

BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model

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Background: Epidemiologic studies suggest an inverse correlation between infections and development of atopy. The purpose of this study was to test the hypothesis whether a preexisting T_{H1}-type immune response elicited by BCG immunization could suppress allergic sensitization and airway hyperreactivity in an animal model.

Methods: BALB/c mice were immunized with BCG and/or sensitized to ovalbumin.

Results: BCG immunization alone resulted in cutaneous type-IV hypersensitivity reactions to tuberculin and granulomatous lesions in the liver. Splenic mononuclear cells (MNCs) produced increased levels of IFN- γ after activation by Concanavalin A (ConA). Ovalbumin sensitization alone resulted in increased production of IL-4 after activation by ConA. Ovalbumin-sensitized animals also demonstrated markedly elevated anti-ovalbumin IgE/IgG1 serum antibody titers and increased airway reactivity after allergen challenges by means of the airways. BCG immunization 14 days before the start of ovalbumin sensitization markedly hindered the development of allergic responses as indicated by (1) increased IFN- γ and normalized IL-4 and IL-10 production by splenic MNCs after activation with ConA, (2) a reduced proliferation rate of splenic MNCs after ovalbumin restimulation, (3) partial prevention of ovalbumin-specific IgE/IgG1 serum antibody titers but elevated (nonallergic) anti-ovalbumin IgG2a serum antibody titers, (4) prevention of airway responsiveness, (5) reduced eosinophilic influx into the airway lumen, and (6) reduced levels of IL-4 and IL-5 in bronchoalveolar lavage fluids.

Conclusion: In this model BCG immunization established a T_{H1}-type immune response that hinders allergic sensitization and the development of increased airway reactivity. (*J Allergy Clin Immunol* 1998;102:867-74.)

Key words: T_{H1}-type immune response, *Bacillus Calmette-Guerin*, allergic sensitization, airway hyperresponsiveness, airway eosinophilia

Abbreviation used

AR:	Airway reactivity
BAL:	Bronchoalveolar lavage
ConA:	Concanavalin A
DTH:	Delayed-type hypersensitivity
MNCs:	Mononuclear cells
OVA:	Ovalbumin
PPD:	Purified protein derivative

Bronchial asthma is characterized by a T_{H2}-type immune response, altered airway reactivity (AR) to inhaled allergens, and chronic inflammation of the airways.^{1,2} The pivotal role of the T_{H2}-type cytokines IL-4 and IL-5 in the pathophysiologic design of this disease has been extensively studied in humans and mouse models. IL-4 serves as a proliferation factor for T_{H2}-type T cells and provides B-cell help for allergen-specific antibody isotype production (IgE, IgG1).^{3,4} IL-5 stimulates eosinophil differentiation, prolongs eosinophil survival by preventing apoptosis, and triggers eosinophil recruitment into the airway lumen.⁵

In most species the capacity to respond in a T_{H1}- or T_{H2}-type fashion is to a large extent genetically determined. However, infection or immunization may skew the cytokine microenvironment toward preferential proliferation of T_{H1} cells and may protect against the development of T_{H2}-dependent allergic disease. Indeed epidemiologic studies showed an inverse correlation between the frequency and intensity of the response to viral and bacterial infections and the development and prevalence of allergic diseases.⁶⁻⁸ One possible explanation for this phenomenon could be that the characteristic cytokine products of T_{H1}-type and T_{H2}-type T cells are mutually inhibitory for the differentiation and effector functions of the reciprocal phenotype. Mycobacteria are among the most potent inducers of a T_{H1}-type response.⁹⁻¹¹ T_{H1}-type cells are characterized by the production of IFN- γ and IL-2 that mediate T-cell cytotoxic activity and delayed-type hypersensitivity (DTH).^{12,13} Moreover, IFN- γ inhibits T_{H2}-type T-cell expansion and enhances IgG2a production.¹⁴⁻¹⁶

We have developed a murine model of allergic asthma in which BALB/c mice are sensitized to ovalbumin and repeatedly exposed to ovalbumin aerosol. In this model several symptoms can be observed that resemble those found in patients with allergic asthma, such as presence of

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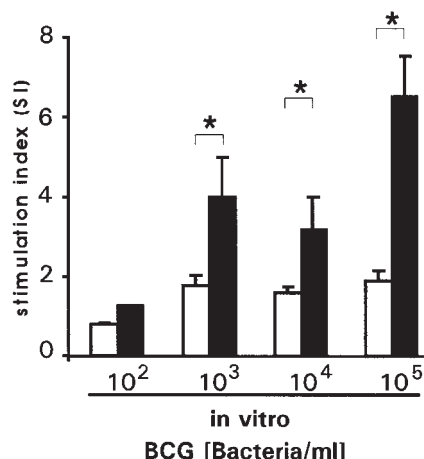


FIG 1. Splenic lymphocytes were prepared 14 days after intravenous application of 10^6 bacteria/mouse. PBMC/well (2.5×10^5) were cultured for 96 hours in the presence of ConA ($2.5 \mu\text{g/mL}$). ^3H -Thymidine incorporation (stimulation index) was assayed in response to increased concentrations of BCG ($10^2 - 10^5$ bacteria/mL; black bars). Controls received PBS intravenously only (white bars). Mitogen stimulation was assayed in parallel. ^3H -Thymidine incorporation increased in response to ConA ($2.5 \mu\text{g/mL}$) by a factor of 64 ± 30 for nonimmunized mice and 68 ± 30 for BCG-immunized mice. The means $\pm$ SEM are presented for each group, with 6 to 9 mice in each group. *Statistical significance, $P \leq .05$.

allergen-specific IgE in the serum, increased AR, and airway inflammation.^{17,18} The aim of this study was to investigate the possible inhibitory effects of a preexisting systemic T_H1 -type immune response elicited by bacterial antigens on the development of allergy in this animal model.

METHODS

Animals

Female BALB/c mice, aged 6 to 8 weeks, were obtained from Bombholtgard (Ry, Denmark). The animals were virus free, as indicated by negative antibody titers to common murine viruses. Groups of 8 mice were caged separately according to their treatment and had free access to ovalbumin-free diet and water.

Immunization

Mice were immunized with an attenuated *Mycobacterium bovis* strain (BCG) commonly used for vaccination in infants (BCG vaccine; Behring, Marburg, Germany). Viable organisms (1×10^6) in 0.1 mL PBS were injected in each mouse intravenously under sterile conditions in accordance with published protocols.^{19,20} Control mice received PBS.

Sensitization

Mice were sensitized by repeated intraperitoneal injections of $10 \mu\text{g}$ chicken ovalbumin (grade VI; Sigma, Deisenhofen, Germany) emulsified in $1.5 \text{ mg Al}(\text{OH})_3$ (Inject Alum; Pierce, Rockford, Ill) on days 14, 28, and 35. Control mice were injected with $\text{Al}(\text{OH})_3$ alone.

Study groups

The following study groups were analyzed: Control mice received an intravenous injection of PBS ($100 \mu\text{L}/\text{mouse}$) on day 1 followed by intraperitoneal injections of $1.5 \text{ mg Al}(\text{OH})_3$ on days 14, 28, and 35. BCG mice were immunized with BCG (intravenous

TABLE I. Cytokine production in BCG-immunized BALB/c mice

Study groups	Cytokine production (pg/mL)		
	IL-4	IL-5	IFN- γ
Intravenous			
—	329 $\pm$ 94	174 $\pm$ 29	1554 $\pm$ 255
BCG	331 $\pm$ 49	140 $\pm$ 10	3457 $\pm$ 268*

Splenic MNC were prepared 14 days after intravenous application of 1×10^6 bacteria/mouse. MNC/well (2×10^6) were cultured for 96 hours in the presence of ConA ($2.5 \mu\text{g/mL}$). Cytokine production in cell-free culture supernatant were measured by ELISA. The means $\pm$ SEM are presented, with 5 mice per study group. Statistical significance was determined by Student's t test.

* $P \leq .05$ was considered significant.

1×10^6 bacteria/mouse) as described followed by intraperitoneal injections of $1.5 \text{ mg Al}(\text{OH})_3$ on days 14, 28, and 35. Ovalbumin mice received an intravenous injection of PBS on day 1 followed by sensitization to ovalbumin by 3 intraperitoneal injections of $10 \mu\text{g}$ ovalbumin emulsified in $1.5 \text{ mg Al}(\text{OH})_3$ on days 14, 28, and 35. The BCG + ovalbumin mice were immunized with BCG and sensitized to ovalbumin as described.

Airway allergen challenge

Before analysis on day 42, all animals received 2 consecutive airway allergen challenges of 1% (weight/volume) ovalbumin diluted in PBS delivered by aerosolization on days 40 and 41, as described elsewhere.¹⁷

Assessment of BCG infection

Lung and liver were homogenized and diluted 1/50 in 7H9 bouillon and grown on 7H11 agar plates at $37^\circ\text{C} + 5\%$ carbon dioxide. Bacterial colonies were counted.

Histologic evaluation of delayed hypersensitivity reaction (type IV) in the skin and liver

Tuberculin sensitivity was tested 28 days after immunization by intracutaneous injection of 100 IU purified protein derivate (PPD; Behring) dissolved in PBS (0.1 mL) into shaved ventral skin. Seventy-two hours later, the animals were killed; the skin of the test area was prepared, fixed in 4% formaldehyde, and stained with hematoxylin and eosin. For analysis of DTH reactions, the liver was prepared and fixed with 4% formaldehyde (weight/volume), and $3\text{-}\mu\text{m}$ sections embedded in paraffin were stained with hematoxylin and eosin.

Tissue culture conditions and stimulation of mononuclear cells

Spleens were prepared at the indicated time points, and small pieces were pressed through stainless steel meshes. Cells were suspended in RPMI 1640/10% FCS culture medium (Biochrom, Berlin, Germany). Mononuclear cells (MNCs) were purified by density gradient centrifugation (Lympholyte M; Cedarlane Laboratories, Hornby, Canada; $1000g$, 20 minutes at room temperature), washed twice in culture medium ($800g$, 10 minutes at room temperature), and suspended in culture medium (RPMI1640/10% FCS). Cells were counted in a Coulter counter (Coulter Electronics, Krefeld, Germany). MNCs/well (2.5×10^5) were incubated in 96-well U-bottom tissue-culture plates (Costar, Cambridge, Mass) at 37°C . Cells were stimulated for 96 hours with mitogen (con-

TABLE II. Cytokine production in splenic mononuclear cells

Study groups		Cytokine production (pg/mL)			
Intravenous	Intraperitoneal	IL-4	IL-5	IFN- γ	IL-10
—	—	294 $\pm$ 96	209 $\pm$ 51	1794 $\pm$ 354	143 $\pm$ 22
BCG	—	245 $\pm$ 51	143 $\pm$ 33	3716 $\pm$ 805*	91 $\pm$ 26
—	OVA	664 $\pm$ 98*	111 $\pm$ 14	1321 $\pm$ 279	203 $\pm$ 35
BCG	OVA	336 $\pm$ 67†	113 $\pm$ 9	4265 $\pm$ 803†	84 $\pm$ 31†

Cytokine production of splenic MNCs was measured in the following study groups: BCG, mice were immunized with BCG (intravenous 10^6 bacteria/mouse); OVA, mice received an intravenous injection of PBS on day 1, followed on days 14, 28, and 35 by sensitization to ovalbumin by intraperitoneal injection of 10 μ g ovalbumin/injection adsorbed to Al(OH)₃; BCG + OVA, mice were immunized with BCG on day 1 and sensitized against ovalbumin on days 14, 28, and 35. Splenic lymphocytes were prepared on day 42. MNC/mL (2×10^6) were cultured for 96 hours in the presence of ConA (2.5 μ g/mL). Cytokine production in cell-free culture supernatants was measured by ELISA. The mean $\pm$ SEM are presented, with 5 to 7 mice in each study group. Statistical significance was determined by Student's *t* test.

*Significantly different as compared with control group ($P < .05$).

†Significantly different as compared to OVA-group ($P < .05$).

canavalin A, 2.5 μ g/mL; Sigma, Deisenhofen, Germany) or $10^2/10^3/10^4/10^5$ BCG bacteria/mL (Behring) or ovalbumin 10 to 100 μ g/mL (Sigma). [³H]-thymidine (Amersham Buchler, Braunschweig, Germany) was added for the last 18 hours (1 μ Ci/well), and its incorporation was measured in a scintillation counter (Beckman, Munich, Germany). The proliferation rate was calculated as the index of [³H]-thymidine incorporation in stimulated cells divided by that in nonstimulated cells.

Bronchoalveolar lavage

Animals were killed 24 hours after the last airway allergen challenge; the trachea was cannulated, and bronchoalveolar lavage (BAL) was performed by 2 lavages with 0.8 mL ice-cold PBS. BAL fluid of each animal was pooled, and the recovered volume and total cell number were determined. Mean recovery volume was 1.4 ± 0.2 mL; no significant difference was detected between the study groups. Cytospins were prepared for each sample by centrifugation of 50 μ L BAL fluid (100g, 5 minutes). After fixation, cytopins were stained with Diff Quik (Baxter Dade, Düringen, Switzerland). Differential cell counts of 2×100 cells were performed. The cells were classified as either neutrophils, eosinophils, lymphocytes, or macrophages by standard morphologic criteria. Cell-free lavage fluids were stored at -20°C until further analysis.

Determination of cytokines

IL-4, IL-5, and IFN- γ were measured by ELISA as previously described.¹⁸ Sensitivities were 30 pg/mL for IL-4 and IL-5 and 50 pg/mL for IFN- γ . Concentrations of IL-10 were determined by an ELISA kit (Laboserv, Giessen, Germany) in accordance with the manufacturer's recommendations. Sensitivity was 16 pg/mL.

Determination of total IgE and allergen-specific antibody titers

Blood was sampled from the lateral caudal veins, clotted at room temperature, and centrifuged (1200g, 10 minutes at room temperature). Serum samples were stored at -20°C until analysis. Total IgE and allergen-specific IgE, IgG1, and IgG2a antibody titers were measured by ELISA as previously described.¹⁸ The antibody titers of the samples were related to pooled standard serum generated from sensitized BALB/c mice, the activity of which was arbitrarily set at 2000 ELISA units (EU)/mL.

Assessment of airway smooth muscle responsiveness

Twenty-four hours after the last allergen challenge through the airways, mice were killed, and the response of tracheal smooth mus-

TABLE III. Detection of BCG bacteria in lung and liver

Study groups		BCG bacteria (CFU/g tissue)	
Intravenous	Intraperitoneal	Lung	Liver
—	—	0	0
BCG	—	7784 $\pm$ 519	11987 $\pm$ 414
—	OVA	0	0
BCG	OVA	6789 $\pm$ 184	8227 $\pm$ 572

Lungs and liver were prepared from the same study groups as described in Table II. Tissues were homogenized, and BCG colony forming units were determined as described in the text. CFU/g tissue are presented as means $\pm$ SEM.

cle segments was assessed by electrical field stimulation as previously described.¹⁸ The frequency that caused 50% of the maximum contraction was calculated from logarithmic plots of the contractile response versus the frequency of electrical field stimulation, expressed as ES₅₀.

Statistical analysis

Results are presented as mean $\pm$ SEM values, unless stated otherwise. Student's *t* test was used to determine the level of difference between groups.

RESULTS

BCG immunization induces a T_{H1}-type-mediated DTH response

Splenic MNCs were prepared 14 days after BCG immunization and restimulated in vitro with BCG. Lymphocyte proliferation increased in a dose-dependent manner in the BCG group but not in the PBS group (Fig 1). This proliferative response was accompanied by a shift in cytokine production towards T_{H1}-type after ConA stimulation, as indicated by elevated IFN- γ production at day 14 (start of ovalbumin sensitization). In contrast, IL-4 and IL-5 production remained unaffected compared with the PBS group (Table I). This effect continued until day 42 after BCG immunization (Table II).

DTH reactions to BCG was assessed 4 weeks after BCG immunization. BCG-immunized mice developed

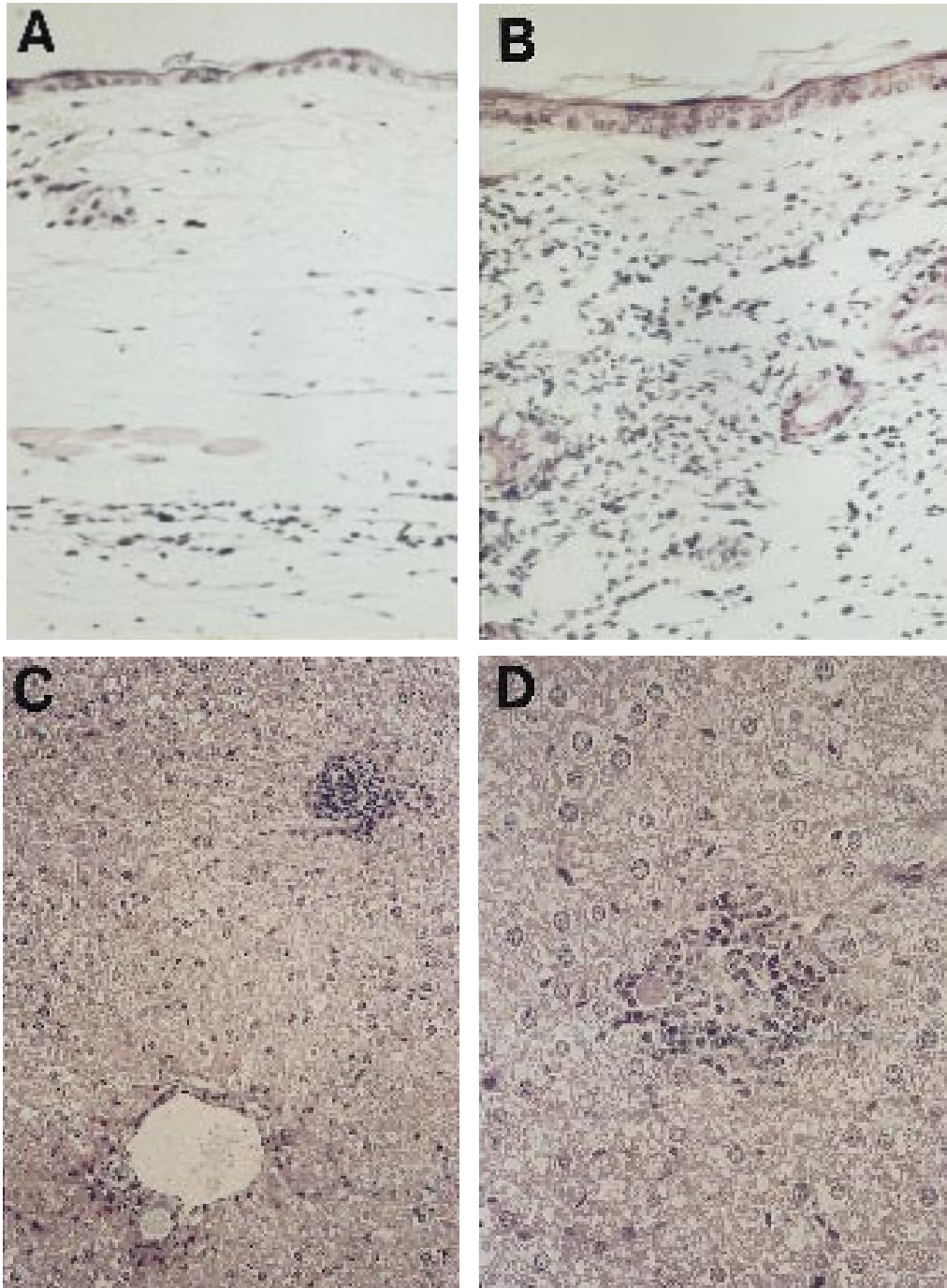


FIG 2. BALB/c mice were immunized by intravenous injection of 10^6 BCG. On day 28, 100 IE PPD were injected intracutaneously. Seventy-two hours later animals were killed, and 3- μ m sections of paraffin-embedded skin and liver were stained with hematoxylin and eosin. **A**, Skin of a nonimmunized animal (original magnification, $\times 20$). **B**, Skin of a BCG-immunized animal (original magnification, $\times 20$). **C**, Liver of a BCG-immunized animal (original magnification, $\times 20$). **D**, Liver of a BCG-immunized animal (original magnification, $\times 40$).

strong DTH responses after intracutaneous challenge with PPD-standard (PPD-S) as indicated by a marked lymphocyte and macrophage influx into the dermis at the site of PPD-S injection. In contrast, no lymphocyte or macrophage influx was detected after PPD-S challenge in

skin sections derived from animals of the ovalbumin and PBS groups (Fig 2, A and B). This T_{H1} -type immune response was accompanied by the formation of granulomatous lesions in the liver of BCG-immunized mice, the site where intravenously administered BCG bacteria are

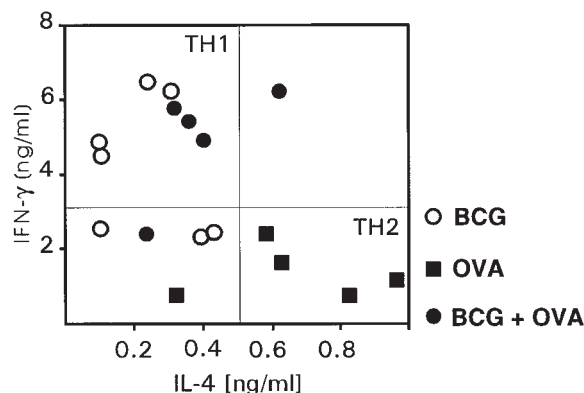


FIG 3. Cytokine production of splenic lymphocytes was analyzed in the following study groups: BCG, BCG-immunized mice; ovalbumin, sensitized to OVA; BCG + ovalbumin (OVA), BCG-immunized and OVA-sensitized mice. IL-4 and IFN- γ were measured by ELISA (described in the text). The results are plotted from individual mice.

predominantly cleared (Fig 2, C and D). In addition, viable BCG bacteria were recovered from the liver and lung from the BCG and BCG + ovalbumin groups (Table III).

BCG immunization suppresses ovalbumin sensitization

Cytokine production of splenic MNCs from animals sensitized to BCG + ovalbumin was measured on day 42 after BCG immunization and compared with the production measured in control mice and animals of the ovalbumin group (Table II). The cytokine pattern of splenic MNCs from animals immunized with BCG was comparable at days 14 and 42 (Tables I and II), indicating the stability of the established T_H1 response over this period. Ovalbumin sensitization alone induced a T_H2 -type cytokine pattern, indicated by elevated levels of IL-4 in MNCs culture supernatants, whereas IFN- γ expression was unaffected. In mice of the BCG + ovalbumin group, the cytokine levels were in the same magnitude as in mice immunized with BCG, especially the IFN- γ production, which was enhanced (Table II).

The individual in vitro IL-4 versus IFN- γ response pattern of animals of the BCG, ovalbumin, and BCG + ovalbumin group on day 42 showed that BCG immunization before ovalbumin sensitization prevented the development of the T_H2 -type immune response in BCG + ovalbumin mice (Fig 3). Furthermore, in BCG + ovalbumin mice allergen-specific proliferation rate of splenic MNCs was reduced to less than 50%, compared with mice sensitized to ovalbumin (Fig 4).

BCG immunization before ovalbumin sensitization had a marked effect on immunoglobulin production. Ovalbumin sensitization alone induced high levels of anti-ovalbumin IgE and IgG1 antibody titers (Table IV). BCG immunization before ovalbumin sensitization prevented allergen-specific IgE antibody titers by about 75% and IgG1 production by about 70%. Ovalbumin sensitization alone induced moderate anti-ovalbumin IgG2a produc-

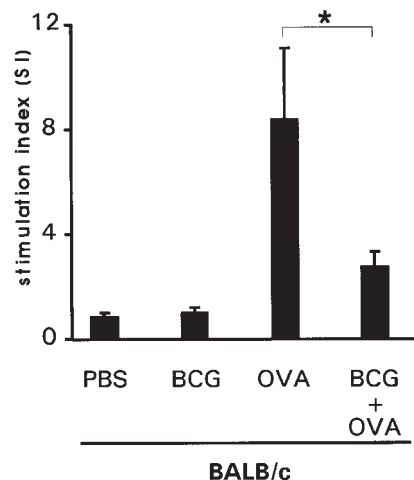


FIG 4. Splenic lymphocytes were prepared from the same study groups as described in Fig 3. PBMC/well (2.5×10^5) were cultured for 96 hours in the presence of ovalbumin (100 μ g/mL). Mitogen stimulation was assayed in parallel. ^{3} [H]-Thymidine increased incorporation in response to ConA (2.5 μ g/mL) by a factor of 40 ± 21 . The means $\pm$ SEM are presented for each group, with 4 to 6 mice in each group. *Statistical significance, $P \leq .05$.

tion, whereas prior BCG immunization approximately doubled anti-ovalbumin IgG2a antibody production.

Prevention of increased airway responsiveness in BCG-immunized mice

Tracheal segments from control tissues (airway allergen challenge alone in nonsensitized mice) and BCG-immunized mice responded with an ES_{50} (50% of maximum airway smooth muscle contractility) of 3.7 ± 0.4 Hz and 3.8 ± 0.4 Hz, respectively (Fig 5). Ovalbumin sensitization followed by airway allergen challenge induced increased airway responsiveness as indicated by a decrease of the ES_{50} values to 2.2 ± 0.2 Hz ($P \leq .01$). BCG immunization before ovalbumin sensitization prevented the development of increased airway responsiveness as indicated by ES_{50} values of 3.3 ± 0.4 Hz ($P \leq .01$ versus ovalbumin mice and not significant versus control animals). The prevention of increased airway responsiveness in the BCG + ovalbumin group was accompanied by a significantly ($P \leq .01$) reduced influx of eosinophils into the airway lumen (Fig 6). In contrast, the levels of lymphocytes and neutrophils influx were not affected compared with the ovalbumin group. In addition, IL-4 and IL-5 production in BAL fluids from BCG-immunized and ovalbumin-sensitized animals were significantly ($P < .05$) reduced in comparison with ovalbumin-sensitized mice (Table V).

DISCUSSION

This study demonstrates that systemic BCG immunization hinders the development of allergic sensitization and prevents the development of increased AR in BALB/c mice. BCG was chosen for this investigation as

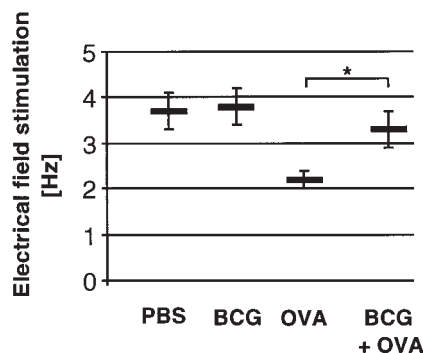


FIG 5. AR was analyzed on day 42 by electrical field stimulation of tracheal smooth muscle preparations and expressed as a percentage of control ES_{50} . The mean $\pm$ SEM ES_{50} for control animals ($n = 23$) was 3.7 ± 0.4 ; this value was taken as 100%. AR was analyzed in OVA-sensitized ($n = 11$) and BCG-immunized before OVA-sensitization ($n = 7$) mice. Data are presented as the means $\pm$ SEM. *Statistical significance, $P \leq .01$.

a prototypic stimulus for a persistent T_{H1} -type immune response,²¹ such as naturally occurring mycobacteria or viral infections.^{22,23} BALB/c mice are well described as a "high-responder strain" to ovalbumin sensitization and also susceptible to mycobacterial infection associated with a T_{H1} -type immune response.^{18,24,25}

BCG immunization before ovalbumin sensitization resulted in a markedly increased production of IFN- γ by splenic MNCs after ConA stimulation (Table II). Because it is unlikely that BCG immunization elicits a direct effect on ovalbumin-specific T cells, the results suggest that BCG immunization hinders the development of ovalbumin sensitization by altering the general immune balance towards T_{H1} -like activity.²⁰ IL-4, a cytokine involved in IgE production, was similarly reduced as IL-10. IL-10 is a cytokine not only secreted mainly by T_{H2} -type lymphocytes but also by other cells on activation.²⁶ Although it has been shown that high levels of IL-10 can be present in infectious models,²⁷ the increase of IL-10 and IL-4 production of MNC from ovalbumin-sensitized animals in vitro in parallel with the depression of IFN- γ suggests that IL-10 is part of the T_{H2} -type response in this model (Table II). The influence of BCG immunization on allergen-specific IgE and IgG1 production has not been described before under experimental conditions. One could speculate that the global T_{H1} -like cytokine milieu induced by BCG alters the direction of ovalbumin-specific humoral immune responses, but we were not able to demonstrate a change of the cytokine pattern of splenic MNC after ovalbumin stimulation (data not shown). Alternatively, inadequate priming of an ovalbumin-specific T_{H2} -response has to be considered the leading cause for this observation. This explanation is supported by the markedly reduced proliferative capacity to ovalbumin in BCG + ovalbumin mice. Furthermore, a study among Japanese school children showed that a strong PPD-skin test reactivity is paral-

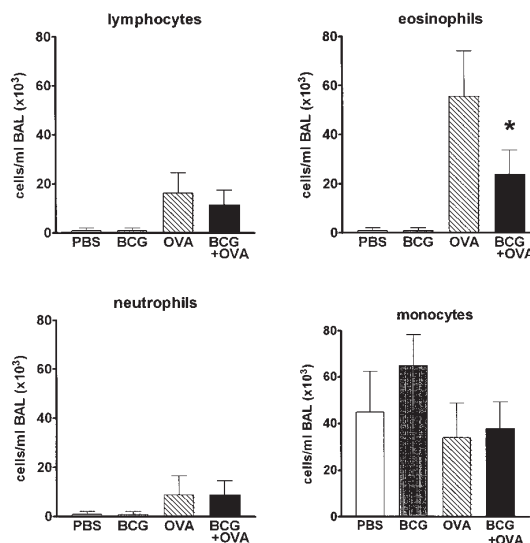


FIG 6. Absolute numbers of leukocytes in BAL fluid/mouse were determined in following study groups: BCG, BCG-immunized mice; OVA, sensitized to OVA; BCG + OVA, BCG-immunized and OVA-sensitized mice. All animals received 2 allergen challenges administered by aerosolization 48 and 24 hours before analysis. BALs were performed 24 hours after the last airway allergen challenge (described in text). Means $\pm$ SD are presented for each group, with 5 to 7 mice in each group. *Statistical significance, $P \leq .05$ between OVA-sensitized and BCG-immunized plus OVA-sensitized mice.

leled by a cytokine pattern skewed towards a T_{H1} -response and lower serum IgE antibody titers.⁸

The altered immune response was associated with marked changes on the development of increased AR. BCG immunization before ovalbumin sensitization suppressed eosinophil influx into the lung and IL-4 and IL-5 production in BAL fluids (Table V; Fig 4). The important *in vivo* role for IL-5 in recruitment of eosinophils into the lungs was demonstrated in several murine models of allergic asthma. Treatment with anti-IL-5 antibodies inhibited the eosinophilic infiltration in allergen-sensitized and allergen-challenged mice.^{28,29} Furthermore, in IL-5-deficient mice, airway eosinophilia could not be induced,³⁰ and IL-5-transgenic mice displayed eosinophilic infiltration and increased AR in the absence of allergen challenges.³¹ Increased AR may be caused by either the interaction of allergen-specific IgE/IgG1 antibodies and allergen³² or an inflammatory component associated with mediators. Because BCG immunization before ovalbumin sensitization did not totally suppress allergen-specific IgE/IgG1 production (Table II), the reduction of airway inflammation is the supposed mechanism of action in this model. In another recently published study the effect of BCG immunization on the development of airway inflammation was abolished by intranasal application of recombinant IL-5.³³ As shown previously,³⁴ even when administered after allergic sensitization, inhaled IFN- γ can prevent increased AR. It has been suggested that IFN- γ prevents the development of

TABLE IV. Allergen-specific immunoglobulin production

Study groups		Serum antibody titers [EU/mL]		
Intravenous	Intraperitoneal	Anti-OVA IgE	Anti-OVA IgG1	Anti-OVA IgG2a
BCG	—	≤7	≤5	≤8
—	OVA	1047 ± 225	2245 ± 695	540 ± 142
BCG	OVA	282 ± 36*	692 ± 198*	1007 ± 255*

Serum antibody production for ovalbumin (OVA)-specific and total immunoglobulin was determined by ELISA. Analysis was of the same study groups as described in Table II. Blood from all animals was drawn on day 42 and stored at -20°C until analysis of serum immunoglobulins. The means ± SEM are presented, with 5 to 7 mice in each group. Statistical significance was determined by Student's *t* test.

**P* ≤ .05 was considered significant.

TABLE V. Cytokine production in BAL fluids

Study groups		Cytokine production in BAL fluid (pg/mL)		
Intravenous	Intraperitoneal	IL-4	IL-5	IFN-γ
—	—	≤30	≤30	≤100
BCG	—	≤30	≤30	≤100
—	OVA	359 ± 66	347 ± 43	≤100
BCG	OVA	184 ± 28*	221 ± 42*	≤100

Cytokine production in BAL fluids was measured by ELISA in the same study groups as described in Table II. The mean ± SEM are presented for each study group, with 5 to 7 mice in each group. Statistical significance was determined by Student's *t* test.

**P* ≤ .05 was considered significant compared with ovalbumin-sensitized animals.

increased AR after ovalbumin sensitization by interfering with eosinophil migration and eosinophil degranulation.^{35,36} Although higher levels of IFN-γ could not be observed in BAL, the recovery of BCG bacteria from lung tissue supports the view that IFN-γ was produced by local T cells.

Taken together, our results reveal an inverse relation between BCG immunization and the development of ovalbumin sensitization in the murine system. Because murine T-helper subsets have similar functions as in the human immune system, it could be speculated that in children with a genetic predisposition towards allergic sensitization, early infections may skew the T_{H1}/T_{H2} balance towards a T_{H1}-cytokine pattern, thereby hindering allergic sensitization and subsequent allergic disease. In the genetically and geographically similar populations of former East and West Germany, different prevalences of allergic sensitization and atopic disease were observed.⁶ Similar results were reported from the Baltic area.⁷ These differences have been attributed to environmental and lifestyle factors, such as exposure to indoor allergens and respiratory infections. Recently, a study among children with asthma demonstrated that AR normalization was 6 times more likely if there was a conversion to a positive tuberculin response between the ages of 6 and 12 years.⁸ The results from this study suggest that exposure to atypical mycobacteria might result in a shift towards a T_{H1} response in this population. However, this phenomenon could also be a surrogate rather than a cause, with

the atopics showing an impaired T_{H1} response to intradermally injected tuberculin.

The administration of BCG in the dose used in our experiments, which clearly is aimed at infection/immunization, is different from vaccination schedules, where the same mycobacterium is used. Reports on the effect of BCG vaccination on allergic sensitization are sparse. Most recently, BCG-vaccinated and nonvaccinated children of preschool age with a family predisposition for atopic diseases have been compared for atopic manifestations. No protective effect of BCG vaccination against sensitization or manifestation of atopic disease could be detected.³⁷ The quality of the T_{H1} response was not verified by tuberculin responses, however.

The results from this study cannot offer a plan of protection from atopy by a T_{H1} response; efforts are being undertaken, however, to induce oral tolerance by confronting the immune system at an early stage with certain antigens to induce a T_{H1}-type-like immune response.³⁸ Another approach would be to focus on the local environment by inhalation, where a sustained immune response can be elicited.³⁹ Because it is known that autoimmune diseases such as insulin-dependent diabetes mellitus are T_{H1} associated and a shift towards T_{H2} type protects the fetus in pregnancy, great caution should prevail when strategies are being considered to shift the cytokine balance away from atopy too early in life.^{40,41}

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