

Role of bacillus Calmette–Guérin as an immunomodulator for the prevention and treatment of allergy and asthma

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Purpose of review

As an essential part of the hygiene hypothesis, the association between exposure to mycobacterial components and the prevention, development and severity of atopic diseases has not been fully understood. The current status on the causal–effect link of this relationship and the potential use of mycobacterial adjuvants as a preventive or disease-modifying modality in allergic diseases is reviewed in this article.

Recent findings

Data obtained from human and animal models indicate a discrepancy regarding the preventive and therapeutic effect of bacillus Calmette–Guérin in atopic diseases. Among the issues that require clarification include whether the distinction in T helper type 1/2 cells described in mice can be fully extrapolated to humans. Other factors involved could be caused by genetic variation, optimal timing, dose, route of delivery as well as environmental factors, which affect the degree of natural exposure to pathogenic or saprophytic mycobacteria.

Summary

Most of the evidence available to date suggests a need for an improved mycobacterial vaccine administered early in life, by means of alternative routes, preferentially mucosal. As switching away from the T helper type 2 immune response by inducing T helper type 1 is unable to explain the underlying mechanisms of action of mycobacterial antigens, it may be worthwhile to investigate whether T regulatory cells are induced in response to different mycobacterial adjuvants.

Keywords

allergy, asthma, atopy, bacillus Calmette–Guérin, IgE, mycobacteria, T cells, toll-like receptors

Abbreviations

BAL	bronchoalveolar lavage
BCG	bacillus Calmette–Guérin
PBMC	peripheral blood mononuclear cell
SLIT	sublingual immunotherapy
TGF-β	transforming growth factor beta
Th	T helper
TLR	Toll-like receptor
T_{Reg}	T regulatory

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Introduction

The prevalence of allergic asthma and other atopic disorders has increased dramatically to almost epidemic proportions in the past few decades, especially in industrialized countries [1,2]. This increase cannot solely be explained by genetic factors, because substantial shifts in the genome do not occur over a few generations. Changes in diagnostic procedures, or exposure to conventional etiological factors of asthma and atopy appear to be unable to explain most of this increase. An attractive explanation for this increase is offered by the hygiene hypothesis, which states that the relative lack of infections early in life could promote the development of allergic diseases in genetically predisposed individuals [1,2]. In line with this hypothesis, the reduced frequency, lesser severity and prevention of infections with certain vaccinations and the frequent use of antibiotics could prevent the development of allergic disease [3].

A breakthrough in understanding the regulation of allergic immune responses came with the discovery of different T-cell effector subsets termed T helper (Th) type 1 and Th2 [4]. Research in the past decade has highlighted the key role played by Th2 cells in orchestrating the chronic inflammation in allergic diseases. Although Th2 cells preferentially produce IL-4, IL-5, IL-9 and IL-13, Th1 cells produce IL-2, IFN- γ and TNF- β . It has to be noted here that receptor affinity and activation thresholds for IFN- γ and Th2 cytokines IL-4, IL-5 and IL-13 show a significant difference. Because Th1 and Th2 responses are to a certain degree antagonistic, it is possible that certain infections that predominantly evoke Th1 responses might limit allergic Th2 responses. However, the role of IFN- γ in tissue injury, particularly in epithelial apoptosis leading to epidermal spongiosis in atopic dermatitis and bronchial epithelial cell shedding in asthma

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should be taken into consideration [5,6]. For example, 1 ng/ml IFN- γ can induce keratinocyte apoptosis, whereas 50 ng/ml IL-4 and IL-13 are required to induce IgE production by B cells and IL-5 to prolong eosinophil life span *in vitro* [5,7]. A further subtype of T cells, with immunosuppressive function and cytokine profiles distinct from either Th1 and Th2 cells, termed T regulatory (T_{Reg}) has been described [8], and evidence for their existence in humans has been demonstrated [9]. Recent studies have shown that in addition to Th1 cells, T_{Reg} cells are able to inhibit the development of allergic Th2 responses and play a major role in allergen-specific immunotherapy [9,10^{••},11]. Mycobacteria elicit particularly strong protective Th1 immune responses; however, it remains to be elucidated whether some of the mycobacterial components trigger T_{Reg} cells. Mycobacterial lipoproteins bind to dendritic cell and macrophage-bound Toll-like receptors (TLRs) and this interaction leads to the prominent synthesis of IL-12, and thus prominent Th1 switching [12] (Fig. 1).

Mycobacterial infections and allergy

In a hallmark study in 1997 [13], an inverse association among Japanese school children between exposure to mycobacteria and the subsequent development of atopy was among the first to provide epidemiological evidence in favour of the hygiene hypothesis and mycobacterial exposure. These results have later been debated: to what extent the results are explained by exposure to mycobacteria, bacillus Calmette-Guérin (BCG) vaccination, or by host factors is unknown [14,15]. Tuberculin reactivity has been found to be unreliable as a marker of exposure to *Mycobacterium tuberculosis* because considerable

genetic variations have been recognized in tuberculin reactivity [16].

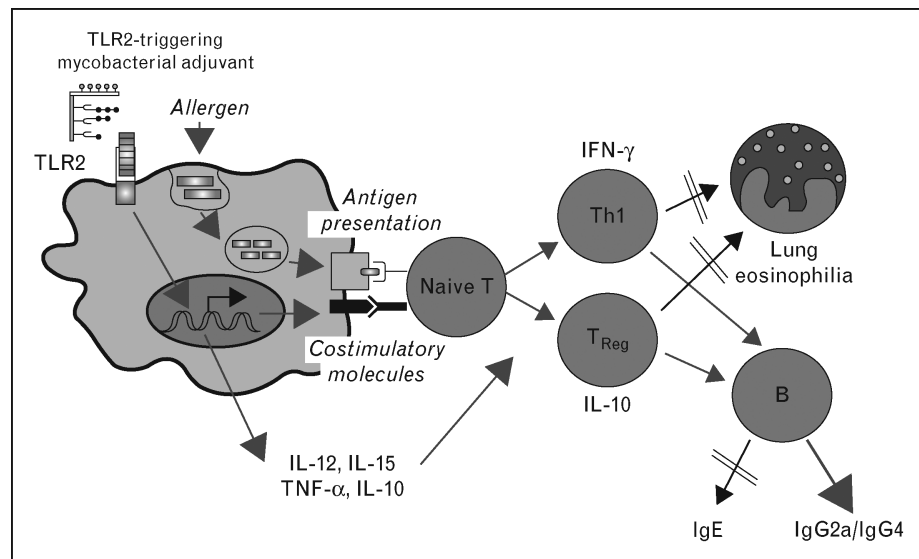
An analysis of health records of children in Guinea-Bissau, West Africa, has demonstrated that vaccinating African children early in life significantly reduces the prevalence of atopy, as demonstrated by skin-prick test positivity to common airborne allergens later in life. However, in that study the BCG response pattern has not been assessed [17]. Furthermore, this study also demonstrated that the greatest reduction in atopy was achieved in those children who were vaccinated during the first week of life. In addition, a protective effect of early BCG vaccination was demonstrated for wheezing, atopic sensitization and symptoms of allergic rhinitis only up to the first 24 months of life, but the effect was not sustained thereafter [18].

A prospective international study evaluated the effect of BCG vaccination at birth on the development of atopy, asthma and allergic diseases at the age of 2 and 5 years by performing an International Study of Asthma and Allergy in Childhood questionnaire and skin-prick testing. In this study, we have shown a protective effect of the BCG vaccine in young children against the development of allergic symptoms, but not IgE sensitization in Turkey and Thailand where BCG is still in routine use [19[•]].

In contrast, a retrospective study performed on children from Sweden [20] found no reduction in atopic disease associated with BCG among children born in Sweden, whereas in the same study among immigrant children,

Figure 1. Allergens engulfed by antigen-presenting cells in the presence of TLR2-triggering mycobacterial adjuvants.

This effects the differentiation of naive T cells mainly to T helper (Th) 1-like cells and in certain conditions T regulatory (T_{Reg})-like cells, because antigen-presenting cells release more IL-12, TNF- α , IL-15 as well as IL-10. The generated T-cell response is dominated by high quantities of IFN- γ and IL-10 production and relatively low quantities of IL-4, IL-5 and IL-13 production. IFN- γ is mainly produced by Th1-like cells and IL-10 by T_{Reg} cells. Both cytokines lead to suppressed lung eosinophilia in asthma models. In addition B cells produce less or no IgE, which is suppressed both by IL-10 and IFN- γ . IFN- γ induces IgG2a isotype antibodies in mice models and IL-10 induces IgG4 in human cell cultures against allergens.



many born in Asia and south America, BCG was associated with a lower prevalence of atopic disease, providing evidence that ethnicity might affect the susceptibility to the immunomodulatory effects of vaccination. In that study, there was no allergy skin testing performed to rule out the existence of any atopy. The discrepancy between the studies could partly be caused by the difference in the timing of BCG vaccination, natural exposure to mycobacteria in early infancy and genetic background.

It is important to distinguish between BCG vaccination and mycobacterial infection. An inverse association between the prevalence of tuberculosis in a given country and the prevalence of atopic disorders has repeatedly been reported [21,22]. The relationship between tuberculosis notification rates and the prevalence of the symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema was investigated in 85 centres from 23 countries in which standardized data were available [21]. These essentially comprised countries in Europe, as well as the USA, Canada, Australia and New Zealand. Tuberculosis notification rates were significantly inversely associated with the lifetime prevalence of wheeze and asthma. An increase in the tuberculosis rates of 25 per 100 000 was associated with an absolute decrease by 4.7% in the prevalence of wheeze. Symptoms of allergic rhinoconjunctivitis in the previous 12 months were also inversely associated with tuberculosis notification rates. Another retrospective study evaluated the occurrence of allergic disease among Finnish adults who had tuberculosis in the first two decades of life [23]. Tuberculosis was inversely associated with allergies and asthma in women, but the opposite trend was observed in men. Because the incidence of tuberculosis is approximately 10 times greater in Japan compared with the Scandinavian countries, the exposure of children to environmental bacteria was not similar. Other environmental mycobacteria could also contribute to the differences.

Bacillus Calmette–Guérin and other mycobacteria in animal models of allergy and asthma

Underlying mechanisms of the impact of mycobacterial antigens on IgE sensitization and asthma have been investigated in several models. In one study [24], high IgE responder Balb/c mice were infected with BCG, 14 days before the start of sensitization with ovalbumin. BCG immunization markedly hindered the development of IgE/IgG1 antibody responses, IL-4, IL-10 secretion in splenocytes, airway hyperresponsiveness, eosinophilic influx into the airway, IL-4 and IL-5 levels in bronchoalveolar lavage (BAL) fluid. In parallel, a marked increase in anti-ovalbumin-specific IgG2a antibody and concanavalin A-induced IFN- γ production from splenocytes was detected.

In a murine model of allergen-induced airway eosinophilia, intranasal BCG infection 4 weeks before allergen airway challenge resulted in a 90–95% reduction in eosinophilia within the lungs compared with uninfected controls. This was associated with reduced IL-5 production by T cells from the draining lymph nodes. Furthermore, BCG-induced inhibition of airway eosinophilia was strongly reduced in IFN- γ receptor-deleted mice and could be partly reversed by intranasal IL-5 application. The suppressive effect of BCG infection on the allergen-specific Th2 response was localized to the lung and did not influence blood eosinophil or allergen-specific IgG1 and IgE serum levels [25]. However, the exact mechanism of how IFN- γ mediates this effect has not yet been established.

There have been several investigations on the interaction between mycobacteria and the innate immune system. It is known that mycobacterial lipoproteins bind particularly to TLR2, which leads to the prominent synthesis of IL-12, thus the prominent Th1 switching and secretion of IFN- γ and TNF- α [12], the cytokines shown to down-regulate Th2 immune mechanisms both *in vivo* and *in vitro* [26–30]. In this context, a mycobacterial muramyl-tripeptide analogue, which triggers TLR2 has been shown to suppress Th2 cytokines, IgE and lung eosinophilia, while enhancing IFN- γ , IL-12, but also IL-10 [31]. Additional experimental data on the inverse link between mycobacteria and atopy came from studies in which exposure to mycobacteria had prominent Th2-limiting effects [24,25,32–34]. In one of those experiments, intranasal BCG inhibited pulmonary eosinophilia and the synthesis of IL-5 by splenocytes in an IFN- γ -dependent mechanism [25]. Intravenous BCG promoted IFN- γ synthesis and inhibited the secretion of IL-4, IL-5, IgE, IgG1 and airway eosinophilia [24]. Similarly, the subcutaneous injection of heat-killed BCG suppressed serum total IgE [33] and chronic changes of allergic airway inflammation [34].

Bacillus Calmette–Guérin and other mycobacteria as therapeutic agents in allergic diseases

Previously, the administration of BCG to asthmatic children allergic to house-dust mite revealed a decline in serum IgE levels, but no effect on IL-2, IL-4, IL-5 and IFN- γ production from peripheral blood mononuclear cells (PBMCs) [35]. Another randomized controlled trial on BCG vaccination in human asthma [36] demonstrated a significant improvement in pulmonary functions and the need for asthma medication, with no apparent suppressive effect on the Th2 immune response. BCG revaccination further improved lung functions and resulted in an apparent increase in the IFN- γ /IL-4 ratio in a subsequent study by the same group [37].

The effect of intradermal BCG vaccination in atopic children induced IFN- γ messenger RNA expression in PBMCs, although there was no expression before. In addition, all patients were found to develop an appropriate tuberculin skin test response 8 weeks after the vaccination, which contradicts the suggestion that atopic children have a decreased ability to mount a delayed-type hypersensitivity response [38]. On the basis of these initial findings, a trial with repeated intradermal injections of heat-killed BCG in adult asthma was initiated [39]; however, it was unfortunately halted prematurely as a result of the occurrence of severe local reactions.

On the basis of evidence obtained from humans and animal models, there seems to be a discrepancy regarding the preventive and therapeutic effect of BCG on atopic diseases. Factors causing this conflict could be: (i) the timing of vaccination; (ii) the route of delivery; (iii) genetic contribution and ethnicity; and (iv) varying degrees of exposure to environmental mycobacteria.

Timing of vaccination

Pregnancy is known to be characterized by intrauterine Th2 predominance, which is clearly essential for the prevention of fetal allograft rejection [40]. Although the development of atopy in the newborn is determined by a variety of factors, an intense Th1 stimulus such as BCG vaccination right after birth could contribute to switching away from the Th2 environment of the placental unit. We may speculate that any mycobacterial inhibition of atopy in humans should have its maximum impact early in life, that is before the establishment of a predominant Th2 response. The study by Aaby *et al.* [17] provided further evidence for less atopy in infants receiving BCG in Guinea Bissau, particularly in the first week of life. The results of another international prospective study [19^{*}] further supported the role of early BCG vaccination in preventing allergy by history. Meanwhile, Omenaas *et al.* [41] reported that purified protein derivative reactivity in young adults receiving BCG as late as 14 years of age was not associated with atopy.

Route of vaccine delivery

After inhalation mycobacteria are phagocytosed primarily by alveolar macrophages. Whereas evidence suggests that systemic delivery is efficient in suppressing an allergic response [24,33], alternative, non-invasive routes such as the intranasal route has given promising results. In support of this suggestion, it has been demonstrated that intranasal infection with BCG was able to suppress the accumulation of eosinophils into the airways, which proved to be superior to the intraperitoneal or subcutaneous routes [25]. Further evidence has been provided by a reduction in nasal eosinophil infiltration by skewing the microenvironment towards Th1 in a murine model of

allergic rhinitis [42]. In addition, the effect of the intraperitoneal and intranasal routes of BCG administration in a murine model of asthma was investigated. Intranasal BCG treatment was the most effective route in reversing antigen-induced asthma symptoms, BAL and peribronchial eosinophilia, as well as BAL fluid IL-5 levels [43].

Genetic contribution and ethnicity

Results from immigrant and African children raised the possibility that BCG vaccination prevents atopy only in certain ethnic groups [17]. In humans, the association or linkage of the natural resistance-associated macrophage protein 1 gene (NRAMP1) with susceptibility to infectious diseases, but also atopy and autoimmune disease, has been demonstrated [44,45]. Although the functions of NRAMP1 remain unknown, it is hypothesised that it may regulate the intraphagosomal concentration of Fe (2+) or other cations. The immune response to mycobacteria seems to be under the control of NRAMP1, and polymorphisms in the genomic region have been suggested to be associated with the risk of atopy in BCG-vaccinated children [44]. In addition, NRAMP1 affected the efficiency of *Mycobacterium vaccae* in diminishing the allergic response in a mouse model, providing a link between genes, environment, and allergic asthma [46,47].

Exposure to environmental mycobacteria

Earlier observations have shown that previous exposure to live environmental mycobacteria primes the host immune system against mycobacterial antigens shared with BCG, and thus modulates immune responses generated by the vaccine [48]. BCG is highly efficient at preventing tuberculosis in neonates vaccinated before exposure to environmental mycobacteria occurs [49], although BCG has been demonstrated to be particularly ineffective and failed to protect against pulmonary tuberculosis in adults in southern India, where various environmental mycobacteria are ubiquitously found [50]. Several studies suggested that immunization with a fast-growing saprophytic mycobacterium, *M. vaccae*, prevented or treated allergic and asthmatic manifestations more efficiently than BCG in murine models [2,33,34,51].

Mycobacterial adjuvants for allergen-specific immunotherapy

Adjuvants such as killed bacteria or components derived from them have been shown to inhibit the development of allergic responses in animals [52–55]. By administering the allergen in conjunction with the adjuvant during specific immunotherapy, the goal is to induce selectively long-lived allergen-specific memory Th1 cells. It is suggested that upon re-encountering allergen, these cells will secrete IFN- γ , thereby suppressing the development of allergen-specific Th2 cells.

We recently investigated whether the administration of BCG as an adjuvant to allergen-specific sublingual immunotherapy (SLIT) has any additive effect on clinical, laboratory and immunological parameters in children with asthma sensitized to house-dust mite. The results demonstrated a higher in-vitro production of IFN- γ from PBMCs of both SLIT and SLIT plus BCG groups compared with controls, who never received immunotherapy. This increase was well correlated with the improvement in bronchial hyperreactivity, clinical parameters and decrease in eosinophil counts in both groups. Meanwhile, the in-vitro production of IL-12 was found to be higher in the BCG receiving group, not resulting in any further improvement in the clinical outcome measures [56^{*}]. Similarly, immunotherapy with *Dermaphagoides pteronyssinus* in conjunction with BCG did not alter lung functions, non-specific hyperreactivity and allergen-specific IgE in asthmatic patients sensitized to house-dust mite [57].

In humans, there is circumstantial evidence to suggest that T_{Reg} cells play a major role in the inhibition of allergic disorders. It has been shown that successful immunotherapy of patients resulted in a state of non-responsiveness of allergen-specific T cells [58], as well as by an enhanced production of IL-10 and transforming growth factor beta (TGF- β) by these cells [9,11]. Adjuvants in allergen-specific immunotherapy vaccine that induce the generation of allergen-specific T_{Reg} cells have been suggested to increase the success of allergen-specific immunotherapy. Supporting this concept, it has been demonstrated that *M. vaccae* is able to induce CD4⁺CD45RB^{low} T_{Reg} cells that secrete IL-10 and TGF- β [51,59,60], and upon transfer can protect recipient allergic mice from airway inflammation. *M. vaccae*-induced CD11c⁺ cells have recently been shown to play a potential regulatory role at the site of inflammation through the secretion of immunomodulatory cytokines such as IL-10, TGF- β and TNF- α [61^{**}].

Conclusion

Most of the evidence available to date suggests a need for an improved mycobacterial vaccine administered early in life. As switching away from the Th2 immune response by inducing Th1 is unable to explain the underlying mechanisms of action of mycobacterial antigens, it may be worthwhile to investigate whether T_{Reg} cells are induced in response to different mycobacterial strains including BCG. Although there is some evidence that exposure to environmental mycobacteria might play a role in the prevention of allergic diseases, further studies are required to identify dose, time and strain differences. In addition, studies should also focus on the purification and characterization of certain mycobacterial components in the development of Th1 or T_{Reg}-type immune responses.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 582–583).

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