

Immunotherapy with mycobacteria

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Purpose of review

To summarize and evaluate critically recent progress with mycobacteria as a potential novel disease modifying treatment strategy in asthma.

Recent findings

The link between exposure to pathogenic or saprophytic mycobacteria and protection from allergic diseases is still controversial, and recent epidemiological studies, which addressed only exposure to *Mycobacterium tuberculosis* or bacillus Calmette–Guérin, did not help to clarify this issue. Moreover, the clear efficacy of mycobacterial treatment seen in animal models has not been reproduced in human asthma, and a recent small study testing the hypothesis that heat-killed *Mycobacterium vaccae* attenuates asthmatic reactions after allergen challenge did not provide convincing results. However, it has been shown that treatment of mice with *M. vaccae* induces the generation of allergen-specific T regulatory cells capable of suppressing allergen-mediated eosinophilic lung inflammation, suggesting that a general deficiency of T regulatory cell activity might be responsible for the increased prevalence of asthma. This hypothesis is supported by findings that a lack of T regulatory cells, as found in genetic disorders of man and mouse attributable to a mutation of *Foxp3*, a transcription factor specifically expressed by T regulatory cells, is associated with manifestations of severe atopy and autoimmunity, precisely the spectrum of diseases linked to the hygiene hypothesis.

Summary

Further studies on the relationship between mycobacteria and atopic disorders are needed, but there is reason to believe that the novel findings and molecular mechanisms associated with mycobacterial infections will further strengthen the currently unproved therapeutic value of immunotherapy with mycobacteria.

Keywords

asthma, hygiene hypothesis, mycobacteria, T regulatory cells

Abbreviations

BCG	bacillus Calmette–Guérin
TGF- β	transforming growth factor beta
Th	T helper
TLR	Toll-like receptor

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Introduction

Despite the introduction of increasingly potent and effective anti-symptomatic drugs, the prevalence of atopic diseases such as asthma and allergic rhinitis has substantially increased over the past few decades. A clear medical need exists, therefore, for new disease-modifying therapies that prevent or reverse the chronic, predominantly T helper (Th) type 2 cell-driven, allergic inflammation. Immunotherapy, through its capacity to produce a long-term, antigen-specific and protective immune response, is the only aetiological treatment that offers the possibility to prevent or even cure atopic diseases [1]. However, the potential of severe side-effects associated with conventional immunotherapy limits its widespread use. Novel strategies to minimize side-effects and improve the efficacy of immunotherapy or alternative approaches such as mycobacterial vaccines are thus of considerable interest in the treatment of atopic diseases.

The explanation for the increased prevalence of atopic diseases that has attracted the most attention is the 'hygiene hypothesis', which suggests that the increase in allergic diseases is caused by a cleaner environment and fewer childhood infections [2–4,5•,6•]. Numerous epidemiological studies provide support for this hypothesis, suggesting that a relatively clean environment, clean food and the use of antibiotics in early life deprives the developing immune system of the microbial antigens that stimulate Th1 cells and thereby favours the development of Th2-driven responses [7–12]. For example, recent studies have demonstrated that exposure to markers of poor hygiene such as hepatitis A, *Toxoplasma gondii* or *Helicobacter pylori* and infection with herpes simplex virus was associated with less asthma, hay fever and allergen sensitization [13–15]. Moreover, an inverse relationship between endotoxin levels in bedding and the incidence of atopic diseases among children living in rural areas was recently reported, and children with a high vaccination coverage seem to be better protected against the development of atopy in the first years of life [16•,17]. Similarly, exposure to *Mycobacterium tuberculosis* or to environmental mycobac-

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teria were proposed to protect from atopy, introducing the concept that the administration of mycobacteria and their products may therefore be used as vaccines aimed at inhibiting Th2 responses [18,19•].

Mycobacteria and the hygiene hypothesis

What is the epidemiological evidence for mycobacteria protecting from allergic diseases? In a survey among 867 Japanese children all immunized with bacillus Calmette–Guérin (BCG), responders to tuberculin had lower levels of total IgE and Th2 cytokines, higher serum levels of IFN- γ and a lower prevalence of atopy and atopic diseases when compared with tuberculin non-responders [20]. However, most subsequent studies failed to confirm this inverse association between tuberculin responses and allergic diseases and total IgE levels [19•]. Such studies included recent reports on a population of 507 children living in Gambia with no significant difference between children with or without a BCG scar with regard to the prevalence of positive immediate skin test responses to locally relevant allergens and the serum levels of total IgE [21]. An Australian study testing the hypothesis that neonatal BCG vaccination is associated with a reduced prevalence of allergic sensitization, asthma, eczema and hay fever during childhood [22] also failed to demonstrate a significant change in the rate of allergic sensitization between vaccinated and non-vaccinated individuals. Similarly, a study in asthmatic children in Turkey [23] was also not able to demonstrate an inverse association between tuberculin reactivity and atopy, adding further doubts about the role of *M. tuberculosis* and BCG in the protection from atopy and the concept that Th1-inducing, mycobacteria-based vaccines may protect from developing allergic diseases.

Should we therefore discard the hypothesis that mycobacteria contribute to the prevention of atopy? Probably not. Such studies involving tuberculin tests and BCG scars address only the role of the *M. tuberculosis* group of organisms. However, these are surprisingly potent inducers of mixed Th1 plus Th2 responses, rather than pure Th1. Tuberculosis patients thus have raised levels of IgE and IgE antibody to mycobacteria and an increased expression of IL-4 by both CD4 and CD8 T cells [24–26]. The hygiene hypothesis is more focused on the more than 80 environmental saprophytes, almost all of which belong to the taxonomically very different ‘fast growing’ subgenus of the mycobacteria [27]. These are common in mud and untreated water, and unlike *M. tuberculosis*, they were present throughout human evolution.

Multiple studies in animal models of allergic inflammation have demonstrated that treatments with either live or dead mycobacteria are capable of inhibiting allergen-

induced lung inflammatory responses. For example, it has been shown that BCG can reduce the formation of specific IgE, eosinophilia and bronchial hyperresponsiveness induced by allergen with an increase in IFN- γ production [28–30]. Similarly, heat-killed BCG, *Mycobacterium vaccae* and purified protein derivative from *M. tuberculosis* have all been shown to suppress allergic and asthma-like responses in mice [31–35,36•]. Furthermore, a recent study [37] demonstrated that treatment of newborn mice with heat-killed *M. vaccae* significantly reduced the number of goblet cells, the thickness of the basement membrane and subepithelial smooth muscle after subsequent allergen sensitization and challenge. In all these studies, the exposure to Th1-inducing mycobacterial infections was suggested to cause a shift from Th2 to Th1 immune responses, which subsequently prevent the development of allergy. However, more recent studies indicated that the immunological processes behind the inhibitory effects of *M. vaccae*, a representative of the saprophytic environmental mycobacteria found in mud, might be much more complex than is predicted by the Th1–Th2 paradigm and an additional non-Th1–Th2 immunological regulatory mechanism may play a much more prominent role. This is supported by the finding that *M. vaccae* induced its inhibitory effect on allergen-induced eosinophilic lung inflammation by an IFN- γ independent mechanism, indicating that the inhibitory effect of this mycobacterium is not caused by a redirection from a predominantly Th2- to a Th1-dominated immune response [36•]. This is also consistent with the fact that Th1-mediated autoimmune diseases such as type 1 diabetes, multiple sclerosis and inflammatory bowel disease are increasing in prevalence in parallel with allergic diseases in industrialized countries [38••]. In accordance with the hygiene hypothesis, it has also been consistently observed that autoimmune diseases in susceptible strains of mice or rats develop earlier and at a higher rate among animals bred in a specific pathogen-free environment than among animals bred in a conventional environment. Furthermore, the frequency of atopic diseases is increased in patients with diabetes and rheumatoid arthritis, suggesting a common mechanism underlying infection-mediated protection against autoimmunity and allergy [38••,39–41]. Moreover, some of these autoimmune diseases can also benefit from treatment with mycobacteria. For example, diabetes is prevented in non-obese diabetic mice by infecting the young mice with mycobacteria, a treatment which also protects against experimental allergic encephalomyelitis [42–44]. The protection against these conditions can be transferred to uninfected animals by CD4 T cells. This raised the question of whether mycobacteria modulate inflammatory responses via effects on regulatory T cells, which have been shown to display anti-inflammatory properties.

Exposure to *Mycobacterium vaccae* induces T regulatory cells

In a recent study, experiments were designed to determine whether treatment of mice with SRP299, a killed *M. vaccae* suspension, could induce the generation of regulatory cells that would subsequently suppress the allergen-mediated inflammatory response. We were able to demonstrate that treatment of mice with heat-killed *M. vaccae* before immunization by ovalbumin confers long-term protection against airway inflammation and gives rise to CD4 regulatory T cells [45••]. Transfer of these cells to ovalbumin-immunized mice before antigen challenge reduced airway eosinophilia but failed to confer protection against cockroach antigen-induced inflammation. This specific inhibition was shown to reside within the CD45RB^{low} subset, and be mediated through IL-10 and transforming growth factor beta (TGF- β), as anti-IL-10 and anti-TGF- β antibody treatments completely reversed the inhibitory effect of CD4⁺CD45RB^{low} T cells. Taken together, the data suggest that *M. vaccae* stimulates an anti-inflammatory network that inhibits both Th1 and Th2 cells and therefore explains recent epidemiological reports that not only Th2- but also Th1-mediated diseases are on the increase.

Further support for the view that the simultaneous increases in the incidence of inflammatory bowel disease, allergies and autoimmunity might stem from a common cause and be linked to T regulatory cells has come from recent analyses of genetic disorders of man and mouse attributable to mutations in the transcription factor Foxp3 [46••,47•,48–50]. Foxp3 is specifically expressed by T regulatory cells and programmes their development and function. The human genetic disease X-linked autoimmunity-allergic dysregulation syndrome is caused by a mutation in the human homologue of Foxp3 and is characterized by global immune dysregulation with autoimmune endocrinopathy and manifestations of severe atopy, including eczema, food allergy and eosinophilic inflammation, precisely the spectrum of diseases associated with the hygiene hypothesis [48–50]. The phenotype of the so-called scurfy mouse, which has a mutation in Foxp3, and the Foxp3-deficient mouse is very similar to the human syndrome. T regulatory cells are absent in both mice, and the disease phenotype was prevented by the transfer of CD4⁺CD25⁺ T regulatory cells. Therefore a failure in the maturation of regulatory T cells is a logical explanation for the epidemiological findings of an increased prevalence of allergic diseases, some autoimmune disorders and inflammatory bowel disease.

Immunotherapy with mycobacteria

Is there evidence that bacterial products associated with the hygiene hypothesis might be used as immunother-

apeutic agents to prevent or treat atopic diseases? In this regard, it has been reported that the administration of probiotics significantly decreased the incidence of atopic dermatitis and improved the skin lesions [51,52]. Clinical studies with *M. vaccae* treatment in adults with asthma and rhinitis or in children with atopic dermatitis demonstrated clinical benefits, as measured by a reduction in use of rescue medication, the severity of disease or inhibition of the allergen-induced late phase response [53,54]. In contrast, however, another study using lower doses of *M. vaccae* in asthmatic patients [55] did not show any beneficial effects. All patients in that study were prescribed inhaled glucocorticosteroid, and it is possible that the induction and possible effector function of regulatory T cells might be suppressed by this treatment. A recent double-blind, randomized study testing the hypothesis that heat-killed *M. vaccae* attenuates asthmatic reactions that occur after allergen challenge also did not provide convincing results, and showed only a trend towards significance for the effects of heat-killed *M. vaccae* on allergen-induced airway responses, although this was tested at only a single early timepoint, 3 weeks after a single dose of immunotherapy in 12 patients [56•]. A single intradermal injection in allergic asthmatic patients had a small effect on the allergen-induced late phase reaction and a tendency for reduced IL-5 synthesis by T cells stimulated with allergen *in vitro*. Similarly, immunotherapy with *Dermaphagoides pteronyssinus* in conjunction with BCG did not alter the non-specific hyperreactivity to methacholine, the ratio of forced expiratory volume to forced vital capacity and *D. pteronyssinus*-specific IgE before and one month after immunotherapy in *D. pteronyssinus*-sensitive allergic asthmatic patients [57]. The clear efficacy of mycobacterial treatment seen in animal models has thus not been reproduced in human asthma. On the other hand, and in line with clinical studies, a recent study investigating the therapeutic potential of heat-killed *M. vaccae* in two mouse models of allergic asthma [58] failed to show any reduction in airway hyperresponsiveness, lung eosinophilia, IgE or IL-5 production after an allergen challenge, suggesting that the therapeutic effect is much smaller compared with the preventive effect by administering the compound before sensitization. However, these data are in contrast to other studies demonstrating a similar efficacy by using *M. vaccae* as preventive or therapeutic treatment [36•]. The reasons for this discrepancy are currently unclear but are most likely caused by the different protocol used, particularly with regard to the temporal relationship between mycobacteria treatments and allergen exposure. Indeed, it has been demonstrated that allergen exposure is absolutely required to generate allergen-specific regulatory T cells, and one of the critical elements determining the successful induction of allergen-specific T regulatory cells in clinical studies might be the level and duration of

natural exposure to allergens during the treatment period with mycobacteria [36•,45••]. However, no data are available so far as to whether treatment with mycobacteria induces the generation of allergen-specific T regulatory cells. On the other hand, thymic-derived regulatory T cells (CD4⁺CD25⁺) have recently been identified and appear to resemble closely their murine counterparts in all their functional properties *in vitro* [59–61]. Moreover, a recent study indicated that regulatory CD4⁺CD25⁺ T cells are present and functional in most atopic patients with allergic rhinitis, and are able to inhibit Th1 as well as Th2 cytokine production [62]. Furthermore, grass pollen immunotherapy has been shown to result in a population of circulating T cells that express the IL-10⁺CD4⁺CD25⁺ phenotype in response to allergen restimulation [63•]. Similarly, IL-10 and TGF- β have been shown to cooperate in the suppression of the immune response to aeroallergens and to control allergic inflammation caused by mucosal allergen exposure in healthy individuals as well as in the specific immunotherapy of house dust mite allergic patients [64•]. This antigen-specific suppressive activity after specific immunotherapy was again confined to the CD4⁺CD25⁺ regulatory T cells.

Conclusion

Taken together, it is reasonable to propose that a general deficiency of regulatory T cell activity might be responsible for the increased prevalence of asthma, and potentially, other diseases that involve immunodysregulation. However, it remains to be elucidated whether it will be possible to use mycobacteria selectively to induce allergen-specific T regulatory cells, which will subsequently lead to the inhibition of allergic disorders in humans. Novel insights into the mechanisms of how mycobacteria exert their anti-inflammatory effect may lead to a better understanding of the immunoregulatory network, and subsequently better designs of clinical studies. There has been intense study over the past year on the interaction between mycobacteria and the innate immune system, and several molecules known to be associated with asthma have been shown to play a crucial role in this interaction. For example, mycobacteria contain multiple ligands that trigger Toll-like receptors (TLRs), in particular TLR2. Blood cells from the children of farmers express significantly higher amounts of messenger RNA encoding TLR2 than do those from non-farmer children, linking the hygiene hypothesis and a reduction in atopic diseases with TLRs [65,66,67••,68••]. Moreover, the natural bacterial-resistant associated macrophage protein-1 (Nrampl) has been proposed as a candidate gene for the susceptibility to autoimmune diseases as well as mycobacterial infections [69,70•,71]. Interestingly, Nrampl-resistant mice after an allergen challenge produced significantly lower levels of Th2 cytokines and total and specific IgE compared with

Nrampl sensitive mice, thereby linking the hygiene hypothesis with genes controlling antibacterial resistance [72•].

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