

Preferential induction of a Th₁ immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization

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ABSTRACT We compared the antigen-specific antibody isotypes and lymphokine secretion by CD4⁺ T cells in BALB/c mice immunized intradermally with either *Escherichia coli* β -galactosidase (β -gal) or plasmid DNA (pDNA) encoding β -gal in a cytomegalovirus-based expression vector (pCMV-LacZ). pCMV-LacZ induced mainly IgG2a, whereas β -gal in saline or alum induced IgG1 and IgE β -gal-specific antibodies. In addition, splenic CD4⁺ T helper (Th) cells isolated from pDNA-immunized mice secreted interferon- γ but not interleukin (IL)-4 and IL-5, whereas Th cells from β -gal-injected mice secreted IL-4 and IL-5 but not interferon- γ after *in vitro* stimulation with antigen. Together these data demonstrate that pDNA immunization induced a T helper type 1 (Th₁) response, whereas protein immunization induced a T helper type 2 (Th₂) response to the same antigen. Interestingly, priming of mice with pCMV-LacZ prevented IgE antibody formation to a subsequent i.p. β -gal in alum injection. This effect was antigen-specific, because priming with pCMV-LacZ did not inhibit IgE anti-ovalbumin antibody formation. Most importantly, intradermal immunization with pCMV-LacZ (but not pCMV-OVA) of β -gal in alum-primed mice caused a 66–75% reduction of the IgE anti- β -gal titer in 6 weeks. Also, pCMV-LacZ induced specific IgG2a antibody titers and interferon- γ secretion by Th cells in the β -gal in alum-primed mice. The data demonstrate that gene immunization induces a Th₁ response that dominates over an ongoing protein-induced Th₂ response in an antigen-specific manner. This suggests that immunization with pDNA encoding for allergens may provide a novel type of immunotherapy for allergic diseases.

Intramuscular (i.m.) or intradermal (i.d.) injection of “naked” plasmid DNA (pDNA) encoding for viral or other protein antigens results in uptake of the pDNA by the muscle (1–5) or skin cells (6) and subsequent synthesis of the protein by these cells. The transfected cells process the antigen and present the immunogenic peptides on major histocompatibility complex class I and/or class II molecules, depending on the cell type. This results in both a cellular and humoral immune response to the antigen (1–6). Immunization with pDNA encoding an antigen (gene vaccination) has generated great interest by investigators searching for better vaccination methods, especially for inducing cellular immune responses to viral infections, including HIV-1 (4). Recently, we showed that i.d. injection of pDNA encoding influenza nucleoprotein (NP) or *Escherichia coli* β -galactosidase (β -gal) in a cytomegalovirus (CMV)-based expression vector (pCMV-NP and pCMV-LacZ, respectively) led to prolonged expression of intracellular antigen by dermal keratinocytes, fibroblasts, and cells with the morphology of Langerhans cells and macrophages (6). We also showed that expression of these antigens does not cause an

inflammatory reaction at the injection site despite the induction of a cellular (cytotoxic lymphocytes) and humoral (IgG antibodies) immune response to the encoded antigens. When we analyzed the specific IgG antibodies for their subclass distribution, we found that the IgG antibodies were almost entirely of the IgG2a subclass. This was surprising because mice immunized with protein antigens, in contrast to viral or bacterial antigens, produce predominantly IgG1 (7) and often IgE (8) antibodies. IgG2a antibody formation is dependent on interferon- γ (IFN- γ) as an IgM-to-IgG2a switch factor and is believed to be typical for a T helper (Th) type 1 (Th₁) response (9). In contrast, IgG1 and particularly IgE antibody production depends on interleukin (IL)-4 secreted by Th₂ cells (10). To investigate the possibility that i.d. immunization with pDNA induced a Th₁ response to the gene product rather than the expected Th₂ response, we studied the primary immune response of BALB/c mice to i.d. pCMV-LacZ injections as compared with injections of the protein β -gal in saline or alum. The data showed that pCMV-LacZ induces a Th₁ and that β -gal in saline or alum induces a Th₂ response to the same antigen. Therefore, we also investigated the secondary immune response of pDNA-primed mice to β -gal in alum and the response of β -gal in alum-primed mice to pCMV-LacZ to determine whether one of these two modes of immunization dominates over the other in a secondary immune response. A dominant response by immunization with pDNA could be useful for switching an undesired Th₂ response to a more favorable Th₁ response in allergic and certain parasitic disorders.

METHODS

Mice. Female BALB/c mice were purchased from The Jackson Laboratory, maintained in the University of California, San Diego, Animal Facility accredited by the American Association for the Accreditation of Laboratory Animal Care, and used at 6–8 weeks of age.

pDNA Preparation. The construction pCMV-based vectors has been described (6). pCMV-LacZ contains the CMV IE1 promoter–intron, the simian virus 40 t-intron, the *E. coli* LacZ cDNA, and the simian virus 40 polyadenylation site. The pCMV-OVA vector encodes hen egg ovalbumin (OVA) cDNA, and the plasmid backbone is identical to pCMV-LacZ. pDNA was purified using a Qiagen megaprep kit (Qiagen, Chatsworth, CA) and was stored at –20°C in 10 mM Tris-HCl/0.1 mM EDTA, pH 8.0. The endotoxin content was reduced by extraction with Triton X-114 (Sigma) to 0.5–5 ng per 1 mg of pDNA (11), as determined by limulus amoebocyte lysate assay (Sigma). Before inoculation, pDNA was precipitated in 100% ethanol, washed with 70% ethanol, and dissolved in pyrogen-free saline.

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Abbreviations: pDNA, plasmid DNA; i.d., intradermal; β -gal, β -galactosidase; IFN- γ , interferon- γ ; CMV, cytomegalovirus; OVA, ovalbumin; Th, T helper.

Immunization. pCMV-LacZ or pCMV-OVA was either injected i.d. at the base of the tail or scratched into the shaved skin of the lower back with a tyne skin test applicator (Connaught Laboratories). The applicators were washed extensively in distilled water, soaked overnight in 0.5% SDS, rinsed with water, soaked overnight in 0.1 M NaOH, rinsed with water, and dried at 37°C for 8 hr. Fifty micrograms of pDNA dissolved in 6 μ l of saline was dripped onto the spikes of the tyne applicator. Mice received two applications (total of 100 μ g of DNA) per inoculation. Both i.d. and tyne applicator pDNA inoculations were given once a week for 3 weeks. For pDNA injections, 100 μ g of pDNA in 50 μ l of saline was injected i.d. three times at weekly intervals. Three weekly injections were previously found to induce a more reproducible response than a single injection (6). For primary immunization with protein, 10 μ g of β -gal (Calbiochem) dissolved in 50 μ l of saline or 1 μ g in 50 μ l of saline containing 3 mg of alum was injected i.d. once at the base of the tail. For booster injections, 1 μ g of β -gal in 0.5 ml of saline containing 3 mg of alum was injected i.p. OVA, 2 μ g in 0.5 ml of saline containing 3 mg of alum, was injected i.p. for primary or booster injections.

Antibody Measurements. Anti- β -gal antibodies of the IgG subclasses IgG1, IgG2a, IgG2b, and IgG3 were measured by ELISA. Microtiter plates were coated overnight with 5 μ g of β -gal per ml of borate-buffered saline (BBS; pH 9.2) and then washed with BBS, and nonspecific binding sites were then blocked with 1% bovine serum albumin in BBS. After washing twice in BBS/0.5% Tween 20 (Sigma), serum samples diluted 1:40 and then 1:4 for 8 steps in phosphate-buffered saline (pH 7.4) were added to the wells. After overnight incubation at 4°C, the plates were washed with BBS/Tween 20 and incubated with alkaline phosphatase-labeled goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology Associates, Birmingham, AL) for 2 hr at room temperature. The plates were washed with BBS/Tween 20, and a solution of *p*-nitrophenyl phosphate (1 mg/ml; Boehringer Mannheim) was added. Absorbance at 405 nm was read 1 hr after addition of the substrate. Each plate included a previously screened standard serum that contained a high titer of anti- β -gal antibodies. The results are expressed in units per ml, calculated based on the units/ml of the standard serum, and represent the mean $\pm$ SE of four animals in each group.

The IgE anti- β -gal or anti-OVA antibodies were measured by a radioimmuno-allergosorbent test as described (12). Briefly, 96-well polyvinyl plates were coated with 10 μ g of β -gal per ml of BBS (pH 9.2) for 1 hr at room temperature. The plates were washed, and nonspecific sites were blocked with 5% nonfat milk in BBS for 2 hr. Sera diluted at 1:10 and 1:20 were added overnight, and after the plates were washed,

^{125} I-radiolabeled purified goat anti-IgE antibodies were added for 4 hr. The plates were washed, and the radioactivity bound to the wells was measured with a scintillation counter. Serial dilutions of a standard serum consisting of a pool of β -gal in alum-immunized mice was included on each plate to ensure reproducible cpm. Also, to determine the effect of competition of IgG antibodies for antigen, sera of groups of four mice were pooled and passed over 0.5 ml of protein G-Sepharose (Pharmacia), which removed essentially all IgG2a, IgG2b, and IgG3 and $\approx$ 90% of the IgG1. Retesting of the IgG-depleted serum and comparison with unabsorbed serum showed higher cpm for the IgG-absorbed sera but no qualitative differences in the IgE antibody titers.

Lymphokine Assays. In each set of experiments, mice were killed 1 week after the last antibody determination for quantitation of the lymphokines secreted by CD4⁺ T cells after β -gal stimulation *in vitro* (13). Three days before sacrifice, the mice were injected i.v. with 10 μ g of β -gal in 50 μ l of saline. Spleens were removed and teased to prepare single-cell suspensions in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. The cells were enriched for CD4⁺ T cells by treatment with monoclonal antibodies to CD8 and CD56 and complement as described (14). This procedure resulted in removal of >95% of the CD8⁺ cells as shown by FACS (Becton Dickinson) analyses. The CD4⁺ T cells were cultured at 2×10^6 cells per ml with an equal number of irradiated (30 grays) T cell-depleted BALB/c spleen cells in RPMI 1640 medium containing 10% heat-inactivated bovine serum, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 1% penicillin-streptomycin, and 20 μ g of β -gal per ml. After 36 or 72 hr of culture, the IFN- γ , IL-4, and IL-5 levels were determined in the supernatants by ELISA assays (15, 16) with anti-IL-4 antibodies (11B11 and BVD6 24G2; PharMingen), anti-IFN- γ antibodies (R46A2 and XMG1.2), and anti-IL-5 antibodies (TRFK4 and TRFK5).

Statistical Analyses. The data were analyzed for statistical significance by ANOVA.

RESULTS

The Primary Immune Response of BALB/c Mice to pCMV-LacZ or β -Gal. The β -gal-specific IgG2a, IgG1, and IgE antibody responses in BALB/c mice immunized i.d. with either pCMV-LacZ or β -gal in saline or alum are shown in Fig. 1. Mice immunized by three i.d. injections of pDNA produced high titers of IgG2a, low titers of IgG1, and very small or undetectable amounts of IgE antibodies. Qualitatively and quantitatively, the same immune response was observed whether 20 or 100 μ g of pDNA was injected i.d. or whether

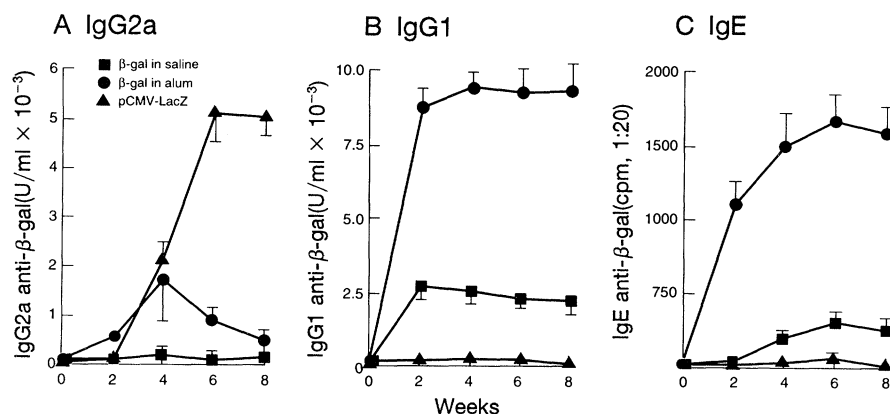


FIG. 1. The primary IgG2a (A), IgG1 (B), and IgE (C) responses of BALB/c mice to i.d. injection of 100 μ g of pCMV-LacZ (▲), 10 μ g of β -gal in saline (■), or 1 μ g of β -gal in alum (●). Means $\pm$ SE of four mice of one of two experiments are shown. The differences in the IgG2a, IgG1, and IgE antibody titers between pCMV-LacZ- and β -gal in alum-injected mice at 4 and 8 weeks were statistically significant ($P < 0.05$).

pDNA was scratched into the skin with a tyne device (data not shown). In contrast to the pDNA-immunized mice, mice injected with the protein β -gal either in saline or in alum produced high IgG1 and IgE antibody titers and significantly less IgG2a antibodies. β -Gal in saline induced a lower IgE titer than β -gal in alum; however, the qualitative nature of the immune response (IgG1 and IgE antibody formation) was the same. IgG2b and IgG3 antibodies were also measured in the serum of these mice. pDNA-immunized mice produced more IgG2b than the protein-immunized mice, whereas both pDNA and protein immunization induced only low titers of IgG3 antibodies (data not shown).

Because IgG2a antibody formation is typical for a Th₁ response and IgG1 and IgE antibody production results from a Th₂ immune response (9), these isotype profiles suggested that pDNA and protein induced different Th cell responses to the same antigen. To confirm this hypothesis, we determined the β -gal-induced IFN- γ , IL-4, and IL-5 secretion by splenic CD4⁺ T cells from the two groups of mice. As shown in Table 1 (experiments A–D), *in vitro* β -gal-stimulated CD4⁺ T cells from naive animals did not secrete lymphokines, whereas CD4⁺ T cells from pCMV-LacZ-immunized mice secreted IFN- γ and no detectable IL-4 or IL-5. In contrast, CD4⁺ T cells from β -gal in saline- or β -gal in alum-immunized mice secreted IL-4 and IL-5 and no detectable IFN- γ . These data corroborate the isotype-restricted IgG2a or IgG1 and IgE antibody responses shown in Fig. 1, and they confirm that pCMV-LacZ induced a Th₁ immune response and β -gal induced a Th₂ immune response to the same antigen.

The Response to a Secondary β -Gal in Alum Immunization of Mice Primed with pCMV-LacZ. To determine the effect of priming of mice with pCMV-LacZ on the Ig isotype response to a secondary immunization with β -gal, pCMV-LacZ-primed mice were injected i.p. with β -gal in alum 6 weeks after the primary pDNA immunization. A group of mice was primed with β -gal in saline as a control for the effect of a booster injection of β -gal in alum. As shown in Fig. 2A, mice primed with pDNA showed an increase from 5000 to 42,000 units/ml of IgG2a antibodies after injection of β -gal in alum. Control mice primed with β -gal in saline, which formed only minimal titers of IgG2a after the primary β -gal injection, also formed almost no IgG2a after the β -gal in alum booster injection. Injection of β -gal in alum did induce IgG1 antibody formation in the pDNA-primed mice (Fig. 2B); however, the titers were not as high as those in β -gal in saline-primed mice. Boosting of pCMV-LacZ-primed mice with β -gal in alum induced only minimal IgE antibody production over the 6-week observation period (Fig. 2C). Injection of β -gal in alum induced a large IgG1 and also an IgE antibody response in the β -gal in saline-primed control mice (Fig. 2C). The IgE response reached a peak at 2 weeks and declined thereafter. The suppression of IgE anti- β -gal antibody formation in pCMV-LacZ-primed mice after a booster injection of β -gal in alum was antigen-specific. As shown in Fig. 2D, mice primed with pCMV-LacZ and injected with OVA in alum formed IgE antibodies to OVA with no increase in IgE antibodies to β -gal.

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

Exp.	Priming	Boosting	IFN- γ , pg/ml	IL-4, pg/ml	IL-5, units/ml
A	None	None	<10	<2	<2
B	pCMV-LacZ	None	741 $\pm$ 170	<2	<2
C	β -Gal/saline	None	<10	12 $\pm$ 2.1	14.7 $\pm$ 4.2
D	β -Gal/alum	None	<10	351 $\pm$ 24	642 $\pm$ 51
E	pCMV-LacZ	β -Gal/alum	1050 $\pm$ 314	4.1 $\pm$ 2.1	46 $\pm$ 10
F	β -Gal/saline	β -Gal/alum	53 $\pm$ 48	126 $\pm$ 43	165 $\pm$ 54
G	β -Gal/alum	pCMV-LacZ	730 $\pm$ 193	307 $\pm$ 128	183 $\pm$ 70

Means $\pm$ SE of splens of four mice per experiment.

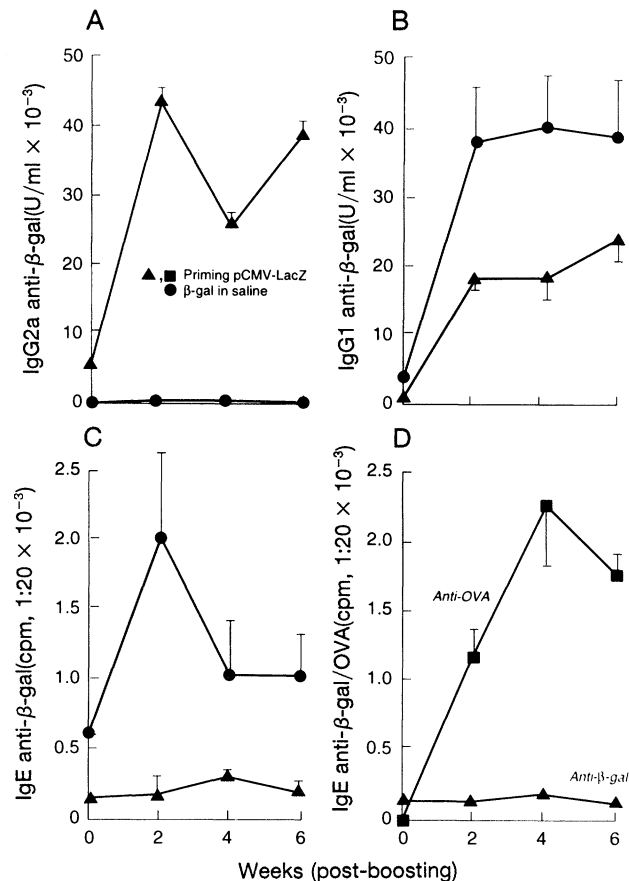


Fig. 2. (A–C) The IgG2a (A), IgG1 (B), and IgE (C) immune response to a secondary immunization with 1 μ g of β -gal in alum of mice primed with 100 μ g of pCMV-LacZ ($\blacktriangle$) or 10 μ g of β -gal in saline ($\bullet$). (D) For antigen specificity, control mice primed with 100 μ g of pCMV-LacZ were injected i.p. with 2 μ g of OVA in alum ($\blacksquare$). Means $\pm$ SE of four mice per group are shown. The differences in antibody titers at 2–6 weeks between pDNA- and protein-primed mice were statistically significant ($P < 0.05$).

At 4 weeks, the mean $\pm$ SE anti-OVA titer in the pDNA-primed mice was 2358 $\pm$ 581 cpm as compared with 2241 $\pm$ 338 cpm in naive mice, indicating that pDNA immunization had no nonspecific suppressive effect on IgE antibody production.

The β -gal-induced lymphokine secretion *in vitro* by splenic CD4⁺ T cells from these mice is shown in Table 1 (experiments E and F). CD4⁺ T cells from mice primed with pCMV-LacZ and boosted with β -gal in alum secreted large amounts of IFN- γ and only small amounts of IL-4 and IL-5, indicating that a subsequent challenge with β -gal in alum did not change the primary pDNA-induced Th₁ response to a Th₂ response. CD4⁺ T cells from the protein-protein in alum-injected control mice secreted mainly IL-4 and IL-5.

The Response to a Secondary pCMV-LacZ Immunization of Mice Primed with β -Gal in Alum. To determine the effect of pDNA immunization on an ongoing primary IgG1 and IgE (Th₂) response, mice primed with an i.p. injection of β -gal in alum were inoculated i.d. with pCMV-LacZ 6 weeks after the primary β -gal in alum injection. Control mice received 1 μ g of β -gal in saline i.d. Another group received pCMV-OVA and served as a pDNA control. As shown in Fig. 3, pCMV-LacZ immunization induced IgG2a antibody formation in the β -gal in alum-primed mice within 4 weeks, the titer being similar to the primary pDNA response (Fig. 1). The low IgG2a titers in the β -gal in alum control mice decreased over the same time period. Injection of pCMV-LacZ also induced an increase in IgG1 antibody formation, whereas the IgG1 titer decreased

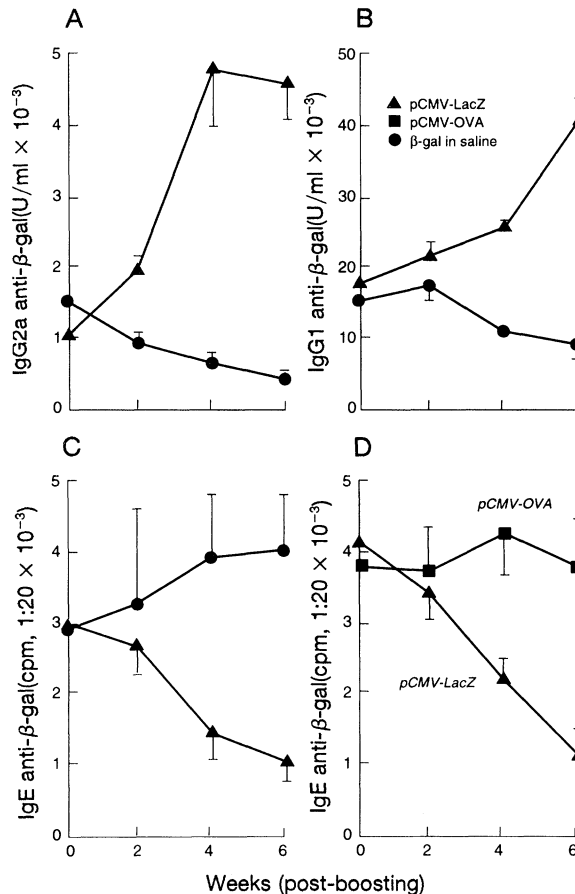


FIG. 3. (A–C) The IgG2a (A), IgG1 (B), and IgE (C) immune response to a secondary pCMV-LacZ (100 μ g; ▲) or β -gal in saline (1 μ g; ●) injected into mice primed with 1 μ g of β -gal in alum. (D) For antigen specificity control, mice were injected i.d. with 100 μ g of pCMV-OVA (■). Means $\pm$ SE of four mice per group are shown. The differences in antibody titers at 4 and 6 weeks between the pDNA- and β -gal-injected mice were statistically significant ($P < 0.05$).

slightly in the control mice. Most interestingly, and in contrast to the β -gal in saline-injected control mice, pCMV-LacZ, whether injected i.d. or administered with the tyne device (data not shown), induced a 66% (Fig. 3C) and a 75% (Fig. 3D) decrease of the IgE antibody titer over a 6-week period in two different experiments. This was not due to the effect of competition for antigen by IgG antibodies in the radioimmuno-allergosorbent assay because removal of IgG2a, IgG2b, and IgG3 and 90% of the IgG1 in pooled sera from the four mice in each group and retesting the bled sera for IgE did not result in qualitatively different results. Also, the suppressive effect on IgE antibody formation was antigen-specific because injection of pCMV-OVA did not result in a decrease of IgE anti- β -gal antibody formation (Fig. 3D).

Six weeks after the pCMV-LacZ booster injection, the mice were killed, and the β -gal-induced IFN- γ , IL-4, and IL-5 secretion by splenic CD4⁺ T cells was determined. As shown in Table 1 (experiment G), CD4⁺ T cells from the β -gal in alum-primed and pCMV-LacZ-boosted mice secreted IFN- γ in amounts similar to the pCMV-LacZ-primed mice (experiment B). The cells secreted IL-4 similar to β -gal in alum control mice, but only one-third of the amounts of IL-5 (experiment G versus D). The Ig isotypes of the antibodies and the lymphokine secretion by the Th cells from the β -gal-primed and pDNA-boosted mice indicates that priming of mice with β -gal in alum did not prevent a secondary Th₁ response (IgG2a antibodies and IFN- γ secretion) after pDNA immunization.

DISCUSSION

The results of this study show that i.d. immunization of BALB/c mice with naked plasmid DNA encoding for *E. coli* β -gal induces a Th₁ immune response, whereas immunization with the corresponding protein, β -gal, in saline or alum induces a Th₂ response. This conclusion is based on a highly restricted IgG2a antibody response to β -gal together with IFN- γ but not IL-4 or IL-5 secretion by *in vitro* β -gal-stimulated splenic CD4⁺ T cells from mice immunized with pCMV-LacZ. Conversely, immunization with β -gal in saline or alum induced IgG1 and IgE but not IgG2a antibodies, and splenic CD4⁺ T cells from these mice secreted IL-4 and IL-5 but no detectable IFN- γ after antigen stimulation. Furthermore, the Th₁ immune response to pCMV-LacZ dominated over any subsequent attempts to induce a Th₂ response to β -gal. When we immunized mice sequentially, first with pDNA and then with protein in alum, the primary pDNA-induced Th₁ response remained essentially the same because such treated mice showed a strong secondary IgG2a response but no IgE antibody formation and their splenic CD4⁺ T cells secreted large amounts of IFN- γ and only very small amounts of IL-4 and IL-5. In contrast, priming of mice with β -gal in alum induced a strong Th₂ response that was not able to prevent a subsequent Th₁ response after injection with pCMV-LacZ. Mice primed with β -gal in alum followed by a secondary immunization with pCMV-LacZ showed an IgG2a antibody response similar to the primary response to pCMV-LacZ. Furthermore, their CD4⁺ T cells secreted not only IL-4 and IL-5 but also large amounts of IFN- γ . Most importantly, boosting with pCMV-LacZ of β -gal in alum-primed mice caused a decrease in specific IgE antibody formation. Together these data show that immunization with pDNA encoding a protein antigen is a powerful tool for initiating a Th₁ response to that antigen. Therefore pDNA immunization may be particularly useful in certain clinical situations where it would be beneficial to down-regulate undesired Th₂ immune responses, such as those that exist in allergic disorders (17) and certain parasitic infections (18).

The suppressive effect of pCMV-LacZ immunization on IgE anti- β -gal antibody production was not only antigen-specific but also selective for the IgE isotype. Priming of mice with pCMV-LacZ prevented IgE formation and diminished IgG1 anti- β -gal antibody production following a secondary β -gal in alum injection. Similarly, boosting β -gal in alum-primed mice with pCMV-LacZ resulted in an increase of the IgG1 antibody titer whereas it caused a 66–75% decrease in the β -gal-specific IgE antibody formation 6 weeks after pDNA injection. Although IgG1 antibody production, like IgE production, is typical for a Th₂ cell response (9), IgG1 formation is much less dependent on, or in some instances even independent of, the requirement for IL-4. Injection of anti-IL-4 antibodies inhibits IgE but not IgG1 formation in mice (19). Although the mechanism of the selective down-regulation of IgE antibody production is not known, it is probably due to the induction of new antigen-specific Th₁ cells that are able to down-regulate the differentiation of new Th₂ cells as has been shown in other systems (9). Furthermore, IgE has a very short serum half-life (20), which is likely responsible for the rapid decrease of the IgE titer once IgE synthesis is suppressed by the secondary pDNA immunization. Whether the decrease of specific IgE antibodies in the serum will also be reflected in a decrease of anaphylactic reactions after antigen challenge remains to be determined.

The reasons why pDNA immunization leads to a Th₁ response and protein immunization leads to a Th₂ response are presently not understood. As we have shown previously (6), the pDNA is taken up by various types of cells in the skin which then produce the gene product. Therefore, β -gal becomes an intracellular antigen that can be processed and presented on

major histocompatibility class I molecules like other intracellular antigens (21). Major histocompatibility complex class I-presented β -gal thus may preferentially induce CD8⁺ antigen-specific T cells (6) secreting IFN- γ , as well as other lymphokines that promote Th₁ cell differentiation and down-regulate the differentiation of CD4⁺ Th₂ cells. Another possibility is that pDNA uptake induces IL-12 secretion by antigen-presenting cells and/or keratinocytes in the skin. Keratinocytes, as well as macrophages, have been shown to produce IL-12 (22, 23) and IL-12 induces IFN- γ secretion which plays an important role in Th₀-to-Th₁ cell differentiation. Such a model would be similar to infection of the skin with herpes simplex virus, which also elicits a Th₁ response to the virus (24). In a recent study, the immune response to intramuscular vaccination with pDNA encoding a herpes simplex virus glycoprotein was compared with the immune response to live or attenuated herpes simplex virus (5). All three immunization procedures elicited predominantly IgG2a antibodies and IFN- γ -producing CD4⁺ T cells; however, the ratio of IgG2a to IgG1 antibodies for pDNA-immunized animals was 3- and 6-fold higher than live or attenuated virus immunization, respectively. Although herpes simplex virus by itself induces a Th₁ response, these experiments show that pDNA immunization is the most effective inducer of IgG2a antibodies to the viral glycoprotein.

The fact that a preexisting Th₂ response to β -gal in alum was unable to prevent a Th₁ response to a secondary immunization with pCMV-LacZ suggests that pDNA immunization may have a clinical application for disorders resulting from a dominant Th₂ response. Strains of mice that are resistant to *Leishmania* infections produce a Th₁ response, whereas susceptible strains show a Th₂ response (25). Manipulations such as IFN- γ injections that "switch" the Th₂ to a Th₁ response in susceptible strains make such mice resistant to *Leishmania* infection (26). A similar situation may occur in humans with some types of parasitic infections such as filariasis and in persons with allergies. Nonatopic humans form a Th₁ whereas atopic patients form a Th₂ response to allergens (27). Classical immunotherapy appears to slowly change the Th₂ to a Th₁ response in the patients (28, 29). However, the allergen must be injected subcutaneously over a long period of time. Furthermore, injection of native antigen as is used in conventional immunotherapy may cause severe anaphylactic reactions. Therefore, transfecting skin cells with pDNA encoding for allergens may be a safer and more efficient form of immunotherapy. Because pDNA remains in the transfected cells for a long time (6), only a few injections of pDNA would be necessary. Furthermore, the fact that scratching the skin with a tyne applicator to which the pDNA was applied resulted in the same transfection of the skin cells as i.d. injection of pDNA could make this form of immunotherapy easily applicable and cost effective. For these reasons it will be crucial to investigate in primates whether immunization with pDNA-encoding allergens will induce IgG antibodies and down-regulate any preexisting or new IgE anti-allergen antibody formation. This experiment will help to determine whether allergen gene vaccination may be applicable for the treatment of allergic disorders in humans.

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