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Inhibition of IgE Antibody Formation by Plasmid DNA Immunization Is Mediated by both CD4+ and CD8+ T Cells

Abstract

Background: We previously showed that immunization of mice with plasmid DNA (pDNA) encoding the *Escherichia coli* β -galactosidase gene (pCMV-LacZ) induces a Th1 response, whereas β -galactosidase (β -gal) in saline or alum induces a Th2 response. Furthermore, the Th1 response dominates over the Th2 response and downregulates preexisting IgE antibody formation. Here, we determined by passive transfer of CD4+ or CD8+ lymphocytes and by immunizing β_2 -microglobulin knockout (β_2 -M KO) mice whether CD4+ and/or CD8+ cells from pDNA-immunized mice suppress IgE antibody production. **Methods:** BALB/c mice were injected with either CD4+ or CD8+ lymphocytes from naive β -gal-in-alum or pCMV-LacZ-immunized mice, then immunized with β -gal in alum, and the IgE antibody formation was determined. Second, C57BL/6 wild-type (WT) or β_2 -M KO mice were immunized with β -gal or pCMV-LacZ, and the IgE antibody production was assessed. **Results:** Passive transfer of both CD4+ and CD8+ lymphocytes from pDNA-immunized mice suppressed the IgE antibody response by 90% compared to transfer of CD4+ T cells from naive or β -gal-in-alum immunized mice. β_2 -M KO mice produced 3 times more IgE than the WT control mice both in the primary and secondary response. **Conclusion:** Both CD4+ and CD8+ subsets of T cells from pDNA-immunized mice can suppress IgE antibody production by affecting the primary response and/or by propagating the Th1 memory response in a passive cell transfer system. Immunization with pDNA-encoding allergens may be an effective new form of immunotherapy for atopic diseases.

Key Words

IgE, suppression
DNA immunization
CD4+, CD8+ suppressor cells
Th1 response to plasmid DNA
immunization

Introduction

We have recently demonstrated that intradermal immunization of BALB/c mice with plasmid DNA (pDNA) encoding the *Escherichia coli* β -galactosidase gene (pCMV-LacZ) induces a Th1 immune response to β -galactosidase (β -gal), whereas the protein β -gal induces a Th2 response

[1]. This was shown by restricted IgG2a antibody production in pCMV-LacZ-immunized mice and antigen-induced interferon- γ (INF- γ) secretion by CD4+ splenic T cells in vitro. In contrast, mice injected with β -gal in saline or alum formed IgG1 and IgE antibodies, and their T helper cells secreted interleukin-4 (IL-4) and IL-5. More recently, we showed that short palindromic immuno-stimulatory se-

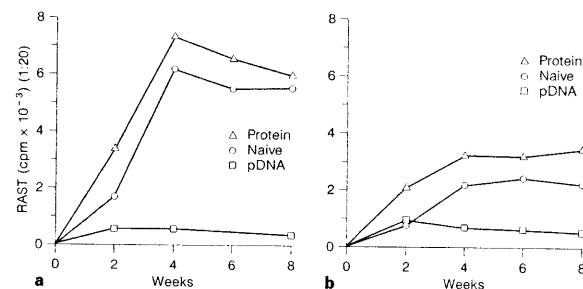
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Fig. 1. The effect of passive transfer of β -gal-specific CD4+ (a) and CD8+ (b) spleen lymphocytes from naive, β -gal-in-alum or pCMV-LacZ-immunized mice on the IgE antibody response. The cells were injected into naive BALB/c mice which were immunized 1 day after the cell transfer with β -gal in alum, and the IgE titers were measured for the subsequent 8 weeks.



quences in the ampicillin resistance gene of the pDNA construct were responsible for the Th1 immune response to β -gal [2]. Preliminary data suggest that transfection with immunostimulatory sequences induces antigen-presenting cells to secrete INF- α , INF- β and IL-12, cytokines that induce Th1 cell differentiation [3, 4]. However, the precise mechanism of the pDNA-induced Th1 immune response is yet to be identified. The protein product is detected in various skin cells including antigen-presenting cells [5] and is most likely processed and presented to T cells on both major histocompatibility (MHC) class I and class II molecules. The MHC class I antigen presentation, together with the immunostimulatory-sequence-induced INF- α secretion by the antigen-presenting cells, could have resulted in differentiation of INF- γ -secreting CD8+ T cells (Tc1) that may be responsible for inducing the Th1 response. To determine the role of CD4+ and CD8+ T cells in the pDNA-induced Th1 response, we investigated the effects of passive transfer of CD4+ and CD8+ T cells from pCMV-LacZ-immunized mice on the immune response to β -gal in alum. In addition, we studied the primary and secondary IgE antibody response to β -gal and pCMV-LacZ in β_2 -microglobulin knockout (β_2 -M KO) mice which have a profound deficiency in CD8+ T cells [6].

Methods

BALB/c mice were immunized intraperitoneally with either 3 μ g β -gal in 3 mg alum or intradermally with 100 μ g pCMV-LacZ [1]. After 8 weeks, the spleen cells were separated into CD4+ or CD8+ cells by negative selection (lysis of one of the Tcell subsets with monoclonal antibodies and complement). Fifty $\times 10^6$ naive or immune cells were injected intravenously into 200-rad-irradiated mice. One day later, the mice were injected intraperitoneally with 2 μ g β -gal in alum, and the

Table 1. IgE response to β -gal or pDNA-LacZ in C57BL/6 and C57BL/6 β_2 -M KO mice

	IgE anti- β -gal*	
	C57BL/6 WT	C57BL/6 β_2 -M KO
Primary IgE		
Immunization with β -gal	5,570	15,373
Immunization with pDNA	0	0
Secondary IgE		
Primary immunization β -gal	1,106	8,545
Secondary immunization β -gal		
Primary immunization pDNA	673	3,443
Secondary immunization β -gal		

*RAST (counts per minute) was carried out on pooled sera from 4 mice, 1:12.5.

IgE antibody titers were determined by radioimmunoassay [1]. C57BL/6 wild-type (WT) or β_2 -M KO (Jackson Labs., Bar Harbor, Me., USA) mice were immunized with either pCMV-LacZ or β -gal in alum and the IgE antibody titers determined.

Results

Passive Cell Transfer

The effect of passive transfer of CD4+ or CD8+ T cells from naive or immunized BALB/c mice is shown in figure 1. Mice injected with CD4+ cells from naive or β -gal-in-alum-immunized mice showed similarly high titers of IgE antibodies 4 weeks after immunization, which declined slowly thereafter. In contrast, mice injected with CD4+ cells

from pDNA-immunized mice showed about 90% lower IgE titers. All three groups of mice injected with CD8+ cells had lower IgE titers than the naive CD4+ control-cell-injected mice. Mice injected with CD8+ cells from naive and β -gal-in-alum-primed mice had about 50% lower IgE titers, whereas the mice injected with cells from the pDNA-primed mice had a 90% lower IgE titer than the CD4+ naive cell controls.

β_2 -M KO Mice

The primary and secondary IgE responses of C57BL/6 WT and β_2 -M KO mice 6 weeks after immunization are shown in table 1. In the primary response, β -gal in alum induced a 3 fold higher IgE titer in the β_2 -M KO mice than in the WT mice. Immunization with pCMV-LacZ did not induce IgE in either type of mouse. After secondary immunization with β -gal in alum, β -gal-primed WT mice had an 8-fold lower IgE titer than the β_2 -M KO mice. The IgE titer was lower than that of the primary response, which is typical for IgE-low-responder mice such as C57BL/6 mice. Priming of both the WT and β_2 -M KO mice with pCMV-LacZ resulted in a 2-fold lower IgE titer than in the β -gal in alum-primed mice.

Discussion

As shown by passive cell transfer, both CD4+ and CD8+ T cells from pCMV-LacZ-immunized mice suppressed the IgE antibody response to β -gal by 90% compared to mice receiving CD4+ cells from naive mice. This suggests that immunization with pCMV-LacZ induced both Th1 and Tc1 cells and that both types of cells suppressed the IgE antibody production either by directing the primary T cell response toward Th1 cell differentiation in the recipient mice and/or by propagating the Th1 memory response. A similar conclusion can be made from the IgE response in CD8+ deficient β_2 -M KO mice. β -Gal in alum induced a 3- fold higher IgE titer in the β_2 -M KO mice than in the WT mice, suggesting a suppressive role of CD8+ cells in WT mice. The suppressive effect was greater in WT than β_2 -M KO mice, presumably because both CD4+ and CD8+ cells had a downregulatory effect in the WT mice. Recently, Hsu et al. [7] reported that transfer of CD8+ but not CD4+ cells, obtained from rats immunized with pDNA-encoding the Der p 5 allergen suppressed the IgE response. The reason for the lack of a suppressive effect of CD4+ cells in the experiments of Hsu et al., but not in ours, may be the result of a species difference or the experimental design, because the suppressive effect was not compared to that of the transfer

of naive cells. Our data, obtained by passive transfer experiments and by immunization of CD8+ deficient mice, indicate that both CD4+ and CD8+ are able to suppress IgE antibody formation.

The induction of a Th1 response by pDNA immunization is not restricted to the β -gal antigen. In previous experiments, we showed that pDNA encoding ovalbumin also does not induce IgE antibodies [1]. Furthermore, in rats, Hsu et al. [7] showed that pDNA encoding the house dust mite allergen Der p 5 prevents induction of IgE synthesis, histamine release and airway hyperresponsiveness after allergen challenge. These data suggest that pDNA immunization in general may induce Th1 immune responses to the encoded antigens or allergens.

The use of immunotherapy – the injection of small but increasing amounts of allergen over a long period of time – has decreased over the past years as a major form of allergy therapy. The reason for this is a low benefit-to-risk ratio, the risk being anaphylactic reactions to the allergen injections. In addition, optimal pharmacological management of allergic disorders is equally beneficial with less risk. However, immunotherapy could regain a major role in the management of allergic diseases if a safe form of allergen administration could be found. A successful immunotherapy would eliminate the need for daily medication. Intradermal immunization with pDNA-encoding allergens fulfill such criteria. The allergen is produced intracellularly, and very little allergen is secreted, which should not induce anaphylactic reactions [5]. Second, at least in mice, pDNA persists in the dermis for a long time and continuously induces antigenic stimulation [5]. It remains to be shown whether pDNA will also induce a strong Th1 and Tc1 response to allergens in humans, and whether allergen gene vaccination will be a feasible new immunotherapy for allergic diseases.

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References

- 1 Raz E, Tighe H, Sato Y, Corr M, Dudley JA, Roman M, Swain SL, Spiegelberg HL, Carson DA: Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Natl Acad Sci USA* 1996;2:5141-5145.
- 2 Sato Y, Roman M, Tighe H, Lee DJ, Corr MP, Nguyen MD, Silverman GJ, Lotz M, Carson DA, Raz E: Immunostimulatory DNA sequences necessary for an effective intradermal gene immunization. *Science* 1996;273:352-354.
- 3 Finkelman FD, Svetic A, Gresser WC, Snapper C, Holmes J, Trotta PP, Katona IM, Gause WC: Regulation by interferon alpha of immunoglobulin isotype selection and lymphokine production in mice. *J. Exp. Med.* 1991;174:1779-1788.
- 4 Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM: Development of Th1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 1993;260:547-550.
- 5 Raz E, Carson DA, Parker SE, Parr TB, Abul AM, Aichinger G, Gromkowski SH, Singh M, Lew D, Yankauckas MA, Baird SM, Rhodes GH: Intradermal gene immunization: the possible role of DNA uptake in the induction of cellular immunity to viruses. *Proc Natl Acad Sci USA* 1994;91:9519-9523.
- 6 Rautel DH: MHC class I-deficient mice. *Adv Immunol* 1994;55:381-421.
- 7 Hsu CH, Chua KY, Tao MH, Lai YL, Wu HD, Huang SK, Hsieh KH: Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. *Nature Med* 1996;2:540.