

Enhanced contact hypersensitivity by delayed T-helper 2 response in BALB/c mice

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ABSTRACT

T-helper (Th) 1/Th2 balance determines the direction of contact hypersensitivity (CHS). To clarify the immunopathogenesis of contact dermatitis, 2,4-dinitrofluorobenzene (DNFB)-induced CHS reaction was compared between the BALB/c and C57BL/6 mice. The two strains were sensitized with DNFB systemically and challenged with DNFB locally. The CHS reaction in BALB/c mice was intense compared with that in C57BL/6 mice at 24 and 48 hours post-DNFB challenge. The dermal lesions were characterized by infiltration of lymphocytes, eosinophils, neutrophils, and macrophages including CD4⁺ and CD8⁺ T cells, and interleukin (IL)-4-producing⁽⁺⁾ and interferon (IFN)-gamma⁺ cells in BALB/c mice. In C57BL/6 mice, the composition of inflammatory cells was same as those in BALB/c mice except for eosinophils, CD4⁺ T cells, and IL-4⁺ cells. There was no increase in the number of mast cells in the two strains. Local and systemic productions of IL-4 and IFN-gamma in BALB/c mice were higher than those in C57BL/6 mice. Although blood IgE values increased in BALB/c mice, but not in C57BL/6 mice, at 48 hours postchallenge, its value was low. The delayed Th2-like response together with Th1-like response in BALB/c mice may induce strong CHS reaction compared with C57BL/6 mice, which may dominantly develop Th1-like reaction. Moreover, mast cell and IgE do not appear to be involved in delayed CHS.

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Key words: Contact dermatitis, contact hypersensitivity, delayed contact hypersensitivity, eosinophil, immunopathogenesis, lymphocyte, mast cell, mouse, Th1, Th2

Allergic contact dermatitis (ACD) is caused by a wide range of chemicals after skin contact clinically. According to a simplified model, the pathogenesis of ACD can be divided into preimmunologic and immunologic phases. The former is characterized by the penetration of a prohaptan into the skin and its subsequent metabolic transformation into a hapten. The immunologic phase involves the interaction of immunocompetent cells, primarily antigen-presenting cells and T cells, and is regulated by a complex network of cytokines.¹

In general, ACD is believed to be mediated by type IV cell-mediated immune reaction. During the induction (afferent) phase, primary T cells' sensitization to the antigen occurs, and, subsequently, by further contact with the antigen, antigen-specific T cells and macrophages induce severe dermal inflammation including erythema and edema.^{2–8}

There are general agreements on the existence of functionally polarized immune responses compromised of T-helper (Th) 1 and Th2 subsets in CD4⁺ T cells.⁹ Th1-type immune responses are characterized

by the production of interferon (IFN)- γ , interleukin (IL)-2, and tumor necrosis factor (TNF)- β and proinflammatory cytokines (e.g., TNF- α), and those cytokines activate macrophages,^{3,4} leading to the formation of cell-mediated immunity. On the other hand, Th2-type immune responses characterized by the production of IL-4, IL-5, IL-6, IL-10, and IL-13 promote production of IgE and IgA, and Th2 cytokines activate eosinophils, mast cells, and basophils.⁹ From the point of view of the Th cell subpopulation, contact hypersensitivity (CHS) reaction in dermis is usually regarded as a Th1 phenomenon based on the evidence that local passive transfer *in vivo* in nonsensitized mice of T-cell lines producing IFN- γ can induce CHS reaction.² In addition, TNF- α , which is mainly produced from macrophages, plays a key role at the effector stage of CHS reaction.⁵ Contradictorily, it has been reported that CHS reaction may be mediated by Th2 cells.^{10–13} For example, CHS reaction developed if mice were transferred with the IL-4-producing immune T-cell population.¹¹ In addition, signal transducer and activation of transcription factor 6, which plays a central role in IL-4- and IL-13-mediated biological responses, deficient C57BL/6 mice, decreased in CHS reaction.¹⁴ Moreover, IgE and mast cells, which mediate immediate allergic response, were involved in the induction of CHS reaction.^{15–17}

Despite extensive studies to identify the molecular and cellular bases of proinflammatory cytokines including Th1 and/or Th2 reactions in the afferent phase,

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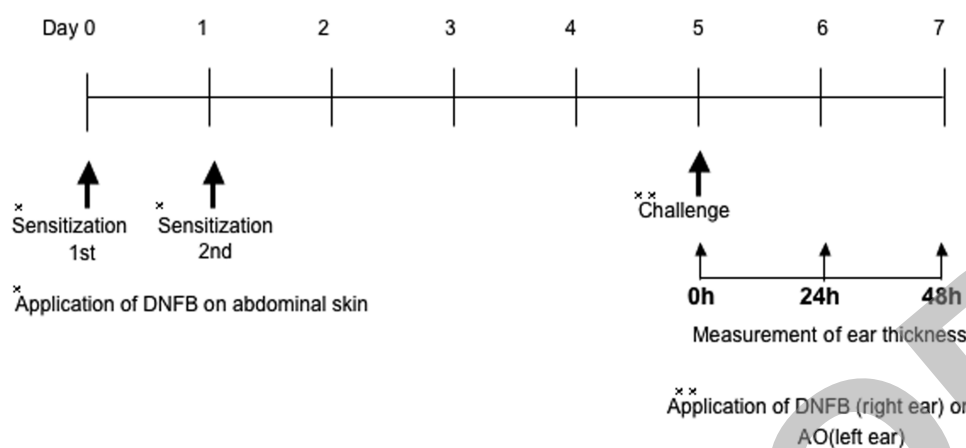


Figure 1. Procedure for induction of contact hypersensitivity by 2,4-dinitrofluorobenzene (DNFB, 0.5%). Ear swelling was measured at 0 (just before the first challenge) and 24 and 48 hours postchallenge of DNFB (0.2%) or acetone-olive oil (AO).

basic inflammatory cell reactions including the Th2 response in the efferent phase of CHS reaction are not well understood. To clarify those points, CHS was induced by 2,4-dinitrofluorobenzene (DNFB), a Th1-predominant allergen¹⁸ in BALB/c mice, which generate a Th2-like immune response, and in C57BL/6 mice, which generate a Th1-like immune response.¹⁹

MATERIALS AND METHODS

Mice

Specific pathogen-free 8-week-old male BALB/c and C57BL/6 mice (total number of mice, $n = 12$ in BALB/c mice and $n = 10$ in C57BL/6 mice) were obtained from SLC Japan Co. (Shizuoka, Japan). The number of mice used in each set of experiments was given in parenthesis. They were kept at $25 \pm 2^\circ\text{C}$ room temperature, $55 \pm 10\%$ humidity, and a 12-hour light/dark cycle (lighting time, 8:00 A.M.–8:00 P.M.) in metal cages sterilized by heating and given autoclaved pellets (CA-1; Clea Japan, Inc., Tokyo, Japan) and water freely. The animal experiments were approved by the Animal Research Ethics Board of the Faculty of Agriculture, Yamaguchi University.

Sensitization and Elicitation Procedures

CHS reaction was induced by the method described previously (Fig. 1).²⁰ In brief, mice ($n = 10$ in each of the two strains) were sensitized to $20 \mu\text{L}$ of 0.5% (w/v) DNFB (Wako, Tokyo, Japan) in 4 in 1 acetone-olive oil (AO; Sigma, St. Louis, MO) on the shaved abdominal skin by two daily applications. Five days after the first sensitization, the ear-swelling assay was performed with DNFB challenge. Mice were challenged on the dorsal and ventral surface of each ear with $10 \mu\text{L}$ of 0.2% DNFB in 4 in 1 AO (right ear) or 4 in 1 AO as control (left ear). The thickness of the right and left ears was measured at 0 hours (just before challenge) and 24 and 48 hours after challenge by a dial thickness gauge

(Ozaki MFG, Co., Tokyo, Japan) and recorded for each ear. The thickness (right ear minus left ear) was expressed as the mean $\pm$ standard error (SEM), in units of 10^{-2} mm.

Sampling

Samples were obtained from both strains of mice. Plasma was obtained at just before the first sensitization and 24 and 48 hours postchallenge for IgE assay ($n = 10$). At 24 and 48 hours postchallenge, after observation of macroscopic changes, all mice were killed by overdose of chloroform. Left and right ears were sampled for histopathology at 24 and 48 hours ($n = 10$ at each time point), and right ears were obtained for immunohistochemistry at 24 hours ($n = 5$) in each strain of mouse. Spleen ($n = 7$) and both sides of the cervical lymph node ($n = 7$) were obtained for cytokine assay (IL-4 and IFN- γ) at 24 hours postchallenge. Tissues were fixed in neutral buffered formalin (pH 7.4) for histopathology and a part of the tissues was frozen in chilled n -hexane (-80°C) for immunohistochemistry.

Histopathology

Longitudinal paraffin sections of both ears were stained by hematoxylin and eosin (H&E), Giemsa, and toluidine blue (pH 3.0). The number of different types of leukocyte was counted on longitudinal sections of 1 cm wide including dorsal and ventral lesions by two different observers.

Immunohistochemistry

Frozen sections were made by cryostat and fixed in acetone. Sections were treated with $10 \mu\text{L}$ of 5% skim milk (Morinaga Co., Tokyo, Japan) mixed with bovine albumin fraction V (Sigma-Aldrich Co., St. Louis, MO) in phosphate-buffered saline (PBS; pH 7.4) to block nonspecific antibody binding and then reacted with following antibodies. Fluorescein isothiocyanate (FITC)–

labeled rat monoclonal antibody (mAb) against mouse CD4 (50 $\mu\text{g}/\text{mL}$, L3/T4; Cedarlane, Ontario, Canada), FITC-labeled rat mAb against mouse CD8 α (20 $\mu\text{g}/\text{mL}$; Cedarlane), and FITC-labeled rat mAb against mouse macrophages (15 $\mu\text{g}/\text{mL}$; Inter-Cell Tech., Hoswell, NJ) were used. As an indirect immunofluorescence method, rat mAb to mouse IFN- γ (50 $\mu\text{g}/\text{mL}$; Genzyme Corp., Cambridge, MA) and rabbit anti-mouse IL-4 polyclonal antibody (100 $\mu\text{g}/\text{mL}$; Biosource International, Camarillo, CA) were used as primary antibody. For secondary antibodies, FITC-labeled goat antibody against rabbit IgG antibody (37.5 $\mu\text{g}/\text{mL}$; Leinco Technol. Int., Manchester, England) and FITC-labeled goat antibody against rat IgG antibody (37.5 $\mu\text{g}/\text{mL}$; Biosource International) were used as secondary antibody. As positive staining control, frozen sections of spleens from normal male BALB/c mice ($n = 2$) were used, and reaction without primary antibody was done in the control of indirect immunofluorescence. All reactions were prepared for 30 minutes at room temperature.

Isolation of Splenic and Cervical Lymph Node Cells

Cells were obtained by the method described previously.²¹ In brief, aseptically removed spleens and lymph nodes were placed in phosphate-buffered-balanced salt solution (PBBS; pH 7.4) containing 0.1% heat-inactivated fetal calf serum (FCS; Gibco, Grand Island, NY). Single-cell suspension was made by teasing tissues apart with scissors and passing through a disposable syringe with a 21G needle (Terumo Co., Tokyo, Japan). Cell suspensions were collected in sterile conical tubes (Fisher Scientific, Pittsburgh, PA) after red blood cells were hemolyzed by Tris-buffered NH_4Cl solution and washed in PBBS followed by centrifugation at $800 \times g$ for 10 minutes at 4°C . Cells were counted using a hemocytometer and diluted in Dulbecco's modified Eagles's medium (DMEM; KC Biologic, Lenexa, KS) with 5% FCS to a density of 5×10^6 splenic cells/mL or 5×10^5 lymph node cells/mL. Viability of cells was $>95\%$ by trypan blue dye exclusion test. Splenocytes (1×10^6 cells in 200 μL DMEM with FCS) or lymph node cells from the challenged and unchallenged sites, respectively (1×10^5 cells in 200 μL of DMEM with FCS), were cultured for 48 hours at 37°C in 5% atmosphere with 10 μL of concanavalin A (ConA; 2.2 μg ; ICN Biomedicals Inc., Aurora, OH) in DMEM and supernatants were obtained and stored at -80°C until use.

Measurements of IFN- γ , IL-4, and IgE by ELISA

The concentration of IL-4 and IFN- γ (Techre Co., Inc., Minneapolis, MN) in supernatants from lymph node

and splenic cells and blood IgE (Morinaga) was measured by mouse ELISA kits. The minimal detectable concentration was 1.2 pg/mL for IFN- γ , 2.0 pg/mL for IL-4, and 500 pg/mL for IgE. If cytokines were not detected, their values were estimated as 0.

Statistical Analysis

The data are expressed as the mean of mice examined $\pm$ SEM. Unpaired Student's t -test was used to evaluate the significance of differences; a value of $p < 0.05$ was considered significant.

RESULTS

Kinetics of Ear Swelling after DNFB Challenge in BALB/c and C57BL/6 Mice

As shown in Fig. 2, ear swelling prominently developed in BALB/c mice compared with C57BL/6 mice at 24 hours ($15.0 \pm 2.5 \times 10^{-2}$ mm versus $3.8 \pm 0.7 \times 10^{-2}$ mm) and at 48 hours ($10.6 \pm 2.3 \times 10^{-2}$ mm versus $1.9 \pm 0.6 \times 10^{-2} \times 10^{-2}$ mm; $p < 0.01$, respectively) postchallenge. In addition, CHS reaction in BALB/c mice increased at 24 hours ($p < 0.01$) and 48 hours ($p < 0.05$) postchallenges compared with 0 hours, whereas that in C57BL/6 mice increased at 24 hours ($p < 0.05$) but not at 48 hours, postchallenge compared with 0 hours.

Macroscopic and Histopathological Changes of Ear Dermal Lesions in BALB/c and C57BL/6 Mice

Prominent dermal hyperemia and edema in BALB/c mice (Fig. 3 *a*, insert) compared with C57BL/6 mice (Fig. 3 *b*, insert) were observed at 24 and 48 hours postchallenges. Histologically, prominent congestion and edema; diffuse infiltration of lymphocytes, neutrophils, macrophages, and eosinophils (Fig. 3 *c*) in dermis and subcutaneous tissues were seen in BALB/c mice. Degranulation of eosinophil granules was sometimes detected. Attachment of those leukocytes to swelled endothelial cells was often seen in capillaries and venules in dermis and subcutaneous tissues. The epidermis was hyperplastic and hypertrophic. In some parts, edema; vesicle formation; microabscess; and infiltration of lymphocytes, neutrophils, and eosinophils in the epidermal layer were recognized. Necrotic changes with or without hemorrhage were rarely seen in the dermis and subcutaneous tissues. On the other hand, inflammation in C57BL/6 mice (Fig. 3 *b*) was less severe compared with BALB/c mice, and infiltrated cell type was mainly lymphocytes and macrophages with some neutrophils and a few or no eosinophils (Fig. 3 *d*). Although mast cells with swelling in the dermis and subcutaneous tissues were observed in both strains of mice (Fig. 3, *e*, *f*, *h*, and *i*), their degranulation was not prominent and eosinophils

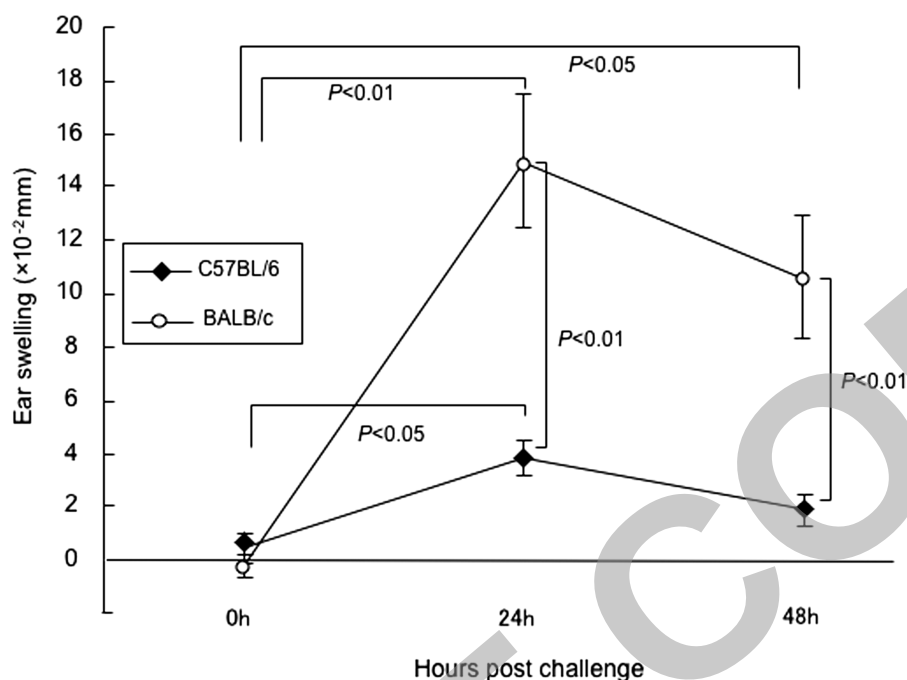


Figure 2. Kinetics of contact hypersensitivity reaction to 2,4-dinitrofluorobenzene (DNFB) in BALB/c and C57BL/6 mice post-DNFB challenge. Each point represents the mean $\pm$ SEM.

around mast cells were not observed. In addition, There was no difference in the number of mast cells between the challenged and unchallenged ears in each group (Fig. 3, e-j).

CD4⁺ and CD8⁺ T Cells, IL-4⁺ and IFN- γ ⁺ Cells, and Macrophages in CHS Reaction at the Site of DNFB Challenge in BALB/c and C57BL/6 Mice

CD4⁺ (Fig. 4 a) and CD8⁺ (Fig. 4 c) T cells, IL-4⁺ (Fig. 4 e) and IFN- γ ⁺ (Fig. 4 g) cells, and macrophages (Fig. 4 i) were mainly detected in BALB/c mice. On the other hand, a small number of CD4⁺ (Fig. 4 b) and CD8⁺ (Fig. 4 d) T cells, IFN- γ ⁺ cells (Fig. 4 h), and macrophages (Fig. 4 j) were observed, and there was no or a few infiltration of IL-4⁺ cells (Fig. 4 f) in C57BL/6 mice.

Number of Infiltrated Cells at the Site of DNFB Challenge in CHS Reaction in BALB/c and C57BL/6 Mice

The number of lymphocytes and macrophages, neutrophils, and eosinophils was significantly increased in BALB/c mice compared with C57BL/6 mice at 24 and 48 hours after challenge (Fig. 5; $p < 0.01$ and $p < 0.05$, respectively). Also, the number of each cell type increased at the challenged site compared with the unchallenged site in each strain of mice except for eosinophils at 24 and 48 hours postchallenge in C57BL/6 mice. There was no increase in the number of mast cells in each strain of mouse at the challenged site compared with the unchallenged site.

Cytokine Productions from Splenic and Lymph Node Cells Stimulated with ConA at 24 Hours Post-DNFB Challenge in BALB/c and C57BL/6 Mice

In the supernatants of splenic cells, production of IL-4 (76 ± 16 pg/mL; Fig. 6 a) and IFN- γ (1176 ± 222 pg/mL; Fig. 6 b) increased in BALB/c mice compared with those of IL-4 (5 ± 2 pg/mL; Fig. 6 a) and IFN- γ (162 ± 51 pg/mL; Fig. 6 b) in C57BL/6 mice ($p < 0.01$). In the supernatants of lymph node cells, productions of IL-4 (3.1 ± 1.1 pg/mL; Fig. 6 c) and IFN- γ (4.1 ± 1.2 pg/mL; Fig. 6 d), respectively, increased in BALB/c mice compared with those of IL-4 (0 pg/mL; Fig. 6 c) and IFN- γ (2.9 ± 0.5 pg/mL; Fig. 6 d) in C57BL/6 mice, although there was no statistical difference. In addition, IFN- γ production from the challenged site increased compared with the unchallenged site in C57BL/6 mice ($p < 0.05$).

IgE Production in Blood before and Post-DNFB Challenge in BALB/c and C57BL/6 Mice

IgE values increased at 48 hours postchallenge compared with at 0 and 24 hours ($p < 0.01$) in BALB/c, but not in C57BL/6 mice (Fig. 7). Also, IgE values were higher in BALB/c mice (5.1 ± 0.4 ng/mL) than those in C57BL/6 mice (2.7 ± 0.5 ng/mL) at 48 hours postchallenge ($p < 0.01$).

DISCUSSION

The present study showed that delayed CHS reaction developed in both strains of mice. However, it was

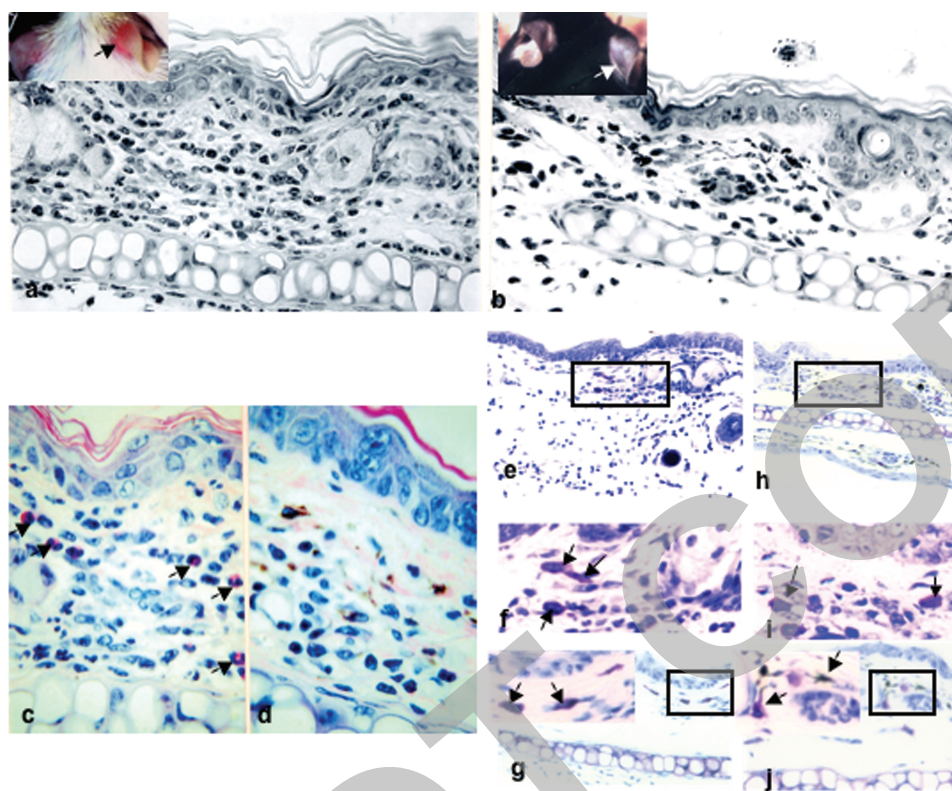


Figure 3. Contact hypersensitivity (CHS) reaction at 24 hours after challenge. At the site of DNFB challenge, an arrow indicates prominent swelling of the right ear with erythema in a BALB/c mouse (a; insert, an arrow), whereas CHS reaction is slight with less erythema in a C57BL/6 mouse (b; insert, an arrow). Infiltration of eosinophils (arrows), neutrophils, lymphocytes, and macrophages in a BALB/c mouse (a), and that of lymphocytes and macrophages in a C57BL/6 mouse (b) are recognized in edematous dermis. Arrows in c indicate eosinophils in BALB/c mice, whereas there is no infiltration of eosinophils in C57BL/6 mice (d). Mast cells in e and f (enlargement of a rectangle of e; arrows) at the site of challenge and those in g and insert (enlargement of g; arrows) at the site of unchallenged in BALB/c mice. On the other hand, mast cells in h and i (enlargement of a rectangle of h; arrows) at the site of challenge and those in j and insert (enlargement of a rectangle of j; arrows) at the site of unchallenge in C57BL/6 mice. (a and b) hematoxylin and eosin (H&E), $\times 300$; (c and d) Giemsa, $\times 450$; (e–j) toluidine blue (TB; e, g, h and j, $\times 150$; f, insert of g, i and insert of j, $\times 450$). Magnification is original.

stronger in BALB/c mice compared with C57BL/6 mice. In BALB/c mice CHS reactions may be induced by Th2-like reaction together with Th1-like reaction, whereas C57BL/6 mice may dominantly develop Th1-like CHS reaction; and the most important finding is that mast cell reaction may not be involved in CHS reaction in the efferent phase in both strains of mice.

The development of Th2-like CHS reaction in BALB/c mice is considered as follows. First, locally increased IL-4 production (Th2 cytokine) from lymph node cells, which are the same side of DNFB challenge, was recognized. Second, there was infiltration of eosinophils, which is induced by Th2 cells.⁹ Third, infiltration of IL-4⁺ cells and IL-4 production from regional lymph node cells were detected. Fourth, a relatively large number of neutrophil infiltrations were observed. It is possible, because it has been reported that they may play an important role of early step in the elicitation and maintenance of Th2-dependent skin inflammation.^{22,23} On the other hand, Th1-like reaction in

BALB/c mice may also develop in some degree, because there was infiltration of macrophages, lymphocytes, and IFN- γ ⁺ cells. Moreover, IFN- γ production by regional lymph node cells at the challenged site was recognized. It has been reported that lymphocytes secreting IFN- γ act as the effector cells,⁷ including activation and proliferation of CD4⁺ Th1 cells⁴ and the skin inflammation in mice deficient in CD8⁺ cytotoxic effector T cells did not develop CHS reaction.²⁴ Taken together, CD4⁺ T cells may be composed of Th1 and Th2 cells and combined action of Th1-like and Th2-like responses may operate in CHS reaction in BALB/c mice, although they are genetically representative of the Th2-dominant strain.¹⁹ This suggests that CHS reaction can be determined by antigen and host interaction. For example, repeated topical application of a hapten may result in shift of a Th1 to a mixed response of Th1 and Th2 in BALB/c mice, suggesting that a strain's Th1/Th2 predominance may be also not be definitive in CHS reaction.²⁵ On the other hand, Th1-like

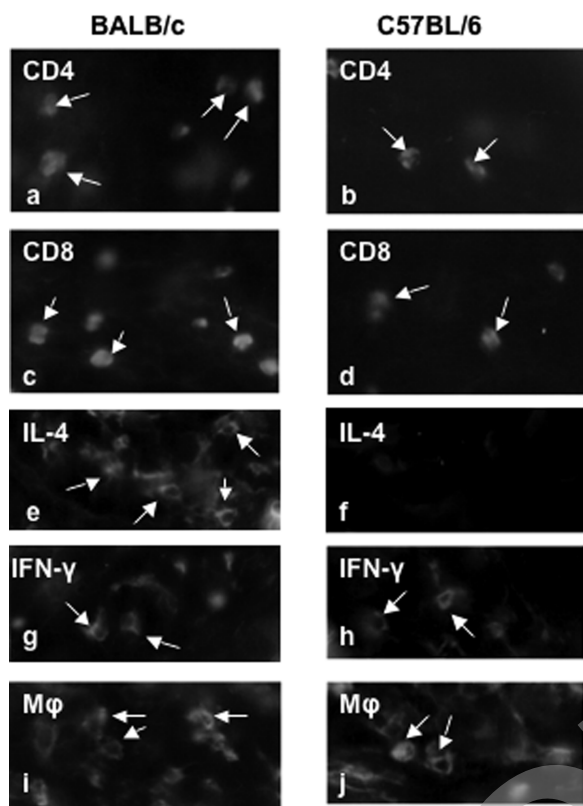


Figure 4. Immunohistochemistry indicates (arrows) CD4⁺ T cells (a and b), CD8⁺ T cells (c and d), IL-4⁺ cells (e and f), IFN- γ ⁺ cells (g and h), and macrophages (i and j) in BALB/c (a, c, e, g, and i) and C57BL/6 (b, d, f, h, and j) mice. (a–j) Immunofluorescence, $\times 450$ (original magnification).

CHS reaction may develop in C57BL/6 mice, because there was an increase in local production of IFN- γ but not IL-4 by lymph node cells at the site of DNFB challenge, and infiltration of macrophages, IFN- γ ⁺ cells, and CD4⁺ T cells, which may be Th1 cells, and CD8⁺ T cells were mainly recognized, whereas there were no or a few IL-4⁺ cells and eosinophils. In the present study, we did not examine any correlation between the IL-4 and IFN- γ concentrations and the severity of the cutaneous cellular infiltration. Additional study is needed to clarify this point including expression of adhesion molecules on infiltrated leukocytes.

The intensity of irritant contact dermatitis using DNFB was stronger in C57BL/6 mice compared with BALB/c mice.²⁶ This discrepancy between the present study and previous studies may be due to the methodological differences (e.g., a single ear paint at different DNFB concentrations). It seems likely, because Morokata et al. have reported that antigen dose defines Th1 and Th2 responses in the allergic airway inflammation of C57BL/6 and BALB/c mice and that local and systemic immune responses occurred independently, although DNFB is one of Th1-dependent anti-

gens.²⁷ Also, different results from the present study have been observed in BALB/c mice with 2,4-dinitrochlorobenzene (DNFB) by long-term dermal sensitization followed by intratracheal challenge.²⁸ This may be caused by the difference of irritant used other than the difference in route of treatment. In addition, DNFB induced weak CHS reaction compared to DNFB within the same BALB/c mice strain (our unpublished data, 1991). Moreover, we have reported recently that acute urticaria-like dermal lesions in ovalbumin-unexposed cutaneous tissues in a mouse model of late allergic rhinitis and we have suggested that immediate allergic reaction due to systemic development of Th2-type response may be involved in the development of dermal lesions.²⁸ These suggest that several endogenous factors also may influence the development of dermatitis including CHS.

Although swelling of mast cells were observed at the site of DNFB challenge in both strains of mice, their role may be minor, because there was no increased infiltration of those cells compared with the unchallenged site. Also, no degranulation of mast cells and infiltration of eosinophils around mast cells were recognized. It seems likely, because we have reported recently that there are increased numbers of mast cells with degranulation accompanied with infiltration of eosinophils in urticaria-like lesions.²⁹ Alternatively, CHS reaction developed at 24 hours post-DNFB challenge at the time of a slight IgE elevation. Furthermore, plasma cells were not detected at the site of challenge. Thus, IgE may not play CHS reaction and its increased value may be DNFB nonspecific, because specific IgE could not be detected at 1 week after the initial sensitization with hapten even in Th2-dominant BALB/c mice.²⁵ Thus, elevated IgE may be induced by systemic IL-4 production being responsible for the development of Th2 reaction. Taken together, delayed type, but not immediate type, of Th2-like response may occur in BALB/c mice.

The controversial results are reported in terms of the role of mast cells in CHS reaction. For example, mast cell-deficient (WBB6F1-W/W^v) mice generate Th2 response that favors local eosinophil recruitment in response to antigens.^{30,31} Others have shown IgE-mediated mast cell activation is required for full CHS response^{17,32,33} and they found that the magnitude of late-phase reaction including ear edema and the accumulation of eosinophils was significantly lower in WBB6F1-W/W^v mice than those in congenic control mice.³¹ The reasons for this discrepancy, at present, are unknown. Several reasons are considered as follows. CHS reaction, in terms of Th1 and/or Th2 predominance including mast cell response in CHS reaction is presumed to be dependent on several factors. For example, it has been reported that different conditions (e.g., exposure to different dose of allergen, frequency

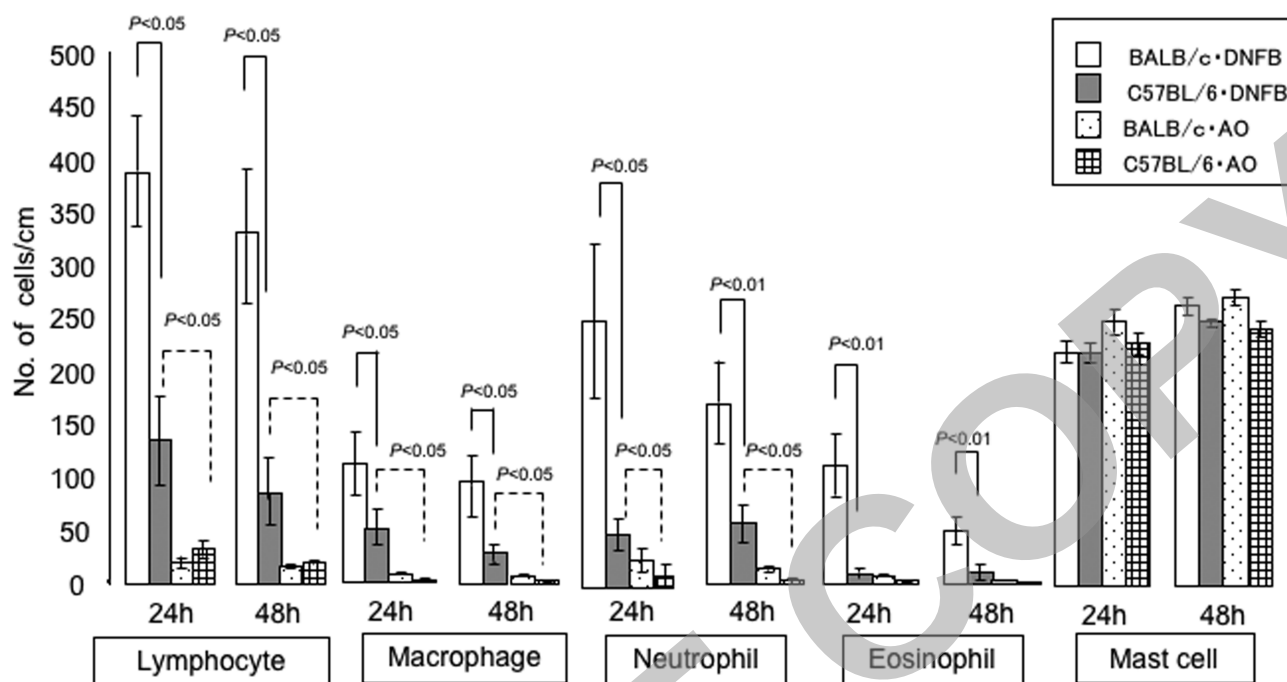


Figure 5. Composition of infiltrated cells in contact hypersensitivity reaction of BALB/c and C57BL/6 mice at 24 and 48 hours post-DNFB challenge. Each point represents the mean $\pm$ SEM.

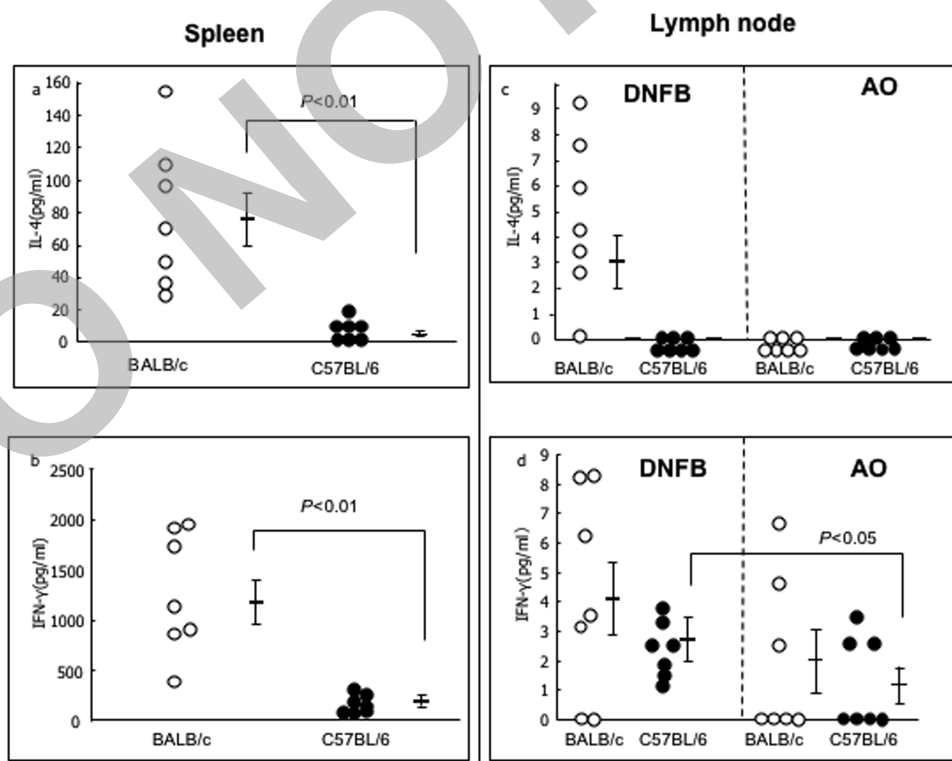


Figure 6. Cytokine values in BALB/c and C57BL/6 mice 24 hours after DNFB challenge. Each point represents the mean $\pm$ SEM.

of exposure, time course after chemicals, and sex difference) induce different Th2 responses in the development of immune-mediated diseases, even within the same mouse strain.³³ Second, we have reported previ-

ously that the number of mast cells is quite different in their location in the skin²⁷ and the number of mast cells are a few in an ear compared with other skin sites, suggesting different response may occur at the skin

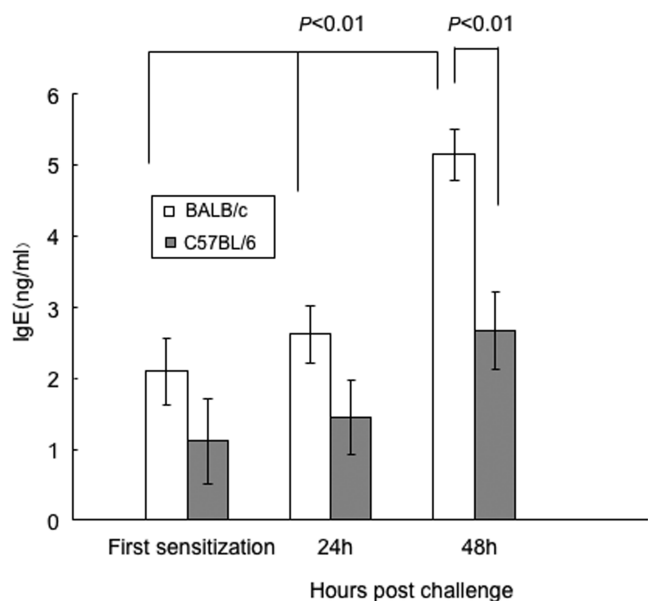


Figure 7. IgE values in both strains of mice at the first sensitization, and 24 and 48 hours postchallenge. Each point represents the mean $\pm$ SEM.

examined. The most likely is that the CHS reaction may include different immune reactions by several factors discussed previously except for the CHS reaction, which is the delayed type.

Interestingly, the present study suggests that Th2-like response in the CHS reaction in BALB/c mice may be induced by delayed type similar to the late allergic airway response (asthma).³⁴ Moreover, the present observations may have important implications for diagnosis of antigen-specific contact dermatitis, because different degrees of CHS reaction may be induced by genetic factors, even though the antigen is identical.

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