

Original Article

Role of T cells in IgE-dependent triphasic cutaneous reaction caused by dinitrofluorobenzene in the mouse ear: Participation of CD8⁺ T cells

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ABSTRACT

Background: Previous studies have suggested that mice passively sensitized with anti-dinitrophenol (DNP) monoclonal IgE antibody exhibit a triphasic cutaneous reaction with an immediate-phase response (IPR) at 1 h, a late-phase response (LPR) at 24 h and a very late-phase reaction (vLPR) at 8 days after challenge with 2,4-dinitrofluorobenzene. The present study was conducted to elucidate the role of T cells in this triphasic cutaneous reaction.

Methods: Mice were passively sensitized by an intravenous injection of anti-DNP monoclonal IgE antibody. Allergic cutaneous reaction was caused by painting an antigen on the ears of mice and measured an enlargement of the ears.

Results: Whereas the magnitudes of IPR and LPR in BALB/c nu/nu T cell-deficient mice were similar to those in BALB/c +/+ mice, vLPR was not observed in nu/nu mice. In addition, FK 506 (3 and 5 mg/kg) and cyclosporin A (30 and 50 mg/kg) clearly suppressed the onset of vLPR without affecting IPR and LPR. Because these findings suggest the participation of T cells in the onset of vLPR, the character of the T cells was investigated by using anti-CD4 or anti-CD8 monoclonal antibody (mAb) and interleukin (IL)-4 and IL-5 receptor α chain gene-deficient mice. When mice were treated with anti-CD4 or anti-CD8 mAb, the magnitude of the

vLPR was augmented by anti-CD4 mAb and suppressed by anti-CD8 mAb, without affecting IPR and LPR. Disruption of the IL-4 gene slightly suppressed IPR, LPR and vLPR, but the lack of the IL-5R α chain gene did not affect these triphasic responses.

Conclusions: The present findings suggest that vLPR is mainly caused by CD8-positive T cells, probably Tc1 cells, and regulated by CD4-positive T cells.

Key words: allergic dermatitis, CD4⁺ T cell, CD8⁺ T cell, FK 506, IgE, mice, triphasic cutaneous reaction.

INTRODUCTION

To clarify the mechanism of allergic cutaneous reactions and to investigate the remedy for allergic skin diseases, two different kinds of mouse model were generally used. One is caused by repeated painting of allergen on the ear skin¹ and the other is caused by the painting of allergen on mice that had been passively sensitized with large amounts of IgE antibody.² Both models show eczematous cutaneous changes and high serum IgE. In the latter model, animals showed a triphasic cutaneous response after challenge with antigen.³ The first reaction was an immediate-phase response (IPR) at 1 h after antigen challenge. The second reaction was observed 24 h after antigen challenge and was named the 'late-phase reaction' (LPR). The third reaction was a very late-phase response (vLPR) that was seen 8 days after antigen challenge. Pharmacologic and histopathologic studies have suggested the mechanisms of the triphasic reaction. The IPR is mainly caused by histamine released from sensitized mast cells⁴ and the LPR is caused by

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pro-inflammatory cytokines.⁵⁻⁷ In addition, a previous study has suggested the participation of T cells in the third phase reaction, the vLPR.⁸ However, the character of the T cells is still unclear. Therefore, the present study was conducted to investigate the character of T cells in the reactions of an IgE antibody mediated triphasic cutaneous response.

METHODS

Animals

Female BALB/c +/+ and BALB/c nu/nu mice (7 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). Female interleukin (IL)-4-knockout mice were produced from a BALB/c background in specific pyrogen-free conditions and age-matched female BALB/c mice (7 weeks old) were obtained from Charles River Japan (Osaka, Japan) and used as control animals. Female IL-5 receptor α chain-knockout mice ((129 $\times$ BALB/c) F2 background) were prepared and maintained as described previously⁹ and age-matched female wild-type mice ((129 $\times$ BALB/c) F2 background) were used as control animals. Experiments were undertaken following the guidelines for the care and use of experimental animals from the Japanese Association for Laboratory Animal Science (1987).¹⁰

Monoclonal IgE antibody and antigen

Mouse monoclonal IgE antibody against dinitrophenyl residue was prepared as reported previously.² The IgE content of the preparation, as estimated by enzyme-linked immunosorbent assay, was 2.9 μ g/mL. 2,4-Dinitrofluorobenzene (DNFB) was purchased from Nacalai Tesque (Kyoto, Japan) and was dissolved in a mixture of acetone and olive oil (3 : 1) at a concentration of 0.15%.

Triphasic cutaneous reaction

Mice were sensitized with an intravenous injection of 1 mL IgE preparation. Twenty-four hours after sensitization, 25 μ L DNFB solution was applied onto both surfaces of both ears. Ear thickness was assessed using a micrometer (Peacock Upright Dial Gauge; Ozaki, Tokyo, Japan) before or after DNFB application. FK 506 (Fujisawa Pharmaceutical, Osaka, Japan) was suspended in 0.5% carboxymethyl cellulose solution and cyclosporin A (Novartis Pharma, Tokyo, Japan) was

dissolved in myglyol 812N. Both drugs were administered twice to mice orally, 1 h before and 72 h after DNFB application.

Antibodies against murine CD4 and CD8

The hybridoma GK1.5¹¹ and 2.43¹² (American Type Culture Collection, Manassas, VA, USA), which produce rat IgG2b monoclonal antibodies (mAbs) to murine T cell CD4 and CD8 antigens, respectively, were grown in ascitic fluid of pristane primed nude mice. The rat IgG2b fractions were purified from ascites by sequential (NH₄)₂SO₄ precipitation and protein A separation. Antibodies were resuspended in phosphate-buffered saline (PBS) at a concentration of 5 mg/mL and stored at -80°C . Almost 98% of CD4⁺ or CD8⁺ T cells were depleted by the intravenous injection of 0.25 mL of each antibody.

Histologic study

Mouse ears were removed 24 h or 8 days after challenge and fixed with 10% neutral formalin (Nacalai Tesque, Kyoto, Japan). Ears were then cut into parasagittal slices, dehydrated and embedded in paraffin following standard procedures. Paraffin sections were stained with hematoxylin and eosin and assessed by light microscopy.

Statistics

Data are expressed as the mean $\pm$ SEM. In experiments containing two experimental groups, either Student's or Aspin Welch's *t*-test was used to evaluate the statistical significance of differences after the variances of the data were evaluated with the *F*-test ($P < 0.05$). For data including three or more experimental groups, either a parametric or a non-parametric Dunnett's multiple range test was used after Bartlett's analysis ($P < 0.05$). Differences were considered significant when $P < 0.05$.

RESULTS

The change in ear thickness by the IgE-dependent triphasic cutaneous reaction in BALB/c +/+ and nu/nu mice is shown in Fig. 1. Typical IPR, LPR and vLPR were observed in BALB/c +/+ mice. In BALB/c nu/nu mice, IPR and LPR were observed similar to those seen in BALB/c +/+ mice, but no vLPR was observed. To confirm the participation of T cells in the vLPR, pharmacologic

studies using the immunosuppressive drugs FK 506 and cyclosporin A were performed (Fig. 2). Whereas neither drug affected the IPR and LPR, both drugs significantly suppressed the vLPR.

The next experiment was conducted to clarify the type of T cell involved in the development of the vLPR.

In the first experiment, to clarify more detailed characteristics of the T cells, the effects of anti-CD4 and anti-CD8 antibodies on this reaction were studied. The administration of anti-CD4 or anti-CD8 mAbs did not affect the development of IPR and LPR (Fig. 3). In contrast, the anti-CD4 antibody enhanced the vLPR and anti-CD8 antibody

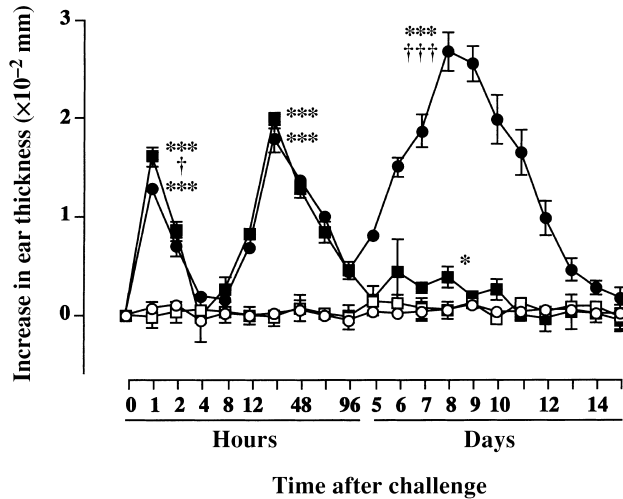


Fig. 1 Time-course study of the IgE-mediated cutaneous reaction in BALB/c nu/nu mice. (○), BALB/c +/+, non-sensitized non-challenge (N-N) group; (●), BALB/c +/+, sensitized challenge (S-C) group; (□), BALB/c nu/nu, N-N group; (■), BALB/c nu/nu, S-C group. Data are the mean $\pm$ SEM of five to six mice. * P < 0.05, *** P < 0.001 between the N-N and S-C groups (Student's t -test). † P < 0.05, †† P < 0.01, ††† P < 0.001 between the same treatment groups (Student's t -test).

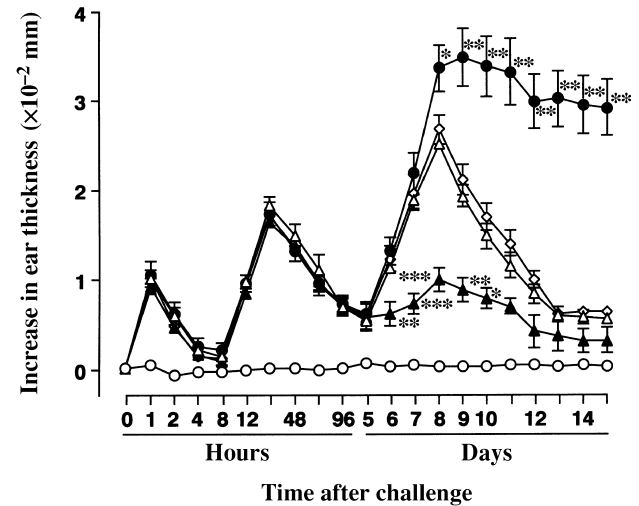


Fig. 3 Effects of anti-CD4 and anti-CD8 antibodies (1 mg each) on the IgE-mediated cutaneous reaction in BALB/c mice. Data are the mean $\pm$ SEM of three to six mice. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with the 2,4-dinitrofluorobenzene (DNFB) group (Student's t -test). (○), vehicle; (△), DNFB; (●), anti-CD4 + DNFB; (▲), anti-CD8 + DNFB; (◇), rat IgG + DNFB.

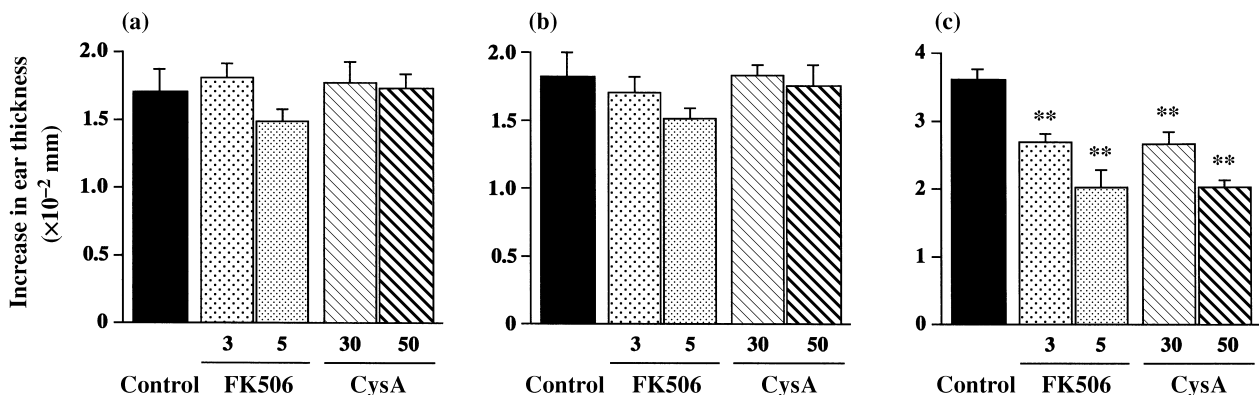


Fig. 2 Effects of FK 506 and cyclosporin A (CysA), given in mg/kg, on the IgE-mediated cutaneous reaction in BALB/c mice at (a) 1 h, (b) 24 h and (c) 8 days. Mice received an intravenous application of anti-dinitrophenol (DNP) IgE antibody 24 h before the skin test. Drugs (FK 506 3 and 5 mg/kg; CysA 30 and 50 mg/kg) were administered twice orally 1 h prior to and 72 h after challenge. Data are the mean $\pm$ SEM of six mice. ** P < 0.01 between the control group and the FK 506- or CysA-treated group (Dunnett's test).

suppressed the vLPR. In addition, the vLPR was prolonged by treatment with the anti-CD4 antibody.

In histopathologic studies, inflammatory signs, including inflammatory cell (mainly lymphocyte) infiltration, vasodilation and the thickness of epidermal cells, decreased after anti-CD8 antibody treatment (Fig. 4).

The next experiment was conducted to clarify the role of T helper (Th) 2 cytokines in this reaction, so IL-4 and IL-5R α gene-deficient mice were used. As shown in Fig. 5a, IL-4 gene-deficient mice showed slightly lowered responses of the IPR, the LPR and the vLPR. In addition, IL-5R α gene disruption did not affect the development of the triphasic cutaneous reaction (Fig. 5b).

In histopathologic studies, a moderate decrease in the number of inflammatory cells (especially eosinophils) in the skin lesion was observed in IL-5R α gene-deficient mice. However, other changes were similar between skin lesions in wild-type and gene-deficient mice (data not shown).

DISCUSSION

In the present study, we demonstrated the phenotypes of the T cells in IgE-dependent triphasic skin reactions. To confirm the role of T cells in the vLPR, T cell-deficient (nu/nu) mice were used. No vLPR was observed in mice,

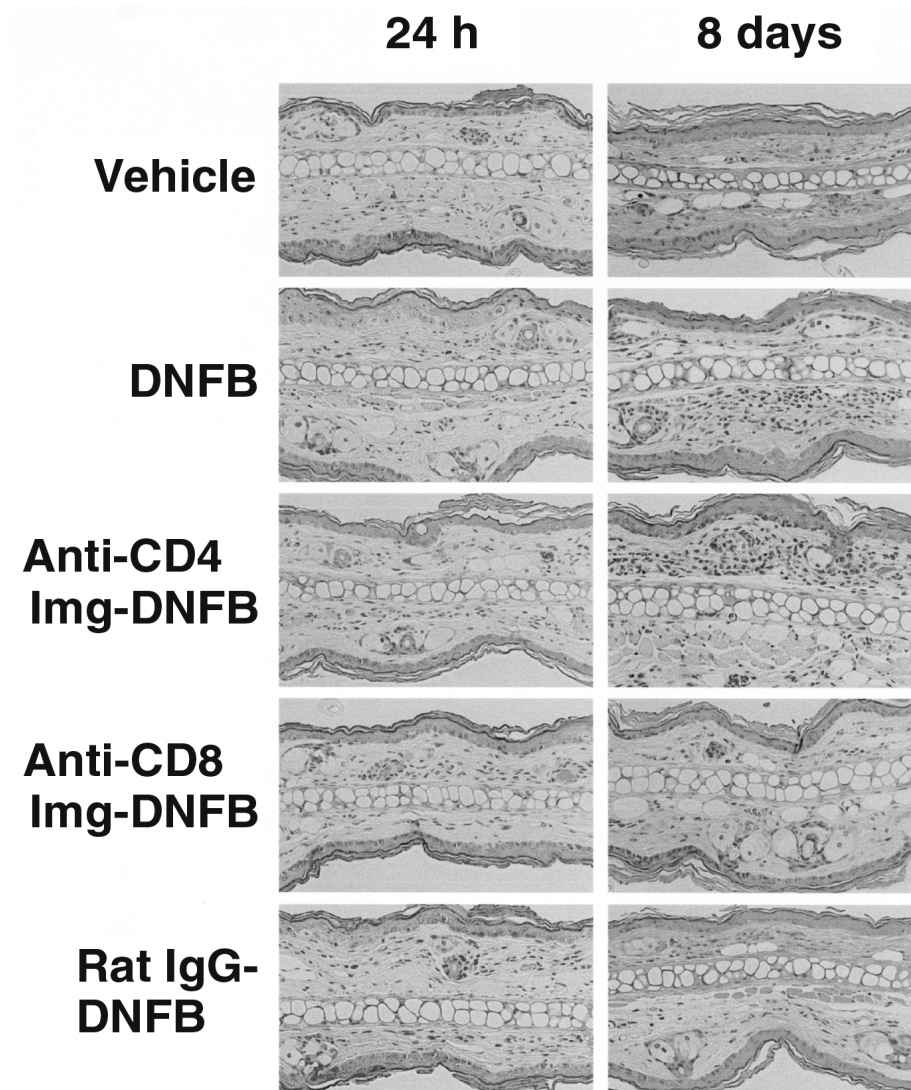


Fig. 4 Effects of anti-CD4 and anti-CD8 antibodies on histopathologic changes caused by the IgE-mediated cutaneous reaction in BALB/c mice (original magnification $\times 100$; H&E). DNFB, 2,4-dinitrofluorobenzene.

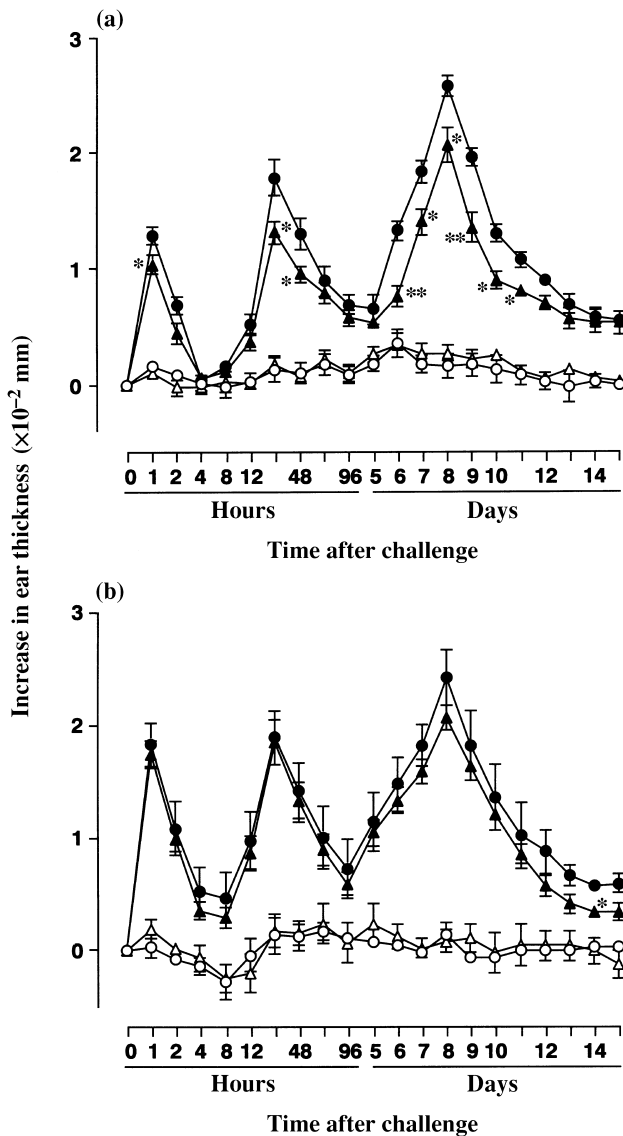


Fig. 5 Time-course study of the IgE-mediated cutaneous reaction in (a) interleukin (IL) 4-knockout, (b) IL-5 receptor α (IL-5R α) chain-knockout and wild-type mice. (○), nonsense wild-type mice; (●) sense wild-type mice; (△), nonsense (a) IL-4 or (b) IL-5R α chain-knockout mice; (▲), sense (a) IL-4 or (b) IL-5R α chain-knockout mice. Data are the mean $\pm$ SEM of three to six mice. * P < 0.05, ** P < 0.01 compared with the wild-type sense group (Student's t -test).

whereas both the IPR and the LPR were induced, almost equivalent to that seen in $+/+$ mice. Therefore, the vLPR in IgE-dependent triphasic skin reactions is deeply dependent on T cells.

In addition, FK 506 and cyclosporin A suppressed the vLPR but not the IPR and the LPR. These findings confirm the role of T cells in the vLPR.

From the above findings, we analyzed the subclass of T cells that play a role in the vLPR. The T cells are classified into CD4⁺ and CD8⁺ cells on the basis of differences in their surface antigens.^{13–15} Because there is some evidence to suggest the participation of CD4⁺ and CD8⁺ T cells in the onset and development of allergic dermatitis,^{15–19} including atopic dermatitis and psoriasis, we studied the role of CD4⁺ and CD8⁺ T cells in IgE-dependent triphasic skin reactions using neutralizing antibodies. When the anti-CD4 antibody was administered, ear swelling of the vLPR was reinforced. In addition, anti-CD8 antibody lowered the magnitude of vLPR, whereas no effect was observed in the first- and second-phase reactions. These findings suggest that CD8⁺ T cells play an inflammatory role in the onset of the vLPR and CD4⁺ T cells regulate the vLPR. These difference concerning the role of CD8⁺ and CD4⁺ T cells may be related to the low efficacy of FK 506 and cyclosporin A on the vLPR. The pharmacologic activity of these two immunosuppressors on each of the CD4⁺ and CD8⁺ T cells should be investigated in the future.

There is much evidence to suggest an important role for Th2 cells in a certain kind of allergic dermatitis, especially atopic dermatitis, including IgE production and eczema symptoms.^{16,17} However, the present findings suggest that the CD4⁺ T cells may have a suppressive effect on the vLPR in IgE-dependent triphasic skin reactions. From these findings, we examined the IgE-induced triphasic cutaneous response in IL-4-deficient mice. The present results suggest all triphasic reactions were slightly lowered in IL-4 gene-deficient mice and IL-5R α gene disruption did not affect them. These findings suggest a small role for Th2 or Tc2 in the onset of the vLPR.

In addition, although a moderate decrease in the number of infiltrating eosinophils was observed in IL-5R α gene-deficient mice, the increase in ear thickness during the vLPR was not altered. These findings suggest no direct correlation between the number of eosinophils and ear thickness.

In delayed-type hypersensitivity (DTH) reactions, the participation of both CD4⁺ Th1 cells and CD8⁺ T cells are well documented.¹⁸ However, the nature of CD8⁺ T cells is still obscure. There is some evidence to suggest that CD8⁺ T cells, especially Tc1 cells, play an important role in DTH mainly by interferon (IFN)- γ production, but not cytotoxic activity.¹⁹ This evidence suggests the existence of similar mechanisms in the onset of the vLPR. Therefore, further experiments to clarify the effect of IFN- γ neutralizing antibody or IFN- γ gene disruption on the vLPR will be necessary.

Regarding the similarity of spontaneous flare-up phenomena in the human skin and the vLPR, we do not have adequate data to make any comment. At least, we have demonstrated a negligible vLPR in DNFB-treated non-sensitized BALB/c +/+ mice in a previous study.³ Whereas many mechanisms may have contributed to the onset of vLPR, CD8⁺ T cells, probably Tc1 cells, and IgE play an important role in the onset of vLPR.

In the present study, we examined the role of T cells in an IgE-dependent triphasic skin reaction. In the vLPR, CD8⁺ T cells played an important role in promoting the reaction. In addition, Th2 cells may play a minor role in the development of the vLPR and CD4⁺ T cells play a regulatory role in the onset of the vLPR.

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