect of BCG immunization was tested, especially those from central and northern Europe and Asia, in which children generally received only a single BCG immunization in the neonatal period. In children from Guinea Bissau, Aaby et al. found less positive SPT to aeroallergens among the BCG-immunized group compared with the non-immunized group (20). However, BCG exposure was defined as positive history of neonatal BCG immunization or the presence of BCG scar only. The lack of TST measurement in this study makes it difficult to correct for the possible additive influence of other mycobacteria in the environment on the results. In early BCG-immunized Estonian children, Annus et al. observed significant inverse relation between TST response and the prevalence of allergic symptoms, but not of allergy SPT to aeroallergens (30). Measurement of BCG immunization in these children was based on school records. In a study in French children to evaluate the effect of early vaccination on allergic disease symptoms, Martignon et al. found lower prevalence of reported symptoms in children who received early BCG immunization compared with non-immunized ones (37). The two studies on the effect of natural *M. tuberculosis* infection also differed with respect to intensity and duration of exposure. In the UK study, Pahari et al. investigated children with a recent history of natural *M. tuberculosis* infection, after their teacher was diagnosed with pulmonary TB (22). In contrast, the South African study investigated children living in a high TB endemic area (with TB notification rate of 341/100,000 confirmed smear positive TB in 2002; 35). These children not only had a high cumulative burden of *M. tuberculosis* infection, but the possible additive influence of MOTTs or *M. bovis*-BCG was not observed (35).

Differences in the efficacy of BCG vaccine strains and the known regional differences in the vaccine efficacy may contribute to the

heterogeneity of study results, as this may lead to variability in immune response (50). There is evidence that this may partly be ascribed to differences in exposure to MOTT. This is demonstrated for instance, by the higher pre-vaccination Th1 cytokine (IFN- $\gamma$ ) responses to MOTT antigens in Malawi than in the UK and greater BCG-attributable increases in IFN- $\gamma$  responses in UK than in Malawi (51). Although it is not known if variability in the efficacy of BCG immunization against TB is related to the immune stimulation required to suppress the development of atopic inflammation, it poses a relevant topic for future research.

Lack of uniformity in the definition of atopy makes it difficult to compare results. This may also account for some of the heterogeneity of study results. While some studies defined atopy as questionnaire-reported symptoms or positive SPT to common environmental allergens, others used both definitions. The utilization of the recently accepted World Allergy Organization (WAO) definition of atopy, as clinical allergic symptom in combination with a positive SPT and/or an elevated serum IgE antibody level makes it possible for future studies to define atopy more uniformly (52). Finally, heterogeneity in genetic background of the different study populations may strongly influence the outcome on the relation between mycobacterial infection and atopic disease. This has been reviewed elsewhere by Smit et al. (53).

In conclusion, this systematic review on the relation between mycobacterial infection and atopy reveals that in contrast to studies in murine models, only a minority of studies in humans observed evidence to support a protective effect of mycobacterial infection on the development of atopic disease. It further reveals a high level of heterogeneity of studies. Among possible explanations for the heterogeneity are differences in type, definition, intensity and duration of mycobacterial exposure or infection, and lack of uniformity in the definition of atopy. Based on these findings, we believe that the causal relationship between mycobacterial infection and atopic disease can only be resolved with large-scale population-based prospective studies.

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# Conflict of interest declaration

C.C.O, C.W.B., N.B. and J.L.L.K declare no conflict of interest in the drafting and decision to publish this manuscript.

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