

Immune effector cells induced by complete Freund's adjuvant exert an inhibitory effect on antigen-specific type 2 T helper responses

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Summary

Background It has been well documented that environmental factors such as antigen-presenting cells and related cytokines could affect the development of T helper cells.

Objective The purpose of this study is to investigate the effect of different adjuvants on T cell development.

Methods Ovalbumin (OVA) combined with aluminum hydroxide (Alum) plus pertussis toxin (PT) or complete Freund's adjuvant (CFA) were used to sensitize mice; the production of IgG and IgE anti-OVA antibodies was then followed. In addition, OVA-specific proliferative responses and cytokine production by spleen cells were also investigated.

Results The data showed that the adjuvants themselves could modify the pattern of immune response: (1) IgG_{2a} anti-OVA antibody was higher in mice sensitized with OVA + CFA compared to that of mice sensitized with OVA + Alum + PT; (2) the ratio of IFN- γ /IL-4 produced by OVA-stimulated spleen cells was higher in mice sensitized with OVA + CFA than that of mice sensitized with OVA + Alum + PT; (3) increased percentage of $\gamma\delta$ T cells was noted in the peritoneal exudate cells of OVA + CFA immunized mice; and (4) the immune response of mice sensitized with OVA + Alum + PT was inhibited by the adoptively transferred ascitic cells from OVA + CFA immunized mice.

Conclusion In general, the data suggested higher IgG_{2a} and the ratio of IFN- γ /IL-4 was noted in mice sensitized with OVA + CFA. Further elucidation of the regulatory mechanism of allergen-specific T helper cells development and exploration of possible agents for immunotherapy might shed light on the management of atopic diseases.

Keywords: T helper cells, complete Freund's adjuvant, $\gamma\delta$ -T cells

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Introduction

Helper T cells can be separated into type 1 T helper (TH₁) and type 2 (TH₂) subsets based on the cytokine profiles. It has been well documented that TH₁ cells secrete IL-2, interferon- γ (IFN- γ), and tumor necrosis factor β (TNF- β); in contrast, TH₂ cells produce IL-4, IL-5, IL-10 [1–11]. TH₂ cells can help the production of IgE and IgG₁ antibody, immune response to parasite infection and immediate type

hypersensitivity [12–14]. Antigen-specific IgE plays an important role in the pathogenesis of allergic diseases such as asthma, rhinitis and atopic dermatitis. Further elucidation of mechanisms involved in the TH₁-TH₂ regulation will help in the future design of therapy for atopic diseases.

Many factors such as the microenvironmental cytokine appeared early in the immune response; the route of antigen administration, the kinds of antigen presenting cells, the ligand density on antigen presenting cells, the antigen dose and the use of adjuvants have been documented to affect the development of T helper cells [1]. For example, adjuvants

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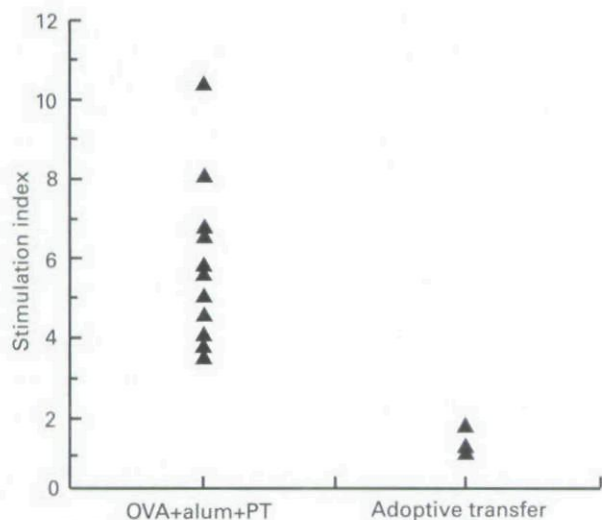


Fig. 9. The proliferative response by splenocytes from mice with or without adoptive transferring of peritoneal exudate cells of mice immunized with OVA combined with CFA. Splenocytes were cultured with 4 µg/ml OVA and the proliferative response was assayed. The result was expressed as SI. 'OVA + Alum + PT' was the control group, OVA+CFA was the experimental group; $n = 4$.

the kinds of adjuvant used in immunization [1]. In previous studies, Alum promoted T cells to TH₂; in another way, CFA tended to help TH₁ development [18]. However, no further studies concerning the real mechanisms of adjuvants on the development of T helper cells have been reported. In this study, OVA plus CFA induced higher TH₁-related cytokines compared to those of mice immunized with OVA plus Alum.

It has been well documented that different kinds of adjuvants may influence the production of IgE. Higher IgE response could be elicited if protein antigens or haptens were immunized with the adjuvant of Alum or *Bordetella pertussis* [26–28]. In contrast, CFA was found to inhibit the production of IgE [29–31]. In addition, pre-immunization with CFA can inhibit the IgE induced by OVA plus Alum [29]. Furthermore, IgE induced by OVA plus Alum can also be inhibited by adoptive transfer of CFA-immunized serum or ascites [31]. In this study, the immune response induced by OVA plus Alum was inhibited by adoptively transferred peritoneal exudate cells from mice immunized with OVA plus CFA. Most interestingly, phenotypic analysis of these peritoneal exudate cells showed the existence of a high percentage of $\gamma\delta$ -T cells, which is consistent with the report of another group [32]. This particular subset of T cells could not be detected in the mice immunized with OVA plus Alum.

One previous study also demonstrated that the adoptive transfer of CD8⁺ T cells can inhibit the production of IgE [33,34]. Furthermore, those CD8⁺ T cells were found with

$\gamma\delta$ -T cell receptor [35]. Although more evidence is needed, it has been shown that lots of $\gamma\delta$ -T cells recognize antigenic determinants of mycobacteria [36]. It has been speculated that CFA might increase the population of $\gamma\delta$ -T cells and subsequently inhibit the production of IgE. It will be interesting to further investigate if the effects of these $\gamma\delta$ -T cells are antigen-specific or non-specific.

This study showed OVA + CFA immunization induced higher TH₁-related cytokine production and antigen-specific IgG_{2a} compared to that of mice immunized with OVA + Alum + PT. In addition, adoptive transfer of immune effector cells induced by OVA+CFA immunization can inhibit the antigen-specific immune response of OVA-Alum. Further, an unusually high percentage of $\gamma\delta$ -T cells was noted in the peritoneal exudate cells of mice immunized with OVA + CFA. Although more studies are needed to further elucidate the real mechanisms of CFA as the development of T helper cells is affected, the possible application of adjuvant or cytokine in immunotherapy should be well anticipated.

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