

Prevention of long-term IgE antibody production by gene gun-mediated DNA vaccination

Isis Ludwig-Portugall, MSc, Evelyn Montermann, BSc, Andrea Kremer, MSc,
Angelika B. Reske-Kunz, PhD, and Stephan Sudowe, PhD *Mainz, Germany*

Background: Vaccination with allergen-encoding DNA represents a promising approach for the treatment of allergic diseases.

Objective: In a mouse model of type I allergy, we analyzed the ability of biolistic transfection to inhibit antigen-specific IgE production and to modulate T_H2 responses.

Methods: BALB/c mice were vaccinated by means of gene gun-mediated DNA immunization with plasmid vector pCMV-βGal, encoding β-galactosidase as a model allergen. Subsequently, mice were immunized by means of repeated intraperitoneal injection of β-galactosidase adsorbed to the adjuvant aluminum hydroxide. Development of IgE, IgG1, and IgG2a antibody titers during the course of immunization was followed, and anaphylactic potential of sera was determined by using RBL-2H3 degranulation assay. Spleen cells of vaccinated mice and unvaccinated control animals were stimulated *in vitro* to analyze cytokine production and induction of CD8⁺ effector T cells.

Results: Gene gun-mediated DNA immunization with pCMV-βGal very efficiently prevented IgE antibody production on a long-term basis. Concomitantly, IgG1 antibody levels in vaccinated mice were strongly reduced, whereas IgG2a antibody production was increased. Analysis of cytokine profiles indicated immune deviation from a T_H2-biased response in control mice toward a mixed T_H1/T_H2 response in vaccinated mice. In addition, substantial numbers of IFN-γ-producing CD8⁺ effector T cells were found in vaccinated mice. **Conclusion:** Gene gun-mediated DNA vaccination prevents the induction of long-lasting IgE antibody production. (*J Allergy Clin Immunol* 2004;114:951-7.)

Key words: IgE, type I allergy, allergen gene transfer, gene gun-mediated DNA immunization, genetic vaccination, mouse, β-galactosidase

Since the first description that immunization with antigen-encoding DNA is effective in the suppression of specific IgE antibody synthesis,¹ genetic vaccination has been considered as an attractive alternative to specific immunotherapy. The latter is at present the only treatment

Abbreviations used

alum: Aluminum hydroxide
βGal: β-Galactosidase
CTL: Cytotoxic T lymphocyte
EGFP: Enhanced green fluorescent protein
pCMV: Plasmid vector harboring the CMV promoter

of allergy that not only medicates the symptoms but also affects the immunologic processes underlying allergic diseases. Until now, a number of clinically relevant allergens has been tested in animal models of type I allergy to evaluate the potency of genetic immunization to interfere with IgE production,² a crucial event in the pathogenesis of atopic diseases.

The vector most frequently used for the administration of allergen genes is naked plasmid DNA, which in this context is applied primarily in saline by means of intramuscular or intradermal needle injection. This route of DNA immunization preferentially induces a polarized type 1 immune response characterized by the predominant production of specific IgG2a antibody, by high IFN-γ release from CD4⁺ T_H cells, and by recruitment of CD8⁺ cytotoxic T lymphocytes (CTLs).^{1,3,4} Both intradermal and intramuscular injection have been reported to be effective in the prevention of IgE antibody production if used prophylactically.^{1,5-13} The suppressive effect has been shown to be correlated with immune deviation from a characteristic T_H2 immune reaction toward a more T_H1-biased response^{1,7-10,12,13} but can also be mediated by inhibitory CD8⁺ T cells.^{7,14,15} However, one major disadvantage of intramuscular and intradermal injection is the high amount of plasmid DNA that is required to initiate an antigen-specific immune response. An alternative method for *in vivo* transfection of somatic cells with DNA is the bombardment of skin with DNA-coated gold particles by using the helium-powered gene gun, referred to as biolistic transfection. This application mode, in contrast to needle injection, allows for the direct introduction of plasmids into epidermal cells and therefore requires 10 to 100 times lesser amounts of DNA to elicit an immune reaction.^{16,17} In general, gene gun-mediated DNA immunization results in substantial production of IL-4 by CD4⁺ T cells and a preponderance of IgG1 antibody synthesis over IgG2a antibody synthesis, suggesting the induction of a type 2 immune response.^{3,4,6,18} The priming of antigen-specific T_H2 cells resulting from

From the Clinical Research Unit of Allergy, Department of Dermatology, Johannes Gutenberg-University.

Supported by the Deutsche Forschungsgemeinschaft, SFB 548.

Received for publication March 26, 2004; revised May 27, 2004; accepted for publication June 4, 2004.

Available online August 3, 2004.

Reprint requests: Stephan Sudowe, PhD, Clinical Research Unit Allergy, Department of Dermatology, Johannes Gutenberg-University Mainz, Obere Zahlbacher Str. 63, D-55131 Mainz, Germany. E-mail: sudowe@mail.uni-mainz.de.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2004.06.013

gene gun-mediated DNA vaccination might favor the generation of IgE-producing B cells and thus might lead to an exacerbation of the allergic reaction after subsequent encounter with the allergen. Therefore it is tempting to conclude that biolistic transfection does not constitute an effective mode of vaccination to prevent IgE antibody production. However, we and others have reported that CD4⁺ T cells of mice immunized by using the gene gun release high amounts of IFN- γ after stimulation.^{1,4,19,20} In addition, considerable numbers of IFN- γ -producing CD8⁺ effector T cells representing the CTL population were observed in gene gun-immunized mice.^{4,21} These 2 features are known to play important roles in the suppression of IgE antibody responses. Therefore we tested gene gun-mediated DNA vaccination by using plasmid vectors encoding the model antigen β -galactosidase (pCMV- β Gal) in a well-established long-term model of type I allergy²² and analyzed the capacity to inhibit IgE antibody secretion resulting from subsequent immunization with β Gal protein adsorbed to the adjuvant aluminum hydroxide (β Gal/alum). In this report we show for the first time that biolistic transfection is effective in the prevention of sustained IgE antibody production.

METHODS

Mice and immunization protocol for induction of IgE antibody production in the type I allergy model

Female BALB/c mice, bred under specific pathogen-free conditions in the animal facilities of the University of Mainz according to German guidelines for animal care, were 6 to 8 weeks old at the beginning of the experiments. High and persistent IgE antibody production was induced by means of repeated intraperitoneal injection of 100 μ L of recombinant β Gal (1 μ g; Sigma-Aldrich, Deisenhofen, Germany) adsorbed to an equal volume of aluminum hydroxide (Pierce, Rockford, Ill). Immunization was performed at intervals of 2 weeks.²²

Plasmid vectors and DNA vaccination schedule

The construction of plasmid vectors encoding the transgenes β Gal or enhanced green fluorescent protein (pCMV- β Gal or pCMV-EGFP, respectively) was described in detail previously.²¹ Plasmids were purified with the GenElute endotoxin-free plasmid purification kit (Sigma-Aldrich), according to the manufacturer's instructions. Plasmid DNA was precipitated in ethanol, resuspended in pyrogen-free water, quantified with a spectrophotometer, and stored at -20°C . Endotoxin levels in plasmid preparations were less than 20 EU/mg DNA, as determined with the Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, Md).

Genetic immunization of mice was performed by using biolistic transfection (4 μ g of plasmid DNA/immunization) with the helium-driven Helios gene gun system (Bio-Rad, Munich, Germany) or by means of intradermal injection intrapinna (100 μ g of plasmid DNA/immunization), as previously described.⁴ To analyze the efficiency of biolistic transfection in the mouse model of type I allergy, mice ($n=4$) were vaccinated by means of a single application or 3 applications every 2 weeks of plasmid DNA administered through the gene gun. Four to 6 weeks after the beginning of the vaccination, mice were immunized with β Gal/alum as described above. Sera were taken at

the indicated time points of the immunization period. After the sixth administration of β Gal/alum, mice were killed, and cytokine production by CD4⁺ T cells, as well as induction of CD8⁺ effector T cells, was analyzed. Representative data of a set of 2 to 4 independent experiments are shown.

Detection of β Gal expression in skin and draining lymph nodes

Mice were biolistically transfected by means of 6 nonoverlapping shots with pCMV- β Gal on the abdomen. After 18 hours, biopsy specimens of depilated abdominal skin were obtained. Draining (inguinal and axillary) lymph nodes were prepared 36 hours after immunization, placed in 2-methylbutane (Carl Roth GmbH & Co, Karlsruhe, Germany), and shock-frozen in liquid nitrogen. All specimens were embedded in Tissue-Tek O.C.T. Compound (Electron Microscopy Sciences, Washington, Pa) and stored at -25°C . Frozen sections (7 μ m) were placed on glass slides, fixed in 0.5% glutaraldehyde in PBS for 10 minutes at room temperature, and incubated overnight at 37°C with X-Gal substrate (0.8 mg/mL X-Gal in PBS, 3.1 mmol/L potassium ferricyanide, 3.1 mmol/L potassium ferrocyanide, and 1 mmol/L magnesium chloride). Skin sections were counterstained for 7 minutes at room temperature with nuclear fast red (Chroma-Gesellschaft, Münster, Germany). All specimens were mounted with DPX Mountant medium (Sigma-Aldrich) and screened for β Gal-positive (blue) cells by using a microscope with digital camera unit (BX50WI; Olympus, Hamburg, Germany). Representative images were acquired and documented with analySIS software (Soft Imaging Systems, Münster, Germany).

Determination of serum antibody titers

Mice were bled through puncture of the retro-orbital plexus. Sera were collected and frozen at -20°C until thawed for determination of β Gal-specific IgG1, IgG2a, and IgE antibody levels by using antigen capture ELISA, as previously reported.²² The antibody titer was defined as the reciprocal serum dilution, yielding an absorbance reading of an OD of 0.2 after linear regression analysis. To directly compare the amount of different IgG isotypes in sera, the ratio of specific IgG1 versus IgG2a was calculated as the quotient of IgG1 and IgG2a antibody titers, which were determined in ELISA assays performed in parallel. For comparison of serum samples obtained at different time points of the immunization period contents of IgG1, IgG2a, and IgE antibody in serum samples were standardized by using high-titer reference sera and are presented as units per milliliter.

RBL-2H3 degranulation assay

To evaluate IgE-mediated immediate-type reactions, we used the rat basophil leukemia cell line RBL-2H3²³ as an *in vitro* model. Cells (3×10^4 /well) were grown overnight at 37°C in 96-well tissue-culture plates (Corning Costar, Bodenheim, Germany) in culture medium (Iscove modified Dulbecco medium [Gibco, Paisley, United Kingdom] supplemented with 10% FCS [PAN Systems GmbH, Nürting, Germany], 50 μ mol/L 2-mercaptoethanol [Carl Roth GmbH & Co], 2 mmol/L L-glutamine [Carl Roth GmbH & Co], 100 U/mL penicillin [Gibco], and 100 μ g/mL streptomycin [Gibco]). Cells were incubated with sera from naive, control, and vaccinated mice at a final dilution of 1:1000 for 2 hours at 37°C . Control wells for spontaneous release without addition of sera were included. Subsequently, cultures were washed twice with Tyrode buffer (130 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl_2 , 1.4 mmol/L CaCl_2 , 5.6 mmol/L glucose, 10 mmol/L Hepes, and 0.1% BSA, pH 7.4). Degranulation of RBL cells was stimulated by addition of recombinant β Gal in Tyrode buffer (10 μ g/mL). Nonsensitized RBL cells were exposed to 5 μ mol/L calcium ionophore A23187 (Sigma-Aldrich) and 100 nmol/L phorbol 12-myristate 13-acetate

(Sigma-Aldrich) to assess maximum release of β -hexosaminidase by means of IgE-independent degranulation. After 1 hour of incubation at 37°C, supernatants were harvested, and the remaining cell layers were lysed in Tyrode buffer supplemented with 1% Triton X-100 (Merck, Darmstadt, Germany). Enzymatic activity of β -hexosaminidase in supernatants and lysates was measured by the addition of the chromogenic substrate *p*-nitrophenyl-N-acetyl- β -D-glucosaminide (Sigma-Aldrich; 1 mmol/L in 0.1 mol/L sodium citrate, pH 4.5) for 1 hour at 37°C. The reaction was stopped by the addition of 0.2 mol/L glycine (pH 10.7). The formation of the product *p*-nitrophenol was assessed with a microplate reader E_{max} (MWG-Biotech, Ebersberg, Germany) by using absorbance at 405 nm. The β -hexosaminidase activity released into the medium (absorbance in the supernatant) and thus the extent of degranulation was expressed as the percentage of total activity (sum of the absorbance in the supernatant and in the corresponding lysate).

ELISPOT assay for determination of IFN- γ -producing CD8⁺ effector T cells

Erythrocytes were lysed from spleen cell suspensions by incubating the cells for 1 minute in lysis buffer (155 mmol/L NH₄Cl, 10 mmol/L KHCO₃, and 100 μ mol/L EDTA-disodium, pH 7.4). Splenocytes were passed through a 100- μ m cell strainer (Becton Dickinson Labware, Franklin Lakes, NJ). The frequency of CD8⁺ effector T cells among spleen cells recognizing the H-2L^d-binding β Gal-derived nonamer peptide TPHPARIGL (β Gal₈₇₆₋₈₈₄; Sigma-Aldrich) was determined by using an ELISPOT assay, as previously described.²² Spots were counted with the EliSpot Reader System (AID, Straßberg, Germany).

Measurement of cytokines in culture supernatants

Splenocytes (5×10^6 /well) were cultured for 72 hours on 24-well tissue-culture plates (Corning Costar) in a volume of 1 mL of culture medium with or without recombinant β Gal (25 μ g/mL). Cytokines were quantified in supernatants by using a sandwich ELISA performed according to a standard procedure, as previously described.⁴ Recombinant mouse cytokines (IFN- γ , IL-4, and IL-5 purchased from PharMingen, San Diego, Calif; IL-13 purchased from R&D Systems, Minneapolis, Minn) were used as standards.

Statistical analysis of data

Statistical evaluation of the experimental data was performed by using the Student *t* test with SigmaStat software. A *P* value of less than .05 was considered statistically significant.

RESULTS

Particle bombardment of mouse skin with pCMV- β Gal leads to extensive production of β Gal, mainly by cells of the epidermal layer (Fig 1, A). In addition, single transgene positive cells were detected in the draining lymph nodes (Fig 1, B). Such cells, previously shown to reveal characteristics of skin-derived Langerhans cells,²¹ are potent inducers of antigen-specific T- and B-cell responses.^{4,21} The humoral immune response after gene gun-mediated immunization of BALB/c mice with pCMV- β Gal is characterized by a predominance of IgG1 antibody production over IgG2a antibody formation.⁴ To classify the immune response after biolistic transfection of BALB/c mice in more detail, we compared Ig synthesis resulting from 3 consecutive DNA immuni-

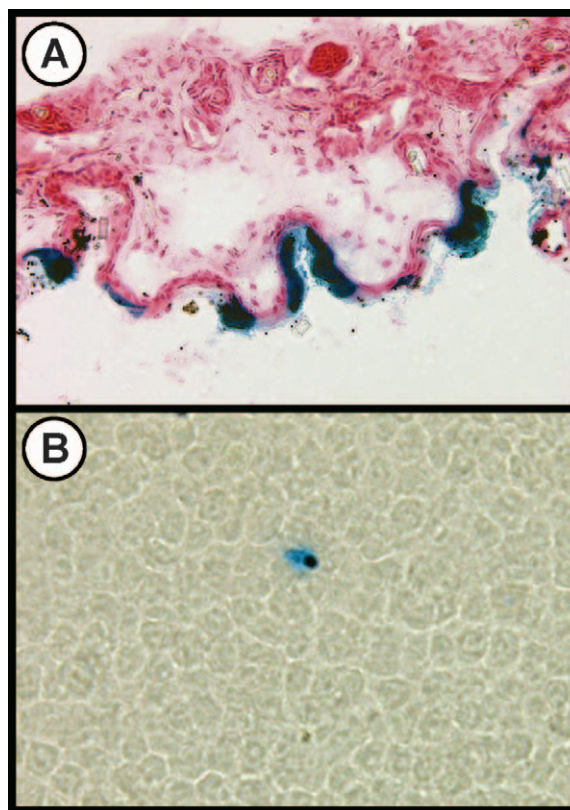


FIG 1. Production of β Gal in skin (A; original magnification 200 $\times$) and draining lymph nodes (B; original magnification 600 $\times$) after biolistic transfection of murine abdominal skin with pCMV- β Gal.

zations with pCMV- β Gal, either by means of particle bombardment or intradermal injection, and antibody secretion caused by 3 weekly intraperitoneal injections of β Gal/alum. The strongest antibody production was observed after protein immunization (Fig 2). The isotype pattern induced after repeated immunization with β Gal/alum exhibited a pronounced preponderance of IgG1 antibody over IgG2a antibody (IgG1/IgG2a ratio: 192.8) and in addition showed very high IgE antibody titers indicative of a characteristic T_H2 response. In contrast, DNA immunization with pCMV- β Gal through the intradermal route elicited no IgE antibody. Production of IgG isotypes was strongly reduced and largely restricted to antibody of the IgG2a subclass (IgG1/IgG2a ratio: 0.2), suggesting a T_H1 response. By comparison, gene gun-mediated DNA immunization generated the highest IgG2a antibody titers of the 3 immunization modes but furthermore induced large amounts of IgG1 antibody, resulting in a dominance of the latter isotype (IgG1/IgG2a ratio: 1.3). IgE antibody titers after biolistic transfection were significantly higher than IgE antibody titers in mice intradermally injected with pCMV- β Gal but represented only 10% of IgE antibody levels of mice immunized with β Gal/alum. Thus the humoral response after gene gun-mediated DNA immunization in fact showed features of a mixed T_H1/T_H2 immune response.

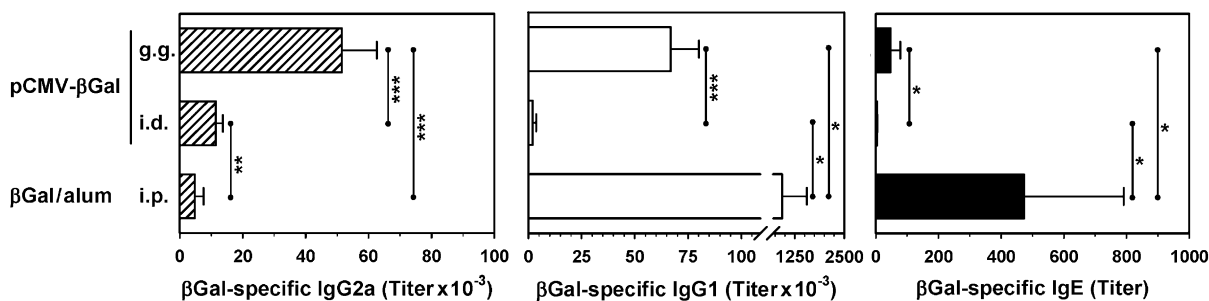


FIG 2. Comparison of β Gal-specific antibody production after immunization with pCMV- β Gal or β Gal protein. BALB/c mice ($n = 4$) were immunized 3 times with pCMV- β Gal with the gene gun (*g.g.*) and by means of intradermal injection (*i.d.*) or with β Gal/alum by means of intraperitoneal injection (*i.p.*). On day 49, levels of β Gal-specific IgG2a antibody (hatched bars), IgG1 antibody (open bars), and IgE antibody (filled bars) were determined by means of ELISA. Data are presented as mean serum titers $\pm$ SD of individual mice. Asterisks indicate significant differences between experimental groups (* $P < .05$, ** $P < .01$, *** $P < .001$).

In a mouse model of type I allergy, we analyzed the effect of DNA vaccination through the gene gun on the development of a long-lasting, antigen-specific IgE antibody response. For this purpose, we biolistically transfected BALB/c mice once either with pCMV- β Gal or with the control vector pCMV-EGFP, encoding for the irrelevant antigen EGFP.²¹ Subsequently, mice were immunized by means of repeated intraperitoneal injection of β Gal/alum in intervals of 2 weeks. After the fourth immunization, IgE antibody titers in unvaccinated BALB/c mice were substantially increased (Fig 3, A). Concomitantly, immune sera recovered from these control mice exhibited pronounced anaphylactic activity in the degranulation assay by inducing mediator release from RBL cells (Fig 3, B). Animals vaccinated with pCMV-EGFP had a comparable IgE antibody response and induced a similar level of degranulation of RBL cells. In contrast, sera from mice vaccinated with the β Gal-encoding plasmid showed considerably reduced IgE antibody titers. In addition, the sera had a significantly decreased capacity to provoke degranulation of RBL cells, being similar to degranulation after incubation with sera from naive mice, which represents the background mediator release in this assay (Fig 3).

To assess whether IgE antibody production in mice vaccinated with pCMV- β Gal was upregulated at any time point in the course of immunization with β Gal/alum, IgE antibody titers were followed up during the entire immunization period. Mice were vaccinated with pCMV- β Gal by means of a single application or 3 applications administered through the gene gun. To compare the intensity of antigen-specific priming of the humoral immune response induced by DNA vaccination and protein immunization, respectively, a third group of mice at the same time received a single intraperitoneal injection of β Gal/alum, whereas a fourth group remained untreated (control). All groups of mice subsequently received 5 injections of β Gal/alum. As depicted in the upper panel of Fig 4, control mice had excessive IgE antibody production after the fourth immunization with β Gal/alum. Mice primed by means of protein injection

showed a rapid boost of IgE antibody formation immediately after the first rechallenging immunization with β Gal/alum, whereas at later time points, IgE antibody levels were comparable with those of the control animals. In contrast, no enhancement of IgE antibody production was observed in biolistically transfected mice after the beginning of immunization with β Gal/alum. Moreover, long-term IgE antibody synthesis in gene gun-vaccinated mice was considerably suppressed (Fig 4, upper panel). Different from IgE antibody production, IgG1 and IgG2a antibody levels in vaccinated mice were boosted after the beginning of immunization with β Gal/alum and remained increased during the course of further immunization.

Comparison of IgG1 and IgG2a antibody content, determined in sera of vaccinated mice and of control mice after the fifth application of β Gal/alum, revealed a profound effect of biolistic transfection with pCMV- β Gal on the production of the different IgG isotypes in the long term as well. IgG1 antibody levels in vaccinated mice were considerably decreased (Fig 4, middle panel), whereas IgG2a antibody production was enhanced (Fig 4, lower panel), thus resulting in reduced ratios of IgG1 versus IgG2a antibody, as determined on the basis of direct comparison of serum titers (control: 122.5; pCMV- β Gal, 1 $\times$: 19.2; pCMV- β Gal, 3 $\times$: 9.2).

Cytokine profiles of spleen cells stimulated *in vitro* with β Gal protein were determined to analyze whether CD4⁺ T_H cell subsets in vaccinated mice and control mice differed in their phenotype and thus might contribute to the generation of divergent Ig isotype patterns. A single vaccination with pCMV- β Gal preceding immunization with β Gal/alum lead to a shift in the production of IFN- γ and IL-4, the surrogate markers for T_H1 and T_H2 responses, respectively; production of IFN- γ was strongly increased, whereas production of IL-4 was considerably reduced (Fig 5). As the result of 3 DNA vaccinations, secretion of IL-5 and IL-13, which are also associated with T_H2 responses, was decreased as well.

In addition, the frequency of β Gal-specific CD8⁺ effector T cells among spleen cells, representing the CTL population, was determined by means of IFN- γ

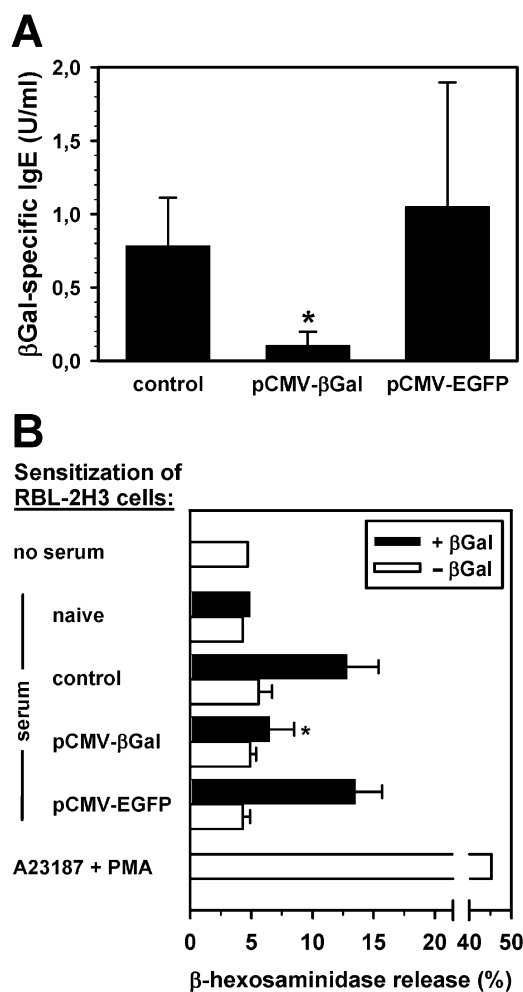


FIG 3. Gene gun-mediated vaccination with pCMV-βGal prevents the induction of IgE antibody production. BALB/c mice ($n = 4$) were vaccinated with a single biolistic transfection with pCMV-βGal or pCMV-EGFP. Subsequently, vaccinated mice, as well as untreated control mice, were immunized 4 times with βGal/alum. Thirteen days after the last immunization, levels of βGal-specific IgE antibody in sera were measured by means of ELISA (**A**), and anaphylactic activity of sera was determined by using the RBL-2H3 degranulation assay (**B**). IgE-dependent activation of RBL cells was induced with βGal protein (filled bars) and compared with degranulation of cells cultured without βGal protein (open bars). Data are presented as means $\pm$ SD of individual mice. Asterisk indicates significant differences between control mice and vaccinated mice (* $P < .01$).

production after stimulation with the H-2L^d-restricted βGal-derived nonamer peptide TPHPARIGL (βGal₈₇₆₋₈₈₄). In contrast to unvaccinated control mice, in which only marginal numbers of IFN-γ-producing CD8⁺ effector T cells were detected, gene gun-vaccinated mice harbored substantial numbers of these T cells (Fig 6). Repeated biolistic transfection increased the frequency of CD8⁺ effector T cells, despite subsequent immunization with βGal/alum, which is similar to earlier results with mice solely immunized with pCMV-βGal by using the gene gun without further treatment with βGal protein.⁴

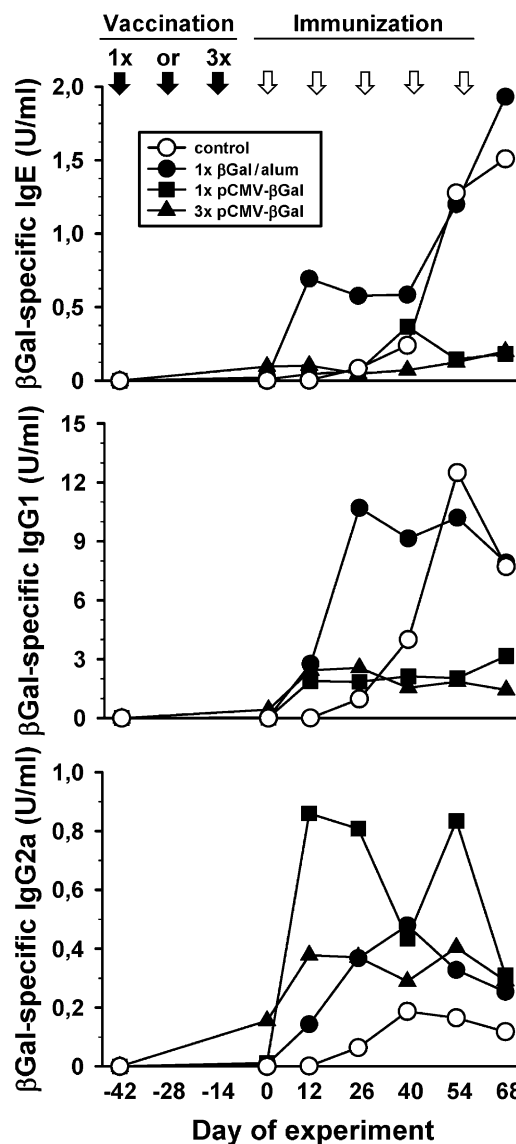


FIG 4. Gene gun-mediated vaccination with pCMV-βGal modulates the humoral immune response. BALB/c mice ($n = 4$) were treated (black arrows) with a single (filled squares) or 3 consecutive (filled triangles) gene gun-mediated vaccinations with pCMV-βGal or with a single injection of βGal/alum (filled circles). Control mice were left untreated (open circles). Subsequently, mice were immunized by means of intraperitoneal injection of βGal/alum (white arrows). On various days of the immunization period, levels of βGal-specific IgE, IgG1, and IgG2a antibody in sera were determined by means of ELISA. Data are presented as means of individual mice.

DISCUSSION

In this report we analyzed the capacity of gene gun-mediated DNA vaccination to modulate the immune response and to prevent IgE antibody synthesis in a mouse model of type I allergy. We demonstrate for the first time that biolistic transfection with plasmid vectors encoding for a model allergen (βGal) represents a suitable tool to

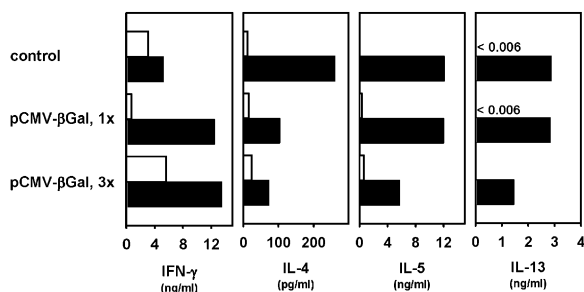


FIG 5. Gene gun-mediated vaccination with pCMV-βGal induces a shift toward a mixed T_H1/T_H2 response. BALB/c mice ($n = 4$) were vaccinated and immunized as described in the legend to Fig 4. Twelve days after the sixth immunization with βGal/alum, splenocytes were cultured with (filled bars) or without (open bars) recombinant βGal. Quantities of cytokines in pooled supernatants were determined by means of ELISA. Data are presented as means of duplicates.

effectively inhibit subsequent induction of IgE production caused by immunization with protein adsorbed to alum.

Concomitant with the suppression of long-term IgE antibody synthesis after immunization with βGal/alum, we observed strongly reduced IgG1 antibody titers in vaccinated mice, whereas IgG2a antibody titers were enhanced, consequently leading to considerably decreased IgG1/IgG2a ratios. The data indicate immune deviation from a T_H2 -biased to a mixed T_H1/T_H2 immune response induced by gene gun-mediated DNA vaccination. This assumption was supported by measurement of cytokine levels released by splenocytes stimulated *in vitro* with βGal protein. In comparison to spleen cells recovered from unvaccinated control mice, activated spleen cells from mice repeatedly vaccinated with pCMV-βGal secreted reduced amounts of T_H2 cytokines, particularly IL-4. On the other hand, production of IFN-γ as the main marker for T_H1 responses was strongly upregulated. These data are coincident with reports from other groups demonstrating successful prophylactic genetic vaccination by using intramuscular or intradermal injection of DNA.^{1,7-10,12,13,22} It is known that IL-4 and IFN-γ act antagonistically on the induction of IgE antibody responses.²⁴ Therefore the inhibition of IgE antibody production observed in gene gun-vaccinated mice might be due to the shift from an immune response dominated by T_H2 cells toward a T_H1 -orientated immune response. In addition, the recruitment of IFN-γ-secreting $CD8^+$ effector T cells in mice vaccinated with pCMV-βGal also might contribute to alleviated IgE antibody responses because it was shown by several groups that $CD8^+$ T cells have inhibitory potential for IgE production.^{7,14,15,25} We are currently investigating whether regulatory $CD4^+$ T cells²⁶ participate in the suppression of IgE antibody formation.

It has been shown previously that prophylactic DNA vaccination is capable of attenuating specific IgE antibody production. This effect, however, could only be achieved after intramuscular^{5-7,9,10,12,14} or intradermal^{1,8,13} inoculation of the DNA. Gene gun-mediated DNA immuniza-

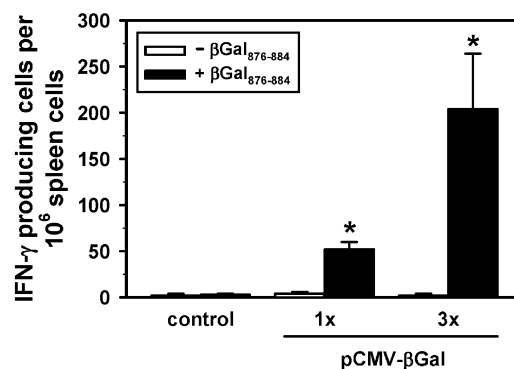


FIG 6. Gene gun-mediated vaccination with pCMV-βGal results in strong recruitment of $CD8^+$ effector T cells. BALB/c mice ($n = 4$) were vaccinated and immunized as described in the legend to Fig 4. Twelve days after the sixth immunization with βGal/alum, frequencies of IFN-γ-producing $CD8^+$ effector T cells among splenocytes were determined by means of ELISPOT assay after incubation with (filled bars) or without (open bars) the H-2L^d-specific peptide βGal₈₇₆₋₈₈₄. Data are presented as means of quadruplicates $\pm$ SD. Asterisk indicates significant differences between control mice and vaccinated mice (* $P < .001$).

tion has been tested only once by Toda et al,⁶ who used Japanese cedar pollen allergen Cry j 1 as an antigen, presumably because of the general notion that particle bombardment is associated with the induction of T_H2 -like immune responses that might prime for enhanced IgE antibody production and thus promote the occurrence of allergic reactions. Indeed, Toda et al⁶ reported higher IgE antibody titers in mice biolistically transfected with allergen-encoding DNA than those seen in control mice. Contrary to these data, we observed a recall IgE antibody response after secondary immunization with βGal/alum solely after initial priming with βGal/alum but not as the result of preceding DNA immunization with pCMV-βGal. The discrepancy in the results obtained by Toda et al⁶ might be explained by different experimental settings because the authors looked for Ig production only at an early time point after the booster injection with allergen in alum but did not follow IgE antibody titers at later time points. In contrast, we observed long-term suppressive activity of gene gun-mediated vaccination with pCMV-βGal on the outcome of the IgE antibody response after repeated challenge with βGal protein. Another factor that might contribute to the observed differences is the nature of the antigen/allergen used in the studies and of the plasmid vector. Gene gun-mediated immunization with pCMV-βGal at first glance initiated a T_H2 -like immune response characterized by a predominance of IgG1 over IgG2a antibody production. However, the IgG response was by far not as polarized as the one after intraperitoneal immunization with βGal/alum. In addition, IgE antibody titers were low, suggesting that the immune response after biolistic transfection with pCMV-βGal represents a mixed T_H1/T_H2 type rather than a characteristic T_H2 type. Furthermore, cytokine production analysis indicated compartmentalization of the T-cell response, being T_H2 -like in the draining lymph nodes and revealing large numbers of

IL-4-producing T cells, but T_H1 biased in the spleen, with a dominance of IFN- γ -producing T cells.⁴ Consequently, the coexistence of T_H1 and T_H2 responses in different lymphoid compartments might favor the inhibition of a subsequent IgE antibody response and thus might contribute to the protective potential of gene gun-mediated vaccination with pCMV- β Gal. Future experiments will be needed to investigate whether intrinsic properties of the protein encoded by the plasmid DNA^{18,27} have any effect on the inhibitory capacity of vaccination with the gene gun.

In conclusion, we demonstrated that prophylactic gene gun-mediated DNA vaccination represents a promising tool to interfere with the generation of T_H2 responses and to prevent IgE antibody production. Because therapeutic DNA vaccination with allergen-encoding vectors for the suppression of already established IgE antibody immune responses has only rarely been documented to be successful^{1,8,11,13} or was reported to be inefficient,^{7,22} it has to be further elucidated whether ongoing IgE production is susceptible for curative intervention by means of biolistic transfection.

The study was done in partial fulfillment of the requirements of the doctoral thesis of I.L.-P.

REFERENCES

1. Raz E, Tighe H, Sato Y, Corr M, Dudley JA, Roman M, 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.
2. Leitner WW, Thalhamer J. DNA vaccines for non-infectious diseases: new treatment for tumour and allergy. *Expert Opin Biol Ther* 2003;3:627-38.
3. Feltquate DM, Heaney S, Webster RG, Robinson HL. Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. *J Immunol* 1997;158:2278-84.
4. Sudowe S, Ludwig-Portugall I, Montermann E, Ross R, Reske-Kunz AB. Transcriptional targeting of dendritic cells in gene gun-mediated DNA immunization favors the induction of type 1 immune responses. *Mol Ther* 2003;8:567-75.
5. 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.
6. Toda M, Sato H, Takebe Y, Taniguchi Y, Saito S, Inoue S, et al. Inhibition of immunoglobulin E response to Japanese cedar pollen allergen (Cry j 1) in mice by DNA immunization: different outcomes dependent on the plasmid DNA inoculation method. *Immunology* 2000;99:179-86.
7. Maecker HT, Hansen G, Walter DM, DeKruyff RH, Levy S, Umetsu DT. Vaccination with allergen-IL-18 fusion DNA protects against, and reverses established, airway hyperactivity in a murine asthma model. *J Immunol* 2001;166:959-65.
8. Jilek S, Barbey C, Spertini F, Corthésy B. Antigen-independent suppression of the allergic immune response to bee venom phospholipase A2 by DNA vaccination in CBA/J mice. *J Immunol* 2001;166:3612-21.
9. Adel-Patient K, Creminon C, Boquet D, Wal JM, Chatel JM. Genetic immunisation with bovine β -lactoglobulin cDNA induces a preventive and persistent inhibition of specific anti-BLG IgE response in mice. *Int Arch Allergy Immunol* 2001;126:59-67.
10. Kwon SS, Kim N, Yoo TJ. The effect of vaccination with DNA encoding murine T-cell epitopes on the Der p 1 and 2 induced immunoglobulin E synthesis. *Allergy* 2001;56:741-8.
11. Peng HJ, Su SN, Chang ZN, Chao PL, Kuo SW, Tsai LC. Induction of specific Th1 responses and suppression of IgE antibody formation by vaccination with plasmid DNA encoding Der f 11. *Vaccine* 2002;20:1761-8.
12. Toda M, Kasai M, Hosokawa H, Nakano N, Taniguchi Y, Inoue S, et al. DNA vaccine using invariant chain gene for delivery of CD4+ T cell epitope peptide derived from Japanese cedar pollen allergen inhibits allergen-specific IgE response. *Eur J Immunol* 2002;32:1631-9.
13. Hochreiter R, Stepanoska T, Ferreira F, Valenta R, Vrtala S, Thalhamer J, et al. Prevention of allergen-specific IgE production and suppression of an established Th2-type response by immunization with DNA encoding hypoallergenic allergen derivatives of Bet v 1, the major birch-pollen allergen. *Eur J Immunol* 2003;33:1667-76.
14. Hsu CH, Chua KY, Tao MH, Huang SK, Hsieh KH. Inhibition of specific IgE response in vivo by allergen-gene transfer. *Int Immunol* 1996;8:1405-11.
15. Lee DJ, Tighe H, Corr M, Roman M, Carson DA, Spiegelberg HL, et al. 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.
16. Fynan EF, Webster RG, Fuller DH, Haynes JR, Santoro JC, Robinson HL. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. *Proc Natl Acad Sci USA* 1993;90:11478-82.
17. Pertmer TM, Eisenbraun MD, McCabe D, Prayaga S, Fuller DH, Haynes JR. Gene gun-based nucleic acid immunization: elicitation of humoral and cytotoxic T lymphocyte responses following epidermal delivery of nanogram quantities of DNA. *Vaccine* 1995;13:1427-30.
18. Hochreiter R, Hartl A, Freund J, Valenta R, Ferreira F, Thalhamer J. The influence of CpG motifs on a protein or DNA-based Th2-type immune response against major pollen allergens Bet v 1a, Phl p 2 and *Escherichia coli*-derived β -galactosidase. *Int Arch Allergy Immunol* 2001;124:406-10.
19. Pertmer TM, Roberts TR, Haynes JR. Influenza virus nucleoprotein-specific immunoglobulin G subclass and cytokine responses elicited by DNA vaccination are dependent on the route of vector DNA delivery. *J Virol* 1996;70:6119-25.
20. Yoshida A, Nagata T, Uchijima M, Higashi T, Koide Y. Advantage of gene gun-mediated over intramuscular inoculation of plasmid DNA vaccine in reproducible induction of specific immune responses. *Vaccine* 2000;18:1725-9.
21. Ross R, Sudowe S, Beisner J, Ross XL, Ludwig-Portugall I, Steitz J, et al. Transcriptional targeting of dendritic cells for gene therapy using the promoter of the cytoskeletal protein fascin. *Gene Ther* 2003;10:1035-40.
22. Sudowe S, Montermann E, Steitz J, Tüting T, Knop J, Reske-Kunz AB. Efficacy of recombinant adenovirus as vector for allergen gene therapy in a mouse model of type I allergy. *Gene Ther* 2002;9:147-56.
23. Barsumian EL, Isersky C, Petrino MG, Siraganian RP. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur J Immunol* 1981;11:317-23.
24. Pène J, Rousset F, Brière F, Chretien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E₂. *Proc Natl Acad Sci U S A* 1988;85:6880-4.
25. Kemeny DM. CD8+ T cells in atopic disease. *Curr Opin Immunol* 1998;10:628-33.
26. Jonuleit H, Schmitt E. The regulatory T cell family: distinct subsets and their interrelations. *J Immunol* 2003;171:6323-7.
27. Lee SW, Sung YC. Immuno-stimulatory effects of bacterial-derived plasmids depend on the nature of the antigen in intramuscular DNA inoculations. *Immunology* 1998;94:285-9.