Mycobacterium bovis BCG Mycobacteria – New Application

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This article is devoted to the memory of the late Prof. W.J.H. Kunicki-Goldfinger
on the tenth anniversary of his passing away

A b s t r a c t

The polarized response of T helper-2 (Th2) lymphocytes to an allergen is considered to be the main cause of the pathogenesis of asthma. In this study, we asked a question whether *M. bovis* BCG mycobacteria which are known for the preferential stimulation of T helper-1 (Th1) immunity, diminish the effector functions of Th2 cells from allergic patients upon stimulation with a common house dust mite Der p-1 allergen. Our results allow a positive answer to this question. We demonstrate that BCG modulates the dendritic cell-dependent allergen presentation process and switches naive T lymphocytes towards an anti-allergic Th1 profile.

K e y   w o r d s: BCG, Der p-1 allergen, Th1/Th2 cytokines

Introduction

The live attenuated *M. bovis* BCG bacilli developed by Calmette and Guérin at the Institute Pasteur in Lille, France, has been a widely used vaccine against tuberculosis since 1921 (Griffin *et al.*, 1999, Hoft *et al.*, 1999, Williams *et al.*, 2000). BCG vaccination of newborns and infants significantly reduces the risk of tuberculosis, by over 50%, on average. A protection has been observed across many populations. However an extraordinary variability in the protective efficacy of BCG vaccines was showed in clinical trials carried out in different parts of the world (Huebner, 1996). More than three billion people throughout the world have been vaccinated with BCG and this vaccine is considered to be one of the safest vaccines available. It appears to be safe even when given to perinatally HIV-1 – infected babies (Lallemant-Le Coeur *et al.*, 1991). However, BCG vaccination did not eliminate the tuberculosis problem and a new more effective vaccine against tuberculosis is required. One newly invented vaccine against tuberculosis, the urease C-deficient recombinant BCG equipped with the membrane-perforating listeriolysin of *Listeria monocytogenes*, has been recently licensed to a pre-clinical trial (Kaufmann, 2005).

The study conducted by Marchant *et al.*, 1999 indicated for the first time that human newborns can develop an acquired T-helper 1 (Th1) cellular response upon immunization with BCG. This response is characterized by a polarized production of type 1 cytokines including interleukin 12 (IL-12), interferon γ (IFN-γ) and IL-2. In this study, we addressed the possible interference of BCG with lymphocyte differentiation into T-helper 2 (Th2) cells upon stimulation with a common allergen from the house dust mite

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Dermatophagoides pteronyssinus (Der p-1). The polarized Th2 response to an allergen is considered to be the main cause of the pathogenesis of asthma. Th2 lymphocytes recognize the allergen peptides *via* T cell receptors and release interleukins which account for the joint involvement of B cells producing anti-allergen IgE (IL-4, IL-13), mast cells (IL-4, IL-10) and eosinophils (IL-5) in allergic inflammation (Romagnani, 2000). In order to investigate whether BCG interferes with the generation of Th2 response to Der p-1 allergen, we stimulated naive T lymphocytes from the peripheral blood of the patients with allergy to Der p-1 and healthy donors with autologous dendritic cells (DC) presenting Der p-1 allergen in the presence or absence of BCG. Dendritic cells are the first immunocompetent cells to encounter inhaled allergens and they possess a unique capacity to initiate response in naive T lymphocytes (Lambrecht and Hammad, 2003). The results show that *in vitro* BCG modulates the dendritic cell-dependent allergen presentation process and switches naive T lymphocytes towards an anti-allergic Th1 profile. This allows speculating that BCG bacilli might be considered as a potential candidate for immunotherapeutic strategies in allergy.

Experimental

Materials and Methods

Patients. Blood was collected from allergic patients sensitive to *Dermatophagoides pteronyssinus* and from healthy donors. All allergic patients had a history of asthma and presented the usual features of house dust mite sensitization: specific IgE antibodies and positive skin prick tests toward *D. pteronyssinus*. The level of total IgE was higher than 100 IU/ml. Healthy donors did not display any of these characteristics; total IgE level was below 100 IU/ml and absence of specific IgE to *D. pteronyssinus* in serum.

Bacteria. *M. bovis* BCG, a skin lyophilized vaccine against tuberculosis; 1.6×10^9 live bacilli/ml was diluted to 1×10^5 and used for pulsation of DC.

Cell preparation. After depletion of platelet-rich plasma, blood was diluted with RPMI 1640 (1:1; Life technologies, Paisley, Scotland) and layered over a Ficoll density gradient (Pharmacia, Uppsala, Sweden). After centrifugation (400 g for 30 minutes) human PBMCs (Peripheral Blood Mononuclear Cells) were isolated, washed and resuspended in PBS (Phosphate Buffered Saline), pH 7.2, supplemented with 0.5% BSA (Bovine Serum Albumin; Sigma, Saint-Quentin Fallavier, France) and 2 mM EDTA. PBMCs were incubated on ice for 30 minutes with anti-CD14 antibodies coupled to magnetic micro-beads (Miltenyi Biotec, Germany), washed and applied onto a column placed in the magnetic field of a MACS separator (Miltenyi Biotec). After elimination of negative cells, the column was removed from the separator and the CD14$^+$ cells were collected and resuspended in RPMI 1640 containing 10% heat-inactivated FCS (fetal calf serum; Life Technologies), 2 mM L-glutamine, and antibiotics (Ticarpen 1%; Smith Kline Beecham, Belgium) before plating (3×10^6 cell / 3 ml per well) into 6-well flat-bottomed culture plates. CD14$^+$ cells were differentiated into monocyte-derived DC for 6 days in medium supplemented with GM-CSF (20 ng/ml; Peprotech, United Kingdom) and interleukin 4 (IL-4) (200 IU/ml; R&D Systems, United Kingdom). Naive CD45RA$^+$ CD4$^+$ T lymphocytes were isolated from the eluted CD14$^-$ cell fraction by using a CD4$^+$ T cell isolation kit (containing CD8, CD11b, CD16, CD19, CD36, CD56 and CD45RO MACS microbeads). T cells were frozen in FCS containing 10% DMSO until used (Pochard, 2005).

Pulsation of DC. Monocyte derived DC (1×10^5 cells/ml per well) were incubated for 24 hours with Der p-1 (1 μg/ml), BCG (1:1), both stimuli concomitantly or LPS (1 μg/ml; Sigma-Aldrich).

T cell-DC cocultures. After washing, 1x 10^5 pulsed DC were incubated with 1×10^6 naive T cells for 5 days. The levels of IL-4, IL-5 and IFN-γ in supernatants of the cultures were determined by specific ELISA (Diaclone). The sensitivity of the assays was 0.5, 5 and 5 pg/ml, respectively.

Statistical analysis. Nonparametric statistical analysis of the cytokine production by naive T cells was performed using the Mann-Whitney U test. The Fisher exact test was used to determine the prevalence differences. Values of p≤0.05 were considered statistically significant.

Results

Immature monocyte-derived DC from allergic patients and healthy donors, pulsed or not with Der p-1, Der p-1/BCG, BCG, or LPS, were co-cultured, for 5 days, with the autologous naive T cells at the ratio of 1:10. The concentrations of IL-4, IL-5 and IFN-γ were measured in the culture supernatants by ELISA. Data in Table I show that naive T cells from all allergic patients produced the Th2 type cytokines, IL-4 and IL-5, when they were stimulated with autologous DC pulsed with Der p-1 antigen, BCG or both stimuli concomitantly. The prevalence of the release of IL-4 and IL-5 by identically stimulated naive T cells from healthy donors was statistically decreased. LPS – pulsed DC used as a control stimulated the IL-4 and IL-5 production by the T lymphocytes from 3 out of 9 allergic patients and no healthy blood donor. The mean concentration of IL-4 produced by T lymphocytes from healthy donors stimulated with the allergen- or BCG-pulsed DC was very low (range 1.6–2.1 pg/ml) and just above the detection test sensitivity (data not shown). The concentration of IL-4 in the co-cultures of naive T cells and allergen- or BCG-pulsed DC from allergic patients was easily measurable (Fig. 1). It is worth noting that the mean production of IL-4 by

Table I
Prevalence of the production of IL-4 and IL-5 by naive T cells from healthy donors and allergic patients,
in response to DC pulsed with Der p-1, Der p-1 + BCG, BCG, or LPS.

Blood donors	Der p-1	Der p-1 + BCG	BCG	LPS
	Number of IL-4 producers / number in the group			
Healthy	3/6*	3/6*	4/6	0/6
Allergic	9/9* p = 0.04	9/9* p = 0.04	9/9 p>0.05	3/9 p>0.05

Blood donors	Der p-1	Der p-1 + BCG	BCG	LPS
	Number of IL-5 producers / number in the group			
Healthy	3/6*	3/6*	4/6	0/6
Allergic	9/9* p = 0.04	9/9* p = 0.04	9/9 p>0.05	3/9 p>0.05

T cells from allergic patients, stimulated simultaneously with allergen and BCG was decreased as compared with the mean IL-4 production driven by DC pulsed with Der p-1 alone. However this difference was not statistically significant.

As it was expected, naive T cells from allergic patients secreted significantly more IL-5 in response to autologous DC pulsed with Der p-1 allergen compared with the cells from healthy donors (p = 0.03) (Fig. 2). In contrast, BCG-treated DC stimulated the IL-5 production slightly more intensively in the autologous T cells from healthy donors than from allergic patients (p = 0.03). It is worth mentioning that naive T lymphocytes from both allergic and healthy subjects when stimulated with autologous DC pulsed with Der p-1
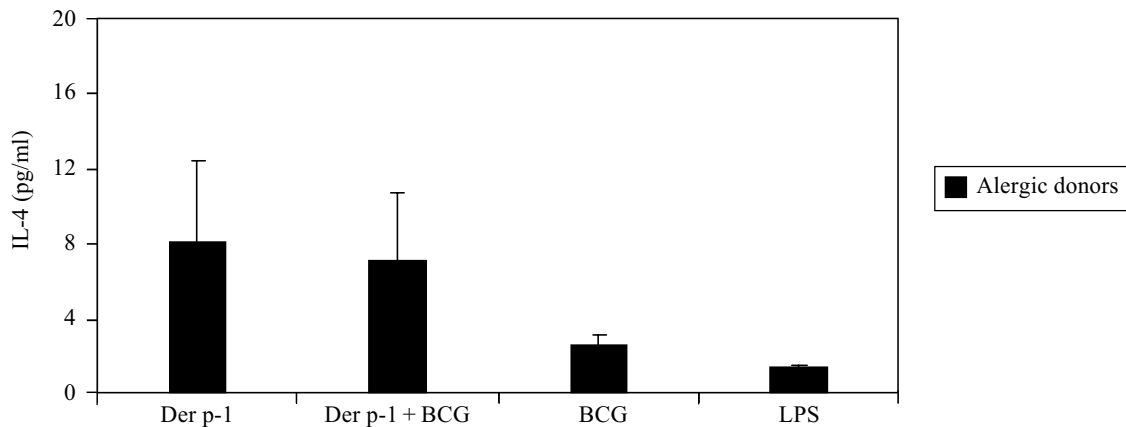

Fig. 1. The concentration of IL-4 in the co-cultures of naive T cells from allergic patients, stimulated
with autologous DC pulsed with BCG, Der p-1, Der p-1 + BCG or LPS.
Results are expressed as the mean ± SEM. Mean intensity value for LPS represents the results
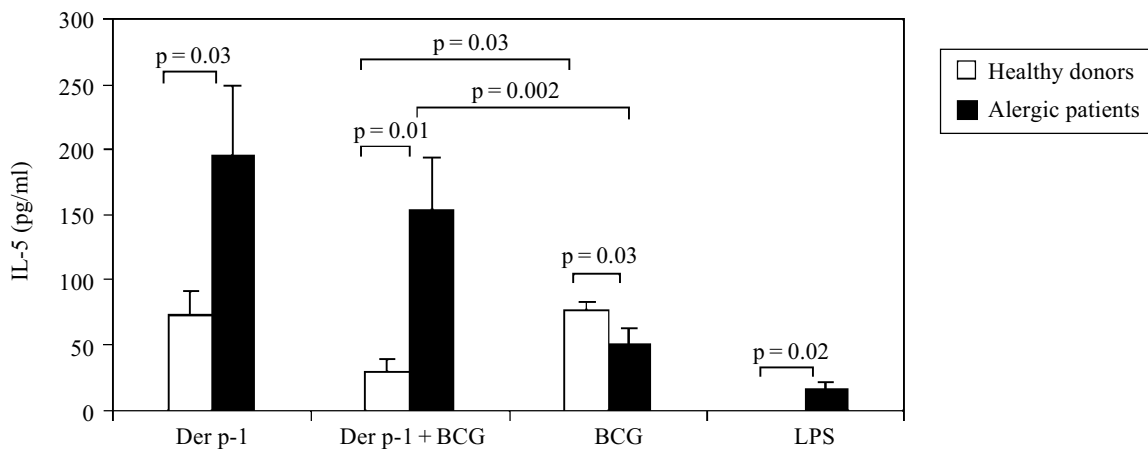for those individuals whose cells produced IL-4 in response to this stimulus.

Fig. 2. The concentration of IL-5 in the co-cultures of naive T cells from allergic patients, stimulated
with autologous DC pulsed with BCG, Der p-1, Der p-1 + BCG or LPS.
Results are expressed as the mean ± SEM. Mean intensity values for LPS represent the results for those individuals
whose cells produced IL-4 in response to this stimulus.

Table II
Prevalence of the production of IFN-γ by naive T cells
from healthy donors and allergic patients, in response to DC
pulsed with Der p-1, Der p-1 + BCG, BCG, or LPS

Blood donors	Der p-1	Der p-1 + BCG	BCG	LPS
	Number of IFN-γ producers / number in the group			
Healthy	6/6	6/6	6/6	6/6
Allergic	9/9	9/9	9/9	9/9

allergen and BCG concomitantly produced less IL-5 than when they were pulsed with Der p-1 allergen alone. However, this differences did not approached statistical significance.

Data on the production of Th1 type cytokine, IFN-γ, are presented in Table II. This cytokine was detected in the cultures of naive T lymphocytes from all allergic and healthy subjects, stimulated with autologous DC pulsed with each stimulus. However, the mean concentration of IFN-γ was significantly lower in the cultures of naive T cells from allergic patients, stimulated with autologous DC pulsed with Der p-1 allergen, BCG or both than in the identically stimulated cultures of T cells from healthy donors (Fig. 3). However, no difference was noticed between the T cells from allergic and healthy subjects, responding by IFN-γ to DC educated with LPS. Moreover, the addition of Der p-1 to BCG-pulsed DC from allergic patients did not reduce their capacity to stimulate IFN-γ production by autologous naive T cells.
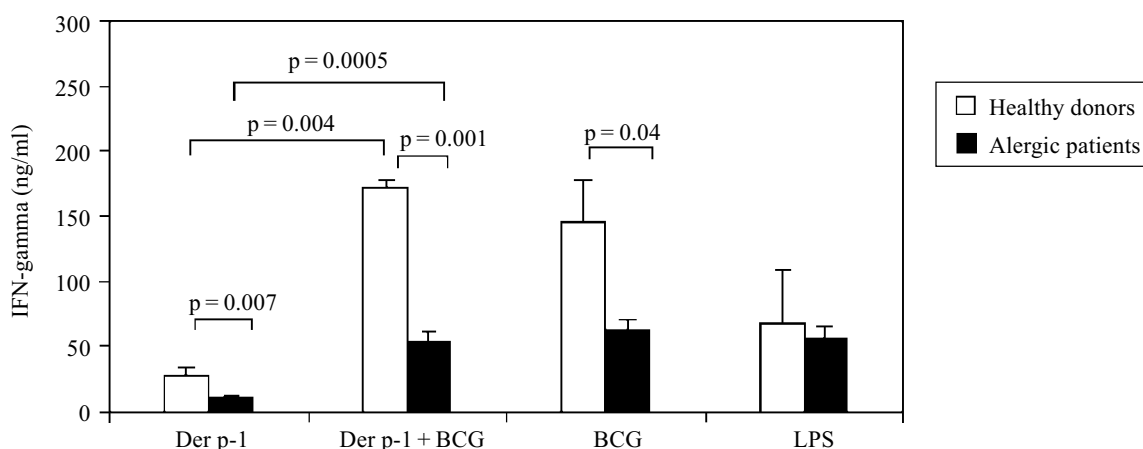

Fig. 3. The concentration of IFN-γ in the co-cultures of naive T cells from allergic patients, stimulated with autologous DC pulsed with BCG, Der p-1, Der p-1 + BCG or LPS.
Results are expressed as the mean ± SEM.

Discussion

Allergy is characterized as the strong immune response to allergens present in the environment. The term of atopy describes the genetic predisposition of organism to produce the increased amount of IgE antibodies against allergens. Atopic allergy is a chronic and systemic inflammatory process that currently affects a high proportion of population. According to the World Health Organization (WHO), 25–30% of the world population suffers from allergy (Geha, 2003). This disease affects up to 20% of the population in developed countries. It is currently estimated that next 15–20% habitants of developed countries produce the allergen-specific IgE antibodies so they represent the group of increased risk to allergy. Atopic diseases are the result of Th2-dominated responses to single or several allergens. The results of this study demonstrated that, at least *in vitro*, autologous BCG-educated dendritic cells modulated the response of naive T cells from the patients with allergy to the main dust allergen, Der p-1. Th2-dominated response of T cells from allergic patients, characterized by the enhanced production of IL-4 and IL-5 and diminished secretion of IFN-γ, was polarized towards Th1 profile. This modulatory effect of BCG-pulsed DC resulted in the increased IFN-γ release and diminished secretion of IL-4 and IL-5. It is possible to speculate that *in vivo*, in the organism,

the decreased secretion of IL-4 and IL-5 will be associated with diminished synthesis of IgE including anti-allergen antibodies of this class which are directly responsible for allergic disorders.

The monocyte derived dendritic cells were used in this study because of their unique capacity to initiate differentiation of naive T cells and their extraordinary potency in the regulation and maintenance of immune responses to antigens and allergens. The contact zone between DC and T cells is highly organized and the signaling between these two types of cells is called "immunological synapse" (Grakoui *et al.*, 1999; Jacobelli *et al.*, 2004). DC regulate immune response of Th1 and Th2 cells through cell-cell interactions and through the release of soluble mediators. In the presence of various antigens/allergens, DC possibly through the release of Th1- or Th2-attracting chemokines selectively attract these cells into inflamed tissue. In this study we demonstrated that BCG mycobacteria directed DC-dependent response of naive T cells from allergic patients to Th1 pathway. Thus, our results suggest that BCG might switch the established Th2 response in allergic patients toward a beneficial Th1 profile. In conclusion, Bacille Calmette – Guérin mycobacteria, which are already used in the treatment of bladder cancer (Herr, 2005), might represent a new therapeutic strategy for the treatment of allergic diseases.

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