

THE IN VIVO DEPLETION OF CD4⁺ T CELLS PREVENTS ANTIGEN-INDUCED
EOSINOPHIL INFILTRATION INTO MOUSE SKIN

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In order to determine the role of CD4⁺ T cells and CD8⁺ T cells in causing antigen-induced eosinophil infiltration into the site of late-phase reaction (LPR), we examined the effect of the in vivo depletion of CD4⁺ T cells and CD8⁺ T cells on antigen-induced eosinophil infiltration into mouse skin. Eosinophil infiltration into the skin of ovalbumin (OA)-sensitized BALB/c mice was biphasic after subcutaneous OA challenge, reaching the first peak at 6 h and the second peak at 24 to 48 h. The in vivo depletion of CD4⁺ T cells by pretreatment with anti-L3T4 monoclonal antibody (mAb) significantly decreased the second peak (at 24 h and 48 h), but not the first peak (at 6 h), of OA-induced eosinophil infiltration into the skin of OA-sensitized mice. However, the depletion of CD8⁺ T cells by pretreatment with anti-Lyt-2 mAb had no significant effect on either the first or second peak of OA-induced cutaneous eosinophilia in the mouse. These results indicate that CD4⁺ T cells, but not CD8⁺ T cells, play an important role in causing antigen-induced eosinophil recruitment of cutaneous LPR.

Key words: CD4⁺ T cells — cytokine — eosinophils — late-phase reaction

INTRODUCTION

Late-phase reaction (LPR) occurs 3 to 8 h after antigen administration and persists for up to 48 h in the skin and airways of sensitized individuals. It has been shown that there is an intense eosinophil infiltration into the site of LPR¹⁻⁴⁾. Previous studies suggested that the eosinophil infiltration in cutaneous LPR was dependent on IgE antibody and mast cells^{1,2)}. In addition to the infiltration of eosinophils, it has recently been shown that there is an increase in CD4⁺ T cells at the sites of LPR in the skin⁵⁾ and airways⁶⁾, suggesting that CD4⁺ T cells might be involved in antigen-induced eosinophil recruitment of LPR. However, there is no direct evidence that T lymphocytes mediate antigen-induced eosinophil infiltration into the site of LPR. Therefore, in order to determine the role of CD4⁺ T cells and CD8⁺ T cells in causing antigen-induced eosinophil

infiltration into the site of LPR, we examined the effect of the in vivo depletion of CD4⁺ T cells and CD8⁺ T cells on antigen-induced eosinophil infiltration into mouse skin. We show that CD4⁺ T cells, but not CD8⁺ T cells, play an important role in causing antigen-induced eosinophil recruitment of cutaneous LPR.

MATERIALS AND METHODS

Mice

BALB/c mice (6-8 weeks old) were immunized intraperitoneally twice with 1 µg of ovalbumin (OA) (Sigma Chemical Co.) in alum at a two-week interval. Ten to 14 days after the second immunization, OA was administered subcutaneously as described below.

Antibodies

Rat anti-L3T4 monoclonal antibody (mAb) GK1.5 (IgG2b)⁷⁾ and rat anti-Lyt-2 mAb 53.6-72 (IgG2a)⁸⁾ were purified from ascites produced from the hybridomas by ammonium sulfate precipitation. Purified rat IgG was purchased from Jackson

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Abbreviations: LPR late-phase reaction; mAb monoclonal antibody; OA ovalbumin; PBS phosphate-buffered saline

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Antigen-induced eosinophil infiltration in mouse skin

Eosinophil infiltration into the skin was induced by subcutaneous antigen challenge in sensitized mice, and the number of eosinophils infiltrating into the subcutaneous tissue was measured by the method described by Lawman et al⁹⁾. Briefly, 0.9 ml of air was injected into the subcutaneous tissue of the left flank of OA-sensitized BALB/c mice using a 1-ml tuberculin syringe with a 26-gauge needle, and then 0.1 ml of OA (50 µg/ml) present in the same syringe was successively introduced into the air bleb. As a control, phosphate-buffered saline (PBS) alone was injected into the air bleb. At various intervals after the injection, the thin membrane of the air bleb was excised after sacrifice by cervical dislocation and was stretched onto a glass slide. After drying, the specimen was fixed with methanol and stained with Wright-Giemsa solution. The number of eosinophils was counted at a magnification of $\times 400$ and expressed as the sum of eosinophils in the five fields.

Effect of anti-CD4 mAb and anti-CD8 mAb

To determine whether T lymphocytes are involved in causing antigen-induced eosinophil infiltration into mouse skin, we examined the effect of the in vivo depletion of CD4⁺ T cells or CD8⁺ cells on antigen-induced cutaneous eosinophilia in sensitized mice. OA-sensitized BALB/c mice were injected intraperitoneally once with 1 mg of anti-L3T4 mAb (GK1.5)⁷⁾ 2 days before the subcutaneous challenge with OA (5 µg). Other OA-sensitized mice were given 1 mg of anti-Lyt-2 mAb (53.6-72)⁸⁾ intraperitoneally twice at a two-day interval and then challenged subcutaneously with OA 2 days later. As a control, OA-sensitized mice were injected intraperitoneally with purified rat IgG (1 mg) 2 days before the subcutaneous OA challenge. The depletion of CD4⁺ T cells and CD8⁺ T cells was confirmed by FACS analysis using spleen cells prepared from the mice 2 days after treatment with the anti-L3T4 mAb or anti-Lyt-2 mAb. Pretreatment with purified rat IgG induced no changes in numbers of either T cell population compared to those in PBS-pretreated mice.

Eosinophil infiltration into the skin was evaluated at 6, 24, and 48 h after the subcutaneous OA challenge in those three groups of mice.

Data analysis

Data are summarized as mean $\pm$ SD. Inhibition of eosinophil infiltration by a given treatment was expressed as % of $1 - [(OA\text{-induced response with the treatment} - \text{PBS-induced response}) / (OA\text{-induced response with a corresponding vehicle} - \text{PBS-induced response})]$. The statistical analysis of the results was performed by the analysis of variance using Fisher's least significant difference test for multiple comparison¹⁰⁾. P values < 0.05 were considered significant.

RESULTS

Time course of antigen-induced eosinophil infiltration into mouse skin

After the subcutaneous OA challenge, there were biphasic infiltrations of eosinophils into the skin of OA-sensitized mice. Eosinophil infiltration into the subcutaneous tissue of OA-sensitized BALB/c mice occurred 4 h after the subcutaneous injection of OA (5 µg), reached the first peak at 6 h after the injection, and then declined at 12 h ($n=5$ mice at each time point) (Fig. 1). The antigen-induced eosinophil infiltration into mouse skin again increased at 24 h and 48 h after the injection, reaching the second peak ($n=5$) (Fig. 1). In addition, there was no significant blood eosinophilia after the subcutaneous injection of OA during the entire course of cutaneous eosinophilia in the OA-sensitized mice.

Effect of depletion of CD4⁺ T cells and CD8⁺ T cells on antigen-induced cutaneous eosinophilia

The in vivo depletion of CD4⁺ T cells by pretreatment with anti-L3T4 mAb decreased the second peak (at 24 h and 48 h), but not the first peak (at 6 h), of antigen-induced eosinophil infiltration into mouse skin. The intraperitoneal pre-injection with anti-L3T4 mAb (1 mg) 2 days before the subcutaneous OA challenge had no significant effect on OA-induced eosinophil infiltration into the skin of OA-sensitized mice at 6 h ($n=6$) (Fig. 2).

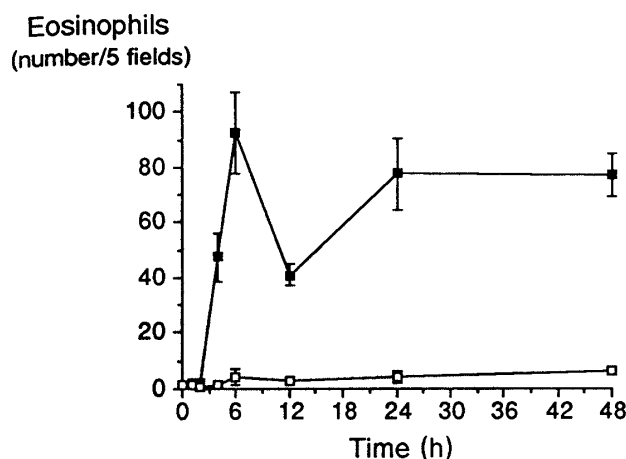


Fig. 1. Time course of antigen-induced eosinophil infiltration into mouse skin.

OA (5 μ g; solid symbols) or PBS (open symbols) was injected into the subcutaneous tissue of OA-sensitized BALB/c mice, and the number of eosinophils infiltrating into the skin was counted at various intervals after the injection. Data are means $\pm$ SD for 5 mice at each time point.

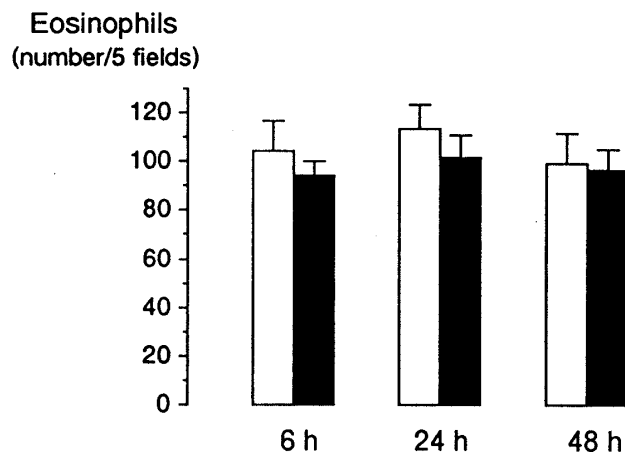


Fig. 3. Effect of depletion of CD8⁺ T cells on antigen-induced eosinophil infiltration in mouse skin.

OA-sensitized mice were injected intraperitoneally twice with anti-Lyt-2 mAb (solid columns) at a two-day interval and then challenged subcutaneously with OA 2 days later. Data are means $\pm$ SD for 6 mice in each group.

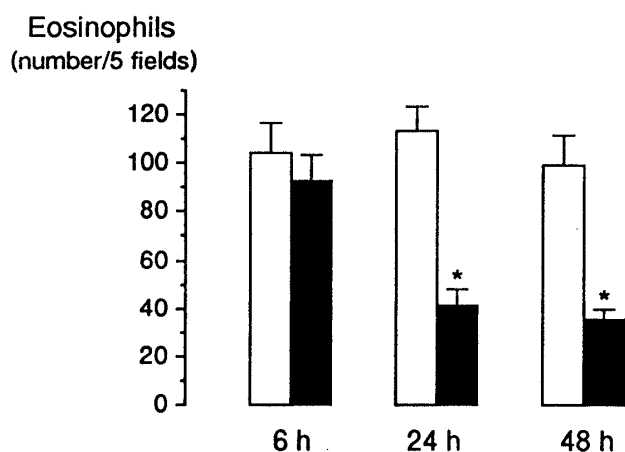


Fig. 2. Effect of depletion of CD4⁺ T cells on antigen-induced eosinophil infiltration in mouse skin.

OA-sensitized mice were injected intraperitoneally with anti-L3T4 mAb (solid columns) or rat IgG (open columns) 2 days before the subcutaneous challenge with OA (5 μ g). The number of eosinophils infiltrating into the skin was then counted at 6, 24, and 48 h. Data are means $\pm$ SD for 6 mice in each group. *Significantly different from the mean value of the corresponding control response (rat IgG), * p < 0.001.

In contrast, the intraperitoneal preinjection with anti-L3T4 mAb significantly decreased OA-induced eosinophil infiltration into the skin at 24 h

by 67% (control rat IgG 113.5 ± 9.8 vs. anti-L3T4 mAb 41.6 ± 6.1 eosinophils/five high power fields, mean $\pm$ SD, $n=6$, $p < 0.001$) (Fig. 2). The intraperitoneal preinjection with anti-L3T4 mAb also decreased the OA-induced eosinophil infiltration at 48 h by 68% (control rat IgG 99.3 ± 12.5 vs. anti-L3T4 mAb 35.2 ± 4.5 eosinophils/five high power fields, $n=6$, $p < 0.001$) (Fig. 2).

However, depletion of CD8⁺ T cells by pretreatment with anti-Lyt-2 mAb (1 mg, twice) had no significant effect on either the first or second peak of OA-induced cutaneous eosinophilia in the mouse (Fig. 3).

DISCUSSION

In this study, we show that CD4⁺ T cells play an important role in causing antigen-induced eosinophil recruitment of cutaneous LPR, as indicated by the preventive effect of the in vivo depletion of CD4⁺ T cells, but not of CD8⁺ T cells, on antigen-induced eosinophil infiltration into mouse skin at 24 h and 48 h (Fig. 2 and 3). Our finding provides direct evidence that CD4⁺ T cells are involved in the induction of antigen-induced eosinophil recruitment in cutaneous LPR.

Frew and Key⁵⁾ found that numerous eosinophil

infiltrates were present at 6 h at the site of allergen challenge in human skin of atopic patients and persisted for up to 48 h; these were associated with a significant increase in the infiltration of CD4⁺ T cells at 6 to 48 h. Another indirect evidence of CD4⁺ T cell involvement in antigen-induced eosinophil infiltration of LPR was an increase in both CD4⁺ T cells and eosinophils in bronchoalveolar lavage fluid at 48 h after segmental bronchial challenge⁶⁾. These findings suggested that there might be some connection between the infiltrating CD4⁺ T cells and the eosinophil recruitment in LPR^{5,6)}, and are also consistent with our findings at 24 h and 48 h. However, our finding indicates that there is no significant direct connection between CD4⁺ T cells and antigen-induced eosinophil infiltration into the skin at 6 h (Fig. 2).

By contrast, our results of the *in vivo* depletion of CD8⁺ T cells suggest that CD8⁺ T cells are not significantly involved in the induction of antigen-induced eosinophil recruitment in cutaneous LPR. Since CD8⁺ T cells have regulatory effects on the immune response^{11,12)}, we performed this experiment to further assess the possible regulatory role of CD8⁺ T cells in antigen-induced eosinophil recruitment into the skin. A previous study demonstrated that there was an increase in CD8⁺ T cells in the bronchoalveolar lavage fluid of asthmatic patients who had only immediate-phase reaction after allergen challenge¹³⁾, also suggesting that CD8⁺ T cells might have an inhibitory effect of LPR. However, our findings do not support the inhibitory role of CD8⁺ T cells in antigen-induced eosinophil recruitment in cutaneous LPR since the depletion of CD8⁺ T cells did not enhance the antigen-induced eosinophil response (Fig. 3).

Our findings suggest that CD4⁺ T cell-derived cytokine(s) mediates antigen-induced eosinophil recruitment in cutaneous LPR. In mice, blood eosinophilia induced by a parasite infection or by the systemic administration of IL-2 was shown to be abolished by treatment with anti-IL-5 mAb^{14,15)}, suggesting that IL-5 is a major cytokine in causing eosinopoiesis and blood eosinophilia. However, which cytokine including IL-5 mediates antigen-induced eosinophil recruitment to the tissue has not yet been studied. Since murine IL-5 has been

shown to be chemotactic for eosinophils¹⁶⁾, IL-5 produced from the infiltrating CD4⁺ T cells could possibly attract the eosinophils to the site of antigen administration in the skin. Therefore, to solve this issue, further studies are needed to determine whether CD4⁺ T cells infiltrate into the site of cutaneous LPR after antigen administration.

In summary, we have shown that CD4⁺ T cells, but not CD8⁺ T cells, are functionally important in causing antigen-induced eosinophil recruitment of cutaneous LPR. This basic knowledge of the mechanism for antigen-induced eosinophil recruitment in LPR should be useful for rational manipulation of allergic inflammation.

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CD4陽性 T 細胞の In Vivo 除去による抗原誘発

マウス皮膚好酸球浸潤の抑制

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I 型アレルギーの遅発反応の好酸球浸潤における CD4陽性 T 細胞および CD8陽性 T 細胞の役割を明らかにするため、マウス皮膚における抗原誘発好酸球浸潤に対する CD4陽性 T 細胞あるいは CD8陽性 T 細胞の in vivo での除去効果を検討した。卵白アルブミン (OA) 皮下投与による OA 感作 BALB/c マウス皮膚の好酸球浸潤は 2 相性で、第 1 相は OA 皮下投与 6 時間後にピークを示し、第 2 相は 24~48 時間後にピークを示した。抗 L3T4 モノクローナル抗体による CD4陽性 T 細胞の除去は第 2 相 (24 および 48 時間後) の好酸球浸潤を減少させたが、第 1 相 (6 時間後) の好酸球浸潤は減少させなかった。しかし、Lyt-2 モノクローナル抗体による CD8陽性 T 細胞の除去は第 1 相および第 2 相の好酸球浸潤に対し有意の効果を与えなかった。これらの結果から、皮膚 I 型アレルギーの遅発反応における好酸球浸潤の惹起には CD4陽性 T 細胞が関与していることが示された。