Allergology International (2002) **51**: 63–73

Review Article

Immunomodulation in the treatment and/or prevention of bronchial asthma

Robert G Townley, Russell J Hopp, Devendra K Agrawal, Thomas B Casale and Michael T Hopfenspirger

Creighton University Center for Allergy, Asthma and Immunology, Omaha, Nebraska, USA

Abstract

The immunologic hallmark of atopic allergy and 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 common environmental allergens. All of us inhale allergens and healthy non-atopics produce allergen-specific IgG1, IgG4 and the Th1 cytokine interferon-α, as well as IL-12 from macrophages. We now 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 airways. In the present review we focus on conventional immunotherapy, mycobacterial vaccines, DNA vaccines using cytosine guanosine, inhibitors of IL-4 and IL-5 and anti-IgE: Omalizumab.

Key words: bacillus Calmette–Guérin vaccine, bronchial asthma, cytosine guanosine oligonucleotide, eosinophils, IgE/anti-IgE, immunotherapy, Th1/Th2 cytokines.

Conventional immunotherapy

The immunologic hallmark of atopic allergy and asthma is an increased production of IgE and T helper (Th) 2 cell cytokines, such as interleukin (IL)-4, IL-5, IL-9 and IL-13, by Th cells reacting to common environmental allergens. All of us inhale allergens and healthy non-atopics produce allergen-specific IgG1, IgG4 and the Th1 cytokine interferon (IFN)-γ as well as IL-12 from macrophages. Conventional immunotherapy is characterized by an increase in IgG1, IgG4, IFN-γ and IL-12. It is very effective and lasts for at least 3 years after discontinuation.[1] Immunotherapy induces a shift from Th2 cytokine production to Th1, inhibition of the early and late-phase allergy skin test reactions. Unfortunately, patients receiving conventional immunotherapy are at risk for anaphylaxis.

Allergen immunotherapy is the only therapeutic modality that can affect the natural course of allergic diseases and may prevent the development of asthma in patients with allergic rhinitis. Allergen immunotherapy is indicated for patients with demonstrated specific IgE antibodies against clinically relevant allergens. Allergen vaccine immunotherapy induces tolerance to allergens. Allergen immunotherapy, as well as guidelines, updates and recommendations of the World Health Organizations was recently summarized by Theodoropoulus and Lockey.[2]

Allergen-induced late responses in the skin and nose following immunotherapy are associated with a decrease in $CD4^+$ cell recruitment and a reduction in local eosinophilia. These changes are associated with an increase in $CD4^+$ cells expressing IFN-γ following allergen provocation. This late increase in IFN-γ-producing cells correlates closely with the clinical response to allergen immunotherapy. Upregulated Th1 responses are protective against allergen-induced responses.[3] The production of IL-12 amplifies the Th1 response. There is a reciprocal association between $IL\text{-}12^+$ cells and $IL\text{-}4^+$ cells.[4]

Immunotherapy also induces tolerance through multiple effects on immune cells, which includes an increase in the generation of allergen-specific $CD8^+$ cells and enhancement of the Th1 response by increasing the

Correspondence: Dr Robert G Townley, 601 N 30th Street, Suite 1630, Omaha, NE 68131, USA.
Email: rtownley@creighton.edu
Received 18 December 2001.

number of IFN-γ-producing cells. Immunotherapy also increases the production of IFN-γ-responding cells, such as mononuclear phagocytes, B cells and NK cells.

Allergen immunotherapy is highly effective in selected patients with IgE-mediated disease. It also provides insight into the basis of allergic disease and assists in further development of therapeutic strategies. It reduces immediate allergen-induced symptoms and reduces the concentration of mediators in the target organ of the allergic disease. It also decreases the nasal epithelial mast cells.[5] Furthermore, allergen immunotherapy has a striking ability to inhibit the late-phase response and results in the decline of IgE levels over several years. This is associated with an increase in allergen-specific IgG and results in a modified T lymphocyte response to subsequent natural allergen exposure. This results in the shift of the balance from Th2 to Th1 responses. Allergen immunotherapy results in suppression of the late nasal response and an increase in cells expressing mRNA for IFN-γ. It results in increased levels of IL-12, which is a potent inducer of Th1-type T cell differentiation. The inhibition of the late cutaneous response is associated with an increase in IL-12 mRNA-positive cells. An attractive hypothesis of the mechanism of immunotherapy is that it results in a shift in favor of a Th1-type response that may occur either as a consequence of selected downregulation, or anergy, of the Th2 response or as immune deviation under the influence of IL-12. Frequently, asthma and allergic rhinitis coexist and the potential for allergic rhinitis to precipitate or exacerbate asthma constitutes an additional reason for immunotherapy in asthma.[1,3,4]

Pollen immunotherapy was also associated with improvement in symptoms of asthma, as well as allergic rhinitis. In placebo-treated control subjects, there was a very significant increase in airway reactivity to methacholine during the pollen season. In contrast, the airway reactivity of asthmatic subjects receiving immunotherapy did not increase during the pollen season.[6,7]

A meta-analysis of 62 randomized, controlled trials on allergen immunotherapy for asthma shows significant and homogenous improvement in asthma symptoms with pollen, animal dander and other vaccines.[8] Parameters that show the most significant and consistent improvement after immunotherapy are allergen-specific bronchial hyperresponsiveness and medication requirements. Improvements in allergen-specific bronchial hyperresponsiveness following immunotherapy is reported in a meta-analysis of randomized, controlled trials.[8]

Normal individuals who are not atopic but are exposed to the same concentrations of allergens as patients with allergy living in the same environment have been perceived as not responding to the environmental allergens and not inducing an immune response. However, when investigators have studied these non-allergic, non-atopic individuals, the immune response to allergens, indeed, does occur and is characterized by type 1-like lymphocyte responses.[9,10] Furthermore, T lymphocytes from normal subjects proliferate in response to grass pollen[11] and produce increased IFN-γ[10] and decreased IL-4[10,11] when compared with grass pollen-sensitive subjects with allergic rhinitis. The principal cytokine responsible for proliferation of Th1-type cells in response to antigen challenge appears to be IL-12.[12] Inhibition of Th2-producing T lymphocytes has been shown to be induced by IL-12, which inhibits IL-4-independent B cell switching from IgG and IgE production.[13] These findings provide further support for the concept that type-1 responses after immunotherapy may be driven by IL-12.

Interleukin-10 may also inhibit the pro-inflammatory responses in the respiratory tract of non-allergic and non-asthmatic subjects. Increased concentrations of IL-10 are found in the airway macrophages and respiratory epithelial cells in normal non-asthmatic individuals.[14,15] Interleukin-10 is an anti-inflammatory cytokine that inhibits cytokine release. Immunotherapy is also associated with an increase of the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)-β.[16,17]

Pollen immunotherapy is effective in selected patients. There is questionable long-term benefit after discontinuation of treatment. Immunotherapy for 3–4 years induced prolonged clinical remission accompanied by a persistent alteration in immunologic activity.[1] This clinical improvement persists for at least 3 years.

Conventional immunotherapy has been reported to enhance production of IL-12 after grass pollen immunotherapy.[4] Immunotherapy may be beneficial through its capacity to increase IL-12 and decrease IL-4 production by Th2 lymphocytes and to reduce Th2 differentiation. Although IL-12 has been shown to be quite effective in reversing the parameters of asthma in a mouse model, its intensely pro-inflammatory effects may potentially worsen the inflammation in the respiratory tract. Therefore, agents that increase IL-12 and IFN-γ, such as Bacillus Calmette–Guérin (BCG) vaccine, and perhaps DNA vaccines that also increase IL-12, such as the cytosine guanosine (CpG) oligonucleotide immunostimulatory sequence found in many bacteria, may be a much

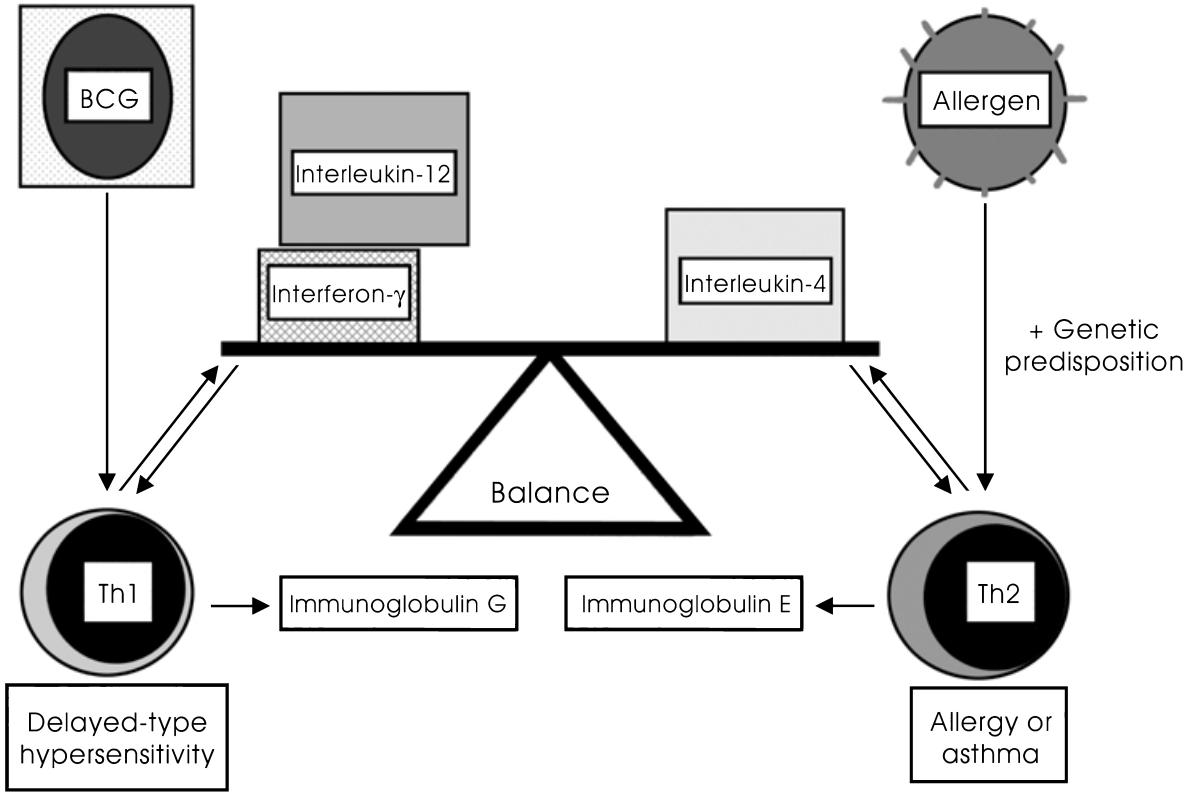

Fig. 1 T helper (Th) 1/Th2 balance with delayed-type hypersensitivity being stimulated by Bacillus Calmette–Guérin vaccine with production of interleukin (IL)-12 and interferon-γ, with allergen sensitivity stimulating IL-4 and IgE production.

safer approach. Immune modulators, such as BCG and CpG, induce the development of Th1 cells that secrete IFN-γ and IL-12 to suppress IL-4 and IL-5 secreting Th2. In this regard, atopy along with allergic rhinitis or asthma symptoms could be prevented or suppressed. This immune balance is depicted schematically in Fig. 1.

Mycobacterial vaccines in a mouse model of asthma

New approaches include the use of DNA vaccine, such as CpG or BCG, either alone or in combination with allergens, to stimulate antigen-presenting cells to produce cytokines that stimulate production of Th1 cells. Bacillus Calmette–Guérin is a live, but attenuated, strain of *Mycobacterium bovis*, which causes tuberculosis (TB) disease in cows. However, there is a great deal of research currently ongoing to develop a better vaccine for tuberculosis. Bacillus Calmette–Guérin has been used as the only TB vaccine for the past 80 years. It produces a strong type-1 immune response in cell-mediated immunity and is a potent stimulator of cytoxic T cells and NK cells. It is the most effective treatment for early bladder cancer.[18] We have examined the effect of BCG and *Mycobacterium vaccae* in a sensitized mouse model.[19] In this model, both BCG and *M. vaccae* significantly attenuated the late allergic response, the airway hyperreactivity to methacholine and bronchoalveolar lavage (BAL) eosinophilia. Plasma IL-12 levels were significantly increased and the IL-4 levels decreased following BCG or *M. vaccae* in ovalbumin (OVA)-sensitized and -challenged mice. The increase in IL-12 was greater after BCG compared with *M. vaccae* administration. In asthma patients, *M. vaccae* has been reported to partially inhibit the late allergic response and to inhibit the dermal manifestations of atopic eczema.[20]

In this allergic murine model of asthma, we have examined many of the important features of asthma, including airway hyperresponsiveness to methacholine, which has long been used to characterize asthma.[21] Tissue and blood eosinophilia are also hallmarks of asthma.[22,23] The late allergic reaction that occurs in approximately 50% of patients with asthma[24] was also examined in this allergic murine model of asthma.

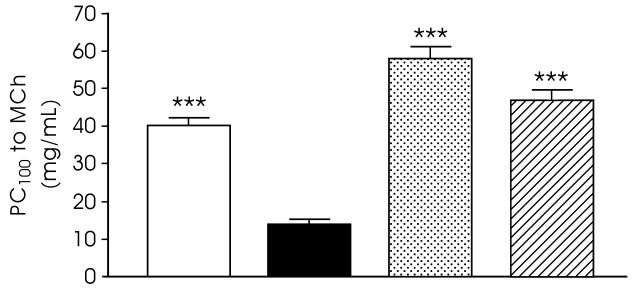

Fig. 2 Effect of mycobacterial antigen treatment on airway hyperresponsiveness to methacholine. Mice were challenged 24 h following ovalbumin (OVA) exposure. A dose–response relationship to methacholine was examined. The dose of methacholine at which a 100% increase in enhanced pause (P_{enh}) values was observed was designated the PC_{100}. Non-sensitized control mice (□), sensitized control mice (■), Bacillus Calmette–Guérin-treated mice (▨) and *Mycobacterium vaccae*-treated mice (▨) are shown. Data are the mean ± SEM for seven to 12 animals in each experimental group. Differences were significant for all groups (***$P < 0.001$) compared with sensitized controls. Reproduced with permission from Hopfenspirger *et al*.[19]

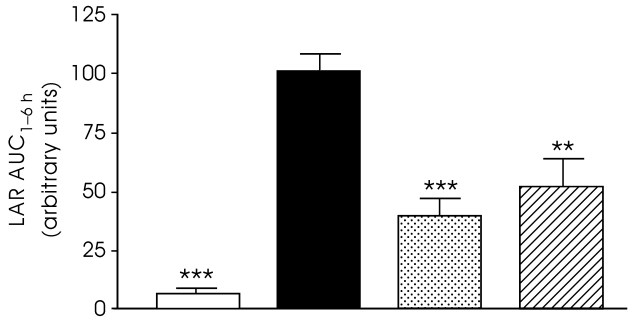

Fig. 3 Effect of mycobacterial antigen treatment on late allergic response (LAR) as calculated by the area under the curve (AUC). Mice were challenged with 2% ovalbumin (OVA) on day 24 and enhanced pause (P_{enh}) values were recorded in individual plethysmograph chambers for up to 9 h. The LAR was observed to begin after 1 h and to resolve by 6 h in sensitized control mice (■). Arbitrary units designate values for each group: non-sensitized control mice (□), Bacillus Calmette–Guérin (BCG)-treated mice (▨) and *Mycobacterium vaccae*-treated mice (▨). Data are the mean ± SEM for seven to 12 animals in each experimental group. Differences were significant for all groups (***$P < 0.001$, **$P < 0.01$) compared with sensitized controls. Reproduced with permission from Hopfenspirger *et al*.[19]

Methacholine challenge performed 24 h postantigen (OVA) challenge demonstrated airways significantly more reactive than those in mice that were either unsensitized

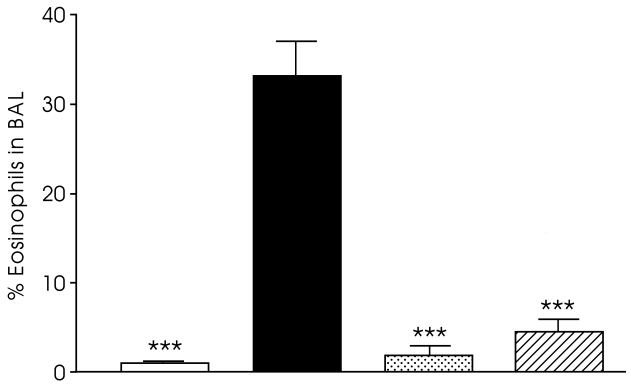

Fig. 4 Effect of mycobacterial antigen treatment on bronchoalveolar lavage (BAL) eosinophilia. Following methacholine challenge on day 25, percent BAL eosinophilia was determined. Non-sensitized control mice (□), sensitized control mice (■), Bacillus Calmette–Guérin-treated mice (▨) and *Mycobacterium vaccae*-treated mice (▨) are shown. Data are the mean ± SEM for seven to 12 animals in each experimental group. Differences were significant for all groups (***$P < 0.001$) compared with sensitized controls. Reproduced with permission from Hopfenspirger *et al*.[19]

or sensitized and given a BCG or *M. vaccae* injection. Bacillus Calmette–Guérin-treated mice demonstrated airway reactivity that virtually matched the results of unsensitized control mice (Fig. 2). Both BCG and *M. vaccae* clearly attenuated the late allergic response to ovalbumin (Fig. 3). The late allergic reaction was observed to begin after 1 h and resolved by 6 h in sensitized control mice. The effect of BCG and *M. vaccae* on BAL eosinophils was very pronounced (Fig. 4). The positive control mice exhibited 33 ± 4% eosinophils. Mice treated with BCG showed virtually complete protection, with only 1 ± 1% eosinophils, and *M. vaccae*-treated mice also showed significant protection, with 4 ± 1% eosinophils. The unsensitized control mice were nearly devoid of eosinophils, with only 1.0 ± 0.2% present. Our results on BAL eosinophilia correlate well with the observations of Erb *et al.*,[25] who also found a negative correlation between BCG and BAL eosinophilia in sensitized unchallenged mice.

We found significantly elevated levels of IL-4 in serum from sensitized control mice compared with the BCG- and *M. vaccae*-treated mice (Fig. 5).[19] Conversely, the sensitized control mice had significantly reduced levels of IL-12 compared with the BCG- and *M. vaccae*-treated mice. The increased production of IL-12 and inhibition of the production of IL-4 following BCG and *M. vaccae* effectively favors a type-1 cytokine profile, thus protecting

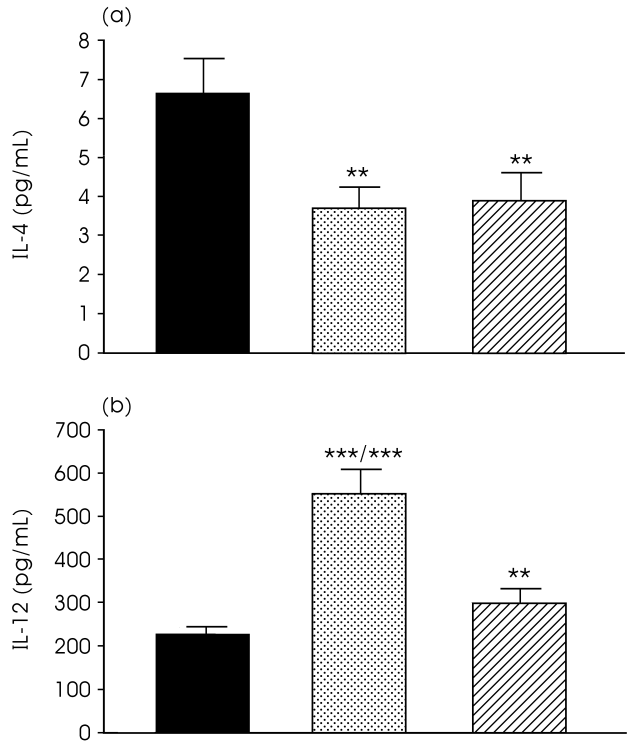

Fig. 5 Effect of mycobacterial antigen treatment on serum interleukin (IL)-4 and IL-12 concentrations. Serum collected from mice on day 25 was measured by ELISA for content of the cytokines IL-4 (a) and IL-12 (b). Shown are sensitized control mice (■), Bacillus Calmette–Guérin (BCG)-treated mice (▨) and *Mycobacterium vaccae*-treated mice (▨). Values for either cytokine were undetectable in non-sensitized control mice. Data are the mean ± SEM for seven to 12 animals in each experimental group. Differences were significant for all groups (***$P < 0.001$, **$P < 0.01$) compared with sensitized controls. In the case of IL-12 concentration, differences between the BCG- and *M. vaccae*-treated groups were also significant. Reproduced with permission from Hopfenspirger *et al*.[19]

against airway hyperreactivity. Bacillus Calmette–Guérin exhibited significantly greater levels of IL-12, even in comparison with mice treated with *M. vaccae*. Most of the other parameters reported above showed a greater effect of BCG compared with *M. vaccae*, but none of these was significantly different, except the IL-12 levels.

The timing of administration of BCG as well as the duration of efficacy of BCG is controversial and may explain some of the differences of the efficacy of BCG in protection against TB as well as asthma and allergy.

We used a protocol that effectively overlapped inoculation and sensitization. In a separate study, Herz *et al*.[26] administered BCG 14 days prior to sensitization and were successful in suppressing both allergic sensitization and development of increased airway reactivity in mice. However, Sano *et al*.[27] concluded that only when both antigen (OVA) and *M. tuberculosis* are presented to the immune system in both temporal and spacial proximity could the Th1/Th2 ratio be affected.

Bacillus Calmette–Guérin vaccines in humans

Several studies suggest a 'window of opportunity' period of time during which Th cells may be made to secrete particular cytokines in response to a particular challenge. Nevertheless, Marchant *et al*.[28] have shown that in infants the effect of BCG on Th1/Th2 populations with the development of a Th1 response can persist for at least 1 year. Marchant *et al*. also showed that memory T cells were still present 1 year following BCG administration to newborn infants. The studies by Holt and Sly[29] that prevention of adult asthma by early intervention during childhood and the value of new immunomodulatory agents highlight the potential value of giving BCG or other agents at birth. Holt and Sly, as well as other investigators, support the notion it is important to prevent immune deviation towards a Th2 response by early intervention before environmental allergens stimulate a Th2 response in genetically susceptible individuals.

Similarly, BCG, when given at birth or in the first 6 months of life, has been associated with a subsequent decreased incidence of positive allergy skin tests in children in Guinea-Bissau, as reported by Aaby *et al*.[30] (Fig. 6). Aaby *et al*.[30] studied 400 children aged 3–14 years. The children underwent skin testing to house dust mite, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and cockroach. Atopy was defined as an allergy skin test 3 mm in diameter or greater. Children receiving BCG before the age of 6 months, and especially in the first 7–31 days, had a significantly lower odds ratio (OR) of developing atopy. For example, the OR of children receiving BCG in the first week of life was 0.1 of having a positive allergy skin test. Those receiving BCG after the age of 6 months had an OR of approximately 0.5–0.6. Similarly, the percentage of children having a positive skin test was approximately 3% for children receiving BCG in the first week of life, in contrast with approximately 15% atopic, as defined by having a positive allergy skin test, for children receiving BCG after 6 months.

Retrospective studies of BCG have been controversial. Thus, the studies performed in Japan[31] and in

- 400 children 3–14 years
- Tested to HDM-DP, HDM-DF and cockroach
- + AST > 3 mm

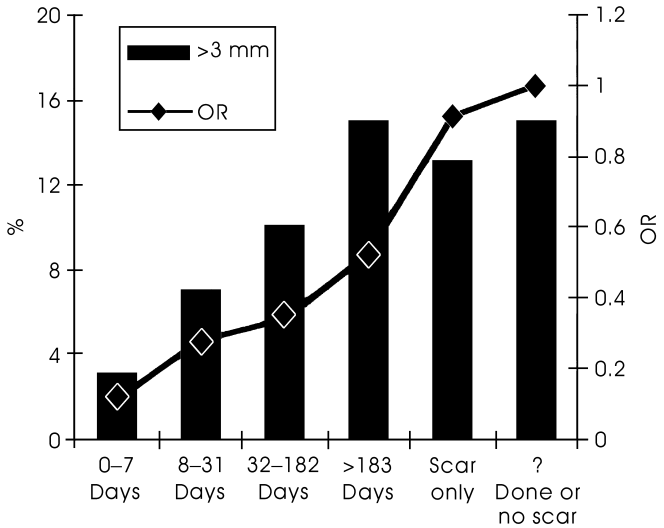

Fig. 6 Early Bacillus Calmette–Guérin (BCG) vaccination and reduction in atopy in Guinea-Bissau.[30] Four hundred children aged between 3 and 14 years were skin tested to house dust mite *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and cockroach. A positive skin test was > 3 mm and is recorded on the left ordinate as percentage and the odds ratio (OR) of developing atopy is recorded on the right ordinate. The abscissa is the time of receiving BCG. Adapted from Aaby *et al*.[30]

Guinea-Bassau[30] showed an inverse correlation between mycobacteria exposure and markers of atopy/asthma. In contrast, the Scandinavian studies[32,33] failed to show any protective effect of mycobacteria towards markers of atopy/asthma. These differences may be explained by genetic background, the dose and strain of BCG used, the type of mycobacteria exposure and the traits of indigenous mycobacteria, as well as the age at time of exposure and other confounding factors.

Alm *et al.*,[32] in Scandinavia, have challenged the conclusions of Shirikawa *et al*.[31] in Japan. Alm *et al*. found early BCG vaccination in children with atopic risk factors did not alter the children's chances of developing atopy themselves. The retrospective study of Alm *et al*. differs from the study of Shirakawa *et al*. in that the subjects in the former study received BCG up until 6 months of age, whereas those in the study of Shirikawa *et al*. received BCG at birth or only up until 2 months of age (Fig. 7). Furthermore, the incidence of tuberculosis was significantly higher in Japan at the time the studies of Shirikawa *et al*. were performed compared with the incidence of tuberculosis in Sweden. Other studies showing conflicting results were those of Omenass *et al*.,[33] who also reported the absence of a relationship between tuberculin reactivity and atopy in BCG-vaccinated young adults. Thus, the timing of BCG delivery may be very important and demonstrates the importance of a memory type-1 cytokine response in newborns.

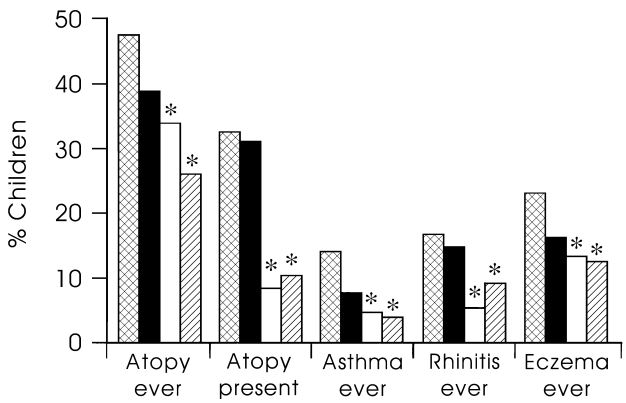

Fig. 7 The inverse association between tuberculin responses and atopic disorder. The ordinate is the percentage of 860 12-year-old children who received Bacillus Calmette–Guérin (BCG) at birth and developed atopy or atopic disease. (▨), children who were tuberculin negative; (■), children who were tuberculin positive at age 6 years but were negative at age 12; (□), children who became tuberculin positive at age 12; (▨), children who were tuberculin positive at both 6 and 12 years of age. *$P < 0.01$ compared with tuberculin-negative children. Adapted from Shirakawa *et al*.[31]

For this reason, our group has undertaken a prospective study in three countries. We studied the effect of BCG on the development of atopy and asthma. This study is currently being conducted in Istanbul, Turkey, Cordoba, Argentina, and Bangkok, Thailand. Children were randomly enrolled from clinics at birth and received the

BCG supplied by their local public health department. They were followed by their private or university physician. A questionnaire concerning family occurrences of allergic diseases was administered at enrollment. A purified protein derivative (PPD) was administered between 9 and 12 months of age and the size of the reaction was measured. The BCG scar was noted. The families were recontacted when children were 2 years of age for an allergy skin test panel, International Standardized Asthma in Allergy Children (ISAAC) questionnaire and a PPD if not done previously. There were a total of 1700 children enrolled in the study: 550 from Thailand and Argentina and 600 from Istanbul (Fig. 8).

Argentina had a significantly higher total of parents with asthma or allergic rhinitis than each of the other countries. Fifty-three mothers had asthma, 89 mothers had hay fever, 53 fathers had asthma and 83 fathers had hay fever. In contrast, those enrolled from Turkey and Thailand had approximately the same number of parents with asthma or allergic rhinitis. Their totals were approximately one-half to one-third the number seen in Argentina (Table 1).

A history of smoking during pregnancy was noted in 68 mothers in Turkey and in 62 mothers in Argentina and Turkey, but in only 17 mothers in Thailand. House pets, however, were noted in 178 homes in Thailand, whereas there were only 53 household with pets in Turkey. These differences were significant from the other site in terms of home pets and smoking during pregnancy.

In Thailand, there was no difference with a PPD positive or negative skin test for the presence of allergy skin test. However, there was a significantly lower incidence ($P = 0.01$) of ISAAC allergic histories in PPD-positive children. Similarly, in Istanbul, there was no difference between children with a PPD positive or negative for the presence of a positive allergy skin test. However, there was a significantly lower incidence ($P = 0.004$) of ISAAC allergic histories in PPD-positive children. In Argentina, there was no significant difference in children with a PPD positive or negative for the presence of a positive allergy skin test. However, in all three countries, there was a trend towards a lower incidence of positive allergy skin test in individuals who were PPD positive.

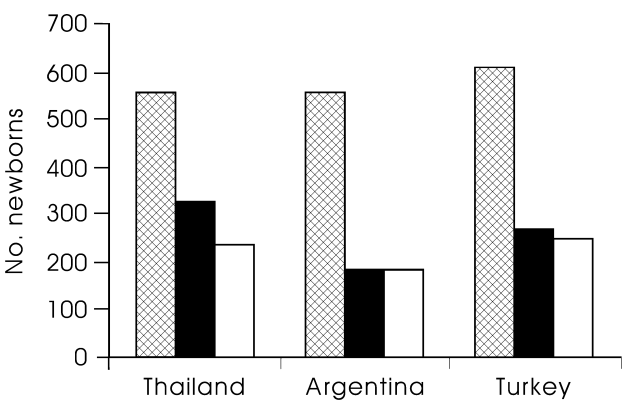

Fig. 8 Enrollment at birth of newborns from the three international sites. The ordinate is the number of newborns receiving Bacillus Calmette–Guérin. (⊠), total number enrolled; (■), the number who were tuberculin skin tested at age 9 months; (□), the number of children who had allergen skin tests at age 2 years.

We tested the hypothesis that BCG can promote a Th1 immune response and decrease Th2 activity. Published retrospectives on BCG and atopy/allergy have been conflicting. We report the 2 year data of a 5 year prospective study of the effect of BCG on asthma, allergy, and atopy (RG Townley *et al.*, unpubl. obs., 2001). Seventeen hundred newborns were enrolled without regard to family history of allergy and approximately 650 have been evaluated at the age of 2 years. Argentina had an incidence of ISAAC allergic histories of 40% compared with 20–30% in the other two countries. In Thailand and Istanbul, there was a lower incidence of ISAAC allergic history in those children with a positive PPD response. The allergy skin test data may suggest the non-discriminatory nature of allergy skin tests in 2-year-old children and suggest our data at 5 years may be more valuable.

DNA vaccines using CpG

Recombinant technology now offers the possibility of entirely new approaches to allergen immunotherapy. Many of the major inhalant allergens have been identified, cloned and sequenced and expressed in a variety of systems.[34]

Table 1 Demographic, familial and environmental characteristics of enrolled subjects

	Thailand	Turkey	Argentina
No. enrolled	550	604	550
Mother with asthma (*n*)	15	11	53*
Mother with hay fever (*n*)	44	30	89*
Father with asthma (*n*)	18	16	53*
Father with hay fever (*n*)	40	19	83*
Both parents with Asthma (*n*)	0	1	3
Mother smoked in pregnancy (*n*)	17	68	62
Indoor smoker (*n*)	138	153	228*
Home pet (*n*)	178	53†	338

*Argentina significantly higher than the other two countries.
†Turkey significantly lower than the other two countries.

Incorporation of the allergen protein cDNA into a plasmid and then injected intradermally results in persistence of the plasmid DNA without replication or incorporation into the host genome.[35,36] This plasmid DNA incorporating an allergen protein into the plasmid resulted in no inflammatory reaction at the site of injection and production of the encoded protein, which is then released from the surface of the cell and integrated into peptide fragments that are presented to the cells major histocompatibility complex (MHC) class 1 molecules.[36] These studies in rats showed the cDNA for dust mite antigen Der p5 was incorporated into the plasmid vector. Expression of the Der p5 could be demonstrated with the antibody response to Der p5, which peaked at 4 weeks and virtually returned to baseline at 6 weeks. These rats demonstrated marked inhibition of the IgE response to Der p5 and decreased airway and basophil sensitivity to Der p5. This study supported the concept that the peptides derived from the encoded protein would be presented by MHC class 1 antigens to $CD8^+$ T lymphocytes, favoring a cell-mediated Th1 response to the protein.

DNA vaccines act by producing antigens in the host. Antigen processing is through MHC classes I and II. In animal studies, immunity is long lasting, broad based and results in antibodies, as well as cell-mediated immunity. DNA vaccines are possible targets for influenza, human immunodeficiency virus, Herpes simplex, human papillomavirus, hepatitis B and C and TB. Other targets include a variety of parasitic diseases, including malaria, schistosomiasis, leishmaniasis, rotavirus, and bacterial toxins.[37] The advantages of intramuscular DNA compared with live virus vaccines are that the DNA vaccines are produced by fermentation, purified chemically, result in a single chemical entity, flexibility to engineer the antigen is markedly enhanced and the technology is one that can result in generic delivery. Early trials with oligonucleotide DNA vaccine using CpG showed they were safe and efficacious in preventing hepatitis B when conjugated with hepatitis B.[37] Gene vaccination using plasma DNA results in induced Th1 response that dominated the Th2 response, whereas anaphylaxis is a risk in classic immunotherapy. This appears to be overcome by plasma DNA encoding the allergen because very low doses of antigen are used in contrast with conventional immunotherapy. In mice, protein immunization induced a Th2 response.[37] In contrast, plasma DNA induced Th1 responses with IgG2A antibody production and IFN-γ.

In studies published by Spiegelberg *et al.*,[38] it was demonstrated that boosting of mice primed with antigen resulted in a 75% decrease of the IgE titer using plasma DNA encoding the antigen. Furthermore, this plasmid DNA using CpG immunization inhibited lung eosinophilia through a mechanism that elicited a Th1 response with induction of IL-12 and IFN-α and IFN-β. It also induced a Th1 immune response to the allergen and appears to be a novel type of immunotherapy.

The advantages of plasma DNA compared with classical immunotherapy are only very small amounts of allergen are secreted and the allergen gene can be modified. Furthermore, DNA vaccines are likely to have a long-lasting effect and allow the elimination of the active site of the enzyme contained in allergens used in classic immunotherapy. Constructs can be made to contain the optimal number and location of the immunostimulatory sequence. The CpG motifs may oppose Th2-type allergic responses and coadministration of CpG oligonucleotide with antigen or allergen may protect against asthma.

Additional studies by Klein *et al*.[39] also showed the effectiveness of CpG oligonucleotide in a mouse model of asthma. In their studies, Klein *et al*.[39] mixed the allergen with the CpG, in contrast with the study of Spiegelberg *et al.*,[38] who covalently linked the allergen with the CpG. Klein *et al*.[39] also showed inhibition of the serum IgE and inhibition of pulmonary eosinophilia, as well as airway reactivity, in mice receiving CpG along with the antigen compared with control mice that received the same antigen but without the CpG.

These studies raise the possibility that childhood exposure of CpG DNA may restore a Th1 immune response and may improve the beneficial effects of immunotherapy, partly due to induction of Th1 cytokines. Currently, studies are in progress to determine the effectiveness in ragweed seasonal allergic rhinitis patients of administering CpG with ragweed allergen.

The promise of major improvements in the practice of allergen immunotherapy by stimulating the innate immune system favorable to a Th1 response to allergens offers a promising alternative to conventional immunotherapy. Studies in experimental animals appear to be very promising, including those involving either BCG vaccine or DNA vaccine incorporating immunostimulatory sequence oligonucleotides. However, these studies performed in experimental animals and *in vitro* could prove to be disappointing in clinical trials. In this regard, it is not possible to predict whether stimulation of the immune system towards a non-allergic response to allergens will prove to be more effective for future immunotherapy.

Inhibitors of IL-4 and IL-5 given by inhalation once a week

Soluble recombinant IL-4 receptor (IL-4R) is currently under investigation and has been shown in preliminary studies (Immunex, Seattle, WA, USA, unpubl. obs.) to be safe and effective in asthmatics. Interleukin-4R combines with IL-4 to block its effect and improve pulmonary function and asthma symptom scores. Early indications are that IL-4 can decrease asthma exacerbation and decrease β-adrenergic receptor agonist rescue use, inhaled steroid use, exhaled nitric oxide (NO) and circulating eosinophils. However, a recent phase III trial has been somewhat disappointing (Immunex, Seattle, WA, USA, unpubl. obs.).

A recombinant humanized monoclonal antibody against IL-5 has been evaluated in patients with mild asthma. It markedly decreased blood eosinophils and reduced the number of sputum eosinophils. However, it had no apparent effect on allergen-induced late-phase reaction or airway hyperresponsiveness. It remains to be seen whether longer-term administration of anti-IL-5 to eliminate tissue eosinophils in the airways is associated with improvement in asthma symptoms and airway hyperresponsiveness.

Antibody against IgE: Omalizumab

Also under evaluation is a strategy to block IgE and its synthesis. A recombinant humanized monoclonal antibody against IgE called rhuMAb-E25, or omalizumab, markedly decreases serum IgE and IgE receptor expression on basophils and inhibited IgE production by B cells.[40] This antibody is specific for the epitope of IgE that binds to the IgE receptor, thereby blocking the binding of IgE to mast cells and inhibiting mediator release. In controlled phase II and phase III trials in patients with moderate to severe asthma, omalizumab significantly improved symptoms, peak flow rates and decreased rescue use of β-adrenergic receptor agonists, inhaled or oral corticosteroid use,[40] exhaled NO,[41] blood and sputum eosinophils and the early and late allergic response.[41]

We evaluated the effects of the anti-IgE antibody omalizumab on exhaled NO in 29 children with allergic asthma.[42] Exhaled NO was assessed during the stable dose of therapy with inhaled beclomethasone for 6 weeks, during the beclomethasone reduction and withdrawal phase of 12 weeks and also during an open extension phase (24 weeks). During the steroid-reduction phase, exhaled NO remained stable in the omalizumab-treated group and rose non-significantly in the placebo group. Significant differences in exhaled NO between groups occurred towards the end of steroid withdrawal. At the end of the open-label extension of omalizumab in the omalizumab-treated group, exhaled NO decreased significantly from baseline, even though beclomethasone was markedly reduced. When placebo patients switched to omalizumab for the open extension, exhaled NO fell significantly, although the doses of beclomethasone remained very low. Our studies suggest omalizumab suppressed airway inflammation during beclomethasone withdrawal. These results demonstrate that omalizumab, like corticosteroids and leukotriene-modifying agents, suppresses exhaled NO. Treatment with anti-IgE antibody reduces both the early and late airway responses to allergen challenge and also leads to significant improvement in asthma control in patients with moderate to severe chronic asthma. However, these findings do not prove that IgE entirely mediates the response to allergen. Further studies are needed to examine the dose or time dependency of anti-IgE effects. It is possible that very low levels of IgE persist in the airway mast cells for long periods after serum IgE has been reduced to very low or undetectable levels. The ability of omalizumab to maintain long-term control in patients with moderate to severe asthma followed for 52 weeks total was reported in a large multinational study.[40] In the extension phase of 24 weeks, more omalizumab-treated patients (33.5%) than the placebo-treated patients (13.5%) were able to complete the extension phase without requiring inhaled corticosteroid treatment ($P < 0.001$).

It is entirely feasible that combining anti-IgE with conventional immunotherapy may provide a more effective, safer and longer-term efficacy than either modality alone. For example, anti-IgE may, by virtue of markedly decreasing the level of IgE in the serum and on mast cells, decrease the severity and incidence of anaphylactic reactions associated with conventional immunotherapy. Conventional immunotherapy, either alone or combined with immunostimulatory sequences of CpG or BCG, may enhance their effectiveness and duration of action through a mechanism mediated by increasing Th1 and decreasing Th2 responses. These immune responses may be further enhanced when combined with anti-IgE. Only future clinical trials will determine whether these concepts prove correct.

We now have many modalities of immunomodulation to decrease the effect of IL-4 or IL-5 or production and

levels of IgE or agents to shift the immune response from a Th2 to Th1 and thereby decrease the allergic inflammatory response in the airways.

References

1 Durham SR, Walker SM, Varga EM *et al*. Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* 1999; **341**: 468–75.

2 Theodoropoulos DS, Lockey RF. Allergen immunotherapy: Guidelines, update, and recommendations of the World Health Organization. *Allergy Asthma Proc.* 2000; **21**: 159–66.

3 Durham SR, Ying S, Varney VA *et al*. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J. Allergy Clin. Immunol.* 1996; **97**: 1356–65.

4 Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* 1997; **99**: 254–60.

5 Hedlin G, Silber G, Naclerio R *et al*. Comparison of the *in vivo* and *in vitro* response to ragweed immunotherapy in children and adults with ragweed-induced rhinitis. *Clin. Exp. Allergy* 1990; **20**: 491–500.

6 Walker SM, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: A randomized, controlled trial. *J. Allergy Clin. Immunol.* 2001; **107**: 87–93.

7 Nelson HS. Future advances of immunotherapy. *Allergy Asthma Proc.* 2001; **22**: 203–7.

8 Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am. J. Respir. Crit. Care Med.* 1995; **151**: 969–74.

9 Wierenga EA, Snoek M, de Groot C *et al*. Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. *J. Immunol.* 1990; **144**: 4651–6.

10 Imada M, Simons FE, Jay FT, Hayglass KT. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: Responses of subjects with allergic rhinitis and normal controls. *Immunology* 1995; **85**: 373–80.

11 Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J. Exp. Med.* 1993; **178**: 2123–30.

12 Manetti R, Gerosa F, Giudizi MG *et al*. Interleukin 12 induces stable priming for interferon gamma (IFN-gamma) production during differentiation of human T helper (Th) cells and transient IFN-gamma production in established Th2 cell clones. *J. Exp. Med.* 1994; **179**: 1273–83.

13 Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J. Clin. Invest.* 1992; **90**: 262–6.

14 Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J. Allergy Clin. Immunol.* 1996; **97**: 1288–96.

15 Borish L, Rosenwasser L. TH1/TH2 lymphocytes: Doubt some more. *J. Allergy Clin. Immunol.* 1997; **99**: 161–4.

16 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994; **265**: 1237–40.

17 Gaglani B, Borish L, Bartelson BL, Buchmeier A, Keller L, Nelson HS. Nasal immunotherapy in weed-induced allergic rhinitis. *Ann. Allergy Asthma Immunol.* 1997; **79**: 259–65.

18 Alexandroff AB, Jackson AM, O'Donnell MA, James K. BCG immunotherapy of bladder cancer: 20 years on. *Lancet* 1999; **353**: 1689–94.

19 Hopfenspirger MT, Parr SK, Hopp RJ, Townley RG, Agrawal DK. Mycobacterial antigens attenuate late phase response, airway hyperresponsiveness, and bronchoalveolar lavage eosinophilia in a mouse model of bronchial asthma. *Int. Immunopharmacol.* 2001; **1**: 1743–51.

20 Arkwright PD, David TJ. Intradermal administration of a killed *Mycobacterium vaccae* suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J. Allergy Clin. Immunol.* 2001; **107**: 531–4.

21 Hopp RJ, Biven RE, Degan JA, Bewtra AK, Townley RG. The usefulness of questionnaire-derived information to predict the degree of nonspecific bronchial hyperresponsiveness. *Allergy Proc.* 1995; **16**: 129–34.

22 Seminario MC, Gleich GJ. The role of eosinophils in the pathogenesis of asthma. *Curr. Opin. Immunol.* 1994; **6**: 860–4.

23 Sugiyama H, Eda R, Okada C, Hopp RJ, Bewtra AK, Townley RG. Eosinophil accumulation and activation in antigen-induced late asthmatic response in guinea pigs. *J. Asthma* 1995; **32**: 37–45.

24 Pelikan Z. Concept of pathogenesis and possible mechanism(s) underlying the late phase reactions-focused on the late asthmatic response (LAR). In: Dorsch W (ed.). *Late Phase Allergic Reactions*. Boca Raton: CRC Press, 1990; 499–518.

25 Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. Infection of mice with *Mycobacterium bovis*–Bacillus Calmette–Guerin (BCG) suppresses allergen-induced airway eosinophilia. *J. Exp. Med.* 1998; **187**: 561–9.

26 Herz U, Gerhold K, Gruber C *et al*. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. *J. Allergy Clin. Immunol.* 1998; **102**: 867–74.

27 Sano K, Haneda K, Tamura G, Shirato K. Ovalbumin (OVA) and *Mycobacterium tuberculosis* bacilli cooperatively polarize anti-OVA T-helper (Th) cells toward a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia. *Am. J. Respir. Cell Mol. Biol.* 1999; **20**: 1260–7.

28 Marchant A, Goetghebuer T, Ota MO *et al*. Newborns develop a Th1-type immune response to *Mycobacterium bovis* bacillus Calmette–Guerin vaccination. *J. Immunol*. 1999; **163**: 2249–55.
29 Holt PG, Sly PD. Prevention of adult asthma by early intervention during childhood: Potential value of new generation immunomodulatory drugs. *Thorax* 2000; **55**: 700–3.
30 Aaby P, Shaheen SO, Heyes CB *et al*. Early BCG vaccination and reduction in atopy in Guinea-Bissau. *Clin. Exp. Allergy* 2000; **30**: 644–50.
31 Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**: 77–9.
32 Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. *Lancet* 1997; **350**: 400–3.
33 Omenaas E, Jentoft HF, Vollmer WM, Buist AS, Gulsvik A. Absence of relationship between tuberculin reactivity and atopy in BCG vaccinated young adults. *Thorax* 2000; **55**: 454–8.
34 Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. Recombinant allergens for diagnosis and therapy of allergic disease. *J. Allergy Clin. Immunol*. 2000; **106**: 409–18.
35 Nelson HS. Combination therapy of bronchial asthma. *Allergy Asthma Proc*. 2001; **22**: 217–20.
36 Hsu CH, Chua KY, Tao MH *et al*. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness *in vivo* by genetic immunization. *Nat. Med*. 1996; **2**: 540–4.
37 Lowrie D, Whaler RG (eds). *DNA Vaccines: Methods and Protocols*. Totowa: Humana Press, 1999.
38 Spiegelberg HL, Broide D, Tighe H, Roman M, Raz E. Inhibition of allergic inflammation in the lung by plasmid DNA allergen immunization. *Pediatr. Pulmonol. Suppl*. 1999; **18**: 118–21.
39 Klein JN, Waldschmidt TA, Businga TR *et al*. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J. Immunol*. 1998; **160**: 2555–9.
40 Soler M, Matz J, Townley R *et al*. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur. Respir. J*. 2001; **18**: 254–61.
41 Fahy JV, Fleming HE, Wong HH *et al*. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am. J. Respir. Crit. Care Med*. 1997; **155**: 1828–34.
42 Silkoff P, Romero FA, Townley RG *et al*. Exhaled nitric oxide in children with asthma receiving omalizumab, a monoclonal Anti-IgE antibody. *Am. J. Respir. Crit. Care Med*. 2002 (in press).