

Review article Series I

DNA immunization: a novel approach to allergen-specific immunotherapy

Spiegelberg HL, Orozco EM, Roman M, Raz E. DNA immunization: a novel approach to allergen-specific immunotherapy. Allergy 1997; 52: 964–970. © Munksgaard 1997.

**H. L. Spiegelberg¹, E. M. Orozco²,
M. Roman², E. Raz²**

Departments of ¹Pediatrics and ²Medicine,
University of California San Diego
School of Medicine, La Jolla, CA, USA

Hans L. Spiegelberg
Department of Pediatrics 08333
University of California San Diego School of
Medicine
9500 Gilman Drive
La Jolla
CA 92093-0833
USA

Accepted for publication 18 March 1997

Immunotherapy, the subcutaneous injection of small but gradually increasing amounts of allergen, has been used as a form of therapy for allergic diseases since the beginning of the century. However, in the last 15 years, the use of immunotherapy has steadily decreased for several reasons. First and most importantly, injection of allergen involves the risk of induction of mild and sometimes even severe anaphylactic reactions. Second, in patients allergic to many allergens, immunotherapy was not very successful. Finally, present-day pharmacologic treatment of allergic disorders allows the control of allergic diseases at a lower risk-to-benefit ratio than immunotherapy (1). However, immunotherapy could regain a place in the therapy of allergies if a nonanaphylactic form of allergen immunization could be found. The advantage of such an allergen immunization would be the same as that of successful classical immunotherapy. It could decrease the severity of the allergic disease and thereby the need for daily use of antiallergic drugs such as β -agonists and corticosteroids. Furthermore, a safe and effective immunotherapy may accelerate the naturally occurring "outgrowth" of allergic disease in children that often occurs in early adulthood. In the past, many attempts were made to develop allergen preparations which do not react with IgE bound to mast cells and trigger anaphylactic reactions;

however, no satisfactory modified allergen preparation was found that gained widespread clinical use.

Recently, research designed to find better and safer vaccines for infectious diseases has yielded two unexpected findings. First, it was found that "naked" plasmid DNA (pDNA) encoding an antigen is taken up *in vivo* by muscle cells, keratinocytes, and fibroblasts as well as by other cells, including antigen-presenting cells (APC), and that these cells synthesize the antigen (2–6). Furthermore, the host responds to such "self-made" antigens with long-lasting antigen-specific cellular and humoral immune responses. The second unexpected finding, which was reported by our laboratory (7), was that the antibody response to pDNA-induced protein antigens in mice is predominantly of the IgG2a isotype, whereas immunization with protein antigens usually induces IgG1 and IgE antibodies (8, 9). These findings suggested that pDNA immunization induced a T helper cell type 1 (Th1) response since murine IgG2a antibodies are induced by interferon- γ (IFN- γ) produced by Th1 cells (10). In contrast to pDNA immunization, injection of the corresponding native proteins induced Th2 cells which produce interleukin-4 (IL-4), the lymphokine that causes the switch from IgM to IgG1 and IgE antibody formation (11). Allergic diseases are the result of

an enhanced Th2 response to the allergens (12, 13). Therefore, the finding that immunization with pDNA encoding allergens induces a Th1 response and no IgE antibodies in mice suggested that immunization with pDNA encoding allergens may offer a novel approach to the development of a new type of allergen immunotherapy, which is the subject of this review.

DNA immunization

After the recent observation demonstrating that intramuscular (i.m.) injection of pDNA encoding an antigen induces antigen synthesis and an immune response to the antigen (2–5), pDNA immunization has been extensively investigated mainly for the development of new and better vaccines (reviewed by Pardoll & Beckerleg [6]). Remarkably, pDNA immunization induced immune responses under almost all conditions tested. We investigated and compared i.m. with intradermal (i.d.) pDNA immunization because we hypothesized that uptake of pDNA and synthesis of antigen by APC may play an important role in the initial phase of the immune response. Moreover, i.d. immunization may be more potent than i.m. immunization since the skin contains an abundance of APC while muscle does not (5).

Indeed, i.d. immunization proved to be very efficient and more effective than i.m. immunization. Injection of as little as 3 µg pDNA i.d. induced IgG antibody formation to a gene product such as influenza nucleoprotein. Even scratching the pDNA into the skin with the pDNA loaded on the spikes of tyne applicators that are used for allergen testing induced a strong immune response. The pDNA stays in the skin cells for a long period of time; 6 months after injection, it was still detectable by the polymerase chain reaction technique and presumably continued to induce antigen synthesis. Of several promoters tested for the preparation of the plasmid vectors, the cytomegalovirus (CMV) promoter proved to induce the most consistent and strongest immune responses and therefore presumably also the strongest antigen synthesis *in vivo* (5).

The immune response consisted of both cytotoxic T cells and IgG antibodies. When we tested the immunoglobulin isotypes of the antibodies, they were predominantly IgG2a, the isotype that is typical of murine Th1 immune responses (11). In mice, infectious agents such as viruses induce IgG2a antibodies (8) and Th1 responses, whereas protein antigens usually induce a Th2 response and IgG1 and IgE antibodies in mice (9). The cause of the unexpected murine Th1 response to protein antigens after pDNA immunization is not under-

stood and is probably manifold. However, two factors may be the most important. First, pDNA immunization “converts” an extracellular antigen into an intracellular antigen, and, second, certain noncoding DNA sequences have a Th1-promoting adjuvant effect, to be described below. Intracellular production of antigens similar to intracellular infectious agents results in predominant major histocompatibility (MHC) class I antigen presentation, and this may be the cause of the strong cytotoxic response observed to the encoded antigens (5). In addition, a small amount of pDNA-induced antigen is secreted by the transfected cells. The secreted antigen is probably processed by conventional APC and presented on MHC class II molecules which induce preferentially antibody production. Therefore, together with the DNA adjuvant effect described below, it is not surprising that pDNA immunization generates both a strong cellular and a strong humoral immune response (5). It is likely that new and powerful pDNA-based vaccines will soon be developed that are safe because they do not contain any traces of contaminating infectious agents, since their production involves neither killed nor attenuated microorganisms. As described in this review, pDNA immunizations may also become applicable as a new form of allergy immunotherapy.

Downregulation of IgE antibody formation by pDNA immunization of mice and rats

To investigate whether i.d. immunization with pDNA downregulates the IgE antibody formation, we first investigated the immune response to the reporter gene *lacZ*, which encodes *E. coli* β-galactosidase (β-gal), and compared it with that to the native protein β-gal (7). We had previously shown that i.d. injection of a “naked” pDNA (pCMV-*lacZ*), consisting of the CMV promoter, the β-gal gene (*lacZ*), the bovine growth hormone poly A termination site, and the ampicillin-resistant gene (*ampR*), into mice induces both a cellular and a humoral immune response (5). IgE high-responder mice, such as BALB/c mice, typically produce IgG1 and IgE antibodies to protein antigens, especially if the protein is injected in alum (9). Therefore, it was surprising that the pCMV-*lacZ*-immunized mice formed mainly IgG2a antibodies. As shown in Fig. 1, injection of β-gal in saline or alum induced high titers of IgG1 and IgE antibodies. In contrast, i.d. immunization with pCMV-*lacZ* induced mainly IgG2a antibodies. The pCMV-*lacZ*-immunized mice also produced IgG2b and IgG3 antibodies but in lower titers. These three isotypes are typical of a Th1 response, a fact which suggested that the

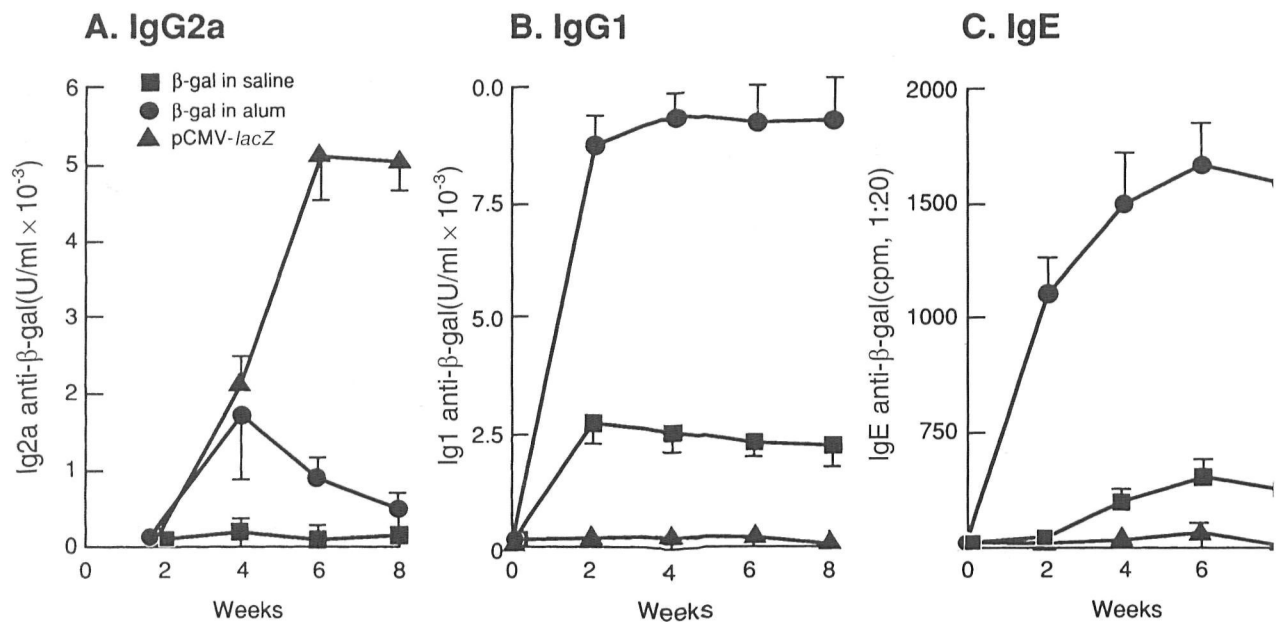


Fig. 1. Primary IgG subclass and IgE immune response of BALB/c mice to i.d. injection of 100 μ g pCMV-lacZ (▲), 10 μ g β -gal in saline (●), or 1 μ g β -gal in alum (■).

pDNA immunization induced a Th1 response to the gene product, whereas protein immunization induced a Th2 response. This was confirmed by measuring the lymphokine secretion of *in vitro* antigen-stimulated CD4⁺ splenic T cells (Table 1). The T cells of the protein-immunized control mice secreted interleukin-4 (IL-4) and IL-5, but almost no IFN- γ . In contrast, the T cells of pCMV-lacZ-immunized mice secreted IFN- γ , but no IL-4 and IL-5.

To determine whether pDNA or protein immunization predominates, we first primed mice with pCMV-lacZ and boosted them 6 weeks later with β -gal protein in alum. As shown in Fig. 2, mice primed with pCMV-lacZ continued to produce IgG2a antibodies after boosting with β -gal in alum. The β -gal in alum even enhanced the IgG2a antibody formation, and, most importantly, pCMV-lacZ

priming prevented the induction of IgE antibody formation subsequent to the injection of β -gal in alum. The inhibition of IgE anti- β -gal antibody formation was antigen specific; mice primed with pCMV-lacZ formed high titers of IgE antiovalbumin (OVA) antibodies after injection of OVA in alum. The lack of specific IgE formation in pCMV-lacZ-primed and β -gal in alum-boosted mice suggested that pCMV-lacZ immunization predominated over and prevented the subsequent Th2 response to β -gal in alum. This was confirmed by secretion of lymphokines of *in vitro* antigen-stimulated CD4⁺ splenic T cells (Table 1). The T cells of pCMV-lacZ-primed and β -gal in alum-boosted mice continued to secrete IFN- γ but formed very little IL-4 and IL-5.

Next, we investigated the effect of pCMV-lacZ boosting of β -gal in alum-primed mice (Fig. 3). Six weeks after priming, the mice showed a low IgG2a but a relatively high IgE titer to β -gal. Boosting with pCMV-lacZ induced a large increase of IgG2a antibodies and a concomitant decrease of the IgE antibody titer. Six weeks after the pDNA boost, the IgE titers decreased by about 75%. The decrease of the IgE titer was antigen specific. Injection of an unrelated pDNA construct containing the OVA gene but otherwise identical to the pCMV-lacZ pDNA did not induce a decrease of the IgE anti- β -gal antibody titer. This experiment indicated that the pre-existing Th2 response to β -gal was unable to inhibit a subsequent Th1 response induced by the pCMV-lacZ boost. Analysis of the lymphokine secretion of *in vitro* antigen-stimulated CD4⁺

Table 1. Lymphokine secretion by β -gal-activated CD4⁺ splenic T cells from pDNA-and/or protein-immunized mice

Experiment	Priming	Boosting	IFN- γ pg/ml	IL-4 pg/ml	IL-5 Units/ml
A	None	None	<10	<2	<2
B	pCMV-lacZ	None	741 $\pm$ 170	<2	<2
C	β -gal/saline	None	<10	12 $\pm$ 2	15 $\pm$ 4
D	β -gal/alum	None	<10	351 $\pm$ 24	642 $\pm$ 51
E	pCMV-lacZ	β -gal/alum	1050 $\pm$ 314	4 $\pm$ 2	46 $\pm$ 10
F	β -gal/saline	β -gal/alum	53 $\pm$ 48	126 $\pm$ 43	165 $\pm$ 54
G	β -gal/alum	pCMV-lacZ	730 $\pm$ 193	307 $\pm$ 128	183 $\pm$ 70

Means $\pm$ SE of spleens from four mice per experiment.

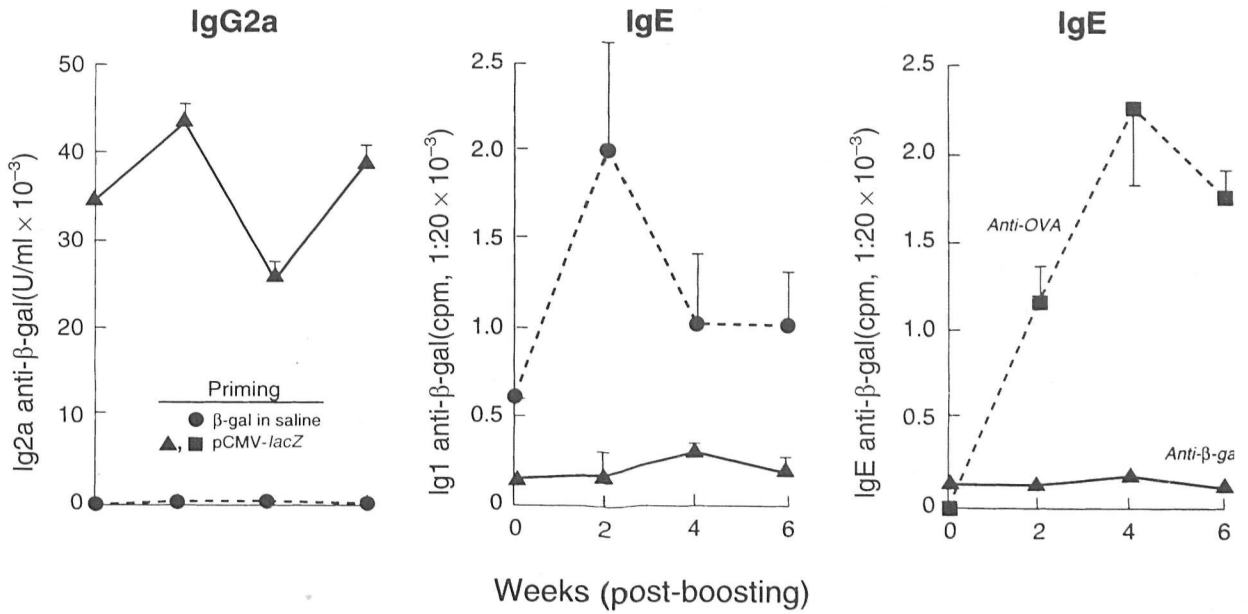


Fig. 2. IgG2a and IgE response of BALB/c mice primed with 100 μ g pCMV-lacZ and boosted with 1 μ g β -gal in alum (▲). Control mice were primed with 10 μ g β -gal in saline (●). For antigen specificity control, mice primed with pCMV-lacZ were injected with 2 μ g ovalbumin (OVA) in alum (■).

splenic T cells showed IFN- γ secretion at quantities similar to that induced by primary pCMV-lacZ immunization (Table 1). IL-5, but not IL-4, secretion was decreased as compared to naive β -gal in alum-injected mice. The IL-4 and IL-5 was presumably secreted by Th2 cells that were generated by the primary immunization with β -gal in alum. However, since the IgE antibody formation in mice is very sensitive to inhibition with IFN- γ both *in*

vitro and *in vivo*, the IFN- γ production by the newly induced Th1 cells appeared to be sufficient to cause the 75% decrease of the ongoing IgE antibody formation within the 6-week observation period (Fig. 3). It remains to be shown whether a pre-existing Th2 response and IgE antibody formation can be completely eliminated over a longer time period by multiple pCMV-lacZ injections and be replaced by a Th1 response.

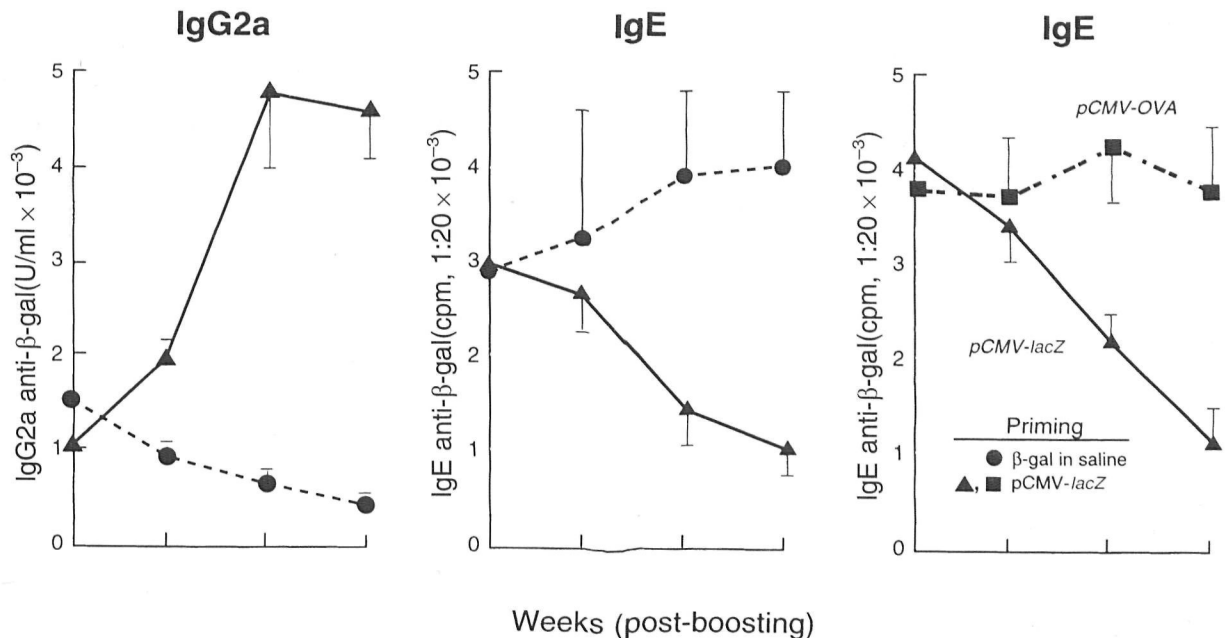


Fig. 3. IgG2a and IgE response of BALB/c mice primed with 1 μ g β -gal in alum and boosted with 100 μ g pCMV-lacZ (▲). Control mice were primed with 1 μ g β -gal in saline (●). For antigen specificity control, mice were boosted with pCMV-OVA (■).

Recently, Hsu et al. (14) reported data on pDNA immunization in rats similar to our results obtained in mice (7). Rats injected i.m. with pDNA encoding the allergen Der p 5 produced IgG2a, but not IgE, antibodies, whereas native Der p 5 induced IgE antibodies. In addition, Slater et al. (15) showed that BALB/c mice primed with the latex allergen Hev b 5 and boosted with pDNA encoding Hev b 5 showed a 23% decrease of the specific IgE antibody titer within 10 days after the pDNA injection. These data show that pDNA immunization encoding different types of protein antigens, including human allergens, induces Th1 immune responses to these antigens, and that pDNA immunization can downregulate a pre-existing Th2 response and IgE antibody formation in both mice and rats.

Possible mechanism of IgE downregulation by pDNA immunization

Initially, we immunized mice with a pDNA construct encoding β -gal containing the *ampR* gene and compared the immune response to that of pDNA encoding β -gal which contained the kanamycin-resistant gene (*kanR*). To our surprise, the *kanR* gene-containing plasmids induced significantly lower anti- β -gal immune responses. The reason for this was the presence of immunostimulatory DNA sequences (ISS) in the *ampR*, but not in the *kanR*, gene (16). It had previously been reported that palindromic single-stranded DNA sequences having the 5'-purine-purine-CpG-pyrimidine-pyrimidine-3' CpG motif have an immunomodulatory effect (17, 18). The *ampR* gene contains two 5'-AACGTT-3' repeats, whereas the *kanR* gene does not contain these DNA sequences. When we inserted one or two of the ISS of the *ampR* gene into the *kanR* gene-containing vector and immunized mice with these constructs, the mice made an immune response to β -gal that was equal to that of mice immunized with the original *ampR* gene-containing vector. In subsequent experiments, we showed that the ISS induce the secretion of IFN- α , IFN- β , and IL-12 from human monocytes and IFN- γ from natural killer cells. These cytokines are known to induce naive T cells to differentiate into either IFN- γ -secreting CD4⁺ Th1 or CD8⁺ Tc1 cells, and it is most likely that the induction of secretion of these cytokines by the ISS plays a major role in the induction of Th1 immune responses to the encoded allergens in mice (16). More recently (M. Roman et al., paper submitted), we investigated whether ISS mixed with the proteins β -gal or OVA would act as a Th1-inducing adjuvant. Indeed, mixing plasmids that contain the *ampR* gene (pUC19) with protein antigens caused a Th1 response to the protein antigens, whereas control mice injected

with β -gal or OVA in saline or alum produced the expected Th2 response (19). These experiments support the notion that the ISS act as an adjuvant which induces IFN- α , IFN- β , IL-12, and IFN- γ secretion, and suggest that the ISS play a major role in the induction of a Th1 response to encoded proteins.

To investigate whether CD4⁺ and/or CD8⁺ T cells from pCMV-*lacZ*-immunized mice caused the suppression of the IgE formation, Lee et al. (20) passively transferred CD4⁺ or CD8⁺ splenic T cells to naive mice and immunized them 2 days later with β -gal in alum. Passive transfer of CD4⁺ or CD8⁺ T cells from pCMV-*lacZ*-immunized mice suppressed the IgE antibody formation by 90% as compared to the transfer of T cells from naive or β -gal in alum-immunized mice. This indicates that the pDNA immunization induced both IFN- γ -producing antigen-specific CD4⁺ and CD8⁺ cells, and that the transferred cells inhibited the IgE formation either by affecting the primary response, by propagating the Th1 response of the transferred cells, or by both actions. Moreover, when β_2 -microglobulin knockout mice, which have few functional CD8⁺ T cells, were immunized with β -gal in alum, they produced threefold more IgE antibodies than the wild-type C57BL control mice (20). pCMV-*lacZ* did not induce IgE antibodies in both types of mice. The threefold higher IgE response in β_2 -microglobulin knockout mice corroborates the passive transfer experiments, and confirms that CD8⁺ T cells play an important role in the suppression of IgE-antibody production. Hsu et al. (14) reported that CD8⁺, but not CD4⁺, T cells from rats immunized with pDNA encoding the house-dust mite Der p 5 allergen inhibited IgE formation in adaptively transferred naive recipient rats. The reason for the lack of a suppressive effect of CD4⁺ T cells in Hsu et al.'s (14) experiments, but not in ours, may be the result of a species difference or the experimental design. Hsu et al. transferred CD4⁺ and CD8⁺ depleted cell fractions and did not compare the transfer of CD4⁺ T cells from naive and pDNA-immunized rats.

Immunization with pDNA encoding allergens suppresses allergic inflammation

Immunization with pDNA encoding allergens inhibits not only IgE antibody formation in mice and rats but also allergic response in the lung after challenge with allergen inhalation. Hsu et al. (14) showed that immunization of rats with pDNA encoding the house-dust-mite allergen Der p 5 prevented histamine release into the bronchoalveolar lavage (BAL) fluid and airway hypersensitivity to allergen inhalation. Similarly, mice

immunized with pCMV-OVA and challenged by OVA inhalation showed significantly reduced numbers of eosinophils in the BAL fluid (43% to 3.5%), the lung tissue, and the bone marrow (10.3% to 1.4%) as compared to control mice (21). Taken together, these findings indicate that immunization with pDNA encoding allergens suppresses not only IgE antibody formation but also anaphylaxis and allergic inflammation in both rats and mice.

Conclusions

The use of classical allergen immunotherapy has greatly decreased over the last 10 years mainly because the risk-to-benefit ratio is not acceptable when compared to optimal pharmacologic management of allergic disorders (1). However, if a safe and potent form of immunotherapy could be developed, immunotherapy could probably regain its role as a major treatment for allergic diseases. The recent discoveries of allergen gene immunization described in this review suggest that it may soon be possible to develop a novel and safer form of immunotherapy.

Immunization of mice and rats with protein antigens or with human allergens such as Der p 5, Hev b 5, or ovalbumin induces a Th2 response and high titers of IgE antibodies. In contrast, immunization with pDNA encoding these same antigens induces a Th1 response, indicated by high titers of IgG2a, but no IgE antibodies, and by IFN- γ -secreting antigen-specific Th1 cells. Furthermore, pDNA-immunized animals when challenged by allergen inhalation had greatly reduced histamine release into the BAL fluid and eosinophil infiltration of the lung tissue, demonstrating an effect on both immediate and late-phase allergic responses (14, 21). Antigen-stimulated CD4⁺ T cells from pDNA-immunized mice secrete IFN- γ , a potent inhibitor of IgE formation. Furthermore, and most importantly, the pDNA-induced Th1 immune response in mice dominated over a pre-existing Th2 response and could suppress ongoing IgE antibody formation in mice (7), a situation analogous to allergic disease in man.

To date, all these preclinical observations have been made in mice and rats. However, it was recently shown that pCMV-*lacZ* encoding β -gal is also taken up by cells in human skin transplanted to immunocompromised nude mice (22). *In vitro* data indicate that the transfected cells secrete only very small amounts of antigen unlikely to induce anaphylactic reactions similar to those induced by classical immunotherapy. Furthermore, the pDNA constructs encoding allergens can be modified to include a membrane anchor DNA sequence, thereby further inhibiting the secretion of allergen and

possible induction of anaphylactic reactions. Enzymatic activities of allergens or potentially cytotoxic elements of a pDNA-induced allergen can also be deleted from the allergen pDNA construct, should this be necessary for the induction of a strong antiallergen immune response.

It has not yet been determined whether pDNA immunization may have a bystander effect and down-regulate Th2 responses to cross-reacting or even unrelated allergens resulting from Th1-inducing cytokine secretion (IFN- α , IFN- β , IL-12, IFN- γ) by the ISS. In children, the use of allergen gene vaccination may cause the "outgrowth" of allergies at an earlier age than after classical immunotherapy or that occurring spontaneously in early adulthood. Although the safety of pDNA vaccination has not been fully investigated, the possibility of incorporation of the pDNA into the host genome where it may activate oncogenes is very small but remains to be investigated. Another adverse reaction could be anti-DNA antibody formation. However, we could not demonstrate anti-DNA antibodies in our experiments in mice.

At present, the safety and clinical efficacy of pDNA vaccination for infectious diseases are being tested in man, and these studies will establish its safety for future pDNA allergen immunizations. Coinjection of allergens with a Th1-inducing adjuvant containing immunostimulatory DNA sequences offers an alternative approach to pDNA vaccination for a new type of allergen immunotherapy. After completion of our preclinical studies, we plan to test pDNA allergen immunization and the use of ISS-DNA-containing adjuvant in man to investigate whether they offer a potent and safe form of immunotherapy for allergic disorders.

Acknowledgment

This work was supported by US National Institutes of Health grant AI 37305.

References

- Adkinson NF, Eggleston PA, Eney D, et al. A controlled trial of immunotherapy for asthma in allergic children. *N Engl J Med* 1997;336:324-31.
- Ulmer JB, Donnelly JJ, Parker SE, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 1993;259:1745-9.
- Fynan EF, Webster RG, Fuller DH, Haynes JR, Santoro JC, Robinson HL. DNA vaccines: protective immunization by parenteral, mucosal, and gene-gun inoculation. *Proc Natl Acad Sci U S A* 1993;90:11478-82.
- Manickan E, Rouse RJ, Yu Z, Wire WS, Rous BT. Genetic immunization against herpes simplex virus. *J Immunol* 1955;155:259-65.
- Raz E, Carson DA, Parker SE, et al. Intradermal gene

- immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses. *Proc Natl Acad Sci U S A* 1994;91:9519-23.
6. Pardoll DM, Beckerleg AM. Exposing the immunology of naked DNA vaccines. *Immunity* 1995;3:165-9.
7. Raz E, Tighe H, Sato Y, et al. Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Natl Acad Sci U S A* 1996;93:5141-5.
8. Coutelier JP, van der Logt JTM, Heessen WA, Waarner G, van-Snick J. IgG2a restriction of murine antibodies elicited by viral infections. *J Exp Med* 1987;165:2373-8.
9. Beck L, Spiegelberg HL. The polyclonal and antigen-specific IgE and IgG subclass response of mice injected with ovalbumin in alum or complete Freund's adjuvant. *Cell Immunol* 1989;123:1-8.
10. Snapper CM, Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987;236:944-7.
11. Mosmann TR, Coffman RL. The Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-73.
12. Parronchi P, Macchia D, Piccini MP, et al. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci U S A* 1991;88:4538-42.
13. Wierenga EA, Snock M, de Groot C, et al. Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-6.
14. 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.
15. Slater JE, Zhang YJ, Arthur-Smith A, Colberg-Poley A. A DNA vaccine inhibits IgE responses to the latex allergen Hev b 5 in mice. *J Allergy Clin Immunol* 1997;99:S504.
16. Sato Y, Roman M, Tighe H, et al. Immuno-stimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* 1996;273:352-4.
17. Yamamoto S, Yamamoto T, Kataoka T, Kuramoto E, Yano O, Tokunaga T. Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN and IFN-mediated natural killer activity. *J Immunol* 1992;148:4072-6.
18. Klinman DM, Yi AK, Beaucage SL, Conover J, Krieg AM. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ . *Proc Natl Acad Sci U S A* 1996;93:2879-83.
19. Raz E, Roman M, Orozco EM, Carson DA. Immuno-stimulatory DNA sequences (ISS) are a Th1 promoting adjuvant. *J Allergy Clin Immunol* 1997;99:S365.
20. Lee DJ, Tighe H, Roman M, Carson DA, Spiegelberg HL, Raz E. Inhibition of IgE antibody formation by plasmid DNA immunization is mediated by both CD4+ and CD8+ T cells. *Int Arch Allergy Immunol* 1997;113:227-30.
21. Broide D, Orozco EM, Roman M, Carson DA, Raz E. Intradermal gene vaccination down-regulates both arms of the allergic response. *J Allergy Clin Immunol* 1997;99:S129.
22. Hengge UR, Walker PS, Vogel JC. Expression of naked DNA in human, pig, and mouse skin. *J Clin Invest* 1996;97:2911-16.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.