

DNA-based vaccination reduces the risk of lethal anaphylactic hypersensitivity in mice

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Background: Anaphylactic hypersensitivity is the most serious clinical concern facing allergists. However, for the majority of anaphylactic hypersensitivities, avoidance is the only therapeutic option presently available.

Objective: This study evaluated the effectiveness of primary gene and protein-immunostimulatory DNA vaccination in the prevention of anaphylactic hypersensitivity in a murine model. **Methods:** Female C3H/HeJ mice were immunized with a plasmid encoding β -galactosidase (β -gal) or β -gal protein plus an immunostimulatory sequence oligodeoxynucleotide. The mice were then T_{H2} sensitized to β -gal by coinjection with alum and pertussis and then intravenously challenged with this model allergen.

Results: Primary gene and protein-immunostimulatory DNA vaccination of subsequently T_{H2}-sensitized mice reduced the risk of death after anaphylactic challenge from 100% to 67% and 58%, respectively ($P < .018$ vs control mice). In addition, gene and protein-immunostimulatory DNA vaccination reduced postchallenge plasma histamine levels by greater than 4-fold ($P < .05$ vs control mice). Consistent with previous studies, these DNA-based vaccination strategies were further shown to blunt the development of T_{H2}-biased immune responses after allergen sensitization. Vaccination with protein alone, the experimental equivalent of a traditional immunotherapy reagent, provided no protection from anaphylaxis nor did it prevent the development of a T_{H2}-biased immune profile after allergen sensitization.

Conclusion: The present series of experiments demonstrate that both gene vaccination and coimmunization with protein and immunostimulatory DNA are effective in attenuating the development of anaphylactic hypersensitivity in subsequently T_{H2} sensitized mice. (*J Allergy Clin Immunol* 2000;106:349-56.)

Key words: Anaphylaxis, gene vaccination, immunostimulatory sequence oligodeoxynucleotides, CpG motifs, immunotherapy

Abbreviations used

BBS:	Borate-buffered saline
β -gal:	β -Galactosidase
ISS-ODN:	Immunostimulatory sequence oligodeoxynucleotide
M-ODN:	Mutated oligodeoxynucleotide
pACB-LacZ:	Plasmid DNA encoding β -galactosidase
pDNA:	Plasmid DNA

In humans, anaphylaxis is a life-threatening hypersensitivity response in which IgE/Fc ϵ RI-mediated systemic mast cell degranulation occurs.¹ However, only a small fraction of allergic individuals have anaphylactic hypersensitivities.¹ The true incidence of anaphylaxis and anaphylactic hypersensitivities is not known, in part because of difficulties in defining what constitutes an anaphylactic reaction and in part because of underreporting.¹⁻³ One study, from Sorenson et al,² proposes that 3.2 cases of anaphylaxis per 100,000 persons occur each year, that 40% are misdiagnosed, and that 5% are fatal. Of particular concern in the case of food allergies is that many anaphylactic reactions occur in individuals after the accidental ingestion of known allergens.³ Because of their lethal potential, effective therapies for the desensitization of individuals with anaphylactic hypersensitivities would be of great clinical utility. Unfortunately, except for stinging insects, traditional protein-based immuno-therapy has no established value in the desensitization of individuals at risk for anaphylaxis, and avoidance remains the only therapeutic option.^{4,5} However, recent reports suggest that plasmid DNA (pDNA) and immuno-stimulatory sequence oligodeoxynucleotide (ISS-ODN) prove to be effective reagents for the prevention and reversal of a number of T_{H2}-mediated hypersensitivity states, including anaphylactic hypersensitivity.⁶⁻¹³

Previous studies in mice have demonstrated that antigen-specific IgE and IgG1 production is robust in response to immunization with protein in alum and poor after pDNA vaccination, whereas high levels of IgG2a are produced with the latter form of antigen exposure.¹³⁻¹⁵ These Ig profiles are reflective of T_{H2}- and T_{H1}-biased B-cell antibody production, respectively, because IgG1 and IgE are produced in response to IL-4, whereas IgG2a is produced in response to IFN- γ . pDNA vaccination has further been shown to prevent IgE production after T_{H2}

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sensitization with clinically relevant allergens, such as latex (Hev b 5), house dust mite (Der p 5), and peanut (Ara h 2).⁶⁻⁸ It has been proposed that immunostimulatory DNA sequences within the backbone of immunization plasmids play a major role in the T_{H1}-biased immune response induced by pDNA vaccines.¹⁸⁻²⁰ Indeed, a number of investigators have demonstrated that coimmunization with antigen and ISS-ODN leads to a T_{H1}-biased immune response that mimics the immune response seen after pDNA vaccination.¹⁸⁻²² Furthermore, protein/ISS-ODN immunization has been shown to prevent IgE synthesis after sensitization with cedar pollen and other proteins.^{9,18} However, although both pDNA and protein/ISS-ODN immunizations are highly effective in inhibiting IgE production and T_{H2} cytokine responses in sensitized mice, IgG1 synthesis is only modestly affected by these vaccination schemes (personal observations).^{9,13}

To prevent the manifestations of allergic hypersensitivity, the T_{H1}-biasing immunomodulatory activity of pDNA and antigen/ISS-ODN vaccination must translate into neutralization of the pathophysiologic response toward allergen exposure. In the case of protein-based immunotherapy, there is no single immunologic parameter that reliably predicts successful anaphylactic desensitization in clinical practice.^{4,23-25} Anaphylactic hypersensitivity responses have even been demonstrated in individuals who have lost their skin test reactivity after receiving immunotherapy for 5 or more years.²⁵ Although DNA-based immunization strategies have been shown to prevent the development of T_{H2}-biased immune responses and to prevent late-phase allergic reactions in mouse and rat models of asthma, it has proven more difficult to prevent the early phase of the immediate hypersensitivity response, which characterizes anaphylaxis.^{6,8-12}

A recent publication demonstrates that pDNA vaccination can prevent relatively mild anaphylactic reactions to peanut allergen.⁷ However, the prevention of death and other severe outcomes remains the principal clinical objective in treating patients with anaphylactic hypersensitivities. Therefore the following series of experiments investigated the efficacy of pDNA and protein/ISS-ODN immunization in the prevention of death caused by anaphylaxis. The results demonstrate that primary immunization with either pDNA or protein and ISS-ODN significantly reduces the risk of death from anaphylaxis after T_{H2} sensitization and antigen challenge. Consistent with previous reports, these DNA-based vaccination strategies are further shown to be effective in the prevention of antigen-specific T_{H2}-biased immune responses. Therefore the present series of experiments further support consideration of the use of pDNA and protein/ISS-ODN immunotherapy for the desensitization of individuals with T_{H2}-mediated hypersensitivities, including those that have the potential to mediate anaphylactic reactions.

METHODS

Mice

Female, 6- to 8-week-old, C3H/HeJ mice were purchased from Jackson Laboratory (Bar Harbor, Me). All mice were maintained in

the University of California, San Diego, Animal Facility, which is certified by the American Association for the Accreditation of Laboratory Animal Care, and all experiments were approved by the animal care committee at our institution.

Reagents

pACB-LacZ contains the CMV IE1 promoter intron, the simian virus 40 t-intron, the *Escherichia coli* LacZ cDNA, and the simian virus 40 polyadenylation site. pACB is the same construct without the *E coli* LacZ insert. Plasmids were purified by using a megaprep kit (Qiagen, Chatsworth, Calif), and endotoxin was removed by extraction with Triton X-114 (Sigma, St Louis, Mo) to below the limits of detection (0.005 EU/mL) by using the limulus amoebocyte lysate assay (Bio-Whittaker, Walkersville, Md). β -Galactosidase (β -gal) and pertussis toxin were obtained from Sigma (St Louis, Mo). Phosphorothioate ISS-ODN and mutated oligodeoxynucleotide (M-ODN) were purchased from Trilink Biotechnologies (San Diego, Calif). The ISS-ODN used in these studies has the sequence 5'-TGACTGTGAACGTTCCGAGATGA-3', and the M-ODN has the sequence 5'-TGACTGTGAACCTTCCGAGATGA-3'. Endotoxin could not be detected in these ODN preparations by using the limulus amoebocyte lysate assay (Bio-Whittaker).

Vaccination, sensitization, and anaphylactic challenge protocols

Mice received intradermal injections with 50 μ g of pACB-LacZ or pACB or 10 μ g of β -gal alone or mixed with ISS-ODN (10 μ g) or M-ODN (10 μ g) in 50 μ L of normal saline solution in the base of the tail on 3 occasions 10 days apart (Fig 1). Twenty-five days after the last vaccination, the mice were T_{H2} sensitized with a mixture of β -gal (100 μ g), pertussis toxin (300 ng), and alum (1 mg) in 500 μ L of normal saline solution injected intraperitoneally on 2 occasions 7 days apart. Mice received an intravenous challenge with 150 μ g of β -gal in 50 μ L of normal saline solution 3 weeks after their last intraperitoneal sensitization. The mice were then observed for 1 hour after intravenous anaphylactic challenge because preliminary experiments demonstrated that challenged mice either died or recovered from challenge in this first hour. Ten minutes before and 2 minutes after challenge, a subset of mice were bled, and plasma was collected in EDTA tubes for histamine analysis using a commercial ELISA kit according to the manufacturer's recommendations (ImmunoTech, Westbrook, Minn). Mice used to assess histamine release were not used in the final analysis of death from anaphylactic challenge.

Antibody assays

Serum β -gal-specific IgG2a and IgG1 were measured by using ELISA, as previously described.^{13,18,19,21} Results are expressed in units per milliliter on the basis of pooled high-titer anti- β -gal IgG2a and IgG1 standards that were given arbitrary concentrations of 4000 U/mL. Ninety-six-well plates were coated with 5 μ g/mL β -gal (Sigma) in 50 μ L of borate-buffered saline solution (BBS; pH 9.2) overnight at 4°C. Plates were then blocked with 1% BSA in BBS at 37°C for 2 hours, washed with BBS/0.5% Tween-20 (Sigma), and incubated with standards and samples overnight at 4°C. Plates were then incubated with alkaline phosphatase-linked anti-IgG1 or IgG2a (Southern Biotechnologies, Birmingham, Ala) at a 1:2000 dilution, washed, and then incubated with *p*-nitrophenyl phosphate (2.63 mg/mL; Boehringer Mannheim). Absorbance at 405 to 650 nm was read at 1 hour. Sample concentrations were calculated by comparison with the standard curve on each plate using the Delta-SOFT II version 3.66 program (Biometallics, Princeton, NJ).

Antigen-specific serum IgE titers were also measured with ELISA, and results are expressed in units per milliliter on the basis

of a pooled high-titer anti- β -gal IgE standard given an arbitrary value of 2560 U/mL. To remove antigen-specific IgG, serum samples were incubated with protein-G sepharose beads according to the manufacturer's recommendations (Pharmacia, Piscataway, NJ). Ninety-six-well plates were coated with β -gal at 5 μ g/mL in 0.05 mol/L carbonate buffer, and nonspecific binding sites were saturated by incubation of plates with 1% BSA in BBS. Protein G-adsorbed 1:10 and 1:40 dilutions of sera were added, and the plates were incubated overnight at 4°C. Plates were then washed and incubated with goat biotinylated anti-mouse IgE at 8 μ g/mL (Pharmingen, San Diego, Calif). Plates were subsequently washed and incubated with horseradish peroxidase-linked streptavidin at a 1:2000 dilution (Zymed, San Francisco, Calif) and then TMB substrate (3,3',5,5'-tetramethyl benzidine; Kirkegaard and Perry Laboratories, Gaithersburg, Md). The color reaction was stopped with an equal volume of 1 mol/L phosphoric acid. Absorbance at 450 to 650 nm was read and compared with the standard curve on each plate using the DeltaSOFT II version 3.66 program.

Splenocyte cytokine profiles

Antigen-specific splenocyte cytokine profiles were assessed, as previously described.^{13,18,19,21} Briefly, mouse spleens were harvested after intravenous challenge, teased to prepare single cell suspensions, and resuspended in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mmol/L glutamine, M2-mercaptoethanol, and 1% penicillin-streptomycin (complete media). Splenocytes were incubated at 5×10^5 cells per well in 96-well plates in a final volume of 200 μ L of complete media with β -gal added at 10 μ g/mL at 37°C in a 5% CO₂ atmosphere. Culture supernatants were harvested at 72 hours and analyzed by using ELISA. Samples were tested for the presence of IL-4, IL-5, and IFN- γ by using ELISA with capture and biotinylated detecting antibodies for IL-4 (Genzyme), IL-5 (Pharmingen), and IFN- γ (Pharmingen). Washing and blocking steps were analogous to those used in the Ig ELISA described previously. Detection of the biotinylated secondary antibody was performed by adding 1:2000 diluted horseradish peroxidase-labeled streptavidin (Zymed) followed by TMB peroxidase substrate reagent (Kirkegaard and Perry Laboratories). The color reaction was stopped with an equal volume of 1 mol/L phosphoric acid, and absorbance was read at 450 to 650 nm. A standard curve was generated by using known amounts of recombinant IL-4, IL-5, and IFN- γ (Genzyme and Pharmingen). Each supernatant was compared with the standard curve on the plate to quantitate cytokine levels using the DeltaSOFT II version 3.66 program.

Statistical analysis

Statistical analysis was conducted using Statview and Mathsoft computer software. Death as an outcome variable was compared between groups by using the Fisher exact test. Two-tailed unpaired Student *t* tests were conducted to compare histamine, antibody, and cytokine histamine levels.

RESULTS

pDNA vaccination provides protection against the development of anaphylactic hypersensitivity

In preliminary studies 6- to 8-week-old, female, C3H/HeJ mice were sensitized with β -gal, alum, and pertussis toxin and subsequently received an intravenous β -gal challenge. Sensitizing doses and dosing intervals were optimized to induce death in 16 of 16 consecutive mice after intravenous challenge. Next, the protective

TABLE I. Anaphylactic challenge outcomes of pACB-LacZ-vaccinated mice

Vaccination	Survival	% Survival	P value
pACB-LacZ	5/15	33	.018
pACB	0/16	0	—
None	0/16	0	—

Mice were vaccinated, sensitized, and challenged as outlined in Fig 1. Mice that died did so within 1 hour of challenge. Surviving mice demonstrated signs of anaphylaxis within the first half hour after challenge but appeared healthy 1 hour later.

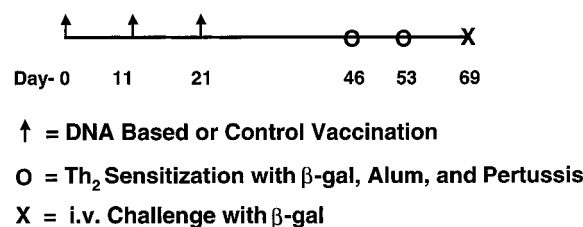


FIG 1. Timetable for vaccination, sensitization, and anaphylactic challenge. C3H/HeJ mice received intradermal vaccinations with 50 μ g of pACB-LacZ or pACB or 10 μ g of β -gal alone or mixed with ISS-ODN (10 μ g) or M-ODN (10 μ g) on 3 occasions 10 days apart. Twenty-five days after the last vaccination, mice were Th₂ sensitized with a mixture of β -gal (100 μ g), pertussis toxin (300 ng), and alum (1 mg) injected intraperitoneally on 2 occasions 7 days apart. Mice then received an intravenous challenge with 150 μ g of β -gal 3 weeks after their last intraperitoneal sensitization.

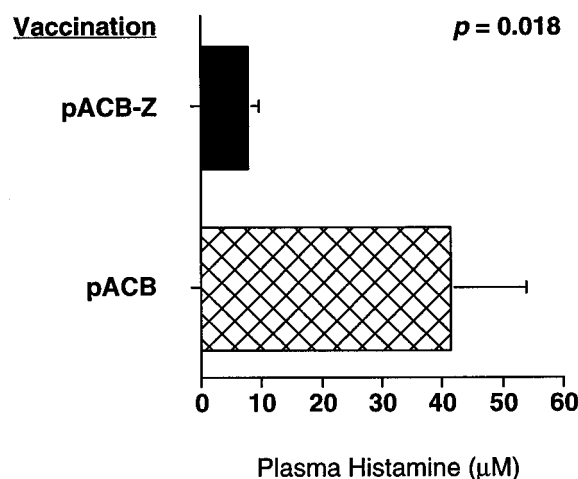


FIG 2. Plasma histamine levels after anaphylactic challenge in pACB-LacZ-vaccinated mice. Mice were vaccinated, sensitized, and challenged as outlined in the "Methods" section and Fig 1. Two minutes after intravenous β -gal challenge, plasma was collected for histamine determination. Results represent mean values $\pm$ SE for 4 mice in each group. Before challenge, plasma histamine levels were less than 10 nmol/L in all mice (data not shown).

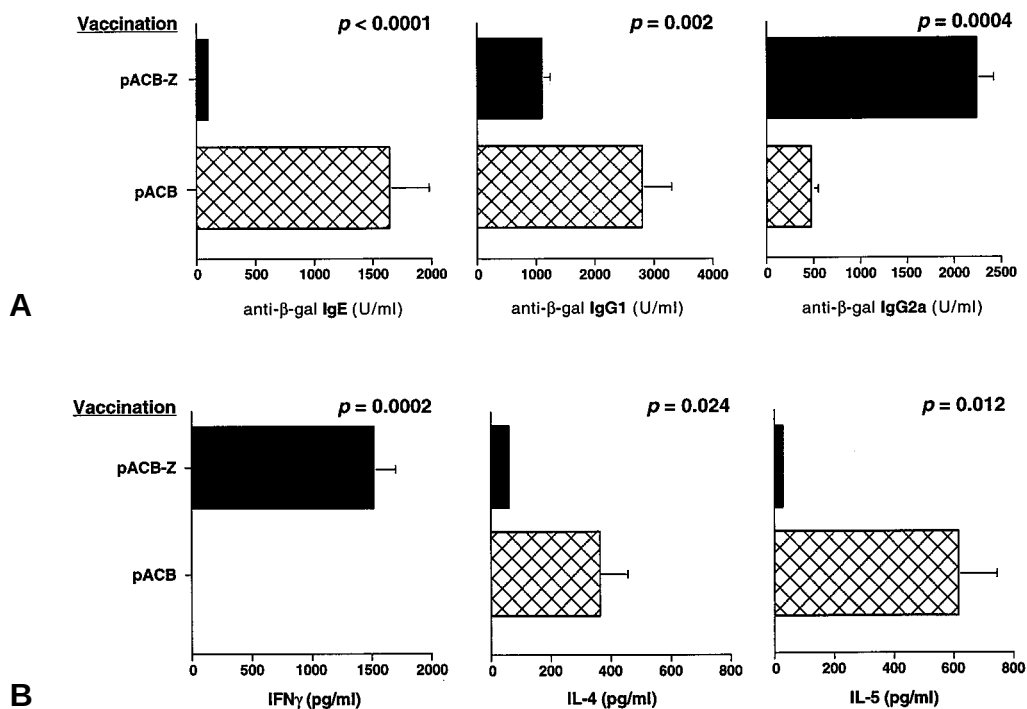


FIG 3. Serum antibody and cytokine responses of pACB-LacZ-vaccinated mice. Mice were vaccinated, sensitized, and challenged as described in the "Methods" section and Fig 1. Results represent mean values $\pm$ SE for 4 mice in each group and are representative of 3 independent experiments. **A**, Antigen-specific antibody levels. One day before challenge, serum was obtained for antibody determination by means of ELISA. **B**, Antigen-specific cytokine responses. After intravenous β -gal challenge, splenocytes were harvested and cultured with and without β -gal. Supernatants were harvested at 72 hours for cytokine ELISA. Culture without β -gal led to negligible cytokine production (data not shown).

TABLE II. Anaphylactic challenge outcomes of β -gal/ISS-ODN-vaccinated mice

Vaccination	Survival	% Survival	P value
β -Gal + ISS-ODN	5/12	42	.01
ISS-ODN	0/12	0	—
β -Gal	0/12	0	—
β -Gal + M-ODN	0/12	0	—

Mice were vaccinated, sensitized, and intravenously challenged as outlined in Fig 1. Mice that died did so within 1 hour of challenge. Surviving mice demonstrated signs of anaphylaxis within the first half hour after challenge but appeared healthy 1 hour later.

effect of pACB-LacZ vaccination on outcomes after sensitization and challenge was evaluated. The timetable for vaccination, sensitization, and challenge is outlined in Fig 1. As shown in Table I, pACB-LacZ vaccination protected 5 of 15 mice from death in 4 experiments, whereas 0 of 16 pACB-vaccinated mice survived challenge ($P = .018$). Despite the protection offered by pACB-LacZ vaccination, mice surviving challenge demonstrated behavioral changes, such as decreased activity and rapid and labored respirations, that were consistent with mild anaphylactic reactions within the first 30 minutes. However, by the end of the first hour, mice surviving the ana-

phylactic challenge appeared normal. To evaluate whether pACB-LacZ vaccination reduced mast cell degranulation from T_{H2} -sensitized and challenged mice, plasma histamine levels were measured before and 2 minutes after intravenous β -gal injection. Before anaphylactic challenge, plasma histamine levels were less than 10 nmol/L in all mice (data not shown). As seen in Fig 2, postchallenge plasma histamine levels increased in all mice. However, they were significantly lower in pACB-LacZ-vaccinated ($7.4 \pm 3 \mu\text{mol/L}$) versus pACB-vaccinated ($41 \pm 13 \mu\text{mol/L}$) mice ($P = .018$).

pDNA vaccination effectively inhibits the development of a T_{H2} -biased immune response after allergen sensitization

Because anaphylactic hypersensitivity is associated with T_{H2} -biased immunity, β -gal-specific antibody and cytokine profiles of vaccinated and sensitized mice were further evaluated. As seen in Fig 3, A, antigen-specific IgE levels were on average 15-fold lower in pACB-LacZ- versus pACB-vaccinated mice ($P < .0001$). In addition, pACB-LacZ-vaccinated mice produced approximately 3-fold less IgG1 ($P = .002$) and 4-fold more antigen-specific IgG2a than pACB-vaccinated

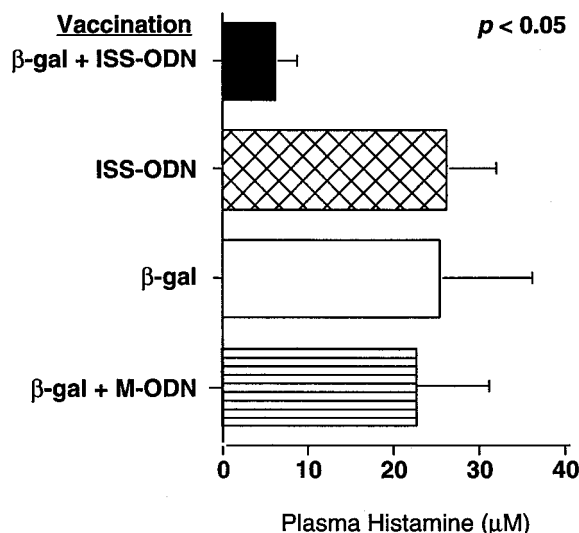


FIG 4. Plasma histamine levels after anaphylactic challenge in β -gal/ISS-ODN-vaccinated mice. Mice were vaccinated, sensitized, and challenged as outlined in the "Methods" section and Fig 1. Two minutes after intravenous β -gal challenge, plasma was collected for histamine determination. Results represent mean values $\pm$ SE for 4 mice in each group. Before challenge, plasma histamine levels were less than 10 nmol/L in all mice (data not shown).

mice ($P = .0004$). Splenocytes from mice vaccinated with pACB-LacZ before sensitization also demonstrated significantly higher antigen-specific IFN- γ ($P = .0002$) and lower IL-4 ($P = .024$) and IL-5 ($P = .012$) responses than control mice (Fig 3, B).

Protein/ISS-ODN vaccination prevents the development of anaphylactic hypersensitivity

Protein/ISS-ODN coimmunization and plasmid vaccination induce similar immune profiles.¹⁸⁻²² Therefore the efficacy of β -gal/ISS-ODN vaccination in protecting against the development of anaphylactic hypersensitivity was next evaluated. Mice were vaccinated with β -gal and ISS-ODN and then sensitized and intravenously challenged with the model allergen (Fig 1). Control mice were immunized with ISS-ODN or β -gal alone or β -gal plus M-ODN. As seen in Table II, in 3 experiments 5 of 12 β -gal/ISS-ODN-vaccinated mice survived intravenous β -gal challenge versus 0 of 36 control mice receiving β -gal or ISS-ODN alone, or β -gal vaccination with M-ODN ($P = .01$ for β -gal/ISS-ODN vaccination vs each control group). Again, surviving mice demonstrated behavioral changes consistent with a mild anaphylactic reaction within the first 30 minutes, but all surviving mice appeared normal 1 hour after intravenous challenge. Plasma histamine levels, before and after challenge, were also measured. Plasma histamine levels were less than 10 nmol/L in all mice before challenge (data not shown). As seen in Fig 4, postchallenge histamine levels increased in all mice, but they were significantly lower in

β -gal/ISS-ODN-vaccinated mice (6.1 ± 2.7 μ mol/L) than in mice vaccinated with β -gal (25.2 ± 11 μ mol/L) or ISS-ODN (26 ± 6 μ mol/L) alone or mice vaccinated with β -gal and M-ODN (29 ± 7.8 μ mol/L; $P < .05$).

Protein/ISS-ODN vaccination effectively inhibits the development of a T_{H2} -biased immune response after allergen sensitization

To establish whether β -gal/ISS-ODN vaccination prevented the development of a T_{H2} -biased immune profile after β -gal/alum/pertussis toxin sensitization, serum antibody and splenic cytokine profiles of β -gal/ISS-ODN-vaccinated and control mice were evaluated. As seen in Fig 5, A, β -gal/ISS-ODN-vaccinated mice had greater than 9-fold lower serum IgE levels than control immunized mice ($P < .035$). In addition, although β -gal/ISS-ODN-vaccinated mice had similar IgG1 levels to control mice, IgG2a levels were 8-fold higher ($P < .007$). β -Gal/ISS-ODN-vaccinated mice also produced significantly more IFN- γ ($P < .03$) and significantly less IL-4 ($P < .04$) and IL-5 ($P < .002$) than control mice (Fig 5, B).

DISCUSSION

The present series of experiments demonstrates that pACB-LacZ and β -gal/ISS-ODN vaccination of subsequently T_{H2} -sensitized mice reduces the risk of death after intravenous β -gal challenge from 100% to 67% and 58%, respectively, with associated reductions in plasma

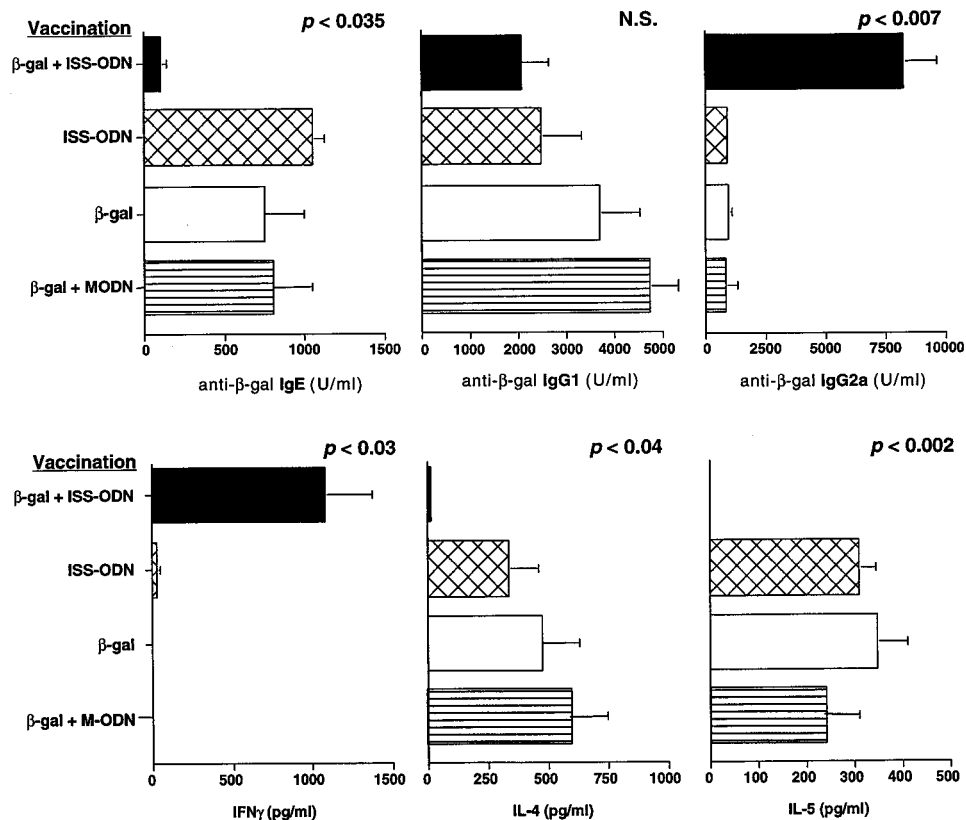


FIG 5. Serum antibody and cytokine responses of β -gal/ISS-ODN-vaccinated mice. Mice were vaccinated, sensitized, and challenged as described in the "Methods" section and in Fig 1. Results represent mean values $\pm$ SE for 4 mice in each group and are representative of 3 independent experiments. NS, No significant difference. **A**, Antigen-specific antibody levels. One day before challenge, serum was obtained for antibody determination by means of ELISA. **B**, Antigen-specific cytokine responses. After intravenous β -gal challenge, splenocytes were harvested and cultured with and without β -gal. Supernatants were harvested at 72 hours for cytokine ELISA. Culture without β -gal led to negligible cytokine production (data not shown).

histamine levels 2 minutes after challenge. This protection occurs in the context of IgE titers, which are dramatically reduced with either form of vaccination. In addition, the IgG subclass and cytokine profiles of pACB-LacZ- and β -gal/ISS-ODN-vaccinated versus control mice demonstrate a persistent T_{H1} bias after T_{H2} sensitization. In contrast to the antianaphylactic protection offered by DNA-based vaccination, vaccination with β -gal alone neither protected mice from the development of anaphylactic hypersensitivity nor from the development of a T_{H2} -biased immune profile after allergen sensitization. Given that β -gal protein is the experimental equivalent of reagents used in clinical practice, the present results suggest that DNA-based vaccination schemes may be more effective than traditional immunotherapy for allergen desensitization in the setting of anaphylactic hypersensitivity.

Although our results demonstrate that both pACB-LacZ and β -gal/ISS-ODN vaccination have dramatic inhibitory effects on IgE production after sensitization,

mice receiving these DNA-based immunizations still demonstrate residual anaphylactic hypersensitivity. This could be explained by production of sufficient allergen-specific IgE to arm mast cells, even in mice receiving DNA-based vaccinations before sensitization. Alternatively, another Ig isotype might contribute to the anaphylactic phenotype of these mice. IgE-dependent mast cell degranulation occurs in mice through Fc ϵ RI cross-linking, as it does in human subjects.^{1,26,27} However, the anaphylactic response can also be elicited in IgE-deficient mice, and IgG1 has been found to mediate mast cell degranulation and anaphylaxis through Fc γ RIII cross-linking.²⁶⁻³⁰ In contrast to IgE and IgG1, IgG2a has been suggested to block anaphylactic responses.²⁹

In the present series of experiments pACB-LacZ and β -gal/ISS-ODN vaccination only modestly inhibits post-sensitization IgG1 production, whereas IgG2a production increases compared with that of control animals. Interestingly, pDNA vaccination against peanut Ara h 2

leads to anaphylactic hypersensitivity in some mouse strains, even though no IgE is produced. Further analysis has shown that anaphylactic hypersensitivity only develops in those strains that respond to pDNA vaccination with an IgG1 response and not in strains in which IgG2a is produced in the absence of IgG1.²⁸ The complex and variable mechanisms by which anaphylaxis occurs in mice makes quantitative analysis of the relative contribution of IgE and IgG1 in mediating and of IgG2a in blocking the anaphylactic response in the vaccinated and control C3H mice used in these experiments difficult. In this vein, it should be noted that with more than 80 years of experience using protein-based immunotherapy in clinical practice, we still do not have completely reliable immunologic markers for its effectiveness.^{4,23-25}

Recently, oral gene vaccination has been shown to provide immunologic protection in a mouse model of peanut allergy.⁷ A subjective anaphylaxis score after intraperitoneal challenge was used to assess protection, and it was found that mice orally immunized with chitosan-DNA nanoparticles encoding Ara h 2 had fewer symptoms of anaphylaxis after sensitization and challenge than did control immunized mice. The series of experiments presented in this article extends this observation, demonstrating that both pDNA and protein/ISS-ODN vaccination reduce the risk of lethal anaphylactic reactions in sensitized mice. Mice immunized with pDNA or protein and ISS-ODN are further shown to have a persistent T_{H1}-biased immune phenotype after T_{H2} sensitization. Finally, traditional immunotherapy with protein allergen alone is demonstrated to be inferior to two different DNA-based vaccination schemes in preventing both the development of lethal anaphylactic hypersensitivity and allergen-specific, T_{H2}-biased, immune deviation.

In further consideration of the viability of DNA-based vaccination for the treatment of anaphylactic hypersensitivity in clinical practice, it should be remembered that except for serum sickness-type reactions with blood products, only IgE-mediated, allergen-specific, anaphylactic reactions have been clearly identified in human subjects.¹ It has even been suggested that IgG4, the likely human equivalent of murine IgG1, may serve as a blocking antibody with respect to allergic hypersensitivity.^{4,23-25} With regard to antigen-specific IgE production in mice, both pDNA and protein/ISS-ODN vaccinations appear to be highly effective in preventing its production. Further investigations are needed to prove that DNA-based vaccination can reverse anaphylactic hypersensitivity in an IgE-dependent model of anaphylaxis. However, if human IgE responses prove to be as readily inhibited by pDNA and protein/ISS-ODN vaccination as IgE responses in mice, then the immunization schemes outlined in this article may prove to be viable approaches to the treatment of anaphylactic hypersensitivity in clinical practice.

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REFERENCES

- Marquardt DL, Wasserman S. Anaphylaxis. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, eds. *Allergy: principles and practice*. 4th ed. St Louis: Mosby; 1993. p. 1525-36.
- Sorensen HT, Nielsen B, Nielsen JO. Anaphylactic shock occurring outside hospitals. *Allergy* 1989;44:288-90.
- Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
- Graft DF, Valentine MD. Immunotherapy. In: Weiss EB, Stein M, eds. *Bronchial asthma*. 3rd ed. Boston: Little Brown & Company; 1993. p. 934-44.
- Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* 1992;90:256-62.
- Hsu CH, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, et al. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. *Nat Med* 1996;2:540-4.
- Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* 1999;5:387-90.
- Slater JE, Zhang YJ, Arthur-Smith A, Colgerg-Poley AM. A DNA vaccine inhibits IgE responses to the latex allergen Hev b 5 in mice [abstract]. *J Allergy Clin Immunol* 1997;99:S504.
- Kohama Y, Akizuki O, Hagihara K, Yamada E, Yamada E. Immunostimulatory oligodeoxynucleotide induces Th1 immune response and inhibition of IgE antibody production to cedar pollen allergens in mice. *J Allergy Clin Immunol* 1999;104:1231-8.
- Sur S, Wild JS, Choudhury BK, Sur N, Alam R, Klinman DM. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 1999;162:6284-93.
- Broide D, Schwarze J, Tighe H, Gifford T, Nguyen M-D, Malek S, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 1998;161:7054-62.
- Kline JN, Waldschmidt TJ, Businga J, Lemish JE, Weinstock JV, Thorne PS, et al. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol* 1998;160:2555-9.
- Raz E. Immune deviation of the allergic response by DNA vaccination. In: Marone G, Austen KF, Holgate ST, Kay AB, Lichtenstein LM, eds. *Asthma and allergic diseases: physiology, immunopharmacology, and treatment*. San Diego: Academic Press; 1998. p. 417-28.
- McDonnell WM, Squires HR Jr. DNA vaccines. *N Engl J Med* 1996;334:42-5.
- Donnelly J, Ulmer JB, Shiver JW, Liu MA. DNA vaccines. *Annu Rev Immunol* 1997;15:617-48.
- Coffman RL, Mosmann TR. Isotype regulation by helper T cells and lymphokines. *Monogr Allergy* 1988;24:96-103.
- Fitch FW, McKisic MD, Lancki DW, Gajewski TF. Differential regulation of murine lymphocyte subsets. *Annu Rev Immunol* 1993;11:29-48.
- Sato Y, Roman M, Tighe H, Lee D, Corr M, Nguyen MD, et al. Immunostimulatory DNA sequences necessary for effective intradermal gene vaccination. *Science* 1996;273:352-4.
- Roman M, Martin-Orozco E, Goodman JS, Nguyen M-D, Sato Y, Ronaghy A, et al. Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. *Nat Med* 1997;3:849-54.
- Klinman DM, Yamshchikov G, Ishigatsubo Y. Contribution of CpG motifs to the immunogenicity of DNA vaccines. *J Immunol* 1997;158:3635-9.
- Horner AA, Ronaghy A, Chen PM, Nguyen M-D, Cho HJ, Broide D, et al. Immunostimulatory DNA is a potent mucosal adjuvant. *Cell Immunol* 1998;190:77-82.
- Davis HL, Weeranta R, Waldschmidt TJ, Tygrett L, Schoor J, Krieg AM. DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis B surface antigen. *J Immunol* 1998;160:870-6.
- Ebner C. Immunological mechanisms operative in allergen-specific immunotherapy. *Int Arch Allergy Immunol* 1999;119:1-5.
- Gehlhar K, Schlaak M, Becker W, Bufe A. Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 1999;29:497-506.

25. Golden DB, Kwitrovich KA, Kagey-Sobotka A, Lichtenstein LM. Discontinuing venom immunotherapy: extended observations. *J Allergy Clin Immunol* 1998;101:298-305.
26. Hazenbos WLW, Gessner JE, Hofhuis FMA, Kuipers H, Meyer D, Heijnen IA, et al. Impaired IgG-dependent anaphylaxis and arthus reaction in Fc γ RIII (CD16) deficient mice. *Immunity* 1996;5:181-8.
27. Miyajima I, Dombrowica D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc γ RIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE or IgG1 dependent passive anaphylaxis. *J Clin Invest* 1997;5:901-11.
28. Li XM, Huang CK, Schofield BH, Burks AW, Bannon GA, Kim KH, et al. Strain dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. *J Immunol* 1999;162:3045-52.
29. Ovary Z, Vaz NM, Warner NL. Passive anaphylaxis in mice with γ G antibodies: competitive effects of different immunoglobulins and inhibition of reactions with antiglobulin sera. *Immunology* 1970;19:715-27.
30. Oettgen HC, Martin TR, Wynshaw-Boris AW, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE deficient mice. *Nature* 1994;370:367-70.