

Polarized TH1 responses by liposome-entrapped allergen and its potential in immunotherapy of allergic disorders

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Summary

Background The conventional immunotherapy used for treating allergic individuals may at times lead to varying degrees of anaphylactic reaction. Liposomes have been proposed as a vehicle for safe and effective allergen-specific immunotherapy as multiple injections of liposome-entrapped allergen (LEA) have been shown to reduce specific IgE response and induce specific IgG response in BALB/c mice.

Objective and methods To elucidate the effect of LEA on polarization of T-cell responses, its effect on the relative production of TH1/TH2 type cytokines (namely IL-2, IL-4 and IFN- γ by commercially available ELISA kits and immunoglobulin profile as measured by ELISA) were studied. Histamine release on challenge of immunized mice was measured to examine the efficacy of LEA in preventing anaphylactic reactions.

Results Measurement of cytokine levels in serum and spleen cell culture supernatants of BALB/c mice injected repeatedly with either free allergen (FA) or LEA (three mice per group) indicate that LEA preferentially induces a TH1-type of response dominated by IFN- γ and IL-2 production. Further, it was also shown that immunization of mice with FA or LEA and subsequent challenge with a large dose of the sensitizing allergen leads to fatal systemic reactions in 50–65% of the animals treated with FA, whereas no mortality was observed in mice injected with LEA. Analysis of IgG subclasses in sera of mice immunized with LEA revealed a sixfold higher production of IgG1 antibodies than mice immunized with FA. Serum IgG2a, IgG2b, IgG3 and IgM responses were also enhanced in the group of mice immunized with LEA in comparison with mice injected with FA.

Conclusion The results indicate that LEA confers protection against anaphylaxis to mice due to their ability to induce a high IFN- γ :IL-4 ratio which may lead to decreased synthesis of IgE and reduced histamine release on challenge with FA.

Keywords: TH1, TH2, immunomodulators, immunotherapy, liposomes

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Introduction

Allergen-specific immunotherapy has been used for over nine decades and current data suggest that one of the mechanisms of action may be the switching of TH2 lymphocyte response to a TH1-type response [1,2]. An increase in IgG blocking antibodies and decrease in IgE antibodies has been proposed as the mechanism for effective immunotherapy.

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Although the conventional method of treatment has been shown to be effective it has its limitations as occasionally it may lead to varying degrees of systemic reactions [3,4]. Several new derivatives and formulations of allergens have been tried in an attempt to increase the safety and efficacy of immunotherapy. Immunoadjuvant property of liposomes provided an opportunity to develop new liposome-based vaccine formulations. Earlier work from the authors' laboratory [5,6] indicated that repeated injections of liposome-entrapped allergen (LEA) given to BALB/c mice potentiates the specific IgG response with a concomitant reduction in specific IgE response.

It has been well documented that synthesis of IgE is critically dependent on the regulatory influences of cytokines secreted by CD4⁺ T helper cells [7]. IL-4 is crucial for polyclonal IgE responses *in vivo* [8] and *in vitro* [9] and IFN- γ strongly suppresses the IL-4-induced IgE synthesis from LPS-stimulated B cells [10]. The ratio of IFN- γ and IL-4 determines the magnitude of specific IgE response.

It is well known that TH1 cells produce IL-2, IFN- γ and lymphotoxin (LT) whereas TH2 cells produce IL-4, IL-5, IL-9, IL-10 & IL-13 [11]. To determine which TH subset is activated by free allergen (FA) or LEA, the effect of repeated injections of FA or LEA on the cytokine levels and immunoglobulin profile were studied.

Materials and methods

Mice

BALB/c mice (4–6-week-old) were purchased from National Institute of Nutrition, Hyderabad, India.

Preparation of allergenic protein (FA)

The extraction of allergenic protein from *Artemisia scoparia* pollen was carried out in 0.1 mol/L ammonium bicarbonate buffer pH 8.0, according to the procedure mentioned for grass pollens [12]. Briefly, defatted pollen in ammonium bicarbonate buffer (1:20) was kept at 4 °C overnight with constant stirring. The supernatant was saturated to 90% with ammonium sulphate and precipitate obtained was dialysed, lyophilized & used for the experiments. Although purified form of allergenic extract (Art VIIb) was used in earlier studies [5,6], the authors' preliminary work demonstrated similar findings with both partially purified and purified allergen. Therefore, a partially purified allergen was used for further experiments.

Preparation of liposomes

Negatively-charged liposomes were prepared by standard detergent dialysis method using liposomat (Dianorm, Munich, Germany). Briefly, the lipid film made up of phosphatidylcholine:cholesterol:sodium cholate:diacetyl phosphate in the molar ratio (7:2:2:1) was dissolved in PBS (Phosphate buffer 50 mmol/L, saline 0.15 mol/L, pH 7.4) containing 5 mg of allergen. After 3 h of dialysis, small unilamellar vesicles with 20–60 nm size were obtained. The untrapped protein was removed by gel filtration chromatography on Sephacryl S-400 (Pharmacia, Uppsala, Sweden). The amount of entrapped protein was determined by Lowry's method [13] after disrupting a small aliquot of liposome preparation with 0.9% sodium dodecyl sulphate.

Measurement of cytokine levels

Cytokine levels were determined in the sera and spleen cell culture supernatants of immunized mice (FA or LEA). Mice were randomly divided into three groups each consisting of three animals. One group (control) was injected with PBS and remaining two groups of mice were injected intraperitoneally (i.p.) with 25 μ g of FA or LEA on the days 0, 10 and 24 of the experiment. Blood ($\approx$ 400 μ L) was drawn carefully from the retro-orbital plexus (according to UFAW Handbook [14]) on day 3 after each immunization for measurement of cytokines, since preliminary studies indicated that optimal responses were obtained at this timepoint (unpublished data). Cytokine responses were determined in the sera of immunized mice with the help of commercially available ELISA kits. For determination of cytokine responses *in vitro*, mice were sacrificed on day 31 and spleens removed under aseptic conditions. Spleen cells were cultured at 2×10^6 cells per mL with or without 2 μ g/mL (preliminary studies demonstrated optimal proliferation at 2 μ g/mL of FA or LEA) of either FA or LEA for three days at 37 °C in a humidified atmosphere of 5% CO₂ in RPMI-1640 medium containing glutamine and Hepes (Sigma, St Louis, MO, USA) supplemented with 100 U/mL penicillin, 100 μ g/mL streptomycin, 2×10^{-5} mol/L of 2-mercaptoethanol and 10% heat-inactivated fetal calf serum (Sigma). Culture supernatants collected after 72 h were assayed for IL-2, IL-4 & IFN- γ by ELISA kits.

IL-2 and IFN- γ levels were measured by Genzyme (Cambridge, MA, USA) ELISA kits with detection limits of 15 pg/mL and 125 pg/mL, respectively, while Endogen (Boston, MA, USA) ELISA kit with a sensitivity of 5 pg/mL was used for measurement of IL-4 levels. The optical density (OD) of standards and samples measured at 450 nm were converted to pg/mL from the standard curve (plotted according to manufacturers instructions).

Measurement of antibody isotypes

Antibody responses were determined on day 31 (i.e. day 7 after second booster) of the experiment by ELISA [15]. Peroxidase-labelled goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 & IgM conjugates were obtained from Seralab (Sussex, UK) whereas peroxidase-labelled goat anti-mouse IgG and IgE were procured from Sigma and Nordic (Immunological Lab., Tilburg, The Netherlands), respectively. OD was measured on a NUNC ELISA reader at 490 nm.

Measurement of histamine release after free allergen challenge

BALB/c mice were randomly divided into three groups of six animals each. Two groups of animals were primed i.p. with 25 μ g of FA or LEA on day 0, and further boosted on day 10 of the experiment. The third group (control) was

injected with PBS. Half the animals from each group were challenged intravenously with 200 µg FA on day 23. The remaining animals of the respective groups were given a 2nd booster dose with similar quantity of FA or LEA on day 24 and challenged similarly on day 37. Blood was drawn from the retro-orbital plexuses 20–30 min after each intravenous (i.v.) challenge and serum histamine levels were quantified by a double isotope radioenzymatic method [16]. Blood was also drawn on days 22 and 36 (i.e. one day prior to each i.v. challenge) for determination of serum-specific IgG and IgE levels by ELISA.

Statistical methods

Data are expressed as the mean $\pm$ SD of three mice. Probability test was not applied due to small number of mice per group.

Results

In vivo administration of LEA to BALB/c mice increased the IFN- γ :IL-4 ratio concomitant with enhancement in the IgG:IgE ratio

Measurement of cytokines and immunoglobulin isotypes in the sera of immunized mice after second booster dose revealed that repeated injections of LEA induced a considerable increase in the serum IFN- γ levels and consequent increase in IFN- γ :IL-4 ratio (three- to fourfold). A marked enhancement in the serum-specific IgG:IgE ratio (nine- to 10-fold) was also observed in comparison with that induced by FA (Fig. 1). This indicated that the immune response to LEA is perhaps dominated by activation of TH1 cells in contrast with the response observed with FA. In this experiment, IL-2 could not be detected *in vivo* under similar conditions probably due to insufficient secretion or too rapid use by these cells. The absolute values of IL-4 and IFN- γ are given in Table 1.

In vitro determination of cytokine levels in the culture supernatants of spleen cells from FA- or LEA-immunized mice

Figure 2 shows the cytokine levels in the culture supernatants of spleen cells from immunized mice (FA or LEA) stimulated with or without 2 µg/mL of FA or LEA. Unstimulated spleen cells of mice pre-immunized with LEA elicited enhanced IL-2 levels, reduced IL-4 levels and markedly increased IFN- γ levels as compared with those pre-immunized with FA. *In vitro* stimulation of spleen cells from mice treated either with FA or LEA elicited a further increase in IL-2 levels (approximately twofold), reduction in the IL-4 response and a dramatic increase in the IFN- γ levels (fourfold for FA and sixfold for LEA) than those of

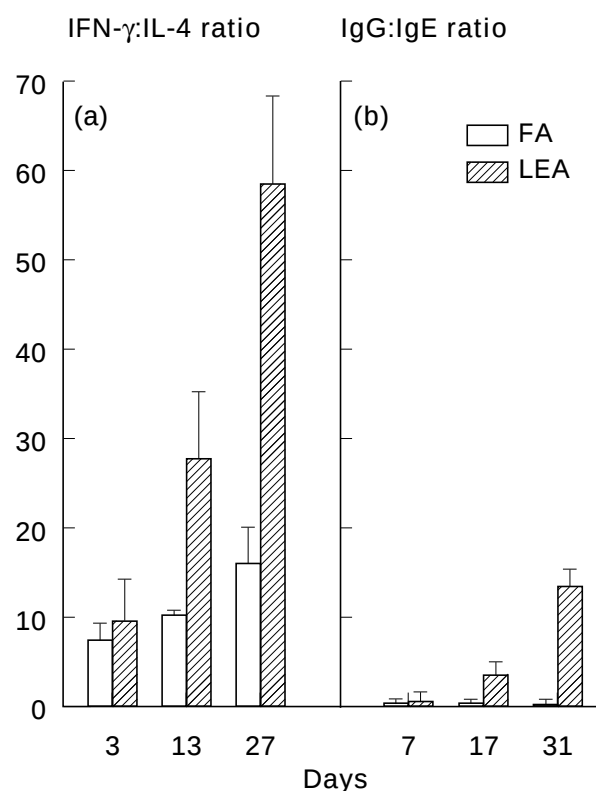


Fig. 1. BALB/c mice were immunized with i.p. injections of FA or LEA of days 0, 10 and 24 of the experiment. (a) IFN- γ :IL-4 ratio in sera. Serum cytokine responses were determined on day 3 after each immunization. Serum IL-4 and IFN- γ responses in control mice were negligible. (b) IgG:IgE ratio in sera. Antibody responses were determined on day after each immunization. Serum-specific IgG and IgE levels in control mice were negligible. Data are expressed as mean $\pm$ SD of the ratios from three mice.

mice immunized with FA. Figure 3 shows substantial enhancement of IFN- γ :IL-4 ratio by stimulation of spleen cells with LEA than by FA.

Comparison of IgG subclass and IgM responses induced by FA and LEA

The IgG isotype distribution in LEA-treated mice is dominated by production of specific IgG1 antibodies although specific IgG2a, IgG2b, IgG3 responses were also enhanced. A considerable enhancement in the IgM response was also observed (Fig. 4). Thus the adjuvant effect of liposomes is particularly reflected in IgG1 subclass.

Comparison of serum-specific IgG:IgE ratio and histamine release in immunized mice (FA or LEA) after challenge

To examine the potential role of LEA in reducing serum IgE

Table 1. Serum cytokine responses in mice sensitized with FA or LEA

Days	IL-4 (pg/mL)		IFN- γ (pg/mL)	
	FA	LEA	FA	LEA
3	15.83 $\pm$ 4.81	11.50 $\pm$ 4.09	115.50 $\pm$ 20.50	98.00 $\pm$ 2.82
13	24.25 $\pm$ 1.76	15.00 $\pm$ 3.53	249.50 $\pm$ 13.43	393.50 $\pm$ 19.09
27	19.33 $\pm$ 4.90	14.75 $\pm$ 2.47	292.00 $\pm$ 16.97	841.00 $\pm$ 22.62

Mice were immunized with 25 μ g FA or LEA via i.p. route on days 0, 10 and 24 of the experiment. Data are expressed as mean $\pm$ SD of the responses from three mice per group.

levels and anaphylactic allergic reactions, the effect of repeated injections of either LEA or FA on sensitization of BALB/c mice was determined. Histamine levels were determined after i.v. challenge of the two groups of mice with 200 μ g FA. Mice immunized with LEA showed a consistent decrease in serum-specific IgE levels and marked

increase in IgG levels in contrast with those treated with FA (Fig. 5). Intravenous challenge of the two groups with 200 μ g of FA resulted in severe to fatal systemic reactions in 50–65% of the animals in the group immunized with FA. No untoward effect was, however, observed in the group of mice treated with LEA.

As shown in Table 2, mice immunized with LEA elicited a high specific IgG:IgE ratio and released comparatively less serum histamine on i.v. challenge with a high dose of FA. However, the animals immunized with FA developed an increased serum-specific IgE response and greater release of histamine after challenge.

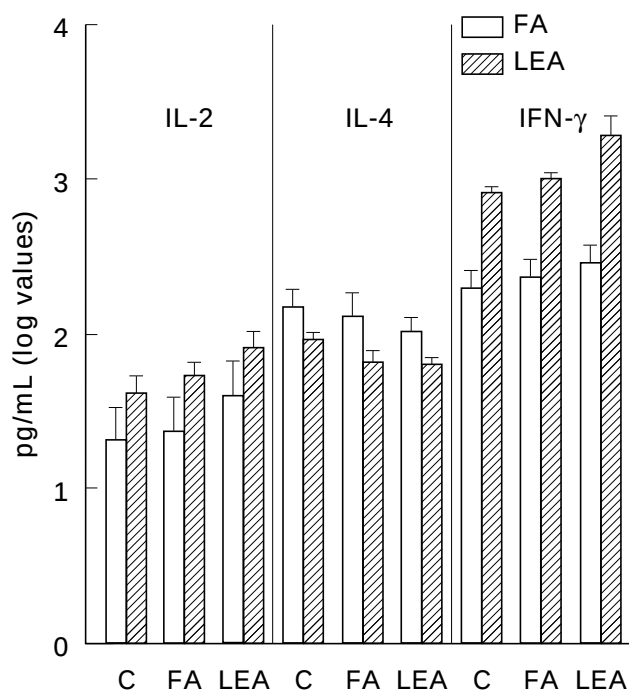


Fig. 2. IL-2, IL-4 and IFN- γ levels (expressed in terms of log values of pg/mL) in culture supernatants of spleen cells from sensitized mice (FA or LEA). On day 7 after second booster spleen cells (2×10^6 cells/mL) were incubated with or without 2 μ g/mL of FA or LEA. IL-2, IL-4 and IFN- γ assays were performed on supernatants collected after 72 h from the above cell cultures by specific ELISA kits. Cytokine production by spleen cells of control mice was negligible in response to *in vitro* stimulation with FA or LEA. Results are expressed in terms of log values of pg/mL as the mean $\pm$ SD of the responses from three mice. Figures represent one of the two similar experiments.

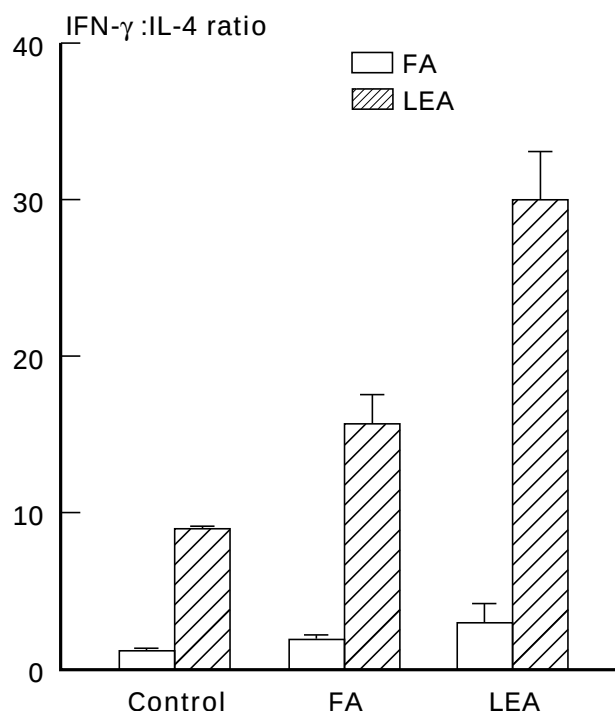


Fig. 3. IFN- γ :IL-4 ratios in culture supernatants of spleen cells from immunized mice (FA or LEA). Results are expressed as the ratio of the IFN- γ and IL-4 responses (pg/mL) from three mice.

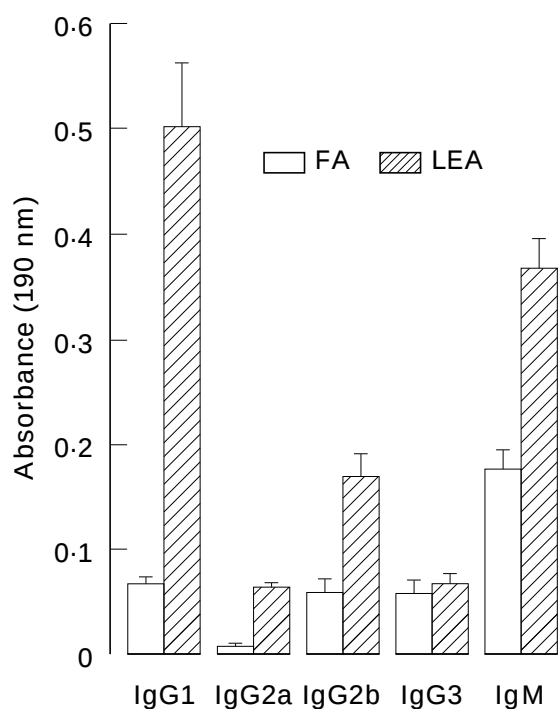


Fig. 4. Levels of specific IgG subclasses and IgM in sera of immunized mice (FA or LEA). Mice were injected with FA or LEA on day 0, 10 and 24 of the experiment. Serum-specific antibody responses were determined on day 31 by ELISA. Results are expressed as the mean $OD_{490\text{ nm}} \pm SD$ of three mice.

Table 2. Relation of serum-specific IgG:IgE ratio in mice immunized with FA or LEA and histamine levels after challenge with free allergen

Day	Mice immunized with:	
	FA	LEA
<i>Specific IgG/IgE</i>		
22	0.44 $\pm$ 0.07	3.59 $\pm$ 1.48
36	0.42 $\pm$ 0.05	13.44 $\pm$ 2.75
<i>Histamine levels (ng/mL)</i>		
23	24.30 $\pm$ 15.00	7.42 $\pm$ 2.60
37	79.33 $\pm$ 6.65	32.00 $\pm$ 11.10

Normal histamine levels: 3.53 ± 0.76 ng/mL. Values of IgG and IgE are expressed in terms of OD determined at 490 nm. Results are expressed as the mean $\pm$ SD of the responses from three mice. Histamine levels of samples are expressed in terms of ng/mL. Values represent the mean $\pm$ SD of the responses from three mice.

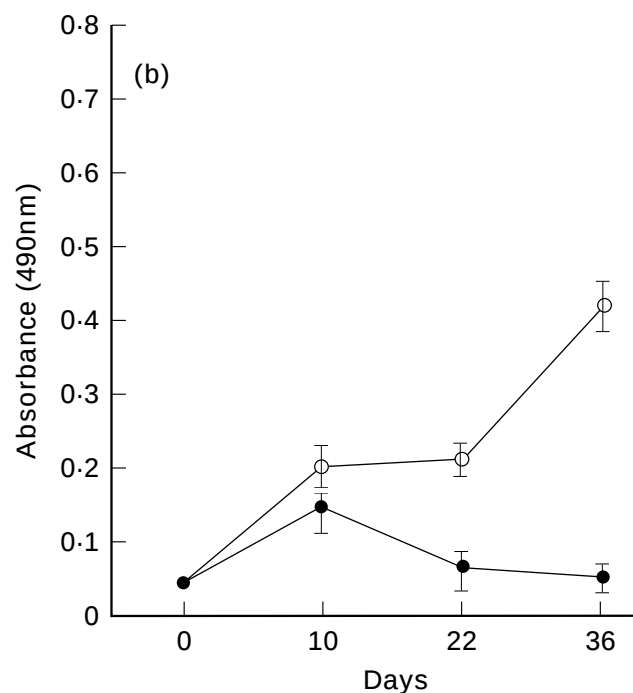
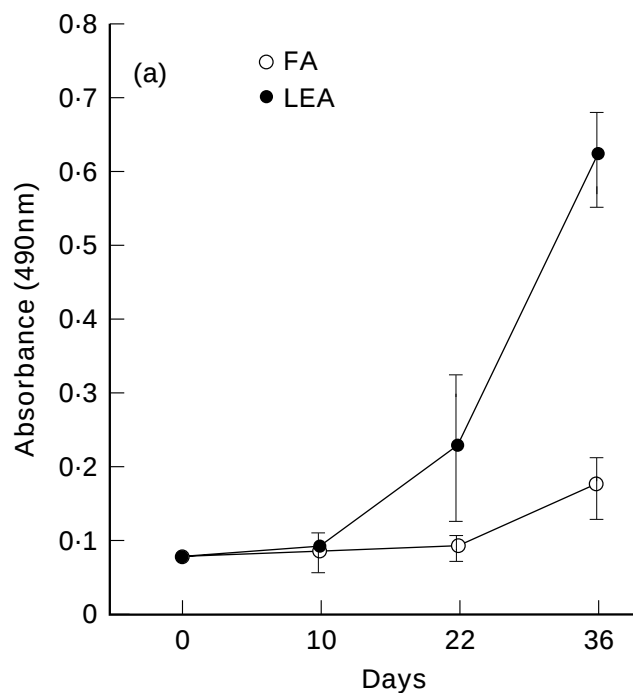


Fig. 5. Specific (a) IgG and (b) IgE levels in sera of mice sensitized with FA or LEA. BALB/c mice were injected with either 25 μ g of FA or LEA on day 0, 10 and 24 of the experiment by i.p. route. Specific IgG/IgE levels were measured by ELISA on day 10, 22 and 36, respectively. Results are expressed as the mean $OD_{490\text{ nm}} \pm SD$ of three mice.

Discussion

In vivo and *in vitro* studies confirm that LEA on repeated injections induces a TH1-type of activation which is evident from the predominant IFN- γ & IL-2 responses and increase in IFN- γ :IL-4 ratio. Cytokine production by unstimulated spleen cells of mice immunized with LEA indicated that LEA modulated the response to TH1-type *in vivo* either by specifically stimulating or recruiting TH1 phenotype. *In vitro* stimulation of spleen cells with FA or LEA probably further consolidates the polarized TH1 response. This suggests that the response once initiated may be maintained and amplified by mutually antagonistic effects. Mosmann and Coffman reported that IFN- γ selectively inhibits proliferation of TH2 cells [17] and effect of IL-4 on B cells. The functional capabilities of TH1 & TH2 cells are in turn reported to correlate with specific immunoglobulin isotypes, TH1 inducing the preferential production of IgG2a/IgG2b, IgG3 & IgM and TH2 that of IgG1 & IgE. Studies on the induction of immunoglobulin isotypes revealed that repeated injections of LEA resulted in an IgG1-dominated response although the levels of IgG2a, IgG2b, IgG3 & IgM antibodies were also enhanced. The significance of a high production of IgG1 antibodies obtained in this study implies that these probably act like blocking antibodies in preventing allergic reactions, a role suggested for IgG4 antibodies in humans after immunotherapy [18,19]. In mice IgG1 is claimed to be immunologically similar to human IgG4 and could possibly act as blocking antibodies in preventing accessibility of soluble allergens to IgE-coated mast cells [20–23].

Although murine IL-4 has the ability to induce the production of IgG1 & IgE antibodies [24], the dose of IL-4 required for the induction of an IgE response is three–10 times higher than that for an IgG1 response [25]. Thus, B cells of mice treated with LEA probably switch to IgG1 because of less availability of IL-4 due to a TH1-dominant response in LEA-stimulated mice. It is possible that substantial IgG1 may also be induced through IL-4-independent mechanisms, namely the TH1 pathway [26,27]. It is evident from the results of this study that LEA induces the production of IgG1 antibodies and suppresses IgE synthesis probably due to the high IFN- γ :IL-4 ratio caused by modulation of the immune response from TH2- to TH1-type. *In vitro* studies with spleen cells also demonstrated a high IFN- γ :IL-4 ratio in mice immunized with LEA. This suggests the induction of TH1 phenotype by LEA. Although cytokines play a major regulatory role in the differential activation of CD4⁺ T-cell subsets, the nature of accessory cells involved in an immune response is also important. Gajewski *et al.* reported that murine TH1 and TH2 cells are optimally stimulated by different types of antigen-presenting cells (APCs) [28]. Moreover, it has also been reported that B cells act as APC to preferentially induce TH2

responses whereas macrophages induce TH1 responses [29]. The authors' earlier studies [30] on macrophage depletion indicated a considerable reduction in stimulation index for LEA in the absence of macrophages. In contrast, the stimulation index with FA was unaffected. Therefore, processing and presentation of FA may possibly involve B cells as APC while LEA may be preferentially presented by macrophages. This may be due to sequestration of allergen by liposomes and their rapid uptake by macrophages.

Although the responses of TH1 and TH2 cells to different APCs have been reported [31] there are controversies that the type of APC does not critically influence the differentiation of TH precursors into one or another phenotype [31]. During antigen exposure, the cytokines released by APC or other cell types appear to play an important role in determining the development of specific TH1 or TH2 responses. Seder *et al.* reported that IL-12 induced by macrophages probably has the ability to promote IFN- γ production by natural killer cells in a T cell-independent manner [32] and IFN- γ in turn favours the development of TH1 cells.

In Type I allergic reactions, release of histamine requires degranulation of mast cells or basophils induced by specific IgE allergen reaction on their surface [33]. The lower release of histamine in LEA-treated mice after challenge suggests less availability of IgE on mast cells for allergen binding. High IFN- γ levels may also inhibit the release of mediators such as histamine from mast cells. Inhibitory effect of IFN- γ generated by splenic T cells on IgE, antigen-dependent histamine and serotonin release by mouse mast cells has been reported [34,35].

In conclusion, data on cytokine and immunoglobulin profile indicated that LEA confers protection to mice due to their ability to modulate the T-cell response from TH2 to TH1 phenotype. This might create a local environment of cytokines particularly a high IFN- γ :IL-4 ratio leading to decreased synthesis of IgE and markedly elevated levels of IgG1. Presence of higher proportions of IL-4- producing and lower proportions of IFN- γ -producing T- cell clones have been reported in peripheral blood of patients with severe atopic disease as compared with that of non-atopic donors [36] indicating a majority of TH2 type cells in atopic individuals. LEA may be able to modulate the T-cell response to a more protective TH1 phenotype. The data also suggested that continuous down-regulation of IgE on repeated injections of LEA could reduce the danger of anaphylactic reactions during immunotherapy. The above findings demonstrate important therapeutic potential for the development of safe and effective liposome-based allergy vaccines in the immunotherapy of allergic disorders.

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References

- McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine responses from a TH2 to a TH1 dominant pattern: comparison of rush and conventional immunotherapy. *Clin Exp Allergy* 1995; 25:828–38.
- Till S. Immunotherapy: readdressing the balance between TH2 and TH1 cells. *Clin Exp Allergy* 1997; 27:981–5.
- Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy and skin testing. *J Allergy Clin Immunol* 1987; 79:660–77.
- Nelson BL, Dupont LA, Reid MJ. Prospective survey of local and systemic reactions to immunotherapy with pollen extracts. *Ann Allergy* 1986; 56:331–4.
- Arora N, Gangal SV. Immunomodulation by liposome-entrapped allergen. *Mol Cell Biochem* 1990; 97:173–9.
- Arora N, Gangal SV. Efficacy of liposome-entrapped allergen in downregulation of IgE response in mice. *Clin Exp Allergy* 1992; 22:35–42.
- Finkelman FD, Holmes J, Katona M et al. Lymphokine control of in vivo immunoglobulin isotype selection. *Ann Rev Immunol* 1990; 8:303–33.
- Finkelman FD, Katona IM, Urban JF Jr et al. IL-4 is required to generate and sustain in vivo IgE responses. *J Immunol* 1988; 141:2335–41.
- Coffman RL, Ohara J, Bond MW, Carty J, Zlotnik A, Paul WE. B cell stimulatory factor-1 enhances the IgE response of liposaccharide-activated B cells. *J Immunol* 1986; 136:4538–41.
- Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J Immunol* 1986; 136:949–54.
- Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and function of helper T cells. *Adv Immunol* 1989; 46:111–47.
- Sridhara S, Singh BP, Kumar L, Verma J, Gaur SN, Gangal SV. Antigenic and allergenic relationships among airborne grass pollens in India. *Ann Allergy* 1995; 74:73–9.
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 93:265–75.
- Petter-Lane W. The laboratory mouse. In: Hume CW, ed. *UFAW Handbook on the Care and Management of Laboratory Animals*. London: Churchill Livingstone, 1976: 193–209.
- Malley A, Hopkins SJ, Turner KJ. Characterization of IgG antibodies response to timothy grass. *Int Arch Allergy Appl Immunol* 1985; 78:57–63.
- Brown MJ, Ind PW, Causon R, Lee TH. A novel double isotope technique for the enzymatic assay of plasma histamine: application to estimation of mast cell activation assessed by antigen challenge in asthmatics. *J Allergy Clin Immunol* 1982; 69: 20–4.
- Mosmann TR, Coffman RL. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 1989; 7:145–73.
- Aalberse RC, Van Milligen F, Tan KY, Stapel SO. Allergen specific IgG4 in atopic disease. *Allergy* 1993; 48:559–69.
- Djurup R. The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985; 40:469–86.
- Chretien I, Pene J, Briere F, De Waal Malefijt R, Rousset F, De Vries JE. Regulation of human IgE synthesis in vitro is determined by the reciprocal antagonistic effect of interleukin 4 and interferon-gamma. *Eur J Immunol* 1990; 20:243–51.
- Gascan H, Gauchat JF, Roncarolo MG, Yssel H, Spits H, De Vries J. Human B cells can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4⁺ T cell clones. *J Exp Med* 1991; 173:747–52.
- Adkinson NF Jr, Sobotka AK, Lichtenstein LM. Evaluation of the quantity and affinity of human IgG 'blocking' antibodies. *J Immunol* 1979; 122:965–72.
- Nakagawa T, Takaishi T, Sakamoto Y, Ito K, Miyamoto T, Skvaril F. IgG4 antibodies in patients with house dust mite sensitive bronchial asthma: relationship with antigen-specific immunotherapy. *Int Arch Allergy Appl Immunol* 1983; 71:122–5.
- Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. *Immunol Rev* 1988; 102:51–75.
- Moon HB, Severinson E, Heusser C, Johansson SGO, Moller G, Persson U. Regulation of IgG1 and IgE synthesis by interleukin 4 in mouse B-cells. *Scand J Immunol* 1989; 30:355–61.
- De Kruff RH, Mosmann TR, Umetsu DT. Induction of antibody synthesis by CD4⁺ T cells: IL-5 is essential for induction of antigen-specific antibody responses by TH2 but not TH1 clones. *Eur J Immunol* 1990; 20:2219.
- Kuhn R, Rajewsky K, Muller W. Generation and analysis of interleukin 4 deficient mice. *Science* 1991; 254:707–10.
- Gajewski TF, Pinnas M, Wong T, Fitch FW. Murine TH1 and TH2 clones proliferate optimally in response to distinct antigen presenting cell populations. *J Immunol* 1991; 146: 1750–8.
- Abbas AK. Cellular interactions in the immune response. The role of B lymphocytes and IL-4. *Amer J Pathol* 1987; 129:23–6.
- Sehra S, Chugh L, Gangal SV. Role of liposomes in selective proliferation of splenic lymphocytes. *Mol Cell Biochem* 1998; 183:133–9.
- Chang TL, Shea CM, Urioste S, Thompson RC, Boom WH, Abbas AK. Heterogeneity of helper/inducer T lymphocytes. III. Responses of IL-2 and IL-4 producing (TH1 and TH2) clones to antigens presented by different accessory cells. *J Immunol* 1990; 145:2803–8.
- Seder RA, Paul WE, Davis MM, Fazekas de St Groth BF. The presence of interleukin 4 during in vitro priming determines the lymphokine producing potential of CD4⁺ T cell from T cell receptor transgenic mice. *J Exp Med* 1992; 176:1091–8.
- Ishizaka T, Ishizaka K, Conrad DH, Froese A. A new concept

- of triggering mechanisms of IgE mediated histamine release. *J Allergy Clin Immunol* 1978; 61:320–30.
- 34 Coleman JW. Conditioned medium from concanavalin A stimulated spleen cells inhibits the IgE dependent sensitization of murine peritoneal mast cells in vitro. *Immunology* 1990; 69:150–4.
- 35 Coleman JW, Buckley MG, Holliday MR, Morris AG. Interferon- γ inhibits serotonin release from mouse peritoneal mast cells. *Eur J Immunol* 1991; 21:2559–64.
- 36 Romagnani S. Regulation and deregulation of human IgE synthesis. *Immunol Today* 1990; 11:316–21.