

Efficacy of intramuscular BCG polysaccharide nucleotide on mild to moderate bronchial asthma accompanied with allergic rhinitis: a randomized, double blind, placebo-controlled study

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Background Atopy is a state of allergy to common antigens and is founded on an immune disturbance of exuberant Th2 activity and IgE production. There is also epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of asthma or atopy. Since Th1 and Th2 immune mechanisms are significantly antagonistic, it is hypothesized that mycobacterial exposure may moderate atopic disease by modification of immune responses.

Methods One hundred and twenty mild to moderate persistent asthmatics accompanied with allergic rhinitis were randomly divided into four groups with one injection every other day for 18 times for group A with 1 ml of normal saline, B with 0.5 mg of Bacillus Calmette-Guérin polysaccharide nucleotide (BCG-PSN) and C with 1 mg of BCG-PSN, 36 times for group D with 0.5 mg of BCG-PSN. Markers for the severity of asthma and rhinitis including the amount of inhaled corticosteroid, bronchodilator and oral H1 blocker-loratadine being used to obtain optimal symptomatic control, symptom scores of asthma and allergic rhinitis, peak expiratory flow (PEF), histamine provocative dose that produces at least a 20% change in forced expiratory volume with in 1 second (PD20-FEV1), blood IgE levels as well as dermatophagoides pteronysinus (DP) and dermatophagoides farinae (DF) skin prick test were measured every visit for 6 months.

Results There were no differences for symptom scores of asthma, daily use of bronchodilator, PEF, PD20-FEV1, blood IgE as well as DF and DP skin prick test among the four groups. Score for allergic rhinitis decreased significantly in groups B, C and D on day 36 and 72 as compared with group A ($P<0.05$). Score for allergic rhinitis increased after day 72 in group B and C while it was significantly lower in group D ($P<0.05$). The patients in group D used less amount of inhaled beclomethosone than other groups ($P<0.05$) from day 72 after the treatment to day 180. Oral loratadine consumption in groups B, C and D was significantly less on day 36 and 72 as compared with their baseline and group A after the treatment ($P<0.05$). Group D maintained significantly lower dosage of oral loratadine until day 150 comparing with its baseline and group A.

Conclusions BCG-PSN has a symptomatic effect on allergic rhinitis. BCG-PSN may reduce the dosage of non-sedative H1 blocker loratadine as well as the dosage of inhaled beclomethosone in the treatment of mild to moderate asthma and allergic rhinitis.

The extensive evidence of Th2 cytokine involvement in the pathogenesis of allergic diseases [1,2] suggests a potential target for immunomodulation. Because the Th1 and Th2 cells are reciprocally regulated, several recent studies on Th2-associated diseases including asthma have shown to reverse the Th2 response into a protective Th1 response [3,4].

Mycobacterium tuberculosis (*M. tuberculosis*) induces potent Th1 responses [5] and some epidemiologic studies [6,7] have suggested that *M. tuberculosis* infection might reduce the risk of developing asthma. Shirakawa et al [6] reported a strong inverse association between delayed hypersensitivity to *M. tuberculosis* and atopy. Recent experimental animal studies [8,9] have shown that Bacille Calmette-guerin (BCG) infection before allergen sensitization inhibited allergen-induced airway eosinophilia and hyperresponsiveness, which are the characteristic features of asthma. In addition, infection with BCG or administration of heat-killed *Mycobacterium vaccae*, [10] even after allergen sensitization, inhibited an established allergic response. However, other studies [11,12] failed to confirm the relationship between BCG vaccination during early childhood and the subsequent development of asthma or atopy.

In China there has been a long history of the repeated injection of inactivated BCG in treatment of asthma. Most of the publications reported a significant improvement in the majority of asthmatic patients [13-15]. However, the findings have been difficult to interpret since the study designs, methodology and presentation of the results are somewhat different from standard scientific practice nowadays. In the present study we investigate the efficacy of another BCG product-BCG polysaccharide nucleotide (BCG-PSN) vaccination on the treatment of mild to moderate persistent bronchial asthma accompanied with allergic rhinitis in a placebo-controlled study with standardized methodology and outcome measurements.

METHODS

Study design

It was a single-center, randomized, double-blind, placebo-controlled study. Patients were 18-45 years old, with mild to moderate persistent bronchial asthma accompanied with various atopic disorders, predominantly allergic rhinitis for more than 1 year. Patients had never been treated with inhaled corticosteroid or were using inhaled beclomethasone ≤ 400 μ g/d or equivalent in the previous half a year, required at least 200 μ g per week of short acting β_2 agonist-salbutamol or equivalent for symptom relief and never used intranasal corticosteroid. They were required to have a baseline forced expiratory volume in 1 second (FEV1) > 60% of the predicted value and a post-bronchodilator FEV1 > 70% predicted. Patients also had to demonstrate 15% reversibility in FEV1 after the inhalation of 400 μ g of salbutamol or a weekly peak expiratory flow (PEF) variability $\geq 15\%$. In 4 weeks of run-in period, the patients maintained their original dose of inhaled corticosteroids and administered inhaled short acting β_2 agonist, salbutamol and oral H1 blocker, loratadine as needed. The patients were then divided randomly into four groups. Group A, as control group, was injected intramuscularly with 1 ml of normal saline solution, group B with 0.5 mg (1 ml) BCG-PSN (Changsha Jiuzhitang Organic Pharmaceutical Company, Hunan Province, China), group C with 1 mg (2 ml) BCG-PSN every other day for 18 times and group D with 0.5 mg (1 ml) BCG-PSN every other day for 36 times. During 6-month treatment period, the patients were allowed to use inhaled beclomethasone, inhaled short acting β_2 agonist (salbutamol) and oral loratadine as needed (Fig. 1). The dose of inhaled beclomethasone was

increased by 50-100 μg when the patient did not meet one of the following criteria at each visit: (i) daytime or nocturnal symptom score ≥ 1 ; (ii) dose of inhaled salbutamol $\geq 100 \mu\text{g}$ per week; (iii) morning and night PEF $\geq 80\%$ predicted value; (iv) weekly PEF variability $< 15\%$. However, the patients who required inhaled beclomethasone $\geq 400 \mu\text{g/d}$ or systemic administration of corticosteroid should be withdrawn from the study.

Skin prick tests

Skin prick tests were done with standard allergen extracts (Soluprick, ALK, Copenhagen, Denmark) of dermatophagoides pteronysinus (DP) and dermatophagoides farinae (DF). The diluant control gave a negative result while a wheal exceeding one-half to one of the histamine hydrochloride (10 mg/ml) response was scored as 1, one to two as 2, two to three as 3 and greater than three as 4. Only those with at least 1 score of skin prick test to DP and DF were enrolled.

Skin test for purified protein derivative of tuberculin (PPD)

A total of 0.1 ml of 0.05% PPD was injected subcutaneously for skin test. A simple millimeter ruler was used to measure the indurations in 2 planes, the largest vertical and horizontal readings, 72 hours after the injection. Induration size ≤ 4 mm was considered as negative, 5-10 mm +, 11-20 mm ++ and > 20 mm +++. Only those with PPD induration size + to ++ were acceptable as past exposure to mycobacteria.

Serum IgE analysis

Peripheral blood taken from the patients at Visit 2, 4 and 8. Level of serum IgE and of specific IgE antibodies against DP and DF were determined by fluorescent enzyme immunoassay (Pharmacia CAP System, Pharmacia, Uppsala, Sweden). This system was previously described in detail. Patient samples were run in single determinations and evaluated against the stored calibration curve, and results were expressed as kIU/L.

Outcome measures

The primary outcome variables were mean daytime and nocturnal asthmatic symptom scores and daily symptom score of allergic rhinitis, daily dosage of inhaled beclomethasone, salbutamol and oral loratidine, as well as mean morning and night-time PEF recordings made with a Mini Wright Peak Flow Meter, as recorded in patient diary cards.

Secondary outcome variables were defined as FEV1 (pre-bronchodilator) and airway responsiveness to histamine bronchoprovocation test (PD20-FEV1) at Visit 2, 4 and 8.

Thirdly, DP and DF skin prick tests were given and blood samples were taken at Visit 2, 4 and 8. Total serum immunoglobulin E (IgE) and specific IgE to DP and DF were assessed.

Ethics

Ethics Committee of Guangzhou Institute of Respiratory Diseases approved the study. All the patients gave informed consent before entering the study.

Statistical analysis

Values are expressed as mean $\pm$ standard error (SE). One-way analysis of variance (ANOVA) was applied to determine statistical differences among four patient groups at each time point using SPSS 10.0 for Windows (SPSS Inc. Chicago, IL, USA). Significance was accepted at $P \leq 0.05$.

RESULTS

The study population consisted of 138 patients at the first visit. After 4 weeks of run-in period, 18 patients did not meet the criteria and were withdrawn. Therefore, 120 patients were allocated randomly into 4 groups with 30 in each group. The patients of four groups were similar with regard to gender distribution, age, asthmatic and rhinitis duration. Daytime and nocturnal symptom score of asthma, daily symptom score of allergic rhinitis, baseline FEV1 % predicted, morning PEF, weekly variation of PEF, daily dosage of inhaled salbutamol and beclomethasone as well as oral loratadine did not show significant differences among the four groups ([Table 1](#)).

Symptoms of asthma and allergic rhinitis

Daytime asthma symptom scores showed significant decrease ($P < 0.05$) in groups B, C and D on day 72. All four groups relieved their daytime asthma symptoms significantly from day 72 to 180 ($P < 0.05$). Nocturnal asthma symptom scores in all four groups decreased significantly after the treatments from day 36 to 180 ($P < 0.01$). Score for allergic rhinitis also decreased significantly in groups B, C and D on day 36 and 72 as compared with group A ($P < 0.05$). The score increased after day 72 in group B and C but stayed significantly lower level ($P < 0.05$) in group D till the end of the study ([Fig. 2](#)).

Usage of medication

The patients in group D used less amount of inhaled beclomethasone than other groups from day 72 to 180 with significant difference ($P < 0.05$) on day 72 and 120. Inhaled salbutamol among four groups showed the tendency toward decrease but the differences were not significant and the amounts of salbutamol did not differ among the four groups at each visit. However, group B, C and D used significant less amount of oral loratadine on day 36 and 72 as compared with their baseline and group A respectively ($P < 0.05$). Group D maintained significantly lower dosage of oral loratadine until day 150 compared with its baseline and group A respectively ([Fig. 3](#)).

Lung function, airway responsiveness, skin prick test and serum IgE

PEF in the four groups increased gradually with significant difference on day 120 (group C and D), 150 and 180 as compared with their baseline respectively ($P < 0.05$). Weekly variation of PEF of the four groups also decreased after the treatment with significant differences on day 72, 90 (group C), 120 (group A, C and D), 150 and 180 comparing their baseline respectively ($P < 0.05$) ([Fig. 4](#)). PD20-FEV1 of histamine, DP and DF skin prick test, total serum IgE and specific serum IgE against DP and DF did not show significant changes before and after the treatment in the four groups. Also, these variables did not show significant differences among the four groups at each visit ([Table 2](#)).

DISCUSSION

This human study suggests that repeated doses of BCG-PSN given intramuscularly to established mild to moderate asthma with allergic rhinitis had a symptomatic effect on allergic rhinitis, and reduced the dosage of inhaled beclomethasone in asthma. This is the first study showing a therapeutic effect of BCG product on human asthma accompanied with allergic rhinitis.

In retrospective human studies¹⁹⁻²¹ which failed to demonstrate protection against the development of asthma or atopy, BCG vaccinations were performed during early childhood and

the effects were evaluated several years later. Therefore, as suggested by von Hertzen and Haahtela,²² it is likely that the relatively weak cell-mediated immune responses induced by BCG vaccination were insufficient to sustain long-term protection against asthma. Although BCG vaccination has been reported to offer more than 70% protection against tuberculosis for at least 10 years,²³ repeated exposure to different allergens over a prolonged period might abrogate the protective effects of BCG vaccination.

The apparent beneficial effects shown in the present study may not be reproducible in Western countries where tuberculosis prevalence is very low and BCG vaccination is not routinely performed. As tuberculosis prevalence in China is high and most Chinese receive BCG vaccination in childhood, all patients in this study showed evidence of past exposure to mycobacteria as all their skin test for PPD were + and ++. Therefore, in these cases, BCG-PSN vaccination might have had a booster effect. Choi et al^{3,4} also showed that BCG vaccination significantly increased pulmonary function and reduced asthma medication use, resulted in an apparent increase in IFN- γ /IL-4 ratio, shifting the Th1/Th2 balance toward Th1, in adult patients with moderate-to-severe asthma.

The ability of mycobacterium to induce the Th1 immune response has been attributed to diverse features of the mycobacterium including its cell wall constituents,²⁴ the proteins it secretes,²⁵ the intracellular environment of the macrophage in which it sequesters, and even the unmethylated copies of DNA it releases.²⁶ However, there were a number of reports regarding the local and systemic adverse responses to the heat-killed *M. bovis* with the development of red tender lumps, itch and blistering reactions, and even abscesses at the sites of injection.^{3,21} Therefore, vaccination-site reactions occurring only in the BCG group may have influenced the blinding of the study. BCG-PSN is purified and extracted from BCG by hot phenol and acetic acid with only 0.66% of protein, which gives little local and systemic reaction. Unlike other human studies^{3,21} with obvious local reactions on the injection site of heat killed BCG that influenced the blinding of the study, no erythema and other reactions occurring on the vaccination site in all groups in this study allow good a blindness among control and treatment groups, and better compliance of the patients to complete the study.

Dose, dose frequency and duration of established asthma and allergic rhinitis are other important considerations. Our dose schedule was based on the recommendation from the pharmaceutical company and previous work. Increase of the dose (from 0.5 mg in group B to 1 mg in group C) of BCG-PSN did not slow down increasing the amount of inhaled beclomethasone, which suggests 0.5 mg of BCG-PSN each injection is good enough to induce therapeutic response. However, increase of the duration of BCG-PSN administration in group D showed a tendency in decreasing the addition of amount of inhaled beclomethasone, better relieving the symptoms of allergic rhinitis and reducing the usage of H1 blocker, loratidine although the symptoms of allergic rhinitis became worse gradually when stopping the administration of BCG-PSN. The patients in this study had a mean duration of mild to moderate asthma and allergic rhinitis of 5-7 years, whereas the patients in another study²¹ had a mean duration of asthma of 25 years, indicating that marked structural changes may have occurred in their airways as a result of the remodeling process which failed to demonstrate significant efficacy with BCG vaccine. Therefore, the efficacy of mycobacterium-based vaccines such as BCG in preventing the development of atopy or influencing its progression may be only applied in newly established disease such as allergic rhinitis or accompanied with short history of allergic asthma, but more studies need to be done and its mechanism remains to be determined.

BCG-PSN contains 77.8% of polysaccharide and 16.67% of nucleic acids. Polysaccharide has been shown to stimulate many of the cellular inflammatory events including macrophage

activation and production of the cytokines IL-12, TNF- α and IL-6.^{27,28} Yamamoto and colleagues²⁹ demonstrated for the first time that DNA from M. bovis BCG activated natural killer (NK) cells and enhanced IFN- γ production in mice. They also determined several sequences of 30-mer oligonucleotides that most potently augment the secretion of IFN- γ and NK activity.³⁰ Fujieda et al³¹ reported that DNA fraction purified from M. bovis BCG inhibited IgE production and enhanced production of IFN- γ and IL-12 in human peripheral blood mononuclear cells from normal and atopic donors. It may be considered that DNA fraction in nucleic acid of BCG-PSN was crucial for the effect of relief of atopic symptoms by inhibiting IgE production. However, in our study we did not find significant improvements of serum and specific IgE and the reactions of DP and DF skin prick test as well as airway hyperresponsiveness after the treatment with BCG-PSN. The reasons for these may be short-course treatment with BCG-PSN as well as many uncontrollable variables such as uneven length of history of asthma and rhinitis, different kinds, length and doses of medications having been used as well as less specificity of IgE to DP and DF peptide fraction. One other consideration of the mechanism of BCG in the treatment of atopy could be the validity of the underlying hypotheses. The Th2 hypothesis of asthma pathogenesis, although convenient, may be considered as an oversimplification. Since the initial description of Th1 and Th2 lymphocyte subsets in mice in 1986 and not long after in humans, further subsets have been described, for example Th3 cells and T regulatory cells that have powerful immunosuppressive properties producing transforming growth factor- β (TGF- β) and IL-10 that may counter regulate the Th2 response.³² The Th1/Th2 concept has also been applied to other leukocyte subpopulations than just CD4 cells, and an increasing number of cell types are recognized as contributing to the classical cytokine patterns. The type 1 response itself may have a role in asthma pathogenesis through provision of signals necessary for Th2 cells to be effectively recruited.^{33,34} One study that specifically investigated the capacity of Th1 cells to counterbalance the pro-asthmatic effects of Th2 cells actually caused severe airway inflammation.³⁵ Further investigations need to be proceeded on the exact mechanisms of BCG vaccination in the treatment of atopy.

In summary, in current human study of mild to moderate persistent asthma and allergic rhinitis we found that BCG-PSN had a symptomatic effect on allergic rhinitis. BCG-PSN could reduce the dosage of non-sedative H1 blocker loratadine as well as the dosage of Inhaled beclomethosone. Further study will be needed to focus not only on improving the active component of BCG-PSN, but also on whether different dosing and course schedules and routes of administration such as directly inhaled to the airways or applied on the skin may lead to efficacy in the treatment of atopic asthma and other allergic diseases.

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