

## EDITORIAL

# Mycobacteria and atopy, 6 years later: a fascinating, still unfinished, business

### Mycobacteria, atopy and the Th1/Th2 explanation of the hygiene hypothesis

The hypothesis that lack of infections in early childhood underlies the epidemic trend of atopic diseases ('hygiene hypothesis') originated from epidemiological observations only [1]. Tentative biological explanations later proposed that allergen-specific T cell memory responses become Th2-skewed in the absence of infections stimulating Th1 immunity [2–4]. This scenario, evoking the Th1/Th2 paradigm, focused on the induction phase and the initial maturation of the immune response against ubiquitous allergens early in life [2]. Accordingly, it was postulated that the protective role of infections stimulating Th1 immunity is limited only to a 'window' period in early infancy [4] and that the decline of infections driving Th1 immunity, including tuberculosis, contributed to the rising trend of allergic diseases [2, 4].

This explanatory model of the hygiene hypothesis fitted the findings in a survey among 867 Japanese children all immunized with BCG, showing that responders to tuberculin had lower serum levels of total IgE and Th2 cytokines (IL-4, IL-10 and IL-13), higher serum levels of IFN- $\gamma$  and lower prevalences of atopy and atopic diseases when compared to tuberculin non-responder subjects [5]. Hence, it was proposed that children with strong tuberculin responses had been protected from atopy by a higher natural exposure to environmental mycobacteria [5].

Two of the postulates of this explanatory model were questioned [6]. Firstly, the existence of a 'window' period limited only to early infancy in infection-driven protection from allergy does not fit three evidences: (1) adult migrants acquiring a western lifestyle can develop atopy [7], suggesting that 'removal' of protection can still release atopic responses in genetically predisposed adult subjects and that protection acquired during early infancy is not a 'once-for-ever' phenomenon; (2) remission of allergic disease can be induced by infections acquired during adulthood [8]; (3) the number of younger siblings, and not only that of older siblings, is inversely associated with the risk of developing hayfever [1].

Secondly, a causal-effect link between positive tuberculin responses and negative responses to allergens among the Japanese children could be challenged [6] by the lack of difference in the examined sociodemographic characteristics, lifestyle indicators and chest radiograph reports of tuberculosis between tuberculin responders' and non-responders' families [5]. The alternative explanation was also considered that very strong tuberculin responses after BCG immunization and very high total IgE serum levels are mutually excluding phenotypes expressed by the same set of genes regulating predisposition to a Th1 or Th2 predominance in the immune responses [5, 6, 9].

### Has the TB–Atopy inverse association been confirmed?

The subsequent epidemiological studies on immunity against mycobacteria and atopic disorders [10–27] added fuel to the debate. Their results can be classified by the issue they addressed, namely the relation of (a) tuberculin responses, (b) BCG immunization, and (c) pulmonary tuberculosis, with the development of asthma, allergic diseases, atopic sensitization and high total serum IgE levels. Although a detailed analysis of each of these studies cannot be accommodated in this editorial, major outcomes are summarized in Table 1.

#### *Tuberculin responses*

No inverse association between tuberculin responses and asthma, allergic diseases, atopic responses and total IgE levels has been reported in any of 11 studies [10–20] following the one on Japanese children [5]. Differences in study design, population examined, BCG preparations, and above all the pattern of natural exposure to *M. tuberculosis* and mycobacteria other than tuberculosis [28] can partially explain failure to confirm previous findings.

#### *BCG immunization*

At least six studies [10, 15, 17, 21, 23, 24] tested whether immunization with BCG can protect from atopic diseases, but none of them found a significant protective effect of BCG administered early in life against the development of asthma or allergy later in childhood.

An additional study reported that the prevalence of skin sensitization to common airborne allergens was lower (21%) among 271 Guinea-Bissau children vaccinated with BCG in infancy in comparison with 53 never vaccinated with BCG (40%; adjusted OR 0.19; CI 0.06–0.59); unfortunately no data on the prevalence of wheezing, asthma or hayfever in the two groups was reported [22]. However, in the present issue of *Clinical and Experimental Allergy*, Ota et al. [20] report on a population of 507 children (age 8–12) living in Gambia, 360 of which with the evidence of a BCG scar. The prevalence of positive immediate skin test responses to locally relevant allergens and the serum levels of total IgE were not significantly different among children with or without a BCG scar [20]. Interestingly, helminthic infections are endemic in Gambia, and their confounding role on parameters related to atopic disorders has been recently documented in a previous epidemiological study in this country [29].

#### *Pulmonary tuberculosis*

Ecological analyses have repeatedly and consistently found an inverse association between the prevalence of tuberculosis in

**Table 1.** Studies on the relation of tuberculin response, BCG immunization, tuberculosis vs. asthma, allergy, atopy and total IgE

|                     |         |               | Study population           |              | Wheezing/asthma                    | Allergic disease | Atopy (SPT/RAST <sup>+</sup> ) | Total IgE |
|---------------------|---------|---------------|----------------------------|--------------|------------------------------------|------------------|--------------------------------|-----------|
| Reference           | Country | (BCG)         | Age (yrs)                  |              |                                    |                  |                                |           |
| Tuberculin response |         |               |                            |              |                                    |                  |                                |           |
| Shirakawa (1997)    | 5       | Japan         | 867 (all) <sup>a</sup>     | 12–13        | Inverse                            | Inverse          | Inverse                        | Inverse   |
| Strannegard (1998)  | 10      | Sweden        | 6497 (294) <sup>e</sup>    | 4–9          |                                    | None             |                                |           |
| Yilmaz (2000)       | 11      | Turkey        | 736 (all) <sup>b</sup>     | 2.5–16       | None                               | None             | None                           | None      |
| Omenaas (2000)      | 12      | Norway        | 574 (all) <sup>f</sup>     | 20–44        |                                    |                  | None                           | None      |
| Kroger (2000)       | 13      | Finland       | 201 (all) <sup>a</sup>     | 10–12        |                                    | None             |                                |           |
| Wong (2001)         | 14      | Hong Kong     | 980 (all) <sup>a</sup>     | 10           | None                               | None             | None                           |           |
| Gruber (2001)       | 15      | Germany       | 774 (92) <sup>e</sup>      | Birth cohort | None                               | None             | None                           | None      |
| Jentoft (2002)      | 16      | Norway        | 386 (all) <sup>c</sup>     | 20–44        | None                               |                  |                                |           |
| Pahari (2002)       | 17      | UK            | 329 (195) <sup>g</sup>     | 11–18        | None                               |                  |                                |           |
| Jang (2002)         | 18      | Korea         | 486 (all) <sup>c</sup>     | 10–13        | None*                              |                  | None                           |           |
| Ozmen (2002)        | 19      | Turkey        | 206 (all) <sup>b</sup>     | 1–14         |                                    |                  | Direct                         |           |
| Ota (2003)          | 20      | Gambia        | 507 (360) <sup>g†</sup>    | 8–12         |                                    |                  | None                           | None      |
| BCG immunization    |         |               |                            |              |                                    |                  |                                |           |
| Alm (1997)          | 21      | Sweden        | 574 (216) <sup>c</sup>     | 3–7          | None                               | None             | None                           |           |
| Strannegard (1998)  | 10      | Sweden        | 6080 (294) <sup>e</sup>    | 4–9          |                                    | None             |                                |           |
| Aaby (2000)         | 22      | Guinea-Bissau | 400 (271) <sup>d</sup>     | 3–14         |                                    |                  | Inverse                        |           |
| Gruber (2001)       | 15      | Germany       | 774 (92) <sup>e</sup>      | Birth cohort | None‡                              | None             | None‡                          | None      |
| Pahari (2002)       | 17      | UK            | 329 (195) <sup>g</sup>     | 11–18        | None                               | None             |                                | None      |
| Gruber (2002)       | 23      | Germany       | 38808 (20383) <sup>a</sup> | 6            | Inverse§                           | None§            |                                |           |
| Krause (2003)       | 24      | Greenland     | 1575 (1065) <sup>a</sup>   | 8–16         |                                    |                  | None                           |           |
| Ota (2003)          | 20      | Gambia        | 507 (360) <sup>g†</sup>    | 8–12         |                                    |                  | None                           | None      |
| Tuberculosis        |         |               |                            |              |                                    |                  |                                |           |
| von Hertzen (1999)  | 25      | Finland       | 2324 (1162)¶               | Adults       | Direct (men)/<br>Inverse (women)** | Inverse††        |                                |           |
| von Mutius (2000)   | 26      | 23 countries  | 235477                     | 13–14        | Inverse‡‡                          | Inverse‡‡        |                                |           |
| Shirtcliffe (2002)  | 27      | 38 countries  |                            | 6–7          | Inverse‡‡                          |                  |                                |           |
| Shirtcliffe (2002)  | 27      | 55 countries  |                            | 13–14        | Inverse‡‡                          |                  |                                |           |

Age at first BCG immunization: <sup>a</sup>neonatal period; <sup>b</sup> = 2 months; <sup>c</sup> < 6 months; <sup>d</sup> *n* = 244, < 6 months; <sup>e</sup> < 12 months; <sup>f</sup> at 14 years; <sup>g</sup> not reported. \*Airway hyper-responsiveness examined by methacholine challenge test. †Evidence of a BCG scar. ‡In the BCG group wheezing bronchitis and atopy transiently less frequent at 1 and 2 years of age, respectively, but not later in life. §BCG immunization weekly inversely associated with bronchial asthma, not associated with atopic dermatitis or hayfever. ¶With tuberculosis; \*\*Persistent asthma more frequent in men with TB, less frequent in women with TB at < 16 years of age; ††Only in women. ‡‡Ecological analysis.

a given country and the prevalence of atopic disorders [26, 27]. One study evaluated retrospectively the occurrence of allergic disease among Finnish adults having had tuberculosis in the first two decades of life. Tuberculosis was inversely associated with allergies and asthma in women, but the opposite trend was observed in men [25].

### Mycobacteria, allergy, and the alternative model explaining the hygiene hypothyresis

Although the observations made in the Japanese study have been not reproduced, it would be premature to discard that, under certain conditions, mycobacteria contribute to protect from atopy. Indeed, this hypothesis is still supported, although very indirectly, by the observation that in countries where tuberculosis is prevalent, allergy is less frequent [25, 26].

The whole subject could now be reappraised in light of a novel explanatory model for the hygiene hypothesis, which recently proposed that infections would actually inhibit allergic, as well as other immune-mediated, inflammation through stimulation

of anti-inflammatory regulatory networks [30]. This model could explain recent epidemiological reports that not only Th2 but also Th1-mediated diseases are on the rise [31, 32]. More research is needed to clarify whether this concomitant rise in Th2 and Th1 diseases can also be seen at the level of an individual or an organ [33, 34]. With respect to allergy the model shifted the attention from the induction and maturation phase of the immune response against allergens toward the effector phase, i.e., the inflammatory consequences of an established atopic sensitization. Interestingly, recent studies in animal models of allergic airway disease have shown that *Mycobacterium vaccae* blocks allergic inflammation not via induction of IFN- $\gamma$  that would lead to redirection of immune responses to Th1 at the induction phase, but by stimulating regulatory T cells [35, 36]. The relevant implications of the revised immunological model for the issues treated here are as follows:

First, according to this model, a healthy or an allergic outcome of the interaction between allergens and humans is not fixed once-for-ever early in life, but retains some flexibility until adulthood. This is in keeping with the concept that prevention of the atopic tendency is neither dichotomous (a yes/no event), nor limited exclusively to a certain 'window' period (very early

in life), but a dynamic and quantitative process, extended to adulthood, and subjected to genetic factors that regulate how early and intense exposure to infections must be to afford permanent protection from atopic sensitization and its inflammatory consequences [6].

Secondly, in hygienic settings, a single shot with BCG, even if very early in life, would not be conceivably sufficient to prevent the development of atopy, and especially its inflammatory consequences. Indeed, by no means can BCG mimic, both qualitatively (route and site of exposure) and quantitatively, the persistent immune stimulation provided by an intense and protracted natural exposure to pathogenic and non-pathogenic mycobacteria.

Finally, as T regulatory cytokines, rather than Th1 cytokines, would protect from allergy, then the response to tuberculin would not be the best parameter to evaluate the putative allergy-protective effect of natural or artificial exposure to mycobacteria.

### Integrating the Th1/ Th2 paradigm with the anti-inflammatory regulatory network

A unifying explanatory model for the hygiene hypothesis would consider the role of infections in influencing both the induction and the effector phase of the immune response against allergens. A strong regulatory network stimulated by chronic infections not only bacterial and viral (Th1 skewing) but also including those from helminths (Th2 skewing), may be the predominant allergy-protective mechanism among populations living in developing countries, but their relevance may be lower in

countries whose populations are much less exposed to chronic infections, including helminthic infections. More research is needed to resolve whether Th1 responses are present to allergens both at the T and the B cell level in protected subjects and more importantly what level of Th1 responses are safe. It is also conceivable that a graded mixture of Treg, Th1 and Th2 responses determine the outcome of allergies in (a) high Th1 and Th2 pathogen load, (b) Th1 pathogen load, and (c) low pathogen load environments (Fig. 1). Similarly, the relevance of a 'window' period in early life for infection-driven protection from atopy would increase progressively as a population advances from a traditional to a westernized lifestyle.

### Implications for further studies

Given these theoretical premises, further studies on the relation between mycobacteria and atopic disorders are needed. New areas of investigation may include measures of anti-inflammatory cytokines other than delayed-type hypersensitivity (DTH) skin responses in response to mycobacterial antigens; evaluation of the influence of pulmonary tuberculosis on the natural history of allergy, other than on immunization with BCG after birth; testing exposure to non-tubercular mycobacteria in the farming environment; and, under safe conditions, the effect of BCG or non-pathogenic mycobacteria administered directly at mucosal level, rather than subcutaneously.

In conclusion, although mycobacteria are not the only players in the hygiene hypothesis, investigations around their relationship with atopic disorders, 6 years after they began, are still an unfinished but fascinating business [37].

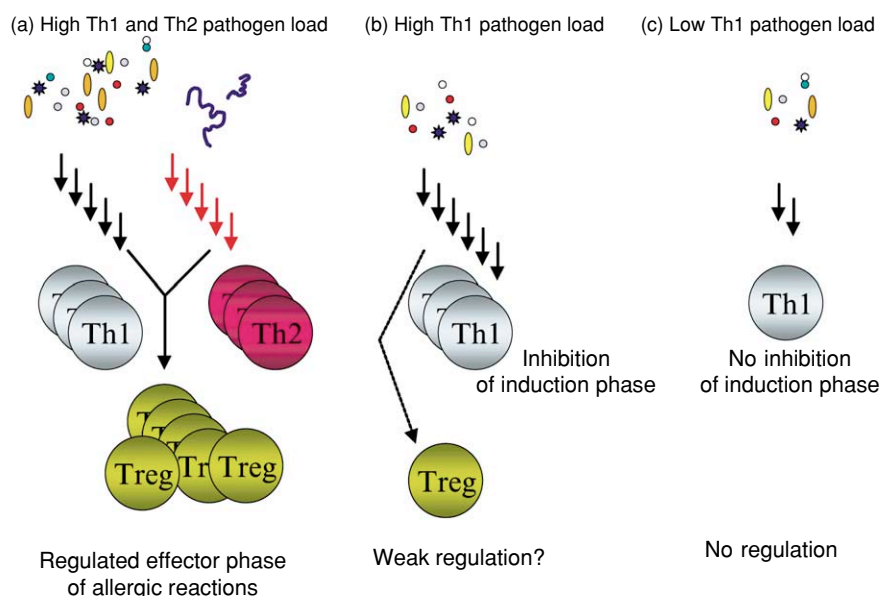


**Fig. 1.** Immunological explanation of the hygiene hypothesis: three different environments. (a) High Th1- and Th2-inducing pathogen burdens are encountered, such as in rural areas in developing countries, a strong anti-inflammatory network characterized, by, among others, regulatory T cells, will ensure that inflammatory reactions to environmental allergens are inhibited at the effector phase as a result of either bystander or specific effects. (b) High Th1-inducing pathogen burdens are encountered such as in early 20th century Europe or traditional farming communities, strong Th1 responses are induced possibly along with a weak regulatory network. Abundant type-1 responses will suppress the induction of Th2 responses to environmental allergens via IL-12 and IFN- $\gamma$ . Any possible Th1 reactions to allergens may be kept under control by the weak regulatory network. (c) Low pathogen burdens and high levels of hygiene are encountered, such as in communities with modern western lifestyle, weak stimulation of Th1 responses and lack of a strong regulatory network will culminate in a strong induction of Th2 responses to environmental allergens which can lead to allergic inflammatory reactions in affected organs.

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