

Note

Adjuvant Activity of Alum in Inducing Antigen Specific IgE Antibodies in BALB/c Mice: a Reevaluation

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The IgE production was compared in the presence and absence of aluminum hydroxide gel (alum). Without alum, the IgE production was induced within a suitable range of the antigen dosage; however, alum enhanced it. Alum did not affect the minimum requirement for the antigen dosage, indicating that alum may not take part in the efficiency of antigen presentation.

Key words: adjuvant; alum; antigen dosage; IgE

Immunoglobulin E (IgE) plays an essential role in type-I allergic diseases.^{1–3)} The production of IgE by B-lymphocytes occurs under the control of type II helper T (Th2) lymphocytes⁴⁾ which stimulate the isotype switching of immunoglobulin *via* its surface transmembrane protein, CD40 ligand, and secreted interleukin-4 (IL-4).⁵⁾ Th2 lymphocytes themselves are differentiated from naive helper T lymphocytes in the presence of IL-4, the origin of which are NK1.1⁺ T lymphocytes.⁶⁾ This differentiation is an alternative pathway with differentiation to Th1 lymphocytes which is stimulated in the presence of IL-12.⁷⁾ Immunity is kept by a balance between Th1 and Th2 lymphocytes.

An adjuvant is a substance that augments immunity and may affect this immune balance. Aluminum hydroxide gel (alum) has often been used under experimental conditions to produce antigen specific IgE in murine cases,⁸⁾ which suggests that alum is an adjuvant stimulating Th2-type immunity. Although the adjuvant activity of alum is sometimes explained by its antigen adsorptive property,^{9–11)} its function, especially to evoke IgE production, is still unclear.

Besides adjuvants, the antigen dosage also has long been discussed as another critical factor for Th2-type immunity.^{12–17)} Alum and the antigen dosage both affect IgE production in experiments, although, the relationship between alum and the dosage of the antigen has not been studied.

To better understand its adjuvant activity, we com-

pared the effects of alum at various antigen doses on immunoglobulin production, especially that of IgE. Twice, with an interval of two weeks, ovalbumin (Sigma, St. Louis, MO, USA), at 10 ng, 100 ng, 1 μ g, 10 μ g, 100 μ g, or 1 mg per mouse, was subcutaneously injected into BALB/c mice with or without alum (Nacalai Tesque, Kyoto, Japan) in 400 μ l of phosphate-buffered saline for immunization (each group contained 5 mice). All animal experiments were performed in accordance with Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science, 1987).

Passive cutaneous anaphylaxis (PCA) reactions were carried out as previously described,¹⁸⁾ and photographs of the resulting back skin on those rats that expressed blue wheal spots were taken. The peak of production was always in the dosage range of 1–10 μ g of the antigen per mouse, whether with or without alum, although the presence of alum augmented the titer of ovalbumin specific IgE (Fig. 1).

Titers of ovalbumin specific IgG1, IgG2a and IgG2b were analyzed by an antigen specific enzyme-linked immunosorbent assay (ELISA), using plates coated with ovalbumin. Whether the antigen was injected with or without alum, the titers of all isotypes increased in an antigen dose-dependent manner (Fig. 2). The effect of alum was not as evident as that in the case of IgE.

The serum total IgE and IgG1 concentrations were measured by sandwich ELISA, using plates coated with the responsible capture antibody (Fig. 3). The change in the serum total IgE concentration was similar to that in the production of ovalbumin specific IgE. Namely, although the presence of alum up-regulated the serum total IgE concentration, the effective antigen dose was always in the range of 1–10 μ g per mouse independently of the presence of alum. The change in serum total IgG1 was similar to that in the production of ovalbumin specific IgG1 which increased in an antigen dose-dependent manner regard-

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Abbreviations: alum, aluminum hydroxide gel; Th1, type I helper T; Th2, type II helper T; PCA, passive cutaneous anaphylaxis; ELISA, enzyme-linked immunosorbent assay

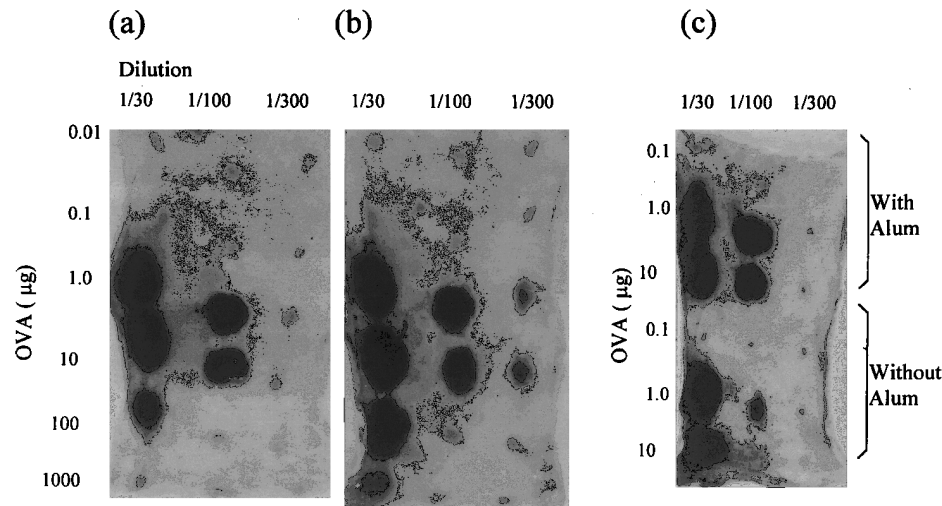
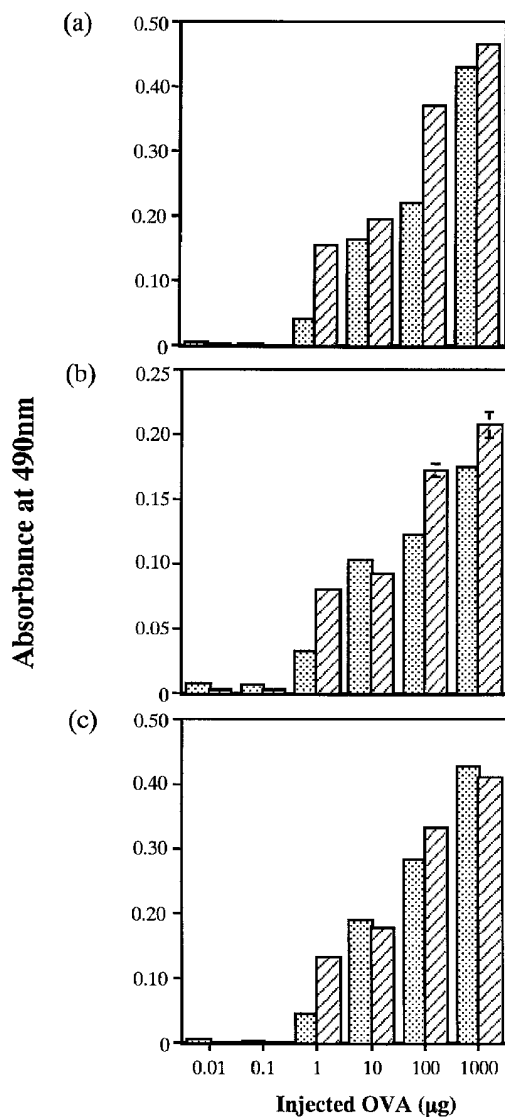


Fig. 1. Ovalbumin Specific PCA Reaction.

Wistar rats anesthetized by Nembutal (Abbott; Chicago, IL, USA) were intracutaneously injected with diluted anti-sera of mice into the skin on the back. After 2 days, the rats were again anesthetized and intravenously injected with 1 mg of ovalbumin (OVA) and 0.5% of Evans Blue (Nacalai Tesque) in 1 ml of PBS. The pooled sera of mice immunized with various amounts of OVA with alum (a) or without alum (b) were analyzed and, for an easier comparison, limited sera from (a) and (b) were reanalyzed on the same skin (c). The amount of OVA for immunization is designated on the left, and the dilution (1:X) of the sera is designated at the top.



less of the presence of alum.

The unresponsiveness of BALB/c mice to alum in the production of ovalbumin specific IgE at higher doses of the antigen was not attributable to the shortage of alum, because excess alum did not improve it (Fig. 4).

Less than 1 μg of ovalbumin seems to have been too little to activate the immune system, because no immunoglobulin isotype was significantly produced, and even a co-injection of alum failed to change the minimum requirement (Figs. 1–3). This indicates that alum did not have the ability to induce the antigen-presentation more efficiently, suggesting that its adjuvant activity is independent of its antigen-adsorptive property. If this ability were included in alum's adjuvant activity, the peak of IgE production would have been shifted to the left in Fig. 3.

Our experiments show that the critical factor for the production of the immunoglobulin isotype was the amount of injected antigen. In respect of the antigen specific IgE production, although alum enhanced this production, it did not have any significant effect on the efficiency of antigen presentation. This finding may be important not only in experiments to produce IgE in mice, but also to solve problems relating to the

Fig. 2. Ovalbumin Specific ELISA.

The titers of ovalbumin (OVA) specific IgG1 (a), IgG2a (b) and IgG2b (c) of the sera of the mice immunized with various amounts of OVA with (dotted bar) or without alum (hatched bar) were analyzed with antigen-coated micro-plates and detecting antibodies for IgG1, IgG2a or IgG2b (Bethyl; Montgomery, TX, USA). The pooled sera were diluted 1:2000 for IgG1, 1:20 for IgG2a and 1:200 for IgG2b before the measurements. Each values is expressed as the average ± SD bar of 3 experiments.

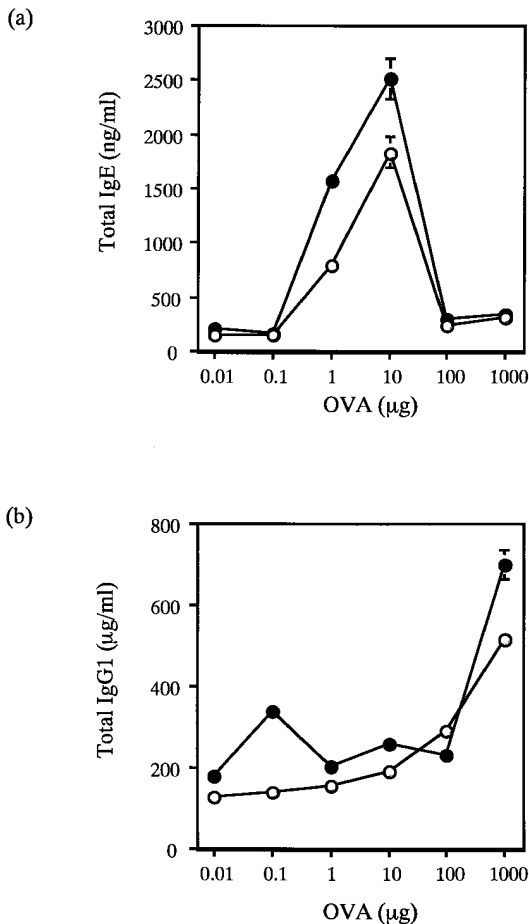


Fig. 3. Total IgE and IgG1.

The concentrations of total IgE (a) and IgG1 (b) in mice sera immunized with various amounts of OVA with alum (filled circles) or without alum (unfilled circles) were analyzed by sandwich ELISA kits (Bethyl; Montgomery, TX, USA). Each value is expressed as the average $\pm$ SD bar of 3 experiments.

prevalence of allergic diseases. Alum maybe have biochemical activity other than its physicochemical effect such as antigen adsorption and enhanced IgE production at a suitable antigen dosage; however, further research is necessary to clarify the nature of this activity.

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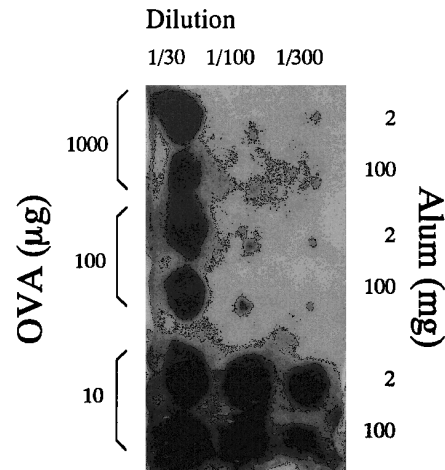


Fig. 4. Effect of the Amount of Alum on the Induction of OVA Specific IgE.

Designated amounts of alum were immunized with relatively high doses of OVA. Mice sera were analyzed by the PCA reaction. The amount of OVA for immunization is designated on the left, and the dilution (1:X) of the sera is designated at the top.

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