

The Potential of *Mycobacterium* to Protect Against Allergy and Asthma

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The increase in the global incidence of atopic disease and asthma during the past few decades has been ascribed to environmental factors, including the reduction in exposure to serious infections. The hypothetical framework to explain the inverse relationship between infections and atopic disease and asthma has been called the “hygiene hypothesis.” Animal and experimental models have identified *Mycobacteria* as important potential candidates in the hygiene hypothesis by demonstrating that exposure to *Mycobacteria* or mycobacterial proteins led to subsequent reduction in different atopic manifestations. Although there are epidemiological studies in support, they have not always been consistent. In this review we appraise epidemiologic evidence on the inverse relationship between mycobacterial exposure and atopic disease, explore the immunological mechanisms involved and evidence that this effect may be dose-dependent, and discuss the challenges facing the use of *Mycobacteria* as vaccine for prevention of atopic disease.

Introduction

The dramatic increase in the incidence of atopic disease (atopic allergy and asthma) during the past few decades in countries with a western lifestyle cannot be accounted for solely by improved awareness of physicians in diagnosis of atopic disease or by media publicity of atopy [1,2]. Because of the short time frame (decades) during which this increase occurred, it is logical to assume that environmental rather than genetic factors are involved in the increased incidence of atopic disease [3]. Even though atopy is not limited to the industrialized world, it is generally assumed that atopic diseases are less prevalent in

nonindustrialized than in industrialized countries [1]. In a study using the same methodology, no significant difference in the prevalence of atopic symptoms and outcome could be demonstrated between Australian (32.5%) and Nigerian children (28.2%) [4]. One category of environmental factors that consistently shows an inverse relationship with atopic disorders is infection. The hypothetical concept to explain this relationship has been called the “hygiene hypothesis.” The hygiene hypothesis suggests that the rise in atopic disease, especially in westernized societies, could be explained by the reduction in exposure to bacterial products and serious infection by microbial agents, which might be explained by declining family size, improved household amenities, high standards of personal hygiene, and the universal use of vaccination in childhood [5].

Animal and experimental models have identified mycobacterial exposure as an important potential candidate in the hygiene hypothesis by demonstrating that exposure to mycobacterial species or proteins led to regulatory mechanisms that restored the immune homeostasis [6–8]. This has prompted the suggestion that absence of certain serious infections may be an underlying factor in the rising prevalence of atopic diseases. However, the exact influence of mycobacterial infections on the prevalence of atopy is less clear, with some epidemiological studies showing an inverse correlation between mycobacterial infection and atopic disease in humans [9,10], while others do not confirm these findings [11].

In this review we appraise the epidemiological data to support the inverse relationship between mycobacterial infection and atopy and discuss the mechanisms involved. In addition, we analyze data supporting a dose-dependent effect of mycobacterial exposure on the development of atopy and the challenges for mycobacterial-based vaccines against atopic disease.

Epidemiological Relation Between Mycobacterial Infection and Allergic Disease

The critical period for establishment of the T helper (Th) cell balance is assumed to be in early childhood, when the Th2-skewed human immune system gradually becomes

Table 1. Characteristics and findings of epidemiological studies on the relationship between mycobacterial infection and atopy

Study	Country of origin	Study type	Sample size	Type of mycobacterium tested	Inverse relationship: yes (+) or no (-)
Shirakawa et al. [10]	Japan	Cross-sectional	867	BCG	+
Alm et al. [13]	Sweden	Case-control	574	BCG	–
Strannegård et al. [14]	Sweden	Cross-sectional	6497	BCG/NTM	–
Yilmaz et al. [15]	Turkey	Cross-sectional	736	BCG	–
Nuhoglu et al. [16]	Turkey	Cross-sectional	252	BCG	–
Aaby et al. [17]	Guinea-Bissau	Cross-sectional	400 (271)*	BCG	+
Wong et al. [18]	China	Cross-sectional	980	BCG	–
Pahari et al. [19]	United Kingdom	Cross-sectional	329	MTB/BCG	–
Grüber et al. [20]	Germany	Prospective	492 (92)*	BCG	–
Krause et al. [21]	Greenland	Cross-sectional	1686	BCG	–
Jang and Son [22]	Korea	Cross-sectional	486	BCG	–
Ozmen et al. [23]	Turkey	Case-control	206	BCG	–
Grüber et al. [24]	Germany	Cross-sectional	38,808	BCG	+
Ota et al. [25]	Gambia	Cross-sectional	507	BCG	–
Marks et al. [26]	Australia	Cross-sectional	751 (309)*	BCG	+
Annus et al. [27•]	Estonia	Cross-sectional	717 (343)*	BCG	+
Mommers et al. [28]	Netherlands / Germany	Case-control	572 (75)*	BCG	–
Cooper et al. [29]	Ecuador	Cross-sectional	1002 (959)*	BCG	–
Townley et al. [30•]	Turkey / Thailand / Argentina	Cross-sectional	1704 (655)*	BCG	+
Da Cunha et al. [31•]	Brazil	Cross-sectional	1612 (1089)*	BCG	+
Obihara et al. [32••]	South Africa	Cross-sectional	337	MTB	+
Bibakis et al. [33•]	Greece	Cross-sectional	806	NTM	–
Martignon et al. [34•]	France	Cross-sectional	718 (647)*	BCG	+

*Number in parentheses indicates participants in whom the association between mycobacterial exposure and atopic symptom was analyzed. BCG—bacille Calmette-Guérin; MTB—*Mycobacterium tuberculosis*; NTM—nontuberculous mycobacteria.

increasingly skewed toward Th1 in nonatopic but not in atopic individuals [12]. Table 1 summarizes the 23 studies on the relationship between mycobacterial exposure and atopic disease in children, with the major results [10,13–26,27•,28,29,30•,31•,32••,33•,34•].

In 21 studies, the inverse relationship between bacille Calmette-Guérin (BCG) immunization and atopic disease was analyzed. In eight of these studies, a significant inverse relationship was observed between *Mycobacterium bovis*–BCG immunization and one or more atopic disease outcome variables [10,13–26,27•,28,29,30•,31•,34•]. Shirakawa et al. [10] observed lower prevalence of atopic symptoms, sensitization to common environmental aeroallergens, higher serum interferon (IFN)- γ , and lower serum interleukin (IL)-4 levels in BCG-immunized children with positive tuberculin skin test (TST) reactivity than in BCG-immunized children with negative TST. Aaby et al. [17]

observed a lower frequency of positive skin prick test (SPT) to house dust mite in children from Guinea Bissau with documented BCG immunization records than in those without. In a German birth cohort study, only a weak inverse relation was observed between BCG immunization in infancy and the prevalence of atopic disease in children [24], while in an Australian birth cohort BCG immunization was associated with less prevalence of symptoms of asthma only in children with a positive family history of atopic disease [26]. Annus et al. [27•] reported lower prevalence of allergic symptoms in BCG-immunized Estonian children with positive TST compared with those who had negative TST. A significant inverse relation between TST reactivity and allergic history was observed in BCG-immunized children from Turkey and Thailand, but not in BCG-immunized children from Argentina [30•]. Da Cunha et al. [31•] observed a lower risk of asthma only in

Brazilian children with neonatal BCG scar and a past history of allergic rhinitis, but not in those without a history of allergic rhinitis. In French children, Martignon et al. [34] observed that a positive BCG immunization history was associated with fewer atopic disease symptoms than a negative BCG immunization history.

Only two of the 23 studies analyzed the inverse relationship between exposure to wild-type *Mycobacterium tuberculosis* and atopic disease. Pahari et al. [19] did not observe an inverse relationship between positive TST reactivity and asthmatic symptoms in schoolchildren from the United Kingdom. In contrast, we observed a significant inverse relationship between positive TST reactivity and both atopic rhinitis symptoms and positive SPT for common aeroallergens in South African children from an area with a very high tuberculosis (TB) burden [32••].

Two of the 23 studies analyzed the relationship between nontuberculous *Mycobacteria* (NTM) and atopic disease, and neither observed any relationship [14,33•].

Despite the consistent evidence from mice models that exposure to *Mycobacteria* ameliorates atopic reactivity and symptoms, the epidemiologic evidence in humans is still far from conclusive. In the following paragraphs we discuss the factors that may explain these differences in findings.

Host Defense Against Mycobacterial Infection

An efficient Th1 immune response is required for the elimination of *M. tuberculosis* and deficiency or mutations of genes involved in Th1 responses leads to extreme vulnerability to mycobacterial infection in laboratory animals and in humans [35]. Activated alveolar macrophages infected with *M. tuberculosis* interact with T lymphocytes via a cytokine network, involving among others IL-12 and IL-18, which stimulate T lymphocytes (principally CD4⁺ T lymphocytes) to release IFN- γ [36]. In animal experiments IFN- γ stimulates the phagocytosis of *M. tuberculosis* in the macrophage, and the release of tumor necrosis factor (TNF)- α , important for granuloma formation and control of the extent of disease [37]. Failure of the host immune response to contain replication of *M. tuberculosis* during initial infection results eventually in active disease, characterized by uncontrolled bacillary proliferation, dissemination of organisms to distal sites, and development of severe symptoms. Alternatively, if the infected host is immune-competent, the host immune system typically will resolve or control the initial infection, using mechanisms that prevent bacterial spread, limit bacterial dissemination, and concentrate immune response mechanisms directly to sites of infection [38].

Activation of cell-mediated immunity against *M. tuberculosis* is associated with the development of a cutaneous delayed-type hypersensitivity (DTH) reaction to tuberculin; these individuals are latent carriers of *M. tuberculosis* bacilli (latent tuberculosis infection), but they do not exhibit overt signs and symptoms of disease,

and are not infectious [38,39]. The TST becomes positive as early as 3 to 6 weeks after initial *M. tuberculosis* infection and may remain positive for the lifetime of the individual [39]. Until the time it will be replaced by more sensitive tests, a positive TST remains an accepted hallmark of infection with *M. tuberculosis*.

Immune System, *Mycobacteria*, and Atopy

The initial step in a chain of events that results in effective host defense against infection with *M. tuberculosis* is innate recognition of mycobacterial products or antigens (Ag). The innate immune system mounts a rapid nonspecific protective response in the early phase of infection and instructs the adaptive T cell-mediated specific immune response [40]. This is achieved through the detection of mycobacterial Ag by Toll-like receptors (TLRs), a family of proteins with a critical role in the detection of, and response to, several pathogens, including *Mycobacteria*. TLR signaling forms an essential link between innate immune system, mainly antigen-presenting cells (APC), such as dendritic cells (DC) or macrophages, and adaptive immune system, mainly T cells or natural killer (NK) cells. Activation of APC is involved in T-cell differentiation and modification of T-cell polarization (especially Th1/Th2 balance), in addition to influencing a variety of immune disorders, including atopy [41•]. TLR signaling can induce the production of inflammatory cytokines and recruitment of various inflammatory cells into the infection site. These activated cells (macrophages or neutrophils) ingest invading pathogens through pattern recognition receptors (PRR) and kill them by producing nitric oxide, reactive oxygen radicals, or defensins [41•]. TLR-activated APCs enhance expression of surface molecules and activate CD4⁺ T cells to differentiate into Th1 or Th2 cells (Fig. 1). Th1 and Th2 cells are not distinct CD4⁺ T-cell subsets, but they simply represent polarized forms of the heterogeneous T cell-mediated immune response [42•]. The two types of immune response can be distinguished on the basis of the cytokines involved; Th1 cells produce IFN- γ , IL-2, and TNF- β and Th2 cells produce IL-4, IL-5, IL-9, and IL-13. These cytokines are mainly responsible for driving further T-cell differentiation. Th1 cells are paramount in the defense against invading intracellular microorganisms, such as *Mycobacteria* and viruses. Allergen-specific Th2 cells, on the other hand, initiate the cascade of reactions involved in the development of atopic disorders. These cells are formed from naive T cells when the APCs are activated in an IL-4-dominated microenvironment [42•]. Th2 cells produce cytokines, which mediate diverse regulatory and effector functions, including stimulating B cells to produce allergen-specific immunoglobulin E (IgE), eosinophil production and recruitment, mucus production, smooth muscle contraction, and tissue homing of Th2 cells [42•]. In addition, Th2 cells cause, through a

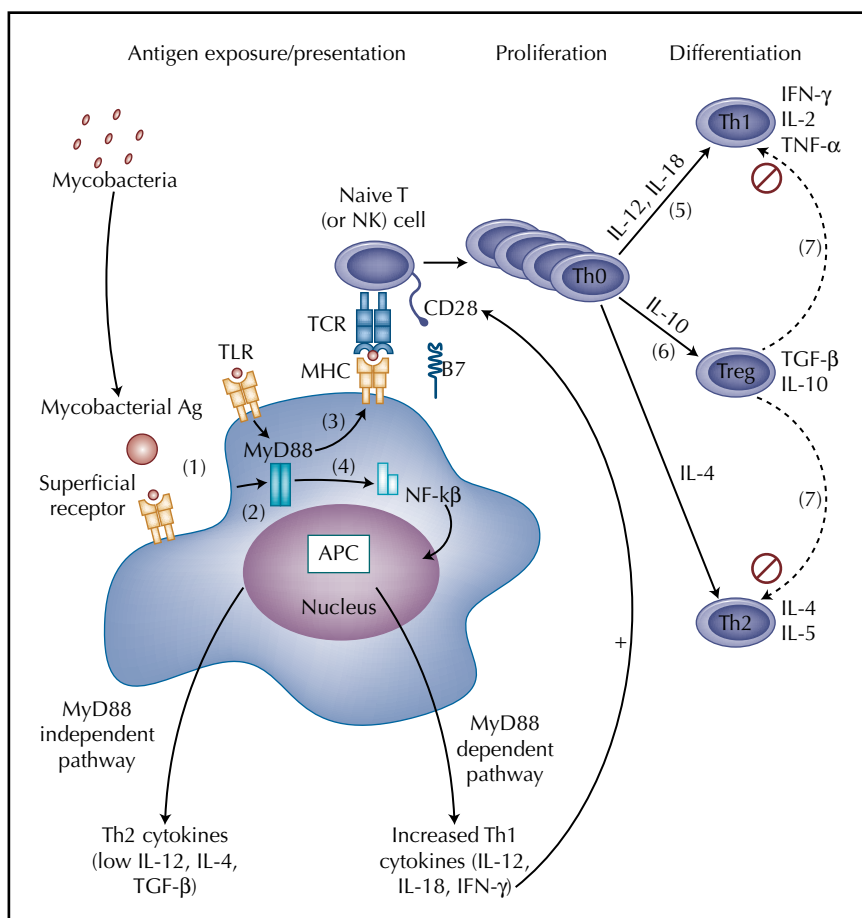


Figure 1. (1) Mycobacterial antigen (Ag) binds with a superficial receptor (mannose, complement, or scavenger receptors) of antigen-presenting cells (APC; dendritic cells [DC]). (2) Toll-like receptors (TLR) recognize Ag through association with pathogen-associated molecular patterns (PAMP) located on cell surface or in phagosomes. (3) Ag fragments are processed and presented to T cells by major histocompatibility complex (MHC) class II molecules, which then are activated. (4) TLR-MyD88-dependent signaling via nuclear factor (NF)- κ B results in strong upregulation of T helper cell (Th1) cytokines, with effects on both immune defense by innate immunity and T-cell proliferation and differentiation. (5) Activated T cells and natural killer (NK) cells in turn stimulate APCs to secrete Th1 cytokines through production of interferon (IFN)- γ . (6) We hypothesize that a very high mycobacterial burden (eg, in high TB-endemic areas), with strong Th1 stimulation, may result in microenvironment changes or accumulation of some yet unknown molecules, which together stimulate cells of innate (DC) and adaptive immunity to induce production of regulatory T cells (Treg). (7) Under high infection pressure, Treg are stimulated to produce anti-inflammatory cytokines (interleukin [IL]-10 and transforming growth factor [TGF]- β), which ensure that inflammatory cells (both Th1 and Th2) are downregulated. In addition, Treg may influence T-cell proliferation. (Data from Yazdanbakhsh et al. [43], Doherty and Arditi [44], Branger et al. [45], Romagnani [46], Bluestone and Abbas [49], Mills [50], and Taylor et al. [51].)

complex cytokine and mediator network, basophil activation resulting in the protection against extracellular organisms, such as parasites [43].

Although some TLR agonists have been shown to be able to provoke Th2 immune responses in certain conditions, the majority stimulate APCs to stimulate Th1-skewed immune responses (Fig. 1). Two members of the TLR family, TLR2 and TLR4, have been demonstrated to mediate *M. tuberculosis*-induced intracellular signaling [44]. In murine models, TLR4 mutant mice were more susceptible to *M. tuberculosis* infection than wild-type mice, suggesting that TLR4 plays a protective role in the host defense against *M. tuberculosis* infection [45].

Missing immune deviation from Th2 to Th1, due to decreased production of Th1 cytokines, is thought to be the result of reduced stimulation of TLRs in westernized society [46]. Although the Th1/Th2 paradigm is a useful model to explain the rising incidence of atopic disorders, it has been challenged on a few important points, such as the existence of a “window of opportunity” in infancy (the first 5 years) in which infection-driven protection from atopy is supposed to occur [47]. If this window of opportunity existed, how can the observation be explained that adult migrants from countries with a

low prevalence of atopy develop atopic disease when they move to a country with a westernized lifestyle? It seems to suggest that abrogation of protection from atopic disease offered by persistent immune stimulation in the presence of infectious agents can still result in development of atopic inflammation in genetically predisposed individuals, even after such a childhood “window of opportunity.” It has also been shown that the remission of atopic disease can be induced by infections acquired after childhood. These observations support the suggestion that a more complex immune mechanism is involved in the protection against atopy. Moreover, if both T-cell types were mutually exclusive or strictly downregulated each other, as initially suggested by the Th1/Th2 paradigm, then the hygiene hypothesis could not logically explain the rising incidences of both Th1-mediated autoimmune diseases and Th2-mediated atopic diseases globally [48].

Recent evidence suggests the importance of reduced immune suppression rather than erratic immune deviation to explain the hygiene hypothesis. According to this view, lower microbial burden does not act by inducing a lower production of Th1-cytokines but by decreasing the activity of regulatory T cells (Treg) [6,43,47]. CD4⁺ CD25⁺ T cells represent most Treg cell subsets. Removal of this

T cell subset from normal mice leads to the spontaneous development of various autoimmune disorders, and the number of CD4⁺ CD25⁺ T cells appears to be reduced in different autoimmune diseases in humans [49]. The mechanism of suppression by Treg is mediated through the production of inhibitory or anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)- β [43,50••]. Treg cells selectively express several TLRs, and can suppress both Th1 and Th2 responses (Fig. 1).

There are indications that anergy, peripheral tolerance, and active immune suppression are related to different stages of Treg cell-mediated immune deviation. Regulatory CD4⁺ CD25⁺ T cells normally inhibit Th2 cytokine expression and proliferation of peripheral blood mononuclear cells (PBMC) from nonatopic individuals in response to allergen [51•]. There is evidence that exposure to bacterial endotoxin or lipopolysaccharide (LPS) enhances the suppressive function of Treg [52]. The higher level of endotoxin, increased blood level of CD14 (another LPS receptor), and TLR2 in European children from a farming environment compared with those from a nonfarming environment was associated with a lower prevalence of atopic diseases. It has been shown recently that the administration of TLR2 or TLR4 agonists led to reduced atopic reaction after sensitization with an allergen [53•].

One important clinical and epidemiologic implication of the revised immunological model of the hygiene hypothesis is that the interaction between infection and allergens is not limited to the window of opportunity in early life, but retains some flexibility well into adulthood. Secondly, the role of pathogens that cause chronic and persistent infection pressure is highlighted [43,47].

Dose-dependent Effect of Mycobacterial Exposure on Atopy

The frequency and persistence of infection with pathogens (infection pressure) in populations may be important in the protection against atopy, although the mechanisms involved are not known. Pathogens that cause chronic infections might have evolved strategies to subvert the host immune responses, by stimulating increased production of anti-inflammatory or immunosuppressive mediators, which normally function to control or terminate the protective immune effector-responses of the host [50••]. This is achieved either through the direct induction of host immunosuppressive cytokines such as IL-10 and TGF- β , which are produced by innate immune cells in response to pathogen-derived antigens, or through the production of Treg. Ag-specific Tregs have been described in several chronic infections including those by *M. tuberculosis* or *Mycobacterium vaccae* [6,43,47]. 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 [54]. Is there any epidemio-

logical evidence to support this suggestion? The Japanese children studied by Shirakawa et al. [10] received BCG immunization three times: at birth, and at the ages of 6 and 12 years. These children had a high prevalence of positive TST (58%), and the lowest risk of atopic disease was observed in the group of children who remained TST-positive at 6 and 12 years. Moreover, the possible influence of natural *M. tuberculosis* infection on the result was suggested. This contrasts with the majority of other studies on BCG immunization and atopy, in which children received BCG immunization at a single occasion in the neonatal period [13–16,18,22,23,28]. The lack of TST measurement in some studies makes it difficult to correct for the possible additive influence of other mycobacterial organisms in the environment on the results [17,27•]. Two studies on the effect of natural *M. tuberculosis* infection on atopy also differed with respect to persistence and duration (burden) of exposure. In a study from the United Kingdom, the children investigated only had a recent history of *M. tuberculosis* exposure (short and intermittent exposure), after their teacher was diagnosed with pulmonary TB [19]. In these children, there was no inverse relationship observed between TST reactivity and atopic symptoms. In contrast, recent studies in South African children, from a very high TB-endemic area (long and persistent exposure), observed a significant inverse relation between TST reactivity and different atopic disease outcome variables [32••,55••]. In these studies previous BCG immunization or NTM did not seem to influence the TST reactivity. This difference in findings leads to the following fundamental questions. 1) Could the effect of *M. tuberculosis* on atopy be dose-dependent? 2) If this were to be so, what are the possible mechanisms involved?

There are indications that triggering the innate immune system through endotoxin exposure and the derived effect (increased or decreased atopic inflammation) may depend not only on the exposure but also on dosage of endotoxin, timing of exposure, other environmental factors, and genetic predisposition. In line with this observation, it has been proposed that the difference in the dosage 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 [47]. In addition, the *Mycobacterium* species may be equally important. For instance, there are suggestions that BCG immunization may not be able to mimic the persistent immune stimulation provided by frequent exposure to pathogenic and nonpathogenic *Mycobacteria*. This is supported by studies showing that *M. bovis*-BCG is a poorer inducer of IL-12 than natural *M. tuberculosis* infection [56]. The size of TST induration has been directly correlated with serum levels of IFN- γ , implying that a large TST size may reflect strong Th1 immunity [57]. A very strong TST response is more frequently associated with *M. tuberculosis* infection than with *M. bovis*-BCG or NTM infection [10,32]. Although BCG immunization may cause a transiently

reactive TST, African children who received BCG immunization as infants have a weak or nonreactive TST at 5 years of age, and BCG immunization had little impact on the interpretation of TST in children during an investigation tracing TB contact [58].

Population differences in the efficacy of BCG vaccine strains and the known regional differences in vaccine efficacy may also contribute to the heterogeneity of study results, because this may lead to variability in immune response [59]. The effect of variability in the efficacy of BCG immunization against TB in relation to the immune stimulation required to suppress the development of atopic inflammation is still not known.

Challenges for Future Prevention of Atopy: *Mycobacteria*-based Vaccines

An important characteristic of atopic diseases is a pathological Th2 response, with overt reaction to environmental factors that are normally innocuous to nonatopic individuals. Among the important basic requirements for *Mycobacterium*-based vaccine for the prevention of atopy is that it must be 1) safe, 2) capable of effectively inducing Th1 response and stimulating immune memory in the host, and 3) consistent in its effectivity. BCG is still the most widely used Th1-stimulating vaccine, known to effectively protect against serious systemic manifestations of TB in childhood, such as miliary TB and TB meningitis. Experimental studies have shown that prior *M. bovis*-BCG vaccination could prevent the development of atopic responses in the airway [8]. Despite these results, the effect of BCG vaccines in protection against atopy in humans is still inconclusive. Recently, genetically modified *M. bovis*-BCG or recombinant BCG (rBCG) vaccines or BCG polysaccharide nucleotide has been shown to reduce atopic disease symptoms [60]. Other mycobacterial-based vaccine candidates thus far are *M. vaccae* and *M. tuberculosis* subunit protein vaccines. Animal studies have shown that prior exposure to *M. vaccae* prevented the development of atopic reaction [7]. This effect was assumed to be due to the strong Th1 immune stimulatory property of the vaccine. Zuany-Amorim et al. [6] showed the suppression of atopic reaction after infection with *M. vaccae*. This was attributed to the stimulation of Tregs and the subsequent effect of the anti-inflammatory cytokines, IL-10 and TGF- β [6]. Comparable results were not observed in other studies [61]. Although promising, largely powered clinical trials are needed to confirm these findings before the vaccines can be applied to the general population.

Janssen et al. [62] explained this discrepancy by arguing that it might not be the Th1-inducing properties of *Mycobacteria*, but simultaneous exposure to both *Mycobacteria* and allergen, which induced an allergen-specific Th1 response strong enough to prevent induction of atopy. When mice with recombinant *Mycobacteria* were challenged with a peptide of Der p I (a major Ag

from house dust mite), a switch was observed from a Th2 to a Th1 profile, which was specific only for the recombinant vaccines expressing allergen peptide. The implication of this observation is that priming of allergen-specific Th1 responses may be crucial in establishing a memory response capable of long-term protection against allergen-specific Th2 response [62]. In support of this, Sano et al. [63] demonstrated in an experimental model that simultaneous *M. tuberculosis* infection and allergen challenge might stimulate a Th1 response strong enough to suppress Th2 activity significantly. They also observed that stimulation of CD4⁺ T cells with ovalbumin (OVA) induced IL-4-secreting Th2-dominant responses, whereas concomitant stimulation with both OVA and *M. tuberculosis* induced Ag-specific Th1 responses, with secretion of IFN- γ . This effect was mediated by IL-12. If this phenomenon, where both *M. tuberculosis* and allergens are simultaneously presented by APC to the T-cell, also applies to humans, then it is most likely to occur where *M. tuberculosis* infection and allergen exposure are both highly prevalent [32,55]. In such a setting, exposures to possible allergens and *M. tuberculosis* would be more likely to occur simultaneously than in a low-prevalence setting. These data indicate that future recombinant mycobacterial vaccine candidates may have to be primed simultaneously with allergens to achieve allergen-specific Th1 effect strong enough to prevent the development of atopic disease and establish enough memory response for long-term protection.

Conclusions

On the basis of the mainly associative and indirect experimental and epidemiological evidence presented in this paper, we believe that it is biologically plausible that *Mycobacteria* (or one or more of their components or products) might be able to protect against the development of atopic disease. Although the exact mechanisms involved in the protective effect are not yet known, we expect that the recent discovery of immune mechanisms (both innate and acquired) involved in the defense against *Mycobacteria* would shed more light on this immune mechanism in the near future. There are suggestions that the level of protection may be dose-dependent or related to the prevalence of both mycobacterial and environmental allergens in the population. If this were true, it might partly explain the differences in prevalence of atopic disease between populations in industrialized and in non-industrialized countries. The *Mycobacterium* species and its associated immunogenicity may also be important, especially in view of future attempts to develop mycobacterial-based vaccines against atopic disease. Future studies could make use of existing situations by studying populations with both a high prevalence of *Mycobacteria* and environmental allergens. Such studies should be population-based, and prospective or longitudinal in design.

Another scenario would be randomized controlled clinical trials mimicking the real-life situation by persistently and prospectively exposing a large group of children and adults to mycobacterial and allergen-specific antigens over a long period of time while monitoring atopic disease outcome variables.

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Papers of particular interest, published recently, have been highlighted as:

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