Immunological effects of BCG vaccine on adults asthmatic patients

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ABSTRACT

Introduction: The immunologic hallmark of atopic allergy e.g. asthma is an increased production of IgE and T helper(h) type 2 cell cytokines (interleukin (IL)-4, IL-5, IL-9 and IL-13 by Th cells reacting to environmental allergens. In contrast, inhalation of allergens by healthy non-atopics produce allergen- specific IgG1, IgG4 and the Th1 cytokine interferon-α, as well as IL-12 from macrophages. There is compelling evidence that allergen- specific Th2 cells accumulate in the target organ of atopic patients e.g. airways of asthmatics and play a crucial role in the pathogenesis of allergy. Thus, preventing or reversing the differentiation of Th cells into Th2 cells appears a logical therapeutic approach to atopic asthma. Nowadays, we have many modalities of immunomodulation to decrease the effect of IL-4 or IL-5 or production and level of IgE or agents to shift the immune response from a Th2 to a Th1 response, thereby decreasing the allergic inflammatory response in the target organ e.g. airways.

Objective: The purpose of this study was to determine whether a Th1 immune response elicited by BCG immunization could suppress allergic inflammation in adult asthmatic.

Material and methods: Thirty four asthmatic patients, 16 extrinsic with positive allergy skin test (AST): group 1, 18 intrinsic with negative AST: group 2 and 21 healthy individuals: group 3 were subjected to this study. Tuberculin test was performed for all groups and subjects with positive results were excluded. BCG vaccine was given for all groups, with assessment of total IgE and Th2 (IL-4), Th1 (IL-2) cytokine response. Significant reduction of total IgE, IL-4 and elevation of IL-2 were seen in group 1 (atopic asthma) following BCG vaccine, while no significant change was observed in group 2 regarding IgE level, whilst IL-4 was significantly reduced and IL-2 was significantly increased after BCG vaccine. Peak expiratory flow rate (PEFR) was significantly improved in group 1 after 8 weeks of BCG vaccination (p <0.01) with positive correlation with IL-2 [(r = 0.60202, p<0.05) (r = 0.628621, p<0.01)] and with negative correlation with IL-4 [(r = 0.65331, p< 0.01) (r =0.62137, p<0.01)] before & after BCG vaccine respectively.

Conclusion: BCG vaccination might improve atopic asthmatic patients probably due to down regulation of a Th2 immune response and this suggests its potential role as a useful therapeutic agent in the treatment of atopic asthma.

Keywords: BCG Vaccine, asthmatic, allergy
INTRODUCTION

The recognition of the divergence of TH cells into predominantly two arms, TH1 and TH2 subsets that are largely mutually exclusive and reciprocally regulated has decisively improved our understanding of the mechanisms that contribute to the pathogenesis of many chronic diseases\(^1\). An increasing body of evidence from mice and humans now supports the concept that the predominance of either reciprocally regulated subset may result in certain chronic diseases\(^2\).

It is well known that that TH2 cells play an important role in the asthmatic mechanisms as they secrete both IL4 which stimulate IgE production and IL5 which activate eosinophils. Alternatively, TH2 cells have a reciprocal inhibition of TH1 cells which produce IFN-γ, the latter has inhibitory action on the IgE production\(^3\).

In the recent decades, there has been an increase in severity and probably in prevalence of atopic disorders including asthma in developed countries. Studies on migrants from developing to developed countries support the importance of etiological environmental changes\(^4\).

Childhood respiratory infections that might strongly modify the developing immune system both systematically and within the lung include measles, whooping cough and tuberculosis. Some of these infections cultivate a TH1 immunological environment with its predominant cytokines\(^5\). Because these cytokines inhibit TH2 cytokine functions\(^6\), the absence of these infections might release TH2 immune mechanisms and thus promote atopic disorders\(^7\).

So it is likely that a set of specific infections especially tuberculosis that strongly promote TH1 immunity has the potential to inhibit atopic disorders by the repression of TH2 immune response\(^8\).

As the new strategies in treating allergic diseases including bronchial asthma are directed towards using agents\(^9,10\) that decrease IgE levels or TH2 cytokines production or increase TH1 cytokines production in order to inhibit the allergen induced eosinophilic recruitment. So, we hypothesize that a vaccine that induces a strong TH1 immune response and a long living memory immunity might prevent the establishment of a biased TH2 cytokine milieu in the lungs of genetically predisposed subjects, and as BCG vaccine is a potent adjuvant for induction of cell-mediated immunity\(^11\) and induces IFN-γ as one of the major cytokines, this action may cause a TH1 type immune response\(^12-14\).

Consequently, this study was conducted aiming to investigate the action of BCG vaccine in adult asthmatic patients through immunization schedules in attempt to modulate the immune deviation away from atopy and thereby interfering with the pathogenesis of this disease\(^15-17\).

MATERIALS AND METHODS

This study included 34 adults asthmatic patients selected from Ain Shams Allergy and Immunology outpatient clinic.

Group I: 16 asthmatic patients with positive skin prick test to one or more allergens.

Group II: 18 asthmatic patients with negative skin prick test.

Both groups were receiving conventional therapy without systematic corticosteroids.

Group III: 21 adult healthy controls with no history of any chronic medical illness.
For all subjects the following were done:

1. Detailed medical history and clinical examination including history of allergy and previous T.B. infection.
2. Tuberculin Test using purified protein derivative (PPD) obtained from (ACSERA), which was injected intradermal on the dorsal side of right forearm and after 48-72 hours the size of the induration was measured. The test was considered.

- Positive (converted) when the induration was ≥ 10 mm in diameter.
- Intermediate (5-9 mm)
- Negative no reaction or <5 m

Table 1: Results of tuberculin test before BCG vaccination

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Intermediate</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Group II</td>
<td>3</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Subjects with negative and intermediate tuberculin test were included in the study while subjects with positive tuberculin test were excluded.

For the included subjects (15 in each group) BCG vaccine (obtained from ACSERA) was injected intracutaneously as 0.1 ml in the proximal lateral part of left upper arm. Successful vaccination was identified by ulceration and secretion at the site of vaccination beginning 2 weeks after vaccination and lasting 2-6 weeks with no lymphadenitis or serious side effects recorded.

For the three groups the following tests were performed before and after 8 weeks of BCG vaccination:

1. PEFR (Peak expiratory flow rate) using peak flow meter
2. Circulating total serum immunoglobulin E (s IgE ) by ELISA
3. Serum interleukin 4 (S. IL4)
4. Serum interleukin 2 (S. IL2)

Step 3 & 4 were done using the commercially available kits from (DIACLONE RESEARCH FANCE) using ELISA technique according to the instructions inserted.

Both groups of asthmatic patients were maintained on conventional asthma therapy of bronchodilators and inhaled steroid and all through the study period no one needed oral or parenteral steroid therapy.

**STATISTICAL METHODS**

The data were collected and processed to a personal computer (P.C) IBM compatible and then the data was analyzed with the aid of the program (SPSS) Statistical package for social science version 6.0 for windows.

The statistical tests used in this study are: student “t” test and Pearson correlation coefficient test “r”. P value < 0.05 was considered significant.
RESULTS

The results of tuberculin test before BCG vaccination showed that positive test was reported in only one patient of extrinsic asthma (6.67%) and reported in 3 patients of intrinsic asthma (16.67%) but 6 healthy controls showed positive tests (28.8%).

Group I (extrinsic asthma): they were 6 males (40%) and 9 females (60%) their ages ranged from 19 to 51 years with mean 34.4 ± 8.76 years.

Group II (intrinsic asthma): they were 7 males (46.5%) and 8 females (53.5%) their ages ranged from 17 to 53 years with mean 35.7 ± 9.19 years.

Group III (controls): they were 7 males (46.5%) and 8 females (53.5%) their ages ranged from 16 to 49 years with mean 35.7 ± 9.19 years.

The results of serum total IgE, IL2 and IL4 of the different groups before BCG vaccination were as follows:

Group I serum total IgE ranged from 105 to 630 IU/L with mean 384.06 ± 131.122 IU/L. Serum IL4 ranged from 14 to 122 pg/ml with mean 78.2 ± 31.46 pg/ml and serum IL2 ranged from 6.5 to 14.2 pg/ml with mean 9.97 ± 3.19 pg/ml.

Group II serum total IgE, ranged from 65 to 270 IU/L with mean 142 ± 56.91 IU/L serum IL4 ranged from 14 to 122 pg/ml with mean 12.4 ± 4.5 pg/ml and serum IL2 ranged from 12 to 24 pg/ml with mean 17.73 ± 3.15 pg/ml.

Group III serum total IgE ranged from 45 to 180 IU/L with mean 102.8 ± 39.07 IU/L with mean 102.8 ± 39.07 IU/L, serum IL4 ranged from 3 to 14 pg/ml with mean 6.66 ± 3.68 pg/ml and serum IL2 ranged from 12 to 33 pg/ml with mean 22.86 ± 6.55 pg/ml.

Table 2: Mean of serum total IgE

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Mean serum. IgE (pg/ml)</th>
<th>Mean serum. IL4 (pg/ml)</th>
<th>Serum. IL2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>34.3±8.76</td>
<td>384.06 ± 131.122</td>
<td>78.2 ± 31.46</td>
<td>9.97 ± 3.19</td>
</tr>
<tr>
<td>Group II</td>
<td>35.7±9.19</td>
<td>142 ± 56.91</td>
<td>12.4 ± 4.5</td>
<td>17.73 ± 3.15</td>
</tr>
<tr>
<td>Group III</td>
<td>35.71±9.1</td>
<td>102.8 ± 39.07</td>
<td>6.66 ± 3.68</td>
<td>22.86 ± 6.55</td>
</tr>
</tbody>
</table>

In all subjects the reactions to BCG vaccine were successful after 2 weeks and lating 2-6 weeks with no lymphadenitis or serious side effects. When reevaluation of patients and control subjects were done (mm in diameter).

Table 3 Results of tuberculin test after BCG vaccination

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Intermediate</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Group III</td>
<td>11</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Regarding the results of serum IgE, IL4 and IL2 after BCG vaccination

Group I, serum total IgE ranged from 100 to 525 IU/L with mean 329.53 ± 95.79 IU/L (i.e.) Serum. IgE was significantly decreased after BCG vaccination (P<0.05).
Serum IL4 ranged from 25 to 130 pg/ml with mean 61.6 ± 30.3 pg/ml being significantly decreased (P < 0.05) Serum.IL2 ranged from 12 to 23 pg/ml with mean 15.26 ± 5.45 pg/ml being highly significantly increased (P > 0.01).

Group II, serum total IgE ranged from 60 to 250 IU/L with mean 138.4 ± 50.58 IU/L which showed no significant difference between pre or post BCG vaccination (P > 0.05).

Serum.IL4 ranged from 6 to 14 pg/ml with mean 8.5 ± 4.48 pg/ml being significantly decreased after vaccination (P < 0.05). After BCG vaccination by 2 months it was found that:

Serum.IL2 ranged from 21 to 39 pg/ml with mean 23.86 ± 9.25 pg/ml being significantly increased after vaccination (P < 0.05).

Group III, serum total IgE ranged from 40 to 170 IU/L with mean 100.13 ± 38.16 IU/L with no significant difference between pre or post BCG vaccination (P > 0.05).

Serum.IL4 ranged from 2 to 7.5 pg/ml with mean 4.13 ± 1.89 pg/ml with no significant difference also (P > 0.05).

Serum .IL2 ranged from 17 to 39 pg/ml with mean 28.86 ± 6.34 with a significant increase in Serum.IL2 post BCG vaccination (P < 0.05).

Results of tuberculin test after 2 months of BCG vaccination

PEER were recorded for the studied subjects at the start of the study (PEEROW), after two weeks of BCG vaccination (PEER 2W), after 4 weeks (PEER4W), and at the end of the study period i.e. after 8 weeks (PEER 8W), to report for the time of maximum improvement.

Table 4: Results of tuberculin test after 2 months of BCG vaccination

<table>
<thead>
<tr>
<th>Group</th>
<th>PEER OW</th>
<th>PEER 2W</th>
<th>PEER 4W</th>
<th>PEER 8W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>55.93 ± 7.68%</td>
<td>54.7 ± 7.44%</td>
<td>60.2 ± 5.38%</td>
<td>65.13 ± 4.45%</td>
</tr>
<tr>
<td>Group II</td>
<td>53.73 ± 7.1%</td>
<td>53.26 ± 5.18%</td>
<td>55.93 ± 5.39%</td>
<td>57.13 ± 5.02%</td>
</tr>
<tr>
<td>Group III</td>
<td>95.33 ± 5.13%</td>
<td>94 ± 3.68%</td>
<td>95.06 ± 4.26%</td>
<td>95.73 ± 3.50%</td>
</tr>
</tbody>
</table>

Table 5. Results of serum. Total IgE, serum IL4 and serum IL2 before and after BCG vaccination in various groups

<table>
<thead>
<tr>
<th></th>
<th>Group I Before</th>
<th>Group I After</th>
<th>Group II Before</th>
<th>Group II After</th>
<th>Group III Before</th>
<th>Group III After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total S.IgE IU/L</td>
<td>384.06 ± 131.122</td>
<td>329.53 ± 95.79</td>
<td>142 ± 56.93</td>
<td>138.4 ± 50.58</td>
<td>102.8 ± 39.07</td>
<td>100.13 ± 38.16</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
<td>P &gt; 0.05</td>
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<tr>
<td>IL4 PG/ML</td>
<td>78.2 ± 31.46</td>
<td>61.6 ± 30.30</td>
<td>12.4 ± 4.5</td>
<td>8.5 ± 4.48</td>
<td>6.66 ± 3.68</td>
<td>4.13 ± 1.89</td>
</tr>
<tr>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>IL2 PG/ML</td>
<td>9.97 ± 3.19</td>
<td>15.26 ± 5.45</td>
<td>17.73 ± 3.15</td>
<td>23.86 ± 9.25</td>
<td>22.86 ± 6.55</td>
<td>28.86 ± 6.34</td>
</tr>
<tr>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
Figure 1: Comparison between mean Serum. Total IgE before and after BCG vaccination in various groups.

Figure 2: Comparison between mean Serum. IL4 before and after BCG vaccination in various groups.
PEER was significantly improved after 8 weeks of BCG vaccination in group I (P<0.05), while PEER in group II after 8 weeks of BCG vaccination was improved but not statistically significant, PEER. In group III after 8 weeks of BCG vaccination PEER showed no statistically significant change.

At the start of the study, in the extrinsic asthmatic group a significant positive correlation was found between PEER and IL2 (r= 0.60202) P < 0.05. Also a highly significant negative correlation was found between PEER and IL4 (r= -0.65331) P <0.01. These correlations were maintained after BCG vaccination as we found that the correlation between PEER and IL2 was statistically highly significant positive correlation (r= 0.628621) P <0.01, and the correlation between PEER and IL4 was statistically significant negative correlation (r= -0.62137) P <0.01.

DISCUSSION

TH2 responses predominate in individuals suffering from atopic disorders. Atopic disorders, in particular, asthma, are steadily increasing (Cookson and Moffatt. 1997). The reasons for the increase are not fully known although it has been noticed that the increase in atopic disorders inversely correlated with a steady decline in the extent to which the population is exposed to major human diseases such as tuberculosis, measles, whooping cough and influenza.

One of the general features of these infectious agents is that they induce characteristic TH1 type immune response which leads to an immunological environment rich in IFN-γ. As IFN- γ is viewed as powerful suppressive mediator of TH2 activity, the lack of frequent exposure to such infections has been proposed to be related to a failure of regulatory T cells (Treg) cell development, resulting in a loss of tolerance to allergens Speculated to increase the risk of developing atopy.

Atopic asthma and pulmonary tuberculosis appear to be inversely related disorders. Predominance of TH2 cytokine cells has been demonstrated in BAL of patients with atopic asthma, whereas in BAL from patients with pulmonary tuberculosis, a predominance of TH1 cells has been found.
The issue of how TH1 cells induced by M. tuberculosis can regulate TH2 cells against distinct antigens has been by 12,23-26.

Previous reports have provided convincing evidence for an inverse association between tuberculin responses and atopic disorders 7 and this agree with the findings in this work as, at the beginning of this study only one patient of the extrinsic asthma group (6.67%) and 3 patients of the intrinsic asthma group (16.67%) showed positive tuberculin test, while 6 healthy controls showed positive tests (28.8%).

The idea that the TH1/TH2 balance could be affected by vaccination with relevant micro-organisms or microbial components is another logical consequence of the current knowledge of the TH1/TH2 paradigm. In that regard, Mycobacterium tuberculosis is one of the most potent immunomodulatory microorganisms and could be expected to strongly effect the cytokine milieu in the lung 27.

The adaptive immune response to allergens and mycobacteria is initiated by DCs. Pattern recognition receptors, including Toll-like receptors (TLRs), on the surface of the DCs recognize foreign material and phagocytose it, or transduce signals into the DCs. T cell receptors recognize the presented fragments of the foreign material on MHC class II of the DCs (signal 1), and co-stimulatory molecules, such as CD80/86 expressed on the cell surface of activated DCs, interact with ligands (such as CD28) on the T cell surface (signal 2). At the same time, polarizing molecules, such as IL-12 produced by DCs (signal 3), determine the type of T cell responses needed. Mycobacterial products are recognized by TLR2 and TLR4, which lead to secretion of IL-12 and IL-10 from DCs, and the development of Th1/Treg cells 27. MBP70, a major secreted protein of BCG, 27 and mycolic acid, a cell-wall component of BCG, 27 effectively suppressed asthmatic reactions in murine models. Although the findings by Kim et al 12 are rare one showing a suppressive effect of BCG-treated DCs on asthma, live BCG vaccination and administration of BCG-treated DCs naturally work in the same manner, because BCG produces adaptive immunity through DCs 28.

However these data could not confirm that mycobacterium tuberculosis reduce predisposition to atopy as the predisposition genetic factors are the most contributing factors to the susceptibility for atopy.

In this study, we addressed the question of whether the BCG vaccination can suppress the development of TH2 responses in adult asthmatic patients with the resultant improvement of asthma symptoms.

At the start of this study, extrinsic asthmatic patients showed a high level of serum IL4 compared to the intrinsic asthmatic group (P< 0.01) and a low level of serum of IL-2 compared to the intrinsic asthmatic group (P< 0.01).

After BCG vaccination serum levels of IL-4 decreased significantly in the extrinsic asthmatic group P <0.05, also decreased significantly in the intrinsic asthmatic group P<0.05, while serum levels of IL-2 showed highly significant increase in the extrinsic asthmatic group P<0.01, and a significant increase in the intrinsic asthmatic group P<0.05 while Serum levels of total IgE showed significant decrease in the extrinsic asthmatic group P<0.05, with no significant change in the intrinsic asthmatic group.

These findings proved that after 8 weeks of BCG vaccination in the asthmatic groups, significant polarized TH1 response was detected by the increase of serum, IL2 with decrease of TH2 response in asthmatic patients as detected by serum, IL4 level reduction.

Improvement of pulmonary function PEER in the extrinsic atopic asthmatic patients after BCG vaccination was much significant than that in the intrinsic non atopic asthmatic patients probably due to the reduction of a TH2 immune response. This suggests the potential pathologic role of TH2 immune response in atopic asthma, and this was proved in this study by the highly significant positive correlation found,
after BCG vaccination between PEFR and serum level of IL-2 and the highly significant negative correlation between PEFR and serum level of IL-4.

Based on the results obtained the most likely explanation is that TH1 cytokine profile induced by BCG could suppress homing, development or expression of TH2.

Ria, et al\textsuperscript{29} published a report showed that IL4 down regulate expression of IL12 and suggested that this down modulation lead to generation of TH2. But the presence of IFN-\gamma mediated inhibits this IL4 induced effect consequently. This leads to development of TH1 response. So, the observed strong inhibition of TH\textsubscript{2} effect response could be due IFN-\gamma\textsuperscript{30}.

The balance between IFN-\gamma and IL4 represents a site for T. cell activation and determines whether TH1 and or TH2 cells are generated\textsuperscript{31}.

Infection with respiratory syncytial virus which induces transient increase of IFN-\gamma did not inhibit TH2 immune response in the airways. Therefore, inhibition of TH\textsubscript{2} immune response might therefore be limited to infections that induce strong and relatively long lasting TH1 response\textsuperscript{32}.

The skewing toward TH1-dominant responses caused by the presence of M. tuberculosis was suggested to be mediated by 1L-12. In mice IL-12 treatment has been reported to abrogate antigen induced pulmonary eosinophilia\textsuperscript{33}.

Mycobacteria elicit particularly strong protective TH1 immune responses. Mycobacterial lipoproteins bind to macrophage which carry T cell-like receptors (TLRs) and this interaction leads to prominent synthesis of IL-2, and hence prominent TH1 switching and secretion of interferon (IFN-\gamma) and tumour necrosis factor (TNF-\alpha). These cytokines, repress TH2 immune mechanisms both in vivo and invitro experiments\textsuperscript{29,34}.

Researches using mycobacterial product-pulsed DCs or DCs co-cultured with allergens and BCG were done\textsuperscript{28, 29, 35-39}. Co-administration of \textit{Dermatophagoides farinae} and BCG to DCs increased the efficiency of IL-10 production from DCs, and effectively decreased IL-5 production from T cells, probably because the allergen and BCG were simultaneously recognized by the same DCs. In addition, co-pulsing of DCs with unrelated antigens may have a mutual helper effect\textsuperscript{28}.

Repression of TH2 immune mechanisms and hence atopy by mycobacterial exposure therefore appears possible.

In view of previous studies the most likely explanation for the results of this work is that the activation of TH1 cells with production of IL2 and INF following active BCG vaccination blocked the expansion of TH2 secreting IL4 as also suggested before by Robinson et\textsuperscript{40}, Ria et al\textsuperscript{29}, Erb et al\textsuperscript{41}.

**RECOMMENDATIONS**

1. Follow up of these groups of asthmatic patients is recommended examining the levels of the IL2, IL4 and IgE to study for how long the immunological effect of BCG vaccination can be maintained in this preliminary study.
2. BCG vaccination of children early in life may potentially be helpful in reducing the risk of developing atopic asthma.
REFERENCES


