Vaccine Potential of Mycobacterial Antigens against Asthma

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Highlights of the Study

- Asthma is a global problem with significant morbidity and mortality.
- T helper (Th)2 and Th17 cells and their cytokines are pathogenic, while Th1 and T regulatory (Treg) cells and their cytokines provide protection against asthma.
- Mycobacterial antigens protect against asthma by shifting the immune responses from Th2 and Th17 to Th1 and/or Treg types.
- The immunomodulatory property of mycobacterial antigens suggests their potential as a vaccine against asthma.

Keywords

Asthma · Mycobacteria · Mycobacterial antigens

Abstract

Asthma is a cause of substantial burden of disease in the world, including both premature deaths and reduced quality of life. A leading hypothesis to explain the worldwide increase of asthma is the “hygiene hypothesis,” which suggests that the increase in the prevalence of asthma is due to the reduction in exposure to infections/microbial antigens. In allergic asthma, the most common type of asthma, antigen-specific T helper (Th)2 and Th17 cells and their cytokines are primary mediators of the pathological consequences. In contrast, Th1 and T regulatory (Treg) cells and their cytokines play a protective role. This article aims to review the information on the effect of mycobacteria and their antigens in modulating Th2/Th17 responses towards Th1/Treg responses and protection against asthma in humans and animal models.

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Introduction

Asthma is a common noncommunicable respiratory disease affecting children, adults, and the elderly all over the world, with high morbidity and mortality in severe cases [1]. Asthma is a cause of substantial burden of disease, including both premature deaths and a reduced quality of life, in people of all ages and in all parts of the world [2]. Worldwide, asthma has been ranked 16th among the leading causes of years lived with disability, and 28th among the leading causes of burden of disease, as measured by disability-adjusted life years [2]. It is characterized by chronic airway inflammation with significant eosinophil and lymphocyte infiltration into the airway submucosa, hyperproduction of mucus, and airway hyperresponsiveness (AHR) to various stimuli [3]. Symptoms of asthma include recurrent periods of wheezing, chest tightness, shortness of breath, and coughing, together with variable expiratory air flow limitation [1].

There has been a dramatic increase in the incidence of asthma in the past decades, especially in the densely pop-

ulated countries of Africa, Asia, and Latin America [4]. According to the global estimates made by the World Health Organization (WHO), about 339 million people suffered from asthma in different regions of the world, and 1,000 people died of it each day in 2018 [4]. The worldwide morbidity and mortality due to asthma is expected to increase in the future, with about 400 million people suffering from it by 2025 [4]. However, this may be an underestimation due to underdiagnosis of asthma [5].

A leading hypothesis to explain the worldwide increase in the cases of asthma is the “hygiene hypothesis” [6]. This hypothesis suggests that the increase in the prevalence of asthma is due to the reduction in exposure to infections/microbial antigens and certain relatively harmless environmental microorganisms or their components due to high standards of personal hygiene and improved household features [6]. Reducing the exposure to environmental/microbial agents will prevent the immune system from developing tolerance to these agents [6]. The main reason for the recent increase in the prevalence of asthma may be an inadequate differentiation of naïve T cells into T helper (Th)1 and T regulatory (Treg) cells because of the decrease in infectious diseases due to improved hygiene [7]. The human immune system tends to deviate to Th2 immune reactions, which are suppressed by Th1 and Treg cells. When this suppression is inadequate, Th2 immune reactions are activated and allergic diseases can result, i.e., the so-called hygiene hypothesis [7]. In this context, a large number of studies on humans and animal models of asthma have reported that immunization with whole-cell mycobacterial preparations and purified mycobacterial antigens can prevent or downregulate asthma features such as AHR and eosinophilic airway inflammation through the induction of Th1/Treg responses [8–11].

This review explains the immunological basis of pathogenesis in asthma mediated by Th2 and Th17 cells and their cytokines, and the role of Th1 and Treg cells and their cytokines in protection against asthma. In addition, studies have demonstrated the protection against asthma using mycobacteria (whole-cell mycobacteria), and their components (secreted antigen mixture as well as purified single and multiple antigens), which primarily induce significant Th1 and/or Treg responses as opposed to Th2 and/or Th17 responses.

Th2 and Th17 Cells and Cytokines in the Pathogenesis of Asthma

In allergic asthma, the most common type, antigen-specific Th2 cells and the cytokines secreted by them, i.e., interleukin (IL)-4, IL-5, IL-9, and IL-13, are primary me-

diators of the pathological consequences leading to airway eosinophilic inflammation, high-serum immunoglobulin (Ig)E concentrations, and AHR [12, 13]. The presence of Th2 cells has been shown in the lungs of patients with allergic asthma by demonstrating the specific presence of IL-4 and IL-5 mRNA in the bronchoalveolar lavage fluid (BALF) of asthmatic patients [14]. The specific contribution of Th2 cells has also been documented in classical allergen challenge studies on humans. Sensitized asthmatics exposed to aerosolized allergen exhibited airflow obstruction, an influx of eosinophils and T lymphocytes, and an increase in Th2 cytokines in their BALF and bronchial mucosa [15]. These results indicate a major role for Th2 cells in the development of allergic asthma in humans.

Studies on mouse models of antigen-induced airway inflammation have also shown a primary role for Th2 cells in asthma [16]. As a result of the activation of allergen-specific Th2 cells, allergen-specific IgE is produced and binds to IgE receptors on the surface of the mast cells. After cross-linking by specific allergen, this induces the activation of the mast cells. Mast cell activation results in the release of preformed mediators, such as histamine and leukotrienes, which directly affect airway smooth muscle and mucous glands, ultimately causing AHR [17]. The specific roles of the individual Th2 cytokines (IL-4, IL-5, IL-9, and IL-13) have been determined by sensitization and airway challenge studies on mice using various strategies: transgenic cytokine overexpression, targeted gene deletion, and blocking anticytokine antibodies. The results of these studies showed a central role for IL-4 in the generation of Th2 cells and in isotype class-switching in B cells for IgE production [18]. IL-5 is an eosinophil-specific regulatory cytokine that plays a role in the differentiation, activation, and survival of eosinophils [15]. IL-9 is involved in the recruitment, proliferation, and activation of mast cells [17]. IL-13 plays a major role in the effector phase of the response and induces the main manifestations of allergic disease including AHR, mucus production, airway smooth-muscle alterations, and subepithelial fibrosis [19].

In addition to Th2 cells, several studies have shown the role of Th17 cells and their cytokines in the pathogenesis of asthma [20–22]. High concentrations of IL-17A were found in induced sputum and bronchial biopsies obtained from patients with severe asthma [20]. The cytokines secreted by Th17 cells (IL-17A, IL-17F, and IL-22) recruit neutrophils to the airway by increasing the secretion of epithelial-derived neutrophilic chemokines, resulting in increased airway inflammation [21]. In addi-

tion, the Th17 cytokines induce mucous-cell metaplasia, and have pleotropic effects on airway smooth muscle that lead to airway narrowing [22].

Th1 and Treg Cells and Cytokines in Protection against Asthma

Compared to the pathologic role of Th2 and Th17 cells and their cytokines, Th1 and Treg cells and their cytokines are considered to have a protective role in asthma [23, 24]. Th1 cells and their cytokines inhibit the development and proliferation of Th2 cells and the secretion of Th2 cytokines, and also suppress overall Th2-mediated airway inflammation [25]. Interferon (IFN)- γ , the signature cytokine produced by Th1 cells, abrogates IgE production and eosinophilia [18]. In animal models, exogenous administration of IFN- γ can result in the suppression of allergic airway inflammation [26]. In addition, the expression of T-bet, a transcription factor important for IFN- γ production, was found to decrease in T cells isolated from the airways of asthmatic patients versus non-asthmatic individuals [27]. The administration of IL-16, a cytokine produced by many cell types with a preferential chemoattractant activity for Th1 cells, resulted in a marked decrease in Th2-type cytokine production, inflammation, and AHR in mice, suggesting that Th1 cells are recruited to the allergic lung via IL-16 and suppressed inflammation [28]. The treatment of sensitized mice by intranasal instillation of IgG, followed by allergen challenge, strongly reduced eosinophilic inflammation and goblet cell metaplasia, and increased Th1 reactivity and IFN- γ levels in the BALF, but inflammatory responses were unaffected in IFN- γ -deficient mice [29].

Treg cells are the primary negative regulators of the immune response [30]. There is compelling evidence that Treg cells inhibit Th2 cell responses, suggesting that asthma can result from an imbalance between Th2 and Treg cells [31]. This is supported by studies where patients with asthma had fewer Treg cells in the peripheral blood than nonasthmatic people [32], and children with asthma had fewer Treg cells in the lungs than nonasthmatic children [33]. The role of Treg cells in protecting against asthma is further supported by studies where the depletion of Treg cells before allergen sensitization enhanced the severity of airway inflammation and AHR [34]. The retransfusion of Treg cells from normal mice into mice suffering from asthma significantly suppressed AHR and reduced the number of eosinophils as well as IL-5 and IL-13 concentrations in the BALF [35].

Treg cells produce 2 important immunoinhibitory cytokines, IL-10 and transforming growth factor (TGF)- β

[36]. Substantial decreases have been reported in the frequencies of allergen-specific IL-10-producing Treg cells and increases in IL-4-producing Th2 cells in allergic versus healthy individuals [37], which highlights the close interplay between the Treg and Th2 effector cells in asthma. The suppressive function of the adoptively transferred antigen-specific Treg cells against asthma is dependent on IL-10 [38]. Furthermore, TGF- β and IL-10 secreted by Treg cells markedly suppresses airway inflammation and AHR in asthma whereas the blocking of TGF- β or IL-10, by using specific antibodies, aggravates airway inflammation and AHR [30].

The Effect of Mycobacterial Exposure on Asthma

Mycobacteria are known to induce strong Th1 and Treg immune responses and weak Th2 and Th17 responses [39–42]. Hence, it may be expected that exposure to mycobacteria may reduce the possibility of developing asthma. In a study from Japan, it was observed that asthmatic symptoms were reduced by up to 50% in school children with positive tuberculin responses (an indication for infection with *Mycobacterium tuberculosis*, the bacteria causing tuberculosis [TB]) compared to tuberculin negatives [43]. Furthermore, remission of symptoms was up to 9 times higher in positive tuberculin responders and the serum IgE level was significantly lower in tuberculin-positive children than tuberculin negatives [43]. The children with a positive tuberculin response had significantly lower levels of Th2 cytokines (IL-4 and IL-13) and higher levels of the Th1 cytokine IFN- γ [43]. In another study, it was reported that TB notification rates were significantly inversely associated with a lifetime prevalence of wheeze and asthma and a 12-month period prevalence of wheeze at rest [44]. An increase in the TB notification rates of 25/100,000 was associated with an absolute decrease in the prevalence of wheeze ever of 4.7% [44]. In a systematic review and meta-analysis of 23 studies (10 cohort, 5 case-control, and 8 cross-sectional studies), the epidemiological evidence, with respect to the effect of *M. bovis* bacillus Calmette-Guérin (BCG) vaccination on asthma, was qualitatively and quantitatively appraised [45]. Three indicators of BCG exposure were considered in this review: BCG vaccination, tuberculin response, and scar diameter. The pooled estimate of association for 23 studies suggested a protective effect of BCG exposure on the occurrence of childhood asthma [45]. Similar results were reported in another meta-analysis showing that BCG vaccination is associated with a protective effect against the risk of asthma [46]. In a later study involving a total of 10,028 children, the evidence of

BCG vaccination was associated with a lower prevalence of allergic diseases including asthma [47]. All the above studies provide epidemiological evidence to support the hypothesis that exposure to mycobacterial antigens prevents asthma, possibly by modulating the immune response. Hence, experimental studies have been performed both on humans and animal models of allergic asthma to test for this possibility.

Modulation of Asthma by Whole-Cell Mycobacteria

Whole-cell mycobacteria belonging to 3 different species (BCG, *Mycobacterium vaccae*, and *Mycobacterium phlei*) have been used to modulate asthma in humans and animal models. In BCG-vaccinated asthma patients from Korea, the presence and size of BCG scars were inversely associated with asthma [48]. BCG vaccination in adults with moderate-to-severe asthma increased the Th1 response, suppressed the Th2 response, improved lung function, and reduced medication [49]. BCG revaccination further improved lung function and resulted in an apparent increase in the IFN- γ /IL-4 ratio, shifting the Th1/Th2 balance toward Th1 [50]. A randomized, controlled, double-blind clinical trial was conducted in adult asthmatic patients to investigate the efficacy of BCG vaccinations for a 12-month cohort study [51]. The results showed a significant improvement in asthma symptoms with significantly increased concentrations of the Th1 cytokine IFN- γ and the Treg cytokine TGF- β_1 in the group of patients receiving BCG 3 times compared to the placebo group and the group that received a single BCG vaccination [51]. In a randomized, single-center, controlled study, young children with moderate asthma were treated with inactivated *M. phlei*. This treatment resulted in the alleviation of asthma symptoms, an improvement in lung function, and a reduction in bronchial hyperresponsiveness and the total serum IgE level [52]. Similarly, in adult patients, inhaled inactivated *M. phlei* improved asthma symptoms, reduced the need for rescue medication, and reduced acute exacerbation of asthma [53]. The late airway response in bronchial asthma patients was reduced to inhaled allergen, with a decrease in serum IgE and Th2 cytokine IL-5 levels, after intradermal injection of a single dose of heat-killed *M. vaccae*. This allowed a major reduction in the use of bronchodilators [54].

In addition to human studies, a large number of reports about animal models of asthma have confirmed the beneficial effects of vaccination with BCG, *M. phlei*, and *M. vaccae* in relieving the symptoms of asthma [55–60]. In neonatal mice, BCG vaccination inhibited AHR, infiltration of eosinophils, and mucous overproduction,

and shifted the predominant Th2-type cytokine response to a Th1-type cytokine response in the BALF and lymphocyte supernatant [55]. Furthermore, the protective effect of BCG vaccination on asthma correlated with increased levels of IFN- γ -secreting Th1 cells in the lung and the IFN- γ concentration in BALF, with no effects on pulmonary Treg or Th17 cells [56]. In another study, BCG vaccination of neonate mice inhibited asthma symptoms following allergen challenge, with reduced IL-4 and IL-17A production and increased IFN- γ secretion in BALF and the lung lymphocytes [57]. To study whether the suppressive effects of BCG on asthma depended on the strain of BCG, mice were vaccinated intraperitoneally with 4 different strains of BCG (Pasteur F1173P2, Tokyo 172, Tice, and Connaught). The results showed that the 4 strains of BCG suppressed asthma to different degrees, but that all strains induced a shift in the Th1/Th2 balance toward Th1 without increasing IL-10-related Treg cell activity [58]. In a study to determine the optimal route of delivery of BCG in mice, it was found that subcutaneous *M. bovis* BCG inoculation had a greater suppressive effect on the development of AHR and eosinophilia than intranasal inoculation did [59]. However, in both groups of vaccinated mice, airway responses to the allergens were significantly lower and the IFN- γ level was significantly higher in the BCG-vaccinated mice than in unvaccinated mice, and the number of airway eosinophils was significantly related to the IFN- γ /IL-5 ratio [59]. In another study, neonatal vaccination with BCG inhibited AHR and inflammation, with reduced IL-17 production and increased IFN- γ production in both the BALF and lung lymphocytes of asthmatic mice, but no reduced levels of Th2 cytokines were observed [57]. The exogenous IL-17 delivered to the airway reversed the antiasthma effects of the neonatal BCG vaccination [60].

The repeated administration of BCG has been shown to induce suppressor/Treg cells [61, 62]. In a newborn mouse model of asthma, mice immunized 3 times with BCG on days 0, 7, and 14 showed inhibition of the de novo allergic inflammatory response, an increase of CD4⁺CD25⁺ Treg cells and Foxp3 expression, accompanied by an increased CTLA-4 expression and cytokine IL-10 and TGF- β concentrations [63]. Similarly, after BCG administration 3 times to mice, the number of Th1 and Treg cells were found to be significantly elevated [64]. Furthermore, the scores for inflammation and mucus secretion in the lung were significantly decreased in the allergen-sensitized group pretreated with BCG versus the control group [64].

In a murine model of asthma, inactivated *M. phlei* alleviated the Th17 response and attenuated airway inflammation and AHR in the lungs [65]. In another study, the administration of inactivated *M. phlei* suppressed airway inflammation and AHR via modulating the balance of CD4⁺CD25⁺ Treg and Th17 cells [66]. Similarly, heat-killed *M. vaccae* reduced asthmatic manifestations in a murine model, and this protection was transferred by spleen cells by an IFN- γ -independent mechanism [67]. Further studies showed that vaccination with heat-killed *M. vaccae* led to the production of CD4⁺CD45⁺RB^{low} Treg cells, which conferred protection against airway inflammation [68]. The inhibition of airway inflammation was mediated through IL-10 and TGF- β , as antibodies against these cytokines completely reversed the inhibitory effect of the Treg cells [68].

Modulation of Asthma by Mycobacterial Antigens

Although live whole-cell mycobacteria, especially BCG, have a suppressive effect on asthma, their use in medical practice may be limited due to the associated adverse reactions [69, 70]. To develop a product that suppresses asthma with minimal adverse effects, culture supernatants containing secreted proteins from *M. tuberculosis* and BCG were used in a mouse model [8]. The results showed that live BCG and culture supernatants containing secreted proteins prevented the development of asthma with altered Th1/Th2 cytokines (increased IFN- γ /IL-5 ratios) [8]. However, culture supernatants are crude mixtures of several hundred secreted mycobacterial proteins with variable contents of individual proteins and batch-to-batch variation with respect to the concentration of individual proteins [71]. To have a standardized and consistent antigenic preparation, the ideal approach would be to identify individual mycobacterial proteins that can modulate asthma.

To determine if individual secreted proteins of *M. tuberculosis* can modulate asthma, studies have been conducted with single proteins purified from the culture supernatants of *M. tuberculosis*, i.e., Ag85 complex, 38 kDa protein, and MPB70 [72], and the recombinant Ag85B produced in *Escherichia coli* [73]. These secreted proteins have been shown to be the inducers of Th1 cytokine IFN- γ from human peripheral blood cells [74]. The findings in the experimental asthma model showed that immunization of mice with the purified and recombinant proteins provided protection against asthma in the ovalbumin-challenge model [72, 73]. With respect to cells and cytokines, mice immunized with individual mycobacte-

rial proteins preferentially activated Th1 cells and showed increased IFN- γ /IL-5 ratios [72, 73]. In another study on a mouse model of asthma, immunization with Ag85A and IL-17A fusion protein (Ag85A-IL17A) resulted in a significant decrease in the concentration of cytokines secreted by Th2 cells (i.e., IL-4 and IL-13) and Th17 cells (IL-17A) and the number of total cells, eosinophils, and neutrophils in the BALF as well as the infiltration of inflammatory cells in the peribronchial region of the lung tissue [75]. Similarly, the administration of Ag85B-DNA protected the mice from induction of allergic airway inflammation, with reduced levels of BALF IgE, decreased eosinophil infiltration, reduced concentrations of Th2-type cytokines, and increased concentrations of Th1 and Treg cytokines [76].

The protective effects of a recombinant construct of an adenovirus expressing 2 secreted proteins, Ag85A and Mtb32 (Ad5-gsgAM), against allergic asthma, showed that Ad5-gsgAM elicited much more Th1-biased CD4⁺ T and CD8⁺ T cells than BCG [77]. Furthermore, Ad5-gsgAM-immunized mice showed significantly lowered airway inflammation in comparison with mice immunized with or without BCG [77]. Total serum IgE and pulmonary inducible nitric oxide synthase were also significantly reduced [77]. The cytokine profiles in the BALF were also modulated, as shown by the increased levels of the Th1 cytokine IFN- γ and Treg cytokine IL-10 and decreased level of the Th2 cytokines IL-4, IL-5, and IL-13 [77].

The above-mentioned studies suggest that allergic asthma could be effectively treated with defined secreted protein antigens of mycobacteria. The mycobacterial antigens of interest can be easily prepared in sufficient quantities by recombinant DNA technologies [78], or else expressed in various delivery systems for the induction of appropriate immune responses for the modulation of asthma [79].

Although the use of purified mycobacterial proteins (vs. whole-cell mycobacteria) is expected to produce fewer side effects [72], all of the above-mentioned proteins, shown to be effective against asthma in animals, are cross-reactive with environmental mycobacteria. Hence, immunizations with these proteins in humans may mean the problem of masking or blocking the antiasthma effects is encountered when tested in the field [80]. According to the masking hypothesis, early sensitization with environmental mycobacteria confers some level of protection against TB that masks the effect of a vaccine given later in life, due to the presence of cross-reactive antigens. The blocking hypothesis postulates that previ-

Table 1. T cell biases according to the cytokines detected in the supernatants of spleen cell cultures from immunized mice restimulated with the same antigens in vitro [84]

Antigen	Th1-biased	Th2-biased	Treg-biased	Th17-biased	No bias
PE35, %	93	0	0	0	7
ESXA, %	93	0	0	0	7
ESXB, %	20	7	27	13	33
Rv2346c, %	80	0	0	0	20
Rv2347c, %	100	0	0	0	0
Rv3619c, %	100	0	0	0	0
Rv3620c, %	47	20	7	20	20

ous immune responses to cross-reactive antigens, because of sensitization to environmental mycobacteria, prevent vaccine taking of a new TB vaccine [81]. The use of *M. tuberculosis*-specific antigens may overcome these effects [82]. Therefore, low-molecular-weight and dominant *M. tuberculosis*-specific antigenic proteins were tested for their ability to induce Th1, Th2, Th17, and Treg cytokine responses in mice using different adjuvants and delivery systems [83]. The results showed that all these proteins, except ESXB, induced primarily Th1-biased responses, but that Rv3619c and Rv2347c induced exclusively Th1-biased responses with all adjuvants and delivery systems (Table 1) [83]. Further experiments in an ovalbumin (OVA)-induced mouse model of asthma showed that immunization with recombinant Rv3619c effectively inhibited the total cell counts and eosinophil infiltration in the BALF; perivascular and peribronchial inflammation and fibrosis, and goblet cell hyper/metaplasia in the lungs; OVA-induced IL-5 in the spleen cells; OVA-specific IgE, IgG, and IgG1 concentrations in the sera; and pERK1/2 in the lung tissue [84]. Of further interest was the finding that immunization of mice with Rv3619c, in combination with low-dose dexamethasone (0.5 mg), resulted in greater inhibition of the total cell count and the eosinophil influx in the BALF than that observed with immunization with Rv3619c alone [84]. Moreover, this combination had a significantly greater inhibitory effect on perivascular and peribronchial inflammation when compared to immunization with Rv3619c alone, comparable with high-dose dexamethasone (3 mg) [84]. These findings suggest that the combination of Rv3619c and a low-dose steroid may be more effective in reducing the asthma phenotype. However, it may be appropriate to further identify other *M. tuberculosis*-specific proteins to prepare a multiantigenic preparation for stronger protection against asthma, as shown with Ag85B-Mtb32 [77].

Conclusion

The studies described in this review provide epidemiological and experimental evidence to suggest that exposure to *M. tuberculosis* and other mycobacteria may reduce the risk of developing asthma. Several *M. tuberculosis* antigens that are cross-reactive with other *Mycobacterium* spp. have shown promise in animal models of allergic asthma. However, whole-cell mycobacteria and cross-reactive antigens may encounter the problem of a masking or blocking effect. Hence, *M. tuberculosis*-specific antigens capable of modulating asthma would be preferred. Rv3619c is such an antigen, but more antigens should be identified to produce multiantigenic preparations to protect against asthma. The advantages with defined antigens include fewer side effects, a reduced cost of production when in sufficient quantities, and delivery using appropriate adjuvants and delivery systems. Further research will also be required with respect to the optimal dosage, delivery system, route of administration, and age of subjects targeted for immunization.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

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