

Immediate contact hypersensitivity induced by repeated hapten challenge in mice

MASARU NATSUAKI, NORIKO YANO, KYOKO YAMAYA AND YUKIO KITANO
Department of Dermatology, Hyogo College of Medicine, Hyogo, Japan

An immediate reaction was investigated during repeated challenge testing for contact hypersensitivity to dinitrofluorobenzene (DNFB) in BALB/c mice. The mice were sensitized to DNFB on back skin and repeatedly challenged with the same hapten on the left ear at 1 week intervals. The ear after the 5th challenge showed biphasic responses which consisted of an immediate and a delayed-type reaction. The reactions were hapten specific. Mast cell-deficient WBB6F1 W/W^V mice did not show any immediate reaction, while congenic normal mice showed both immediate and delayed-type reactions. Histologically, numerous dermal mast cells were found in the left ear of repeatedly challenged BALB/c and WBB6F1 normal mice, while there were few mast cells in the ear of WBB6F1 W/W^V mice. Anti-DNP IgE antibodies were detected in BALB/c, WBB6F1 normal and W/W^V mice after repeated challenge with DNFB. Intradermal injection of anti-IgE antibodies in the repeatedly DNFB-challenged ear elicited an immediate reaction. These results suggest that immediate contact hypersensitivity develops through the production of anti-DNP IgE antibodies and an increase in dermal mast cells after repeated challenge with DNFB.

Key words: mouse; contact hypersensitivity; immediate reaction; mast cells; anti-DNP IgE antibodies. © Munksgaard, 2000.

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Contact hypersensitivity (CH) is a type of delayed hypersensitivity reaction that is caused by percutaneous penetration of a hapten and a cellular immune response, associated with T lymphocytes, to the hapten (1). Our previous studies have shown that local immunological memory of CH is induced at affected sites and persists for a period as long as 1 year in mice (2, 3). We also reported that both the severity and memory of CH were amplified by repeated hapten challenge at the same site (4). These data may explain the clinical observation that skin lesions of chronic contact dermatitis are more readily exacerbated by stimulation with a specific antigen than intact skin.

However, in a study of repeated hapten challenge in mice, we found an immediate contact hypersensitivity (ICH) reaction, which appeared after several min to 1 h, in addition to the delayed-

ed-type reaction (DTR) following challenge (5). This observation indicated that repeated exposure to a hapten can induce contact urticaria as well as contact dermatitis. In the present study, we determined that dermal mast cells at the repeatedly challenged site play a key rôle in the ICH in mice.

Materials and Methods

Mice

BALB/c and WBB6F1 (+/+ and W/W^V) female mice aged 6 to 8 weeks were purchased from Charles River Japan, Inc. (Kanagawa, Japan) and Japan SLC (Hamamatsu, Japan), respectively. Each experimental group consisted of 5 mice. All experiments were repeated at least 2× with similar results.

Chemicals

The following haptens were used: 2,4-dinitrofluorobenzene (DNFB, Wako Pure Chemicals, Osaka, Japan) and 4-ethoxymethylene-2-phenyl-2-

Abbreviations: CH: contact hypersensitivity; DNFB: 2,4-dinitrofluorobenzene; DNP: dinitrophenyl; DTR: delayed-type reaction; ICH: immediate contact hypersensitivity; Ox: 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; PCA: passive cutaneous anaphylaxis.

oxazolin-5-one (Ox, Wako Pure Chemicals, Osaka, Japan).

Induction of contact hypersensitivity

Mice were sensitized on shaved back skin with 25 μ l of 0.5% DNFB in a 4:1 acetone: olive oil solution (A/O) or 50 μ l of 5% Ox in A/O on day 0. On days 5, 12, 19, 26, 33, each left ear was repeatedly challenged with 20 μ l of 0.2% DNFB or 0.8% Ox in A/O. The ear thickness just before and after the last challenge was measured with a dial thickness gauge (Ozaki Co. Ltd, Tokyo, Japan), and the difference was defined as ear swelling and expressed in units of 10^{-2} mm (mean $\pm$ SEM). In the time-course study, the ear thickness was measured at various times after challenge.

Dermal mast cell count

The ears of mice were amputated at various times after the last challenge, fixed in formalin, and embedded in paraffin. Thin ear sections (4 μ m) were stained with toluidine blue and dermal mast cells were counted by microscopic observation ($\times 400$) of the central region of each ear section. The number of dermal mast cells was defined as the mean $\pm$ SD calculated after observation of 10 ear sections.

Measurement of serum anti-DNP IgE antibody

Serum was collected from the mice at various times after the last challenge and was stored at -70°C until just before use. The anti-dinitrophenyl (DNP) IgE antibody titer in serum was measured by the passive cutaneous anaphylaxis (PCA) method. For this test, 100 μ l of each serum diluted with saline was injected intradermally into the shaved back skin of male F344 rats (12 weeks old). After 24 h, DNP-bovine serum albumin (1 mg/ml) with 0.25% Evans blue was injected intravenously. The animals were sacrificed 30 min after injection and infiltration of dye into the skin around the injection sites was observed. The PCA titer was expressed as the reciprocal of the maximum serum dilution causing a blue spot more than 5 mm in diameter.

Intradermal injection of anti-IgE or anti-IgG antibodies

Gout anti-mouse IgE and gout anti-mouse IgG antibodies were purchased from Seikagaku Corporation (Tokyo, Japan). Each antibody was injected intradermally into left or right ear of mouse (10 μ l /ear). The ear thicknesses just before injection

and 1 h later were measured and the difference was taken as ear swelling, which was expressed in units of 10^{-2} mm (mean $\pm$ SEM).

Statistical analysis

The statistical significance of differences in the mean values of each experimental group was calculated with the Student *t*-test. Differences were considered to be significant at $p < 0.05$.

Results

ICH is induced at the repeatedly challenged site and is hapten-specific

BALB/c mice were challenged with DNFB to the left ear on days 5, 12, 19, 26, and 33. The changes of ear thickness are shown in Fig. 1. The ear showed a biphasic response which consisted of the immediate reaction and DTR. The ICH reaction peaked at 1 h and the DTR at 24 h after challenge, respectively. In contrast, when the right ears of the mice that had been repeatedly challenged on the left ear were also challenged on day 33, neither immediate reaction nor DTR was recognized. To confirm hapten-specificity, mice that had been repeatedly challenged with Ox to the left ear were finally challenged with DNFB on day 33. As shown in Fig. 1, no reaction was recognized.

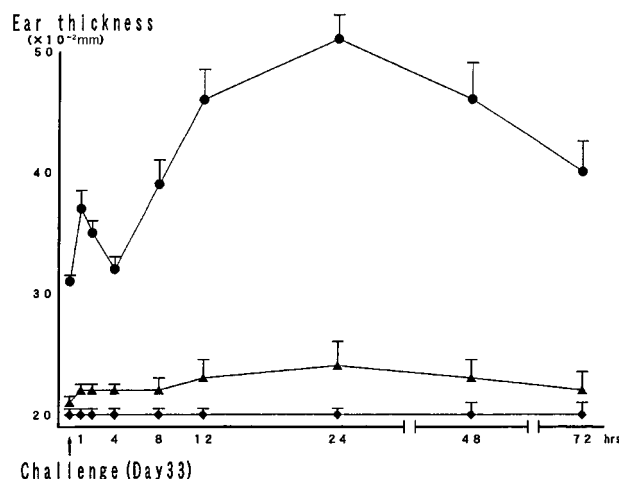


Fig. 1. Time-course study of the ear thickness in BALB/c mice. BALB/c mice were sensitized and repeatedly challenged with DNFB on days 5, 12, 19, 26, 33 on the left ear and firstly challenged on day 33 on the right ear. The ear thickness of each left ear (●) and each right ear (▲) was measured at various times after the last challenge. Ox-sensitized mice were repeatedly challenged with Ox on days 5, 12, 19, 26 and finally challenged with DNFB on day 33. The ear thickness was measured at the same time points (◆).

ICH does not occur at more than 3 months after repeated challenge

BALB/c mice were challenged with DNFB repeatedly and then were challenged with the same hapten at various intervals after the challenge on day 33. The ear swelling responses (immediate reaction and DTR) are shown in Fig. 2, separately. The mice challenged at 1 week or 1 month after day 33 showed both immediate reaction and DTR in ear swelling responses. However, the mice re-challenged at 3 or 6 months after repeated challenge did not show a detectable immediate reaction, but showed only the DTR. In these mice, we carefully checked ear swelling responses from 15 min to 4 h after challenge, but no early-phase reaction was recognized.

Mast cell-deficient mice do not show the ICH

We investigated whether the mast cell-deficient mice showed the ICH or not in our repeated challenge system. WBB6F1 mice were challenged with DNFB on the left ears on days 5, 12, 19, 26, and 33. The changes in ear thickness are shown in Fig. 3. Mast cell-deficient W/W^V mice did not show the immediate reaction, while congenic normal (+/+) mice showed both the immediate reaction and the DTR.

Number of dermal mast cells and serum anti-DNP IgE antibody level in various experimental groups

In the experimental groups described above, we evaluated the number of dermal mast cells and the

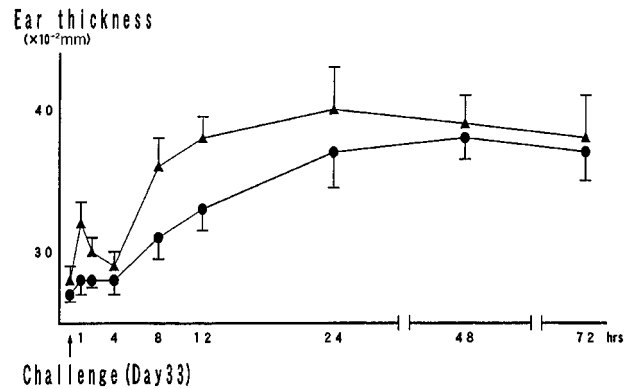


Fig. 3. Time-course study of the ear thickness in WBB6F1 mice. WBB6F1 mice were sensitized and repeatedly challenged with DNFB on days 5, 12, 19, 26, 33 on the left ear. The ear thickness in the congenic normal (+/+) (▲) or W/W^V (●) mice was measured at various times after the last challenge.

serum level of anti-DNP IgE antibodies. As shown in Table 1, numerous dermal mast cells were found in the ears of repeatedly challenged BALB/c and WBB6F1 +/+ mice, while there were few mast cells in WBB6F1 W/W^V mice. Table 1 also shows that the number of dermal mast cells decreased during the interval after the last challenge on day 33. The serum titer of anti-DNP IgE antibodies in mice repeatedly challenged with DNFB was 40 to 80 even in WBB6F1 W/W^V mice or in mice at 6 months after repeated challenge. These data indicate that the development of ITR in CH depends on the number of dermal mast cells.

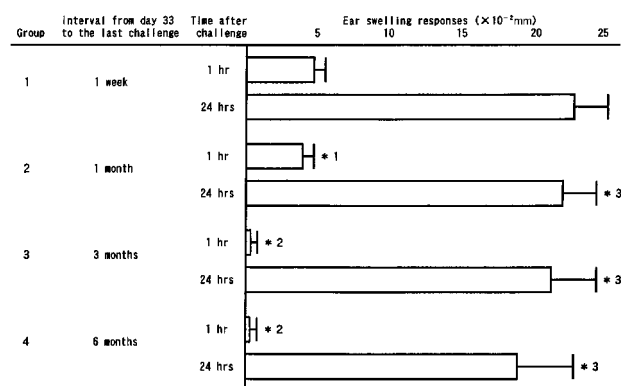


Fig. 2. Ear swelling responses after the last challenge at various intervals to day 33. BALB/c mice were repeatedly challenged with DNFB on days 5, 12, 19, 26, 33 and finally challenged at 1 week, 1 month, 3 months and 6 months after day 33. Ear swelling responses were measured at 1 h (immediate reaction) and 24 h (DTR) after the last challenge. Significant reductions in the immediate reaction were observed in groups 3 and 4 versus group 1 (*2, $p < 0.01$, *1, not significant). In the DTR, significant differences were not seen in groups 2, 3 and 4 versus group 1 (*3, not significant).

Intradermal injection of anti-IgE antibodies induces the immediate reaction in the repeatedly challenged ears

To confirm whether the ICH depends on IgE, we injected anti-IgE or anti-IgG antibodies into the repeatedly challenged ears. The left ears of mice repeatedly challenged with DNFB showed significant ear swelling responses compared with those in the right ears at 1 h after intradermal injection of anti-IgE antibodies, while the left ears did not show significant responses compared with those in the right ears after injection of anti-IgG antibodies (Fig. 4, groups 1, 2). The responses in the negative controls (groups 3, 4) represent non-specific ear edema caused by the injection. These results suggest that the ICH in mice is IgE-dependent. The difference in ear swelling responses between the left and right ears (group 1) also indicates that the development of a detectable immediate reaction depends on the number of dermal mast cells in the ears.

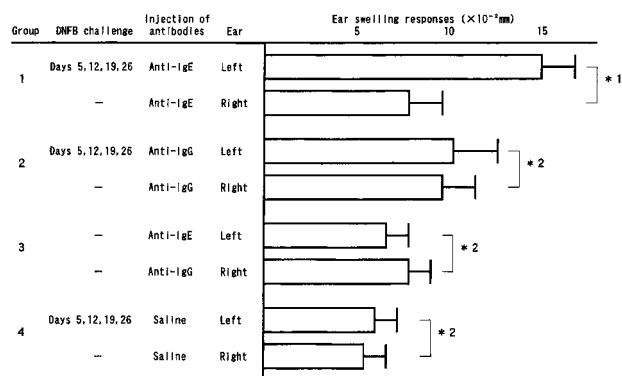


Fig. 4. Ear swelling responses after intradermal injection of anti-IgE or anti-IgG antibodies. Sensitized mice were challenged with DNFB on days 5, 12, 19 and 26 on the left ears. Anti-IgE or anti-IgG antibodies were intradermally injected into either the left or the right ear on day 33. Ear swelling responses were measured at 1 h after the injection. Groups 3 and 4 are negative controls. In group 1, significant responses were recognized in the left ears compared with the right ears (*1, $p < 0.05$, *2, not significant).

Discussion

Allergic contact dermatitis occurs in a subject sensitized by a hapten when the skin comes into contact with that hapten. Generally, delayed hypersensitivity underlies the local inflammatory reaction that occurs 1 to 2 days after challenge. The inflammatory reaction is caused by cytokines that are released by antigen-specific, sensitized T-cells (1).

Our present experiments showed that repeated application of a hapten induced not only a DTR but also an ICH. At the repeatedly challenged skin sites, the dermal mast cell count was about $3 \times$ higher than the count in normal mice. Nagai et al. (6) reported that repeated application of a hapten (DNFB) induced IgE production in the regional lymph nodes and the spleen, resulting in an increase in total IgE and hapten-specific IgE in the

serum. We also detected anti-DNP IgE antibodies in the blood of mice to which DNFB was repeatedly applied.

However, the fact that no immediate reaction occurred in the ear without an increase in mast cells suggests that not only IgE antibodies in the blood but also a local increase in mast cells is important for an immediate response to occur. In BALB/c mice, an immediate reaction occurred after the 5th challenge, whereas a 6th challenge after an interval of 3 or 6 months caused only a DTR and no immediate reaction. These animals had an anti-DNP IgE antibody titer comparable to that in mice with ICH, but the dermal mast cell count of the left ear was decreased to 2/3 of that in mice with ICH. It is still unknown to what extent dermal mast cells are required for the development of a detectable immediate reaction.

Our results suggest that a local increase in mast cells is maintained when a hapten is repeatedly applied, but that the mast cell count decreases over time after contact with the hapten ceases. The strong DTR that occurred despite a decreased mast cell count showed that the cell-mediated immune response in our experimental system was not dependent on dermal mast cells. In fact, as we have reported previously, the immunological memory of a CH reaction persists for a prolonged period (3).

According to the study of Van Loveren et al. (7) on the early-phase reaction in murine CH, the 1st challenge after sensitization with a hapten induces an early reaction in advance of a delayed reaction, with the peak of this reaction being around 2 h after application of the hapten. They suggested that an IgE-like T-cell factor is involved in this reaction. Their findings seem to differ from the results obtained in the present study. Our unpublished data demonstrated that an immediate reaction to DNFB was induced when DNFB was challenged on the repeatedly Ox-challenged ear after intradermal injection of the sera from repeat-

Table 1. Dermal mast cell counts and anti-DNP IgE antibodies

Group no.	Strain	Hapten	Challenge	Time of evaluation	Dermal mast cell (mean $\pm$ SD)	Anti-DNP IgE (mean PCA titer)
1	BALB/c	DNFB	—	day 33	12.1 $\pm$ 2.9	5>
2	BALB/c	DNFB	days 5,12,19,26	day 33	38.8 $\pm$ 1.3* ¹	80
3	BALB/c	Ox	days 5,12,19,26	day 33	35.3 $\pm$ 3.8	5>
4	WBB6F1 +/+	DNFB	days 5,12,19,26	day 33	43.5 $\pm$ 7.5* ²	40
5	WBB6F1 W/W ^V	DNFB	days 5,12,19,26	day 33	8.8 $\pm$ 2.1	40
6	BALB/c	DNFB	days 5,12,19,26,33	1 week later from day 33	36.5 $\pm$ 4.2	80
7	BALB/c	DNFB	days 5,12,19,26,33	1 month later from day 33	33.2 $\pm$ 3.5	80
8	BALB/c	DNFB	days 5,12,19,26,33	3 months later from day 33	23.1 $\pm$ 3.7* ³	80
9	BALB/c	DNFB	days 5,12,19,26,33	6 months later from day 33	21.8 $\pm$ 5.7* ⁴	80

*¹ $p < 0.001$, group 1 versus group 2.

*² $p < 0.001$, group 4 versus group 5.

*³ $p < 0.01$, group 7 versus group 8.

*⁴ $p < 0.01$, group 7 versus group 9.

edly DNFB-challenged mice. Thus, a serum factor such as IgE antibody must be associated with the ICH. However, the early reaction observed by Van Loveren et al. (7) and the ICH observed in our study were both dependent on mast cells, so the possibility that both reactions are essentially the same cannot be denied.

Kitagaki et al. (8) showed that frequent application of a hapten causes the peak skin reaction to shift from a delayed to an immediate one, and they showed that the local cytokine profile at the site of frequent application is shifted from Th1 to Th2 (9). In our experiments, DNFB was applied 1× a week. This induced both immediate and delayed reactions, and no shift of the reaction peak to the immediate type was observed. Differences in the properties and the application frequency of haptens may help to explain these differences in the reaction.

Clinically, immediate reactions to contact allergens such as fragrance mix, balsam of Peru (*Myroxylon Pereirae*), and paraben mix have been reported (10–13) and some of these patients had combined responses of both immediate and delayed reactions. Immediate contact reactions to fragrance compounds have seemed to be non-immunologic (10–12), though Katsarou et al. (13) suggested that allergen-specific immune responses might be involved in such reactions.

We previously reported that, during topical immunotherapy in patients with alopecia using squaric acid dibutylester (SADBE) as the hapten, immediate reactions (contact urticaria) occurred in addition to delayed contact dermatitis (14). The immediate reactions occurred in patients who were frequently exposed to the hapten. It was also confirmed that the dermal mast cell count was increased in these patients (our unpublished data). These findings suggest that phenomena can be observed clinically which resemble the results of our experiments in mice. Recently, it was reported that those who are repeatedly exposed to latex products tend to develop immediate reactions to latex (contact urticaria or anaphylactic shock) and that some of these patients also had contact dermatitis from latex (15–17). However, there are also some patients with allergic contact dermatitis from natural latex without contact urticaria (18, 19). Thus, it seems that both immediate and delayed reactions to an antigen are induced in some patients, and only either an immediate or a delayed reaction induced in others, by repeated exposure to the antigen.

Our results suggest that, in patients with local contact urticaria caused by repeated contact with certain allergens, increased dermal mast cells may play a key rôle in the development of immediate

skin responses to such allergens. This increased local mast cell count can be expected to decrease after cessation of repeated contact with the allergens, such that the immediate reaction can be expected to diminish or to disappear. Therefore, it is just as important for patients with contact urticaria as for those contact dermatitis patients to avoid all their allergens.

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Address:

Masaru Natsuaki
Department of Dermatology
Hyogo College of Medicine
1-1 Mukogawa-cho, Nishinomiya
Hyogo 663-8501
Japan