

A recombinant BCG vaccine generates a Th1-like response and inhibits IgE synthesis in BALB/c mice

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SUMMARY

The tubercle vaccine, bacille Calmette–Guérin (BCG), is a strong inducer of T-helper type 1 (Th1) responsiveness, and it has been suggested that recombinant BCG (rBCG), which produces and secretes antigens, may be used to prevent allergic diseases. The effects of rBCG vaccination on allergic responses in a murine model were examined in this study. A BCG–*Escherichia coli* shuttle vector was developed with the promoter and signal sequence of the α -antigen of *Mycobacterium bovis*, and the vector was tested using *E. coli* β -galactosidase as the model antigen and allergen. This vector enabled the expression of the *E. coli* β -galactosidase gene in BCG, which was detected in its protein extract by immunoblotting analysis. Vaccination of mice with a single dose of 10^6 recombinant BCG generated a β -galactosidase-specific antibody response. The splenocytes of vaccinated mice compared with controls produced significantly higher amounts of interferon- γ (IFN- γ) ($P < 0.01$) and interleukin-2 (IL-2) ($P < 0.05$) and lower amounts of IL-5 ($P < 0.01$). Mice vaccinated with rBCG had significantly less ($P < 0.01$) serum IgE compared with controls. These results together demonstrate that rBCG secreting antigens or allergens may be utilized for the induction of a Th1-like response and the down-regulation of IgE antibody response.

INTRODUCTION

Bacille Calmette–Guérin (BCG), a live attenuated *Mycobacterium bovis*, is used in many countries as a vaccine against human tuberculosis and is a strong inducer of a T-helper type 1 (Th1)-like response.¹ Both cellular and humoral immune responses are observed in animal models vaccinated with BCG.^{2,3} Therefore, this micro-organism constitutes an ideal live vector for the presentation of foreign antigens or allergens to the immune system. In addition, BCG offers several other advantages as a recombinant allergen (rAg) delivery system.⁴ It is relatively safe and can be administered at any time after birth because it is unaffected by maternal antibodies. Also given as a single inoculum, it can sensitize to tuberculin for 5–50 years. A dose of the BCG vaccine is substantially less expensive than many other current vaccine formulations. One of the drawbacks of the BCG vaccine, however, which makes this vaccine unacceptable in the United States, is the induction of persistent tuberculin positivity in vaccinated individuals. Other disadvantages of BCG include necrotic reactions in

those individuals with the Koch phenomenon and a huge variation in its efficacy for protection against tuberculosis.

Atopic diseases, such as allergic asthma and allergic rhinitis, are characterized by an imbalance in the Th cell responses.⁵ Studies in both mice and humans have demonstrated that IgE-mediated allergic disorders are mediated primarily by Th2 lymphocytes.^{6–9} Th1- and Th2-like cells reciprocally regulate each other through the cytokines they secrete. For example, interferon- γ (IFN- γ) and interleukin-12 (IL-12) inhibit Th2-like cells, and IL-4 and IL-10 down-regulate the activity of Th1-like cells.^{10,11} Therefore, it should be possible to design prophylactic and therapeutic modalities that promote a Th1-like immune response and thereby down-regulate the Th2-like immune response.^{11,12} As selection of the appropriate T-cell population occurs during the early stages of the immune response in a naive host, it has been hypothesized that an allergen vaccine, which stimulates a Th1-immunity, may control the T-cell selection process¹³ and prevent allergic sensitization. In support of this hypothesis, the administration of a multi-epitopic recombinant allergen vaccine induces specific deviation of an immune response from a Th2-like to a Th1-like response in a murine model.¹⁴

As BCG induces a predominantly Th1-like immunity, BCG-based vaccines may be useful in designing allergy vaccines. An inverse association between atopy and delayed-type hypersensitivity (DTH) responsiveness to tuberculin suggests that exposure to mycobacteria may decrease the tendency to

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develop atopic disorders.¹⁵ Also *M. vaccae*, which promotes cell-mediated responses to antigens common to all mycobacteria and switches off tissue necrosis in humans, has been shown to inhibit an established allergic response to ovalbumin in BALB/c mice.¹⁶

Recombinant expression systems have been developed for the presentation of heterologous antigens by BCG and have been shown to induce antibody and T-cell responses against these antigens in experimental models.^{17–19} The infection of mice with BCG suppresses allergen-induced airway eosinophilia.²⁰ Also, a *Mycobacteria*-based epitope delivery system expressing Der p1 peptides induced IFN- γ production in mice.²¹ However, the presence of multiple epitopes on individual allergens may limit the therapeutic application of an epitope-based strategy. To explore the potential of a live recombinant BCG (rBCG) allergy vaccine, in this study an antigen-secreting BCG vector was developed using β -galactosidase (β -gal) as a model antigen. The results indicate that the rBCG- β -gal vaccine induces an antigen-specific deviation of immune response from a Th2-like to Th1-like, as determined from the cytokine profile and antibody response in mice.

MATERIALS AND METHODS

Animals

Female BALB/c mice, 6–8 weeks old, were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained in pathogen-free conditions in the animal centre at the James A. Haley Veterans Hospital. All procedures were reviewed and approved by the committee on animal research at the James A. Haley VA Medical Center and the University of South Florida College of Medicine, Tampa, Florida.

Construction of the BCG–*E. coli* shuttle vector and cloning of the β -gal gene

Plasmid BCG α was constructed by using the parent vector p16R1 (5.1 kb)²² from the American Type Culture Collection (ATCC, Rockville, MD). Alpha antigen signal peptide and its promoter were amplified from BCG genomic DNA by the polymerase chain reaction (PCR) using Vent polymerase (New England Biolabs, Beverly, MA). The forward and reverse primers were as follows: forward primer, 5'-dTAT GGT ACC GCC CGA ATC GAC ATT TG-3'; reverse primer, 5'-dATA GGA TCC CGC GCC CGC GGT TGC CGC TC-3'. The oligonucleotides incorporated *Kpn*I and *Bam*HI sites into the PCR product. The *Escherichia coli* origin of replication *oriE* was amplified from the plasmid vector pUC18 using the forward primer 5'-dCCG GAT CCC TGG CGT TTT TCC AT-3' and the reverse primer 5'-dAAA AAG GTA CCG CTA CCA GCG G-3', incorporating *Bam*HI and *Kpn*I sites. The shuttle vector pBCG α was constructed by ligating the amplified products to the parent vector at the *Kpn*I site. Clones harbouring the shuttle vector were selected on LB-hygromycin (200 μ g/ml; Sigma Chemicals, St Louis, MO) agar plates. Plasmid vector pBCG α - β -gal was constructed by inserting the β -gal coding sequences derived from the plasmid pCBT4- β gal²³ at the *Bam*HI site.

Growth of BCG and transformation of shuttle plasmid

Mycobacterium bovis BCG (Pasteur strain) obtained from ATCC was grown in 7H9 Middlebrook medium (Difco, Detroit, MI) containing 10% albumin dextrose solution (ADC;

Difco, Detroit, MI) and 0.05% Tween-80 (T-80; Fisher, Atlanta, GA) to prevent the clumping of the bacteria at 37° with shaking. For transformation, BCG cultures were grown to densities of $\approx 10^7$ CFU/ml, sedimented at 4000 g, washed twice by resuspension and centrifugation (4000 g) in 10% glycerol at 4°, and finally suspended in 1/20 of the original culture volume of cold 10% glycerol. Then 200 μ l of the cold BCG suspension was mixed with plasmid DNA (1–2 μ g) in a prechilled 0.2-cm electroporation cuvette and transformed using a Bio-Rad Gene Pulser electroporator (Bio-Rad Laboratories, Hercules, CA) at 2.5 kV and 25 μ F. After electroporation, 800 μ l of 7H9 Middlebrook medium supplemented with ADC enrichment was added to the electroporation mixture followed by incubation at 37° for 2–3 hr. Aliquots (50–200 μ l) were plated on 7H10 Middlebrook plates supplemented with oleic acid, albumin, dextrose, catalase (OADC; Difco) and hygromycin at a concentration of 50 μ g/ml. Plates were incubated for 3–4 weeks at 37° and the colonies that emerged were used to inoculate liquid cultures. Recombinant BCG harbouring the plasmid DNA was confirmed by isolating plasmid using a standard plasmid isolation procedure²⁴ with bacteria grown in the presence of 1% glycine (Fisher Scientific).

Expression of β -gal and analysis of the expressed protein

The expression of β -gal gene was detected by the addition of the chromogenic substrate X-gal (20 mg/ml; Fisher Scientific) to the culture supernatant. Recombinant BCG was grown to the mid-logarithmic phase in a 7H9 Middlebrook medium supplemented with ADC enrichment containing hygromycin for analysis of protein expression. After washing the cells with phosphate-buffered saline (PBS) plus 0.05% Tween-80, the cell suspension was concentrated 20-fold in PBS and sonicated to lyse the cells. Cell lysates were analysed by 0.1% sodium dodecyl sulphate (SDS)–10% polyacrylamide electrophoresis (PAGE).²⁵ Western blot analysis for the expressed protein was carried out as described previously.²⁶

Immunization

Recombinant BCG cultures were grown in a shake flask to optical densities of $\approx 1.2 \times 10^6$ U. The CFU/ml was determined by the method described by Stover *et al.*¹⁷ Groups of six 6–8-week-old BALB/c mice were vaccinated by the intraperitoneal (i.p.) route with 10^6 rBCG CFU in 200 μ l of PBS–Tween-80. An aliquot of the rBCG was also plated over a 7H10 agar supplemented with hygromycin (50 μ g/ml) and X-gal (1 μ g/ml) to confirm the stability of the plasmid in the BCG. The control group was vaccinated with non-recombinant BCG.

For immunization with β -gal, control and experimental groups of mice were immunized i.p. with 10 μ g of purified β -gal and 1 mg of alum on week 6 after the rBCG immunization.

Determination of anti- β -gal response in mice

Sera were collected from the tail veins of the immunized mice at 4-, 6-, 8-, 10- and 12-week intervals and pooled for each group to monitor the antibody response by enzyme-linked immunosorbent assay (ELISA) with purified β -gal protein (Boehringer Mannheim, Mannheim, Germany). Briefly, ELISA plates (Costar, Cambridge, MA) were coated with 1 μ g

of protein per well in a 0.1 M carbonate buffer, pH 9.6, at 4° overnight. Following three washes with PBS–Tween-20 (PBST) buffer (PBS pH 7.4 and 0.05% Tween-20), plates were incubated with blocking buffer [PBS, 0.1%, Tween-20, 1% bovine serum albumin (BSA)] for 2 hr at 37°. After three washes, the plates were incubated with the serially diluted sera in a blocking buffer. After incubating the plates at 37° for 2 hr, plates were washed with washing buffer three times and incubated with goat anti-mouse IgG–peroxidase conjugate (Boehringer Mannheim) for 2 hr at 37° at 1:1000 dilution. Colour was developed with substrate tetra methyl benzidine (TMB) (Pharmingen, San Diego, CA) and absorbance was read at 450 nm. The negative control consisted of pooled normal BALB/c mouse sera. Individual titres were expressed as the log₁₀ of the highest dilution that gave an absorbance twice as high as that of the negative control.

For the specific antibody assay, mice were bled on day 21 from the day of β -gal immunization and sera collected. Total IgE and antigen-specific IgG1 and IgG2a were estimated by a sandwich ELISA using purified anti-mouse monoclonal antibodies (mAb) (Pharmingen). Briefly, 96-well plates were coated overnight with antibodies at 4°. The plates were washed with PBST and then blocked with 200 μ l/well of PBS, pH 7.4 containing 1% BSA, for 1 hr at 37°. After washing, the serum samples in the dilution buffer (0.5% Tween-20 and 0.5% BSA in PBS) were added at 100 μ l/well for 2 hr incubation at 37°. After washing, biotinylated secondary antibody was added and incubated at 37° for 1 hr. Streptavidin–peroxidase conjugate (1:10000 dilution) was added after washing and further incubated at 37° for 1 hr. Finally, the plates were washed and colour developed by the addition of TMB at room temperature for 30 min.

To determine the β -gal-specific IgE antibodies, a microtitre plate was coated overnight at 4° with 100 μ l of β -gal protein (10 μ g/ml). Non-specific sites were blocked following three washes with PBST. Mouse sera were incubated to the antigen-coated wells at a final dilution of 1:50 and bound IgE was detected with biotinylated anti-mouse IgE (02112D; Pharmingen). Biotin anti-mouse IgE (02122D) reacts specifically with the mouse IgE of Igh^a and Igh^b haplotypes and does not react with other IgG isotypes. Diluted streptavidin–peroxidase conjugate was added, the bound enzyme was detected with TMB, and the absorbance was read at 450 nm.

Spleenocyte culture and assay for cytokines

Mice were killed and their spleens were removed aseptically. The single-cell suspension was prepared by forcing tissue through a 73.7- μ m plastic mesh (Sigma). Red blood cells were lysed by the addition of 900 μ l of water followed by 100 μ l of 10 $\times$ PBS and 9 ml of RPMI-1640 media with 10% fetal calf serum. Avoiding the lysed red blood cells, the cell suspension was transferred to another 15-ml tube and centrifuged at 500 *g* at 4° to pellet the cells. The process was repeated twice or until all the red blood cells were removed. After washing, cells were counted and plated at 1 $\times$ 10⁶ or 10 $\times$ 10⁶ cells/plate in the same medium as above. Cells were incubated with 10 μ g/ml of β -gal protein for 48 hr. Supernatants were collected from the cultures and the production of IFN- γ , IL-2 and IL-5 was determined by using ELISA assays (R&D Systems, Minneapolis, MN) and Endogen (Woburn, MA) following the manufacturers' instructions.

Statistical analysis

Data from cytokine and antibody assays were expressed as the mean $\pm$ standard deviation (SD). Unpaired two-tailed Student's *t*-tests were used to determine significant differences between BCG and rBCG groups. Differences were considered statistically significant when the *P*-value was less than 0.05.

RESULTS

Expression of the β -gal gene in a pBCG α vector

The vector pBCG α (Fig. 1a) is 5870 bp long and has a unique *Bam*HI site for the insertion of the foreign DNA sequence, α -antigen promoter and the signal sequence. The *E. coli* β -gal gene and SV40 polyadenylation sequences were cloned at the *Bam*HI site of the vector. The vector was stable in both *E. coli* and BCG, as confirmed by the re-isolation of the recombinant plasmid several times. The β -gal gene was confirmed through digestion and PCR (data not shown).

The expression of β -gal was verified by the addition of chromogenic substrate X-gal to culture supernatants (data not shown). SDS–PAGE analysis of the recombinant BCG cell lysates showed a major protein band of 110 000 MW, which was not present in the normal BCG lysates (Fig. 1b), suggesting that β -gal is produced in high amounts from recombinant BCG. A specific mAb to β -gal reacted with recombinant β -gal protein produced by BCG in a Western blot analysis (Fig. 1c). These results suggest that foreign genes can be expressed in BCG using a pBCG α vector.

Vaccination of mice with rBCG generates a strong humoral response and stimulates IFN- γ production

The vaccination of mice with 10⁶ CFU of recombinant BCG expressing β -gal generated high antibody titres against the β -gal protein (Fig. 2a). The antibody response was detectable at week 4 and gradually increased thereafter, peaking at week 10 (ELISA titre 1:25 000) and then decreasing slightly at week 12. Mice vaccinated with naive BCG failed to generate any humoral response against the β -gal protein. Twelve weeks after vaccination, spleen cells cultured *in vitro* from the rBCG-vaccinated mice produced higher levels of IFN- γ (31.2 pg/ml) than the control group vaccinated with non-recombinant BCG (7.1 pg/ml) (Fig. 2b). These results suggest that a long-lasting β -gal-specific immune response is generated in mice by vaccination with rBCG expressing β -gal.

Vaccination with rBCG- β -gal alters cytokine profiles of splenocytes

Mice were vaccinated with rBCG and on week 6, immunized with alum-precipitated β -gal protein. On day 7, after the β -gal immunization, spleen cells were removed and cultured with the β -gal protein *in vitro*. Spleen cells from the rBCG vaccinated group compared with the control group produced higher levels of IFN- γ (*P* < 0.01) and IL-2 (*P* < 0.05) (Fig. 3a,b). The control group showed higher production of IL-5 (*P* < 0.01) (Fig. 3c) and reduced IFN- γ and IL-2 levels compared with the treated ones. These results suggest that vaccination of mice with rBCG secreting β -gal up-regulates the production of Th1-like cytokines.

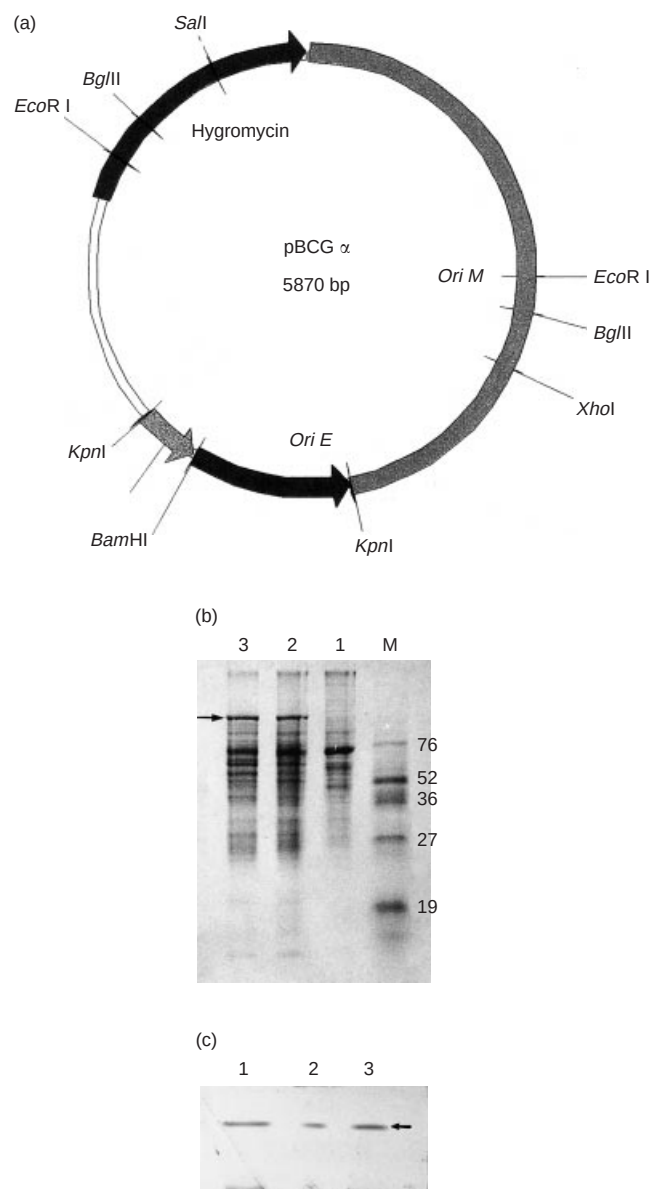


Figure 1. (a) Construction of the BCG-*E. coli* shuttle vector pBCGα. The plasmid vector pBCGα (5.870 kb) was constructed by cloning the 210-bp fragment containing the α-antigen promoter and signal sequence from *M. bovis* (BCG) and the 560-bp origin of replication from *E. coli* at the *KpnI* site in the plasmid vector p16R1.²² (b) Expression of β-gal in pBCGα. The whole cell extract of control BCG (lane 1) and rBCG was subjected to 0.1% SDS–10% PAGE analysis. Lane 2 and 3 show the expression of β-gal as a 110-kDa protein. M denotes molecular weight markers ($\times 10^3$). (c) Immunoblot analysis of the expressed protein. BCG and rBCG cell lysates were analysed for expression by Western blot analysis. Recombinant β-gal was readily identified by rabbit anti β-gal mAbs (lanes 2 and 3). Lane 1 is purified β-gal.

Vaccination with rBCG-β-gal alters the pattern of antibody production

Mice were vaccinated with rBCG followed by immunization with the β-gal protein in alum to determine the effect of rBCG vaccination on total serum antibody. The antibody assay of serum at day 21 indicated that mice given a single vaccination

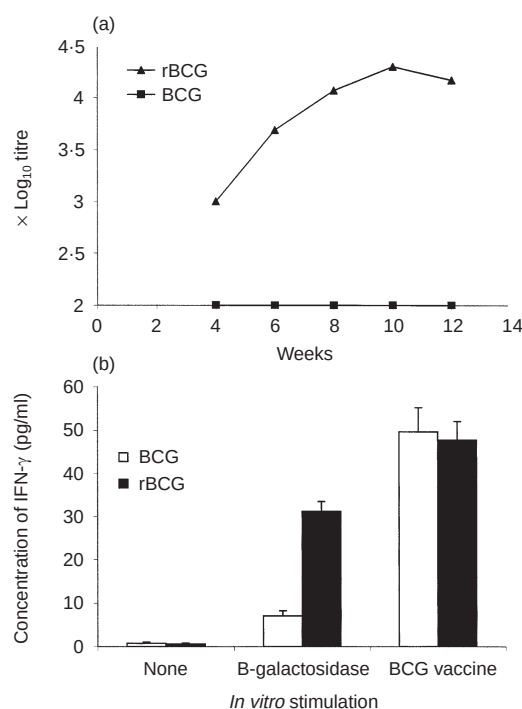


Figure 2. (a) Immunization with rBCG induces an anti-β-gal antibody response in mice. Vaccination of mice with 1×10^6 CFU of recombinant BCG secreting β-gal generates high antibody titres against expressed protein (▲). The antibody response detectable from week 4 increased thereafter and peaked at week 10 (ELISA titre, 1:25 000). Mice vaccinated with equal numbers of non-recombinant BCG failed to generate any antibody response (■). Experiments were performed twice and identical results were observed. (b) Vaccination of mice with rBCG stimulates IFN-γ production. Mice were vaccinated with either 1×10^6 CFU rBCG (solid bars) or non-recombinant BCG (open bars). On week 12 their spleen cells were cultured *in vitro* (1×10^6 cells) and stimulated with β-gal and the BCG vaccine (Glaxo, Greenford, UK). IFN-γ levels in supernates from 48-hr cultures were determined by ELISA. Mice vaccinated with rBCG showed nearly five times more production of IFN-γ than control mice did. Data are mean $\pm$ SD ($n=6$). The experiment was repeated twice with similar results. Data from a single experiment are shown.

of rBCG had reduced total IgE levels ($P<0.01$) as well as antigen-specific IgE ($P<0.01$) and IgG1 ($P<0.05$) compared with control mice vaccinated with non-recombinant BCG (Fig. 4). There was a significant increase ($P<0.05$) in the antigen-specific IgG2a levels in mice vaccinated with rBCG. The results demonstrate that vaccination with recombinant BCG in mice down-regulates IgE production and increases the level of antigen-specific IgG2a antibody.

DISCUSSION

The potential of rBCG as a live vaccine vector has been suggested for a number of diseases, but the possibility of an rBCG allergy vaccine has not been investigated. The present study investigated the potential for down-regulation of the allergen-specific Th2-like response using a live recombinant *M. bovis* (BCG) vaccine in a mouse model. A major finding of this study was that vaccination with a rBCG expressing β-gal induces a state of immune deviation in allergen-sensitized

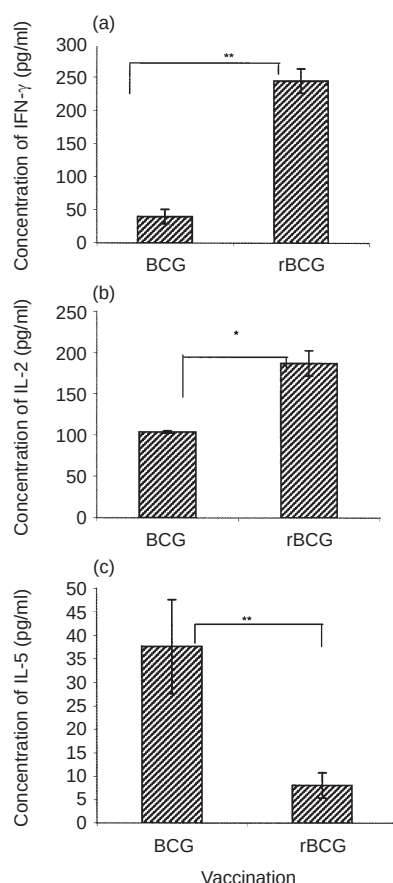


Figure 3. Production of IFN- γ (a), IL-2 (b) and IL-5 (c) by splenocytes cultured with β -gal. BALB/c mice were vaccinated with 1×10^6 CFU of either rBCG or BCG and on week 6 immunized i.p. with $10 \mu\text{g}$ β -gal in 1 mg alum. Seven days later spleens were harvested from these mice and a single-cell preparation was cultured *in vitro* (10×10^6 cells) in the presence of $10 \mu\text{g}/\text{ml}$ of β -gal. Culture supernatants were collected after 48 hr and cytokine levels were determined by ELISA. Data are mean $\pm$ SD ($n = 6$). * $P < 0.05$, ** $P < 0.01$.

mice. The immune deviation is manifested by (1) enhanced production of Th1-like cytokines, IFN- γ and IL-2, instead of Th2-like cytokines, and (2) down-regulation of the IgE antibody response with concomitant elevation of the IgG2a antibody response.

The BCG shuttle vector, pBCG α , can efficiently express the foreign antigens inside BCG under the control of the α -antigen promoter. The β -gal activity could be detected in rBCG culture supernatants, suggesting that the α -antigen signal peptide present upstream to the antigen coding sequences guides the protein outside the membrane. (data not shown). The α -antigen signal sequence has been used previously to express proteins efficiently in BCG.^{27,28} The system involving secretion compared with intracellular expression has some advantages because it facilitates the excretion of large amounts of recombinant antigens, which is favourable to better stimulation of the host immune system and for the release of a foreign protein, which may be toxic for or degraded in BCG.²⁹

The recombinant protein is immunologically similar to the purified protein because it binds to the anti- β -gal mAb gener-

ated against the purified protein. Single vaccination of mice with 10^6 CFU of rBCG is sufficient to generate high antibody titres against that antigen secreted by the recombinant BCG. This result is consistent with other reports where high antibody titres were obtained 2–4 weeks after vaccination with rBCG.^{27,30} The response generated by the rBCG is higher than the antibody response generated by BCG alone and lasts longer than the antigens of BCG itself.³⁰

Mycobacteria are rapidly phagocytosed and contained within the endosomal compartment of phagocytic cells.^{31,32} This makes it an effective agent to induce B and T cells to produce IgG antibodies and IFN- γ , respectively. Vaccination of mice with the rBCG secreting β -gal also stimulated the production of IFN- γ , as shown in Fig. 2b. Spleen cells from mice injected with rBCG and cultured in the presence of β -gal protein produced at least five times more IFN- γ compared with the mice injected with naive BCG. The result suggests that rBCG vaccination leads to the production of antigen-specific T cells, which secrete lymphokines such as IFN- γ .

IFN- γ is a potent inducer of a Th1-like response and most strategies to switch allergen-specific Th2 responses to Th1 involve IFN- γ and IL-2. The vaccination of mice with rBCG followed by alum-precipitated antigen immunization leads to a switch to the Th1 response. Mice vaccinated with rBCG produced more IFN- γ and IL-2 compared with mice receiving BCG alone, when their spleens were cultured *in vitro*. However, mice receiving BCG alone produced more of the inflammatory cytokine IL-5. As demonstrated earlier using IFN- γ receptor-deficient mice, BCG-induced inhibition of Th2-like immune responses and airway eosinophilia is mediated by IFN- γ .²⁰ An IFN- γ -rich environment, which is characteristic of infectious agents that induce a Th1-type immune response, is often viewed as a powerful suppressive mediator of Th2 activity. It has been suggested that decreased exposure to such infections, which induce a Th1 response, increases the risk of developing atopic diseases.^{15,33–36}

An analysis of the antibody isotype profiles in mice vaccinated with rBCG revealed a significant increase in the antigen-specific IgG2a levels with a concomitant decrease in the total IgE and antigen-specific IgE and IgG1. Infection of BALB/c mice with rBCG can result in high titres of antigen-specific IgG2a and less of IgG1 and IgG2b.²⁸ This antibody response was protective against *Schistosoma mansoni* infection in mice.²⁸ Vaccination of BALB/c mice with BCG has also been shown to reduce allergen-induced airway eosinophilia and reduce levels of IL-5.²⁰ These results are consistent with another strain of mycobacteria (*M. vaccae*), which has previously been shown to inhibit antigen-specific IL-5 production and decrease total serum IgE.¹⁶ Use of rBCG not only inhibited the production of IL-5 and IgE but also produced more antigen-specific IgG2a and decreased IgG1 production.

Finally, mycobacteria *per se*, i.e. without allergen, are able to induce a Th1-like immune response and may limit the subsequent prevalence of allergic disorders in a non-specific way. However, it may be difficult to predict whether mycobacteria alone would suffice as a vaccine against allergies as it is likely to depend on the level of exposure and sensitization to specific allergen(s) at the time of vaccination. On the other hand, a prophylactic vaccination may utilize major ubiquitous antigens, such as Fel d1 and Der p1, to which infants and children are exposed indoors.

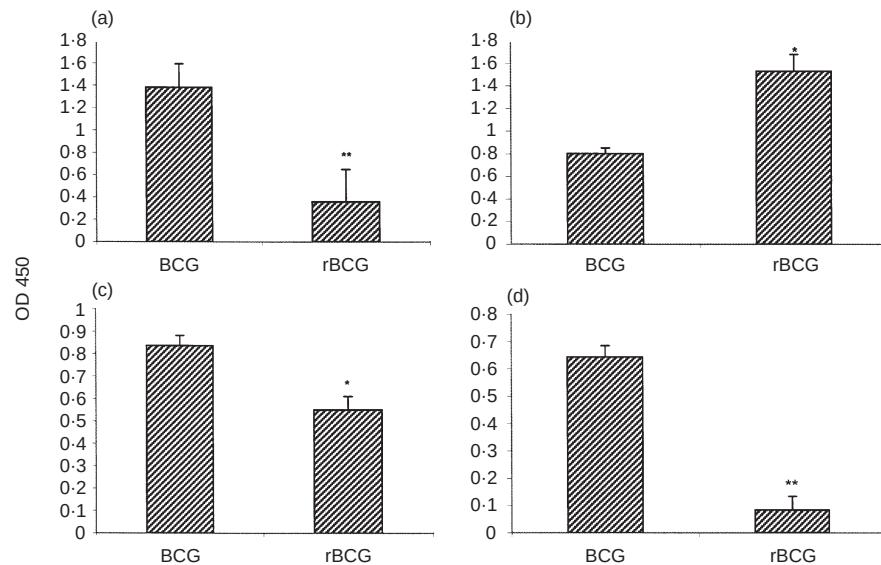


Figure 4. Effect of rBCG vaccination followed by immunization on serum antibody production. Mice were vaccinated with either rBCG or BCG and immunized with β -gal in alum, as described in the Materials and Methods. On day 21 after immunization, sera were collected and analysed by ELISA for total IgE (1:50 serum dilution) (a), antigen-specific IgG2a (1:200 serum dilution) (b), IgG1 (1:200 serum dilution) (c) and IgE (1:50 serum dilution) (d). All serum dilutions were made in serum dilution buffer. Mice vaccinated with rBCG exhibited less IgE and IgG1 production and an increase in IgG2a antibody levels. Data are mean $\pm$ SD ($n=6$). * $P<0.05$, ** $P<0.01$.

The data presented suggest that recombinant BCG-secreting antigens could be utilized to formulate antigen-specific vaccines against allergic diseases. Although here a single antigen was used as a model, in principle it should be possible to modify the vector system to accommodate and express multiple antigens. Indeed, such a feature will increase the potential of rBCG as a vaccine vehicle for immunization against allergic diseases.

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