

Prophylactic and therapeutic intervention in IgE responses by biolistic DNA vaccination primarily targeting dendritic cells

Stephan Sudowe, PhD,^a Isis Ludwig-Portugall, PhD,^a Evelyn Montermann, BSc,^a
Ralf Ross, PhD,^b and Angelika B. Reske-Kunz, PhD^a Mainz and Giessen, Germany

Background: Allergen gene transfer represents an alternative approach to specific immunotherapy with allergen extracts. Gene gun-mediated DNA immunization with plasmid vectors expressing a transgene under control of the promoter of the *fascin* gene (pFascin) allows for antigen production predominantly by dendritic cells and resulted in the generation of CD8⁺ cytotoxic T lymphocytes as well as in the development of a type 1 immune response.

Objective: We compared the *in vivo* efficiency of biolistic transfection with pFascin and plasmids containing the cytomegalovirus promoter (pCMV) in a mouse model of type I allergy.

Methods: BALB/c mice were sensitized with the model allergen β -galactosidase to induce a distinctive type 2 immune response. The effect of prophylactic as well as therapeutic biolistic transfection with β -galactosidase-encoding plasmids on the development of antibody titers was followed, and anaphylactic potential of sera was determined. Spleen cells were stimulated *in vitro* to analyze cytokine production and induction of CD8⁺ effector T cells.

Results: Protective allergen gene transfer with pFascin efficiently prevented specific IgE production accompanied by immune deviation toward a T_H1-polarized immune response as well as by the induction of IFN- γ -producing CD8⁺ effector T cells, being comparable to vaccination with pCMV. In a therapeutic setting, biolistic DNA vaccination with pFascin or pCMV transiently protected allergen-sensitized mice against the strong increase in specific IgE production caused by subsequent allergen challenge.

Conclusion: We demonstrate for the first time that restricting transgene expression primarily to dendritic cells after DNA vaccination suffices to cause inhibition of IgE production prophylactically and therapeutically. (J Allergy Clin Immunol 2006;117:196-203.)

Key words: Immunoglobulin E, type I allergy, allergen gene transfer, gene gun-mediated DNA vaccination, dendritic cells, fascin, mouse, β -galactosidase

Abbreviations used

alum:	Aluminium hydroxide
APC:	Antigen-presenting cell
β Gal:	β -Galactosidase
CTL:	Cytotoxic T lymphocyte
DC:	Dendritic cell
EGFP:	Enhanced green fluorescent protein
LN:	Lymph node
pCMV:	Plasmid vector harboring the cytomegalovirus promoter
pFascin:	Plasmid vector harboring the murine fascin promoter
RBL:	Rat basophil leukemia

Allergen gene transfer represents a promising alternative to the current protein-based desensitization protocols used in specific immunotherapy, to date the only curative treatment of allergy. Since the first description that vaccination with antigen-encoding DNA is effective in the inhibition of specific IgE antibody synthesis,¹ several clinically relevant allergens²⁻¹⁴ or their antigenic epitopes¹⁵⁻¹⁷ have been analyzed in animal models of DNA immunization with regard to their expression and immunogenicity. Allergen genes have been applied as naked plasmid DNA on various routes, mainly by intramuscular or intradermal injection,^{2-7,9-17} but also by biolistic transfection via the gene gun^{5,8} or as plasmid DNA-polymer complexes by oral delivery.^{18,19} However, the efficiency of allergen gene transfer for the purpose of the treatment of allergic sensitization was variable. Prophylactic DNA immunization by injection has frequently been reported to be effective in inhibiting specific IgE production.^{1,2,5-7,9,15-17} In contrast, curative DNA vaccination with allergen-encoding vectors for the suppression of already established IgE immune responses has been rarely documented to be successful^{1,10,17} or was reported to be inefficient.^{6,20}

Dendritic cells (DCs) as professional antigen-presenting cells (APCs) are of crucial importance for the initiation of transgene-specific immune responses by all methods of DNA delivery because of their primary stimulatory capacity.²¹ Maturing and mature DCs are the only hematopoietic cells that express the actin-bundling protein fascin.²²⁻²⁴ Expression of fascin in nontransformed cell

From ^athe Clinical Research Unit Allergy, Department of Dermatology, Johannes Gutenberg-University Mainz; and ^bImmunology, Justus Liebig-University, Giessen.

Supported by the Deutsche Forschungsgemeinschaft, SFB 548.

Received for publication January 13, 2005; revised August 4, 2005; accepted for publication August 29, 2005.

Available online November 14, 2005.

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/\$32.00

© 2005 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2005.08.058

types other than DCs is restricted to neuronal tissues, endothelial cells, and, possibly, follicular DCs.^{25,26} We demonstrated that the activity of the promoter of the human *fascin* gene reflects the endogenous fascin production, being high in CD83⁺ mature DCs and moderate in neuronal cells, but negligible in CD83[−] immature DCs, monocytes, and HaCaT keratinocytes.²⁷ Furthermore, we have shown that cutaneous biolistic transfection of mice with reporter gene constructs containing the promoter of the murine *fascin* gene as regulatory element (pFascin) allowed for transgene expression and antigen synthesis focused to skin-derived DCs, but not to keratinocytes or fibroblasts in epidermis/dermis.²⁸ Gene gun-mediated DNA immunization of mice with pFascin resulted in the induction of substantial numbers of CD8⁺ cytotoxic T lymphocytes (CTLs) as well as in the development of a type 1 immune response, which was characterized by high frequencies of IFN- γ -producing T_H cells both in spleen and draining lymph nodes (LNs) and a predominance of specific IgG2a in the humoral response.²⁹ In contrast, administration of plasmid constructs with the ubiquitously active cytomegalovirus immediate early promoter (pCMV) resulted in a strongly enhanced formation of antibodies, yielding higher levels of IgG1 than IgG2a.²⁹ Furthermore, we observed a dominance of IFN- γ -producing T_H cells in the spleen, but large numbers of IL-4-producing T_H cells in the draining LN.²⁹

We recently reported that in a mouse model of type I allergy, specific IgE production induced by repeated intraperitoneal immunization with low doses of the model antigen β -galactosidase (β Gal) adsorbed to the adjuvant aluminium hydroxide (alum) was considerably reduced by prophylactic biolistic transfection with pCMV.³⁰ Given the differences in immune responses elicited by particle bombardment with pCMV and pFascin, respectively, we examined the efficiency of gene gun-mediated DNA vaccination with pFascin to modulate IgE responses in comparison with DNA vaccination with pCMV. In advancement of our previous work, we show that gene gun-mediated DNA vaccination with pFascin, resulting in transgene expression focussed primarily to DCs by means of transcriptional control, is sufficient to exert effective suppression of IgE responses in a prophylactic setting, comparable to vaccination with pCMV. Furthermore, we show for the first time that therapeutic biolistic application of both the conventional vector and the DC-targeting plasmid to antigen-sensitized mice inhibits the boost in specific IgE production triggered by subsequent provocation with antigen.

METHODS

Mice and immunization protocol for induction of IgE production

BALB/c mice were bred and maintained in the Central Animal Facilities of the University of Mainz in specific pathogen-free conditions on a standard diet. The "Principles of Laboratory Animal Care" (National Institutes of Health publication number 85-23, revised 1985) were followed. The experiments were approved

by the Ethics Commission according to the German Law on the Protection of Animals. Female BALB/c mice, 6 to 8 weeks of age at the beginning of the experiments, were sensitized by intraperitoneal injection of 1 μ g recombinant β Gal (Sigma-Aldrich, Deisenhofen, Germany), dissolved in 100 μ L PBS and adsorbed to an equal volume of Inject Alum (Pierce, Rockford, Ill) as adjuvant according to the manufacturer's instructions. Immunizations were repeated several times as indicated in intervals of 2 weeks.²⁰

Plasmid vectors and DNA vaccination protocols

The construction of plasmid vectors encoding the transgenes β Gal or enhanced green fluorescent protein (EGFP) under control of the fascin promoter (pFascin- β Gal) and the CMV promoter (pCMV- β Gal, pCMV-EGFP), respectively, has been described in detail previously.²⁷ Purification of the plasmids was conducted by using 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 by using a spectrophotometer, and stored at -20°C . Endotoxin levels in plasmid preparations were <20 EU/mg DNA as determined by *Limulus* amoebocyte lysate assay (BioWhittaker, Walkersville, Md).

Genetic immunization was performed by biolistic transfection of 4 μ g plasmid DNA using the helium-driven Helios gene gun system (Bio-Rad, Munich, Germany) or by intradermal injection of 100 μ g plasmid DNA intrapinna as described.³⁰

Determination of serum antibody titers

At various times of the immunization period, mice were bled by 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 by antigen capture ELISA as reported.²⁰ The antibody titer was defined as the reciprocal serum dilution yielding an absorbance reading of OD = 0.2 after linear regression analysis. Contents of IgG1, IgG2a, and IgE in serum samples were standardized by using high titer reference sera.

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

At the end of the experiments, mice were killed by cervical dislocation, and single cell suspensions of spleen and draining mesenteric LN were prepared as described.³⁰ 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 an ELISPOT assay as described.²⁰ Spots were counted and evaluated by using the EliSpot Reader System (AID, Straßberg, Germany).

Determination of cytokines in culture supernatants

Splenocytes or LN cells (5×10^6 /well) were incubated for 72 hours on 24-well tissue culture plates (Coming Costar, Bodenheim, Germany) in a volume of 1 mL culture medium with or without recombinant β Gal (25 μ g/mL). Cytokines were quantified in culture supernatants by using a sandwich ELISA performed after a standard procedure as described previously.²⁹

Statistical analysis of data

Statistical evaluation of the experimental data was performed by Student *t* test using SigmaStat software (Systat Software Inc, Richmond, Calif). Statistically significant differences ($P < .05$)

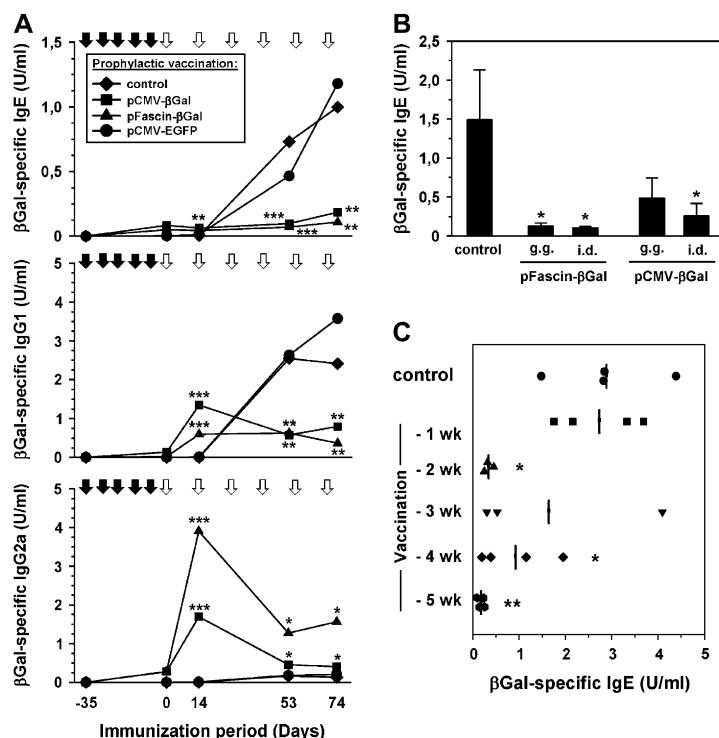


FIG 1. Immunoglobulin production following prophylactic vaccination. **A**, Mice ($n = 4$) were vaccinated by 5 biolistic transfections (closed arrows) with the indicated plasmids or left untreated (control) before sensitization with β Gal/alum (open arrows). Data represent mean β Gal-specific immunoglobulin levels in sera. **B**, Mice ($n = 3$) were vaccinated biolistically (g.g.) or intradermally (i.d.) 3 times with plasmids or left untreated (control) before sensitization with β Gal/alum (day 0). Data represent mean β Gal-specific IgE levels $\pm$ SDs on day 61. **C**, Mice ($n = 3-4$) were vaccinated at the indicated time points by a single biolistic transfection with pFascin- β Gal or left untreated (control) before sensitization with β Gal/alum (day 0). Data represent β Gal-specific IgE levels of individual mice on day 63; mean levels are depicted as bars. * $P < .05$; ** $P < .01$; *** $P < .001$.

between control groups and experimental groups are designated by asterisks.

RESULTS

Analysis of reporter gene expression *in vivo* 14 to 18 hours after biolistic transfection of abdominal skin of BALB/c mice with pFascin- β Gal revealed no production of β Gal in the epidermis, in contrast with bombardment with pCMV- β Gal yielding extensive transgene expression.³⁰ Only very few β Gal-positive cells were observed in serial skin sections, mainly located in deeper layers of the dermis not penetrated by gold particles (see this article's Fig E1, A, in the Online Repository at www.jacionline.org). Also, single β Gal-producing cells were identified in the draining inguinal and axillary LNs (see this article's Fig E1, B, in the Online Repository at www.jacionline.org). We have previously shown by using the EGFP reporter gene that these cells represent Langerhans cells having emigrated from the skin to the regional LN.²⁷

In contrast with intraperitoneal immunization with β Gal/alum, inducing a characteristic T_H2 response and high β Gal-specific IgE titers,^{20,30} gene gun-mediated

DNA immunization with pFascin- β Gal elicited only little production of IgE, being tendentially lower than in mice immunized with pCMV- β Gal (see this article's Fig E2, A, in the Online Repository at www.jacionline.org). Consistent with these data, sera recovered from mice injected with β Gal/alum showed high anaphylactic potential in a rat basophil leukemia (RBL)-2H3 basophil degranulation assay *in vitro*, whereas sera from mice treated with pFascin- β Gal or pCMV- β Gal exhibited only weak capacity to trigger β -hexosaminidase release from RBL-2H3 cells (see this article's Fig E2, B, in the Online Repository at www.jacionline.org).

To compare the effect of prophylactic genetic vaccination with pFascin- β Gal and pCMV- β Gal, respectively, on the development of a subsequent antigen-specific IgE response, BALB/c mice were pretreated with plasmid DNA for various times and on different routes before immunization with β Gal/alum. Fig 1, A, shows the kinetics of antibody production in mice vaccinated 5 times at weekly intervals with pFascin- β Gal, pCMV- β Gal, or the control vector pCMV-EGFP, coding for an irrelevant antigen, and subsequently sensitized with β Gal/alum. Whereas nonvaccinated control mice established substantial IgE production, animals vaccinated with pFascin- β Gal

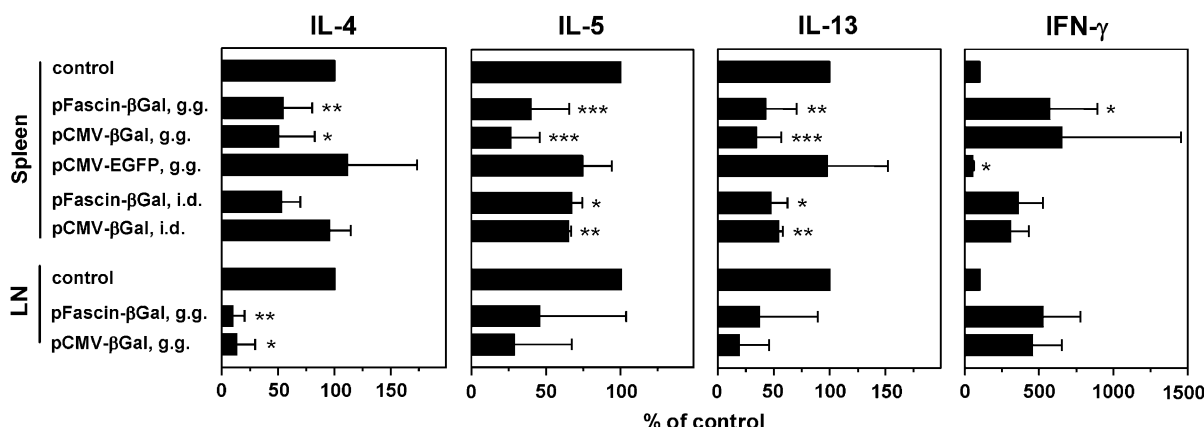


FIG 2. Cytokine production. Mice were vaccinated biolistically (g.g.) or intradermally (i.d.) and sensitized as described in Fig 1, A or B. Splenocytes and mesenteric LN cells, prepared after the fifth to sixth sensitization, were cultured with β Gal-protein. Cytokines were quantified in duplicates in pooled culture supernatants. Data represent means $\pm$ SDs of relative cytokine content in cultures of experimental groups (compared with control) obtained in 2 to 4 independent experiments. * $P < .05$; ** $P < .01$; *** $P < .001$.

like pCMV- β Gal exhibited considerably suppressed IgE titers at the end of the immunization period. The inhibitory effect of DNA vaccination was antigen-specific, because β Gal-specific IgE titers in mice vaccinated with pCMV-EGFP and thus displaying EGFP-specific IgG production (data not shown) were not significantly different from those of nonvaccinated mice. Animals vaccinated with the 2 β Gal-encoding plasmids showed an extensive induction of β Gal-specific IgG1 and IgG2a immediately after boosting with β Gal-protein (Fig 1, A), which was probably a result of the activation of memory B cells originating from the primary response after biolistic transfection. This assumption is supported by the fact that the isotype pattern after the first immunization with β Gal/alum reflected the isotype pattern initially induced by DNA vaccination: IgG2a titers were higher in mice primed with pFascin- β Gal, whereas vaccination with pCMV- β Gal provoked stronger IgG1 secretion after protein challenge. During the further course of sensitization with β Gal/alum, gene gun-mediated DNA vaccination modulated the IgG response by promoting IgG2a production but inhibiting IgG1 formation in comparison with control mice.

In another experimental setting, we compared the effect of different routes of DNA vaccination on the development of the IgE response, namely biolistic transfection via the gene gun and intradermal injection into the ear pinna (Fig 1, B). Prophylactic vaccination with pFascin- β Gal resulted in strong reduction of β Gal-specific IgE synthesis for both modes of application, being slightly but not significantly more pronounced than after DNA vaccination with pCMV- β Gal. The mean inhibition of IgE production determined in several independent experiments after biolistic transfection with pFascin- β Gal ($n = 11$ experiments) and pCMV- β Gal ($n = 8$ experiments) was $87.8\% \pm 11.0\%$ (ranging from 65.0% to 96.2%) and $80.0\% \pm 12.9\%$ (ranging from 57.5% to 95.8%),

respectively. Furthermore, sera from control mice solely immunized with β Gal/alum as well as sera from mice vaccinated with the irrelevant vector pCMV-EGFP induced considerable antigen-specific basophil activation (see this article's Fig E3 in the Online Repository at www.jacionline.org). In contrast, concurrent with the ELISA data sera from mice that had been vaccinated with the 2 β Gal-encoding plasmids triggered significantly lower degranulation by RBL-2H3 cells.

In addition, we investigated whether a single prophylactic vaccination with pFascin- β Gal is sufficient in the prevention of IgE production. In fact, β Gal-specific IgE titers were significantly reduced in the majority of the vaccinated animals provided that the vaccination took place at least 2 weeks before the immunization with β Gal/alum (Fig 1, C). However, individual mice were not or only partially protected against the development of the IgE response, suggesting that a single application was not completely reliable to protect from an IgE response.

To elucidate the reasons for the inhibitory effect of DNA vaccination with pFascin- β Gal on IgE production, we compared control mice and vaccinated mice with respect to the type of the T_H immune response as well as the magnitude of CTL induction. Analysis of cytokine production of spleen and mesenteric LN cells, stimulated *in vitro* with β Gal, revealed a general reduction in the production of the T_H2 -associated cytokines IL-4, IL-5, and IL-13 after prophylactic vaccination with β Gal-encoding plasmids, but not with pCMV-EGFP (Fig 2). At the same time, we observed an enhancement in IFN- γ synthesis by activated T cells from mice vaccinated with pFascin- β Gal and pCMV- β Gal, respectively, suggesting an induction of T_H1 cells.

Furthermore, we analyzed MHC class I-restricted induction of CD8 $^+$ effector T cells by using a modified ELISPOT assay. The frequency of activated β Gal-specific CD8 $^+$ T cells among splenocytes from mice solely immunized with β Gal/alum was low (Fig 3). In contrast, by

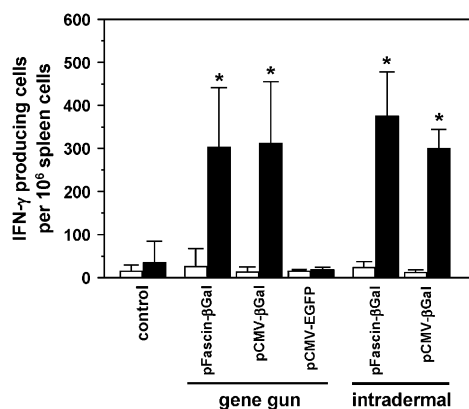


FIG 3. CD8⁺ effector T cells. Mice were vaccinated and sensitized as described in Fig 1, A and B. Frequencies of IFN- γ producing CD8⁺ effector T cells among pooled splenocytes, prepared after the fifth to sixth sensitization, were determined in quadruplicates following incubation with (closed bars) or without (open bars) a H-2L^d-specific peptide. Data represent means $\pm$ SDs of cell counts obtained in 2 to 6 independent experiments. * $P < .001$.

prophylactic vaccination with pFascin- β Gal, large numbers of CD8⁺ effector T cells were recruited in immunized mice. Furthermore, spleen cells from gene gun-vaccinated mice but not control mice showed high MHC class I-restricted cytolytic activity against β Gal-expressing target cells in a cytotoxicity assay (see this article's Fig E4 in the Online Repository at www.jacionline.org). The frequency of CD8⁺ effector T cells as well as the activity of CTLs after biolistic transfection with pFascin- β Gal was in a comparable range to that of pCMV- β Gal. Intradermal vaccination with pFascin- β Gal or pCMV- β Gal induced similar numbers of CD8⁺ effector T cells as particle bombardment with the respective plasmids (Fig 3).

To analyze whether therapeutic genetic vaccination with pFascin- β Gal has the potential to modulate an established IgE response, we first sensitized BALB/c mice by 2 or 3 repeated immunizations with β Gal/alum, which induced elevated β Gal-specific IgE titers (Fig 4). Afterward, mice were vaccinated by 2 periods of treatment consisting of 5 and 3 weekly applications of pFascin- β Gal or pCMV- β Gal via gene gun and intradermal injection, respectively, intervened by a single immunization with β Gal/alum. During the course of vaccination, IgE titers were not significantly different from titers of control mice, which received no DNA immunization, suggesting that genetic vaccination was not effective in the control of ongoing IgE production. Challenge of the animals after the second round of DNA vaccination by subsequent injections of β Gal/alum boosted IgE titers in untreated mice as well as in mice vaccinated with the control vector pCMV-EGFP (Fig 4, experiments 2 and 3). In contrast, in mice vaccinated with pFascin- β Gal and pCMV- β Gal, the strong increase in IgE titers was reduced. In the first experiment, IgE titers were suppressed during the entire immunization period with β Gal protein, whereas in 2 out of 3 experiments, the inhibitory effect of biolistic transfection with pFascin- β Gal or pCMV- β Gal was temporary, and

IgE titers started to increase during the provocation phase between the fourth to sixth immunization with β Gal/alum. The differential suppressive capacity of gene gun-mediated vaccination on long-term IgE production observed in the 3 experiments might be a result of the different protocols applied for primary sensitization, being stronger in experiments 2 and 3. As control for effective DNA vaccination, we performed intradermal injections of pCMV- β Gal, which in fact resulted in continuously decreased IgE production in 2 experiments, but gave rise to increasing IgE titers after the fourth provocation with β Gal/alum in experiment 3a. Moreover, we investigated whether the inhibition of IgE production in the provocation phase could be prolonged by intermediate vaccinations and performed exemplarily 2 additional biolistic transfections with pFascin- β Gal (Fig 4, experiment 3b). On day 310 of the immunization period, the IgE titers in these mice were significantly different from IgE titers of animals without additional vaccination ($P < .05$) and significantly reduced in comparison with IgE levels of unvaccinated control mice ($P < .01$).

DISCUSSION

In this study, we used a well characterized mouse model of type I allergy²⁰ to evaluate the prophylactic and therapeutic capacity of DNA vaccination with a plasmid vector that controls transgene expression by the fascin promoter and thus transcriptionally targets antigen production primarily to DCs for downregulation of antigen-specific IgE production. As a novel finding, we demonstrate that gene gun-mediated DNA vaccination with pFascin- β Gal inhibits β Gal-specific IgE production as effectively as the conventionally used pCMV vector.

It has been shown previously that prophylactic DNA immunization is capable of attenuating specific IgE production. This effect, however, could only be achieved after intramuscular^{2,5,6,9,15,16} and intradermal^{1,7,17} inoculation, respectively, of the DNA. Gene gun-mediated vaccination has been tested in a single publication by Toda et al.⁵ The authors concluded that particle bombardment with allergen-encoding DNA harboring the CMV promoter did not suppress specific IgE synthesis, but even enhanced the production of IgE and IgG1 after challenge with the allergen. However, we recently reported long-term suppressive activity of prophylactic gene gun-mediated vaccination with pCMV- β Gal on the outcome of the specific IgE response after challenge with β Gal-protein,³⁰ which is in accordance with the finding by some investigators that biolistic DNA immunization elicits a mixed T_H1/T_H2 response rather than a distinct T_H2 response.³¹ In the current communication, we show that a single prophylactic vaccination with pFascin- β Gal sufficed to protect the majority of mice from excessive β Gal-specific IgE production, initiated by subsequent immunization with β Gal-protein. The induction of the inhibitory mechanism required a latent period from 7 to 14 days. This is in agreement with data obtained by Adel-Patient et al⁹ showing a significant decrease of IgE production by intramuscular

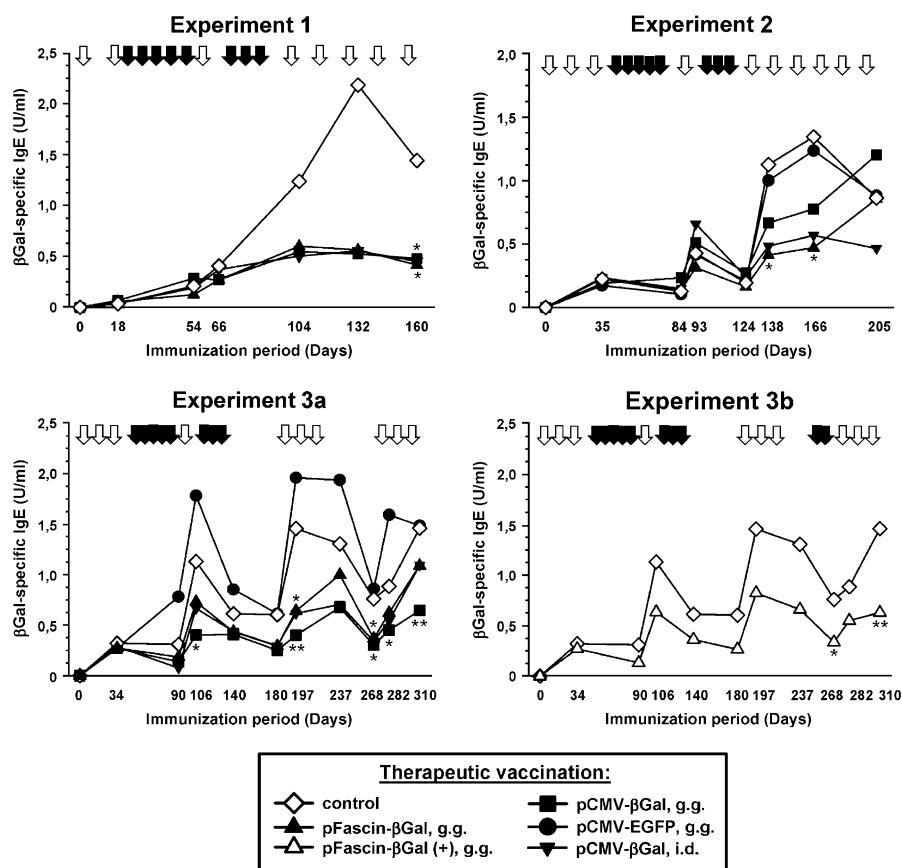


FIG 4. IgE production following therapeutic vaccination. Mice (experiment 1: $n = 4-6$; experiments 2 and 3: $n = 4-8$) were sensitized/challenged with β Gal/alum (open arrows) and vaccinated biolistically (g.g.) or intradermally (i.d.) with the indicated plasmids (closed arrows) as depicted. Control mice were left unvaccinated. In experiment 3b, a group of mice (pFascin- β Gal[+]) was vaccinated by 2 additional biolistic transfections on day 250 and day 257. Data represent means of β Gal-specific IgE levels. * $P < .05$; ** $P < .01$.

injection of pCMV only after a minimum interval of 14 days between genetic and protein immunization. By performing 3 to 5 consecutive biolistic transfections with pFascin- β Gal, we achieved extensive and persistent inhibition of IgE production, comparable to or even slightly higher than the suppression observed after gene gun-mediated vaccination with pCMV- β Gal. The reduction was similar to that determined after intradermal inoculation of pCMV- β Gal, a vaccination mode, which in the β Gal model system has been previously shown to be effective in the downregulation of IgE production¹ and thus represents the gold standard for our experiments. A reason for the discrepancy between the conclusions drawn by Toda et al⁵ and our data might be the use of different experimental settings. Toda et al⁵ determined immunoglobulin production solely at an early time point after treatment of mice with the alum-precipitated allergen. On day 7 after the second immunization with allergen/alum, specific IgE production was augmented by a preceding DNA vaccination. In our experiments, there was also a slight increase in β Gal-specific IgE production after the first contact with β Gal/alum, but IgE levels were low after the fourth immunization. Thus, it appears that the inhibitory effect of gene

gun-mediated DNA vaccination can be observed only at a later time point in allergen immunization, suggesting that prolonged modulation of the immune response is required.

Simultaneous with the inhibition of IgE synthesis IgG1 production decreased on a long-term basis, whereas IgG2a production was strongly enhanced. These findings suggest that in animals vaccinated with pFascin- β Gal, antigen-specific immune deviation from a T_H2 -oriented to a T_H1 -oriented immune response had occurred. This conclusion was supported by *in vitro* analysis of cytokine production by $CD4^+$ T cells recovered from spleens and draining LNs. This result is in line with data obtained by other groups demonstrating successful prophylactic genetic vaccination with pCMV^{1,6,7,9,15-17} when administered intradermally or intramuscularly. Hence, the strong T_H1 -promoting activity after gene gun-mediated vaccination transcriptionally targeting DCs might account for the suppressive capacity as well as the profound counterregulatory effects on the development of a T_H2 -polarized immune response.

A further explanation that might account for the efficient prevention of IgE production in mice vaccinated with pFascin- β Gal is the potent recruitment of IFN- γ

secreting CD8⁺ effector T cells. It has been previously shown that CD8⁺ T cells, induced by DNA vaccination, might possess inhibitory capacity for IgE production.^{6,32,33} So far, it is not known whether these CD8⁺ T cells mediate their suppressive function on IgE synthesis only by the secretion of IFN- γ or whether other mechanisms play an important role, such as the induction of IL-12 release from DCs by an IFN- γ -independent process that consequently leads to T_H1 polarization.³⁴ In addition, the induction of regulatory T cells by DNA vaccination and their relevance in the control of the IgE immune response in the mouse model of type I allergy needs to be investigated and is topic of ongoing experiments.

Contrary to successful preventive intervention before the induction of allergic responses, ongoing and excessive antigen-specific IgE production seems to be largely refractory to therapeutical manipulation. Inhibition of an established IgE response by injection of DNA was reported only by a few groups.^{1,6,10,17} However, achievement of the suppressive effect was apparently dependent on the strength of the immunization protocol used for priming. In our model of type I allergy, we chose an immunization protocol with multiple injections of β Gal/alum, which induced strong and sustained specific IgE secretion and thus resembled closely the clinical situation in patients with allergy. In this therapeutic setting, we show for the first time a distinct inhibitory effect of gene gun-mediated vaccination with pFascin- β Gal and pCMV- β Gal after re-challenging a pre-existing IgE response, but only marginal impairment of ongoing IgE production. However, long-term maintenance of the suppressed status by gene gun-mediated DNA vaccination appears to be critical, because perpetual challenge with β Gal/alum led to increased IgE titers in 2 out of 3 experiments, in which 3 immunizations with β Gal/alum instead of 2 were used to increase the starting level of IgE. In support of this notion, we exemplarily demonstrated for biolistic transfection with pFascin- β Gal that additional intervening vaccinations during the provocation phase with the intention to activate T_H1 memory cells prolonged the inhibition of IgE production.

In the current study, we have shown for the first time that direct transfection of and thus selective allergen synthesis primarily by DCs is sufficient to induce an immune response, which provides inhibitory capacity in the mouse model of type I allergy comparable to that induced by pCMV. The use of pFascin has several general advantages over the use of pCMV: (1) a nonviral promoter reduces the risk of interference of viral elements; (2) limitation of transgene expression mainly to DCs reduces the risk of unfavourable and/or unforeseen immune reactions; tolerizing effects that may result from transfection of other cell types by constructs with ubiquitously active viral promoters are excluded; and (3) restriction of transgene expression predominantly to matured DCs as achieved by the fascin promoter prevents tolerance induction known to be associated with antigen presentation by immature DCs. In conclusion, the approach of transcriptionally targeting DCs using the fascin promoter to focus endogenous antigen production preferentially to primary stimulatory

APCs represents an alternative strategy for immunomodulation and might provide new opportunities for allergen immunotherapy.

REFERENCES

1. Raz E, Tighe H, Sato Y, Corr M, Dudler 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. 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.
3. Slater JE, Paupore E, Zhang YT, Colberg-Poley AM. The latex allergen Hev b 5 transcript is widely distributed after subcutaneous injection in BALB/c mice of its DNA vaccine. *J Allergy Clin Immunol* 1998;102:469-75.
4. Li X, 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.
5. Toda M, Sato H, Takebe Y, Taniguchi Y, Saito S, Inouye 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.
6. 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 hyperreactivity in a murine asthma model. *J Immunol* 2001;166:959-65.
7. 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.
8. 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.
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. 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.
11. Bauer R, Himly M, Dedic A, Ferreira F, Thalhamer J, Hartl A. Optimization of codon usage is required for effective genetic immunization against Art v 1, the major allergen of mugwort pollen. *Allergy* 2003;58:1003-10.
12. Wolfowicz CB, HuangFu T, Chua KY. Expression and immunogenicity of the major house dust mite allergen Der p 1 following DNA immunization. *Vaccine* 2003;21:1195-204.
13. Jacquet A, Magi M, Haumont M, Jurado M, Garcia L, Bollen A. Absence of immunoglobulin E synthesis and airway eosinophilia by vaccination with plasmid DNA encoding ProDer p 1. *Clin Exp Allergy* 2003;33:218-25.
14. Chatel JM, Song L, Bhogal B, Orson FM. Various factors (allergen nature, mouse strain, CpG/recombinant protein expressed) influence the immune response elicited by genetic immunization. *Allergy* 2003;58:641-7.
15. 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.
16. Toda M, Kasai M, Hosokawa H, Nakano N, Taniguchi Y, Inouye 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.
17. 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.

18. 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;5:387-91.
19. Chew JL, Wolfowicz CB, Mao HQ, Leong KW, Chua KY. Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice. *Vaccine* 2003;21:2720-9.
20. Sudowe S, Montermann E, Steitz J, Tütting 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.
21. Takashima A, Morita A. Dendritic cells in genetic immunization. *J Leukoc Biol* 1999;66:350-6.
22. Ross R, Ross XL, Schwing J, Längin T, Reske-Kunz AB. The actin-bundling protein fascin is involved in the formation of dendritic processes in maturing epidermal Langerhans cells. *J Immunol* 1998;160:3776-82.
23. Ross R, Jonuleit H, Bros M, Ross XL, Yamashiro F, Matsumura F. Expression of the actin-bundling protein fascin in cultured human dendritic cells correlates with dendritic morphology and cell differentiation. *J Invest Dermatol* 2000;115:658-63.
24. Mosialos G, Birkenbach M, Ayeahunie S, Matsumura F, Pinkus GS, Kieff E, et al. Circulating human dendritic cells differentially express high levels of a 55-kd actin-bundling protein. *Am J Pathol* 1996;148:593-600.
25. Mosialos G, Yamashiro S, Baughman RW, Matsudaira P, Vara L, Matsumura F, et al. Epstein-Barr virus infection induces expression in B lymphocytes of a novel gene encoding an evolutionarily conserved 55-kilodalton actin-bundling protein. *J Virol* 1994;68:7320-8.
26. Pinkus GS, Pinkus JL, Langhoff E, Matsumura F, Yamashiro S, Mosialos G, et al. Fascin, a sensitive new marker for Reed-Sternberg cells of Hodgkin's disease: evidence for a dendritic or B cell derivation? *Am J Pathol* 1997;150:543-62.
27. Bros M, Ross XL, Pautz A, Reske-Kunz AB, Ross R. The human fascin gene promoter is highly active in mature dendritic cells due to a stage-specific enhancer. *J Immunol* 2003;171:1825-34.
28. 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.
29. 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.
30. Ludwig-Portugall I, Montermann E, Kremer A, Reske-Kunz AB, Sudowe S. Prevention of long-term IgE antibody production by gene gun-mediated DNA vaccination. *J Allergy Clin Immunol* 2004;114:951-7.
31. Payne LG, Fuller DH, Haynes JR. Particle-mediated DNA vaccination of mice, monkeys and men: looking beyond the dogma. *Curr Opin Mol Ther* 2002;4:459-66.
32. 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.
33. 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.
34. Thomas MJ, Noble A, Sawicka E, Askenase PW, Kemeny DM. CD8 T cells inhibit IgE via dendritic cell IL-12 induction that promotes Th1 T cell counter-regulation. *J Immunol* 2002;168:216-23.

Availability of Journal back issues

As a service to our subscribers, copies of back issues of *The Journal of Allergy and Clinical Immunology* for the preceding 5 years are maintained and are available for purchase until inventory is depleted. Please write to Elsevier Inc, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887, or call (800) 654-2452 or (407) 345-4000 for information on availability of particular issues and prices.