

Full Paper

Delayed Type Allergic Itch-Associated Response Induced by Toluene-2,4-diisocyanate in Hairless Mice

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Received March 31, 2003; Accepted June 23, 2003

Abstract. To develop an allergic dermatitis model showing persistent scratching in mice, toluene-2,4-diisocyanate (TDI) was repeatedly painted onto the skin of hairless HR-1 mice, and induction of itch-associated scratching behavior was observed. When HR-1 mice were epicutaneously sensitized with 1% TDI and then challenged by repeated painting the cervicodorsal skin with 0.1% TDI once every 10 days until the 10th challenge, delayed type scratching responses peaked at 1–2 days after challenge. TDI at 0.1% hardly induced scratching in non-sensitized HR-1 mice. The delayed scratching response was influenced by neither an H₁ nor 5-HT_{1/2} receptor antagonist. On the other hand, intradermal injection of histamine and serotonin induced frequent scratching in HR-1 mice. In conclusion, repeated application of TDI can induce delayed type allergic scratching. Although HR-1 mice are high responders to both histamine and serotonin, induction of the delayed response depends on neither of these chemical mediators. This delayed response may be useful in analyzing the mechanisms of allergic pruritis.

Keywords: allergic dermatitis, scratching, toluene-2,4-diisocyanate, hairless mouse, itch

Introduction

Pruritus is a major uncomfortable symptom associated with various skin disorders such as allergic contact (1) and atopic (2) dermatitis. Scratching worsens cutaneous diseases, suggesting that analyzing how pruritus occurs and the development of anti-pruritic drugs using experimental animal models are indispensable. To date, itch-associated scratching responses have been induced in mice via intradermal injections of histamine, compound 48/80, substance P, leukotriene B₄, and other irritants (3–5). Scratching in allergic dermatitis model mice has also been induced by passive cutaneous anaphylaxis (6). The respective responses are simple and reliable, and they may be useful for analyzing at least part of the mechanisms of pruritus in allergic skin disorders. However, in contrast to chronic dermatitis in

patients such as atopic dermatitis, these responses in experimental models described above are acute and not chronic.

On the other hand, toluene-2,4-diisocyanate (TDI), a cause of occupational asthma (7), is a low molecular weight antigen that can induce cutaneous delayed-type hypersensitivity in animal models, especially those of mice (8, 9). Tominaga et al. (10) have reported that when TDI is painted onto the ear skin of sensitized mice, swelling is induced at the site that peaks 20 h later. In addition, repeated painting of mouse skin with TDI induces not only delayed, but also immediate skin inflammation associated with increased amounts of TDI-specific IgE antibody in the serum (11), suggesting that cellular and humoral types of immunity are involved in the induction of dermatitis. We speculated from these findings that repeated application of TDI can induce chronic dermatitis with persistent scratching.

We developed an allergic dermatitis model mouse

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that persistently scratched in response to repeatedly painting the skin with TDI. We then observed the induction of scratching behavior and edema formation. We used hairless mice (HR-1) because the skin can be easily examined without shaving, which can artifactually produce lesions or stimulate the skin, although this strain of mouse has not been well characterized in terms of the relationship between hairlessness and immunity. TDI was repeatedly painted onto the cervicodorsal skin of the sensitized mice once every 10 days until the 10th challenge, and induction of scratching and edema were observed for up to 97 h after each challenge. Then, we pharmacologically analyzed the involvement of histamine and serotonin in the responses. In addition, we compared cutaneous responsiveness to intradermal injections of histamine and serotonin in HR-1 mice with those of ICR and ddY mice. The skin of the latter two strains is sensitive and non-sensitive to histamine, respectively.

Materials and Methods

Animals

We developed a TDI-induced allergic dermatitis model using 5-week-old (21–24 g) male HR-1 mice (Hoshino Experimental Animal Center, Yashio). Responsiveness to histamine and serotonin were compared among 5-week-old ddY (26–28 g), ICR (26–28 g; Japan SLC, Hamamatsu), and HR-1 mice. The animals were fed with a standard laboratory diet and provided with water ad libitum in an air-conditioned room at a temperature of $23 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ humidity.

This study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Antigen

Toluene-2,4-diisocyanate (Wako Pure Chem., Osaka) antigen was dissolved in ethyl acetate at a concentration of 0.1% or 1%.

Reagents

Reagents and their sources were as follows: mepyramine maleate, histamine dihydrochloride, serotonin hydrochloride, and naloxone hydrochloride dihydrate (Sigma Chem., St. Louis, MO, USA) and methysergide dimaleate (Novartis Pharma Co., Basel, Switzerland).

Sensitization and challenge

The abdominal skin of HR-1 mice was sensitized by painting with $20 \mu\text{l/site}$ of 1% TDI once every 2 days on days 0–4. From 14 days after the first sensitization, 0.1% (Group A) and 1% (Group B) TDI were epicutan-

ously applied to the cervicodorsum of the sensitized mice at a volume of $10 \mu\text{l/site}$ once every 10 days until the 10th challenge. Time-course changes in scratching frequency and volume of edema were measured until 97 h after the respective painting challenges as described below.

To assess whether TDI produces scratching behavior and edema formation even in non-sensitized HR-1 mice, 0.1% and 1% TDI were epicutaneously applied to the cervicodorsum of non-sensitized mice at a volume of $10 \mu\text{l/site}$, and then scratching frequency and edema volume were evaluated until 97 h later.

Counting scratching frequency

The scratch response was defined as a movement of the hind limb that was precisely directed to the injected or painted cervicodorsal site. Histamine- and serotonin-induced scratching frequency was counted at intervals of 10 min until 60 min after the respective injections. Frequency of induced scratching behavior was counted before, 0–1, 4–5, 15–16, 24–25, 48–49, 72–73, and/or 96–97 h after each of the respective 10 painting challenges.

Measurement of edema volume

Degree of edema at the challenged site is expressed as volume of edema that was measured 1, 5, 16, 25, 49, 73, and 97 h after the respective 10 challenges. Volume of edema was determined by measuring the longitudinal and transverse diameters of the reaction site and skin thickening, which was the difference in skin thickness between before and after challenge, measured with slide calipers (Kanon, Tokyo).

Effect of mepyramine, methysergide, and naloxone on TDI-induced scratching frequency

The classical H_1 receptor antagonist mepyramine (10 mg/kg , i.p.), the $5\text{-HT}_{1/2}$ receptor antagonist methysergide (1 mg/kg , i.p.), and the opioid receptor antagonist naloxone (1 mg/kg , s.c.) were administered 0.5 h before and at 23.5 and 47.5 h after the respective first, second, and third challenges with 0.1% TDI (Group A). Mepyramine significantly suppressed histamine (100 nmol/site)-induced scratching response at 10 mg/kg in HR-1 mice (data not shown). One mg/kg methysergide (p.o.) blocks scratching responses induced by 100 nmol/site of serotonin (12). According to other reports, naloxone inhibits itching but not pain at this dose (12, 13).

Histamine- and serotonin-induced scratching behavior

Histamine (100 nmol/site per animal), serotonin (100 nmol/site per animal), or saline was intradermally

administered to the cervicodorsum of HR-1, ddY, and ICR mice at a volume of 20 μ l/site per animal, and then scratching frequency was evaluated until 60 min after injection as described above. The cervicodorsum of the ICR and ddY mice was shaved one day before injection.

Statistical analyses

Data were statistically analyzed using one-way analysis of variance (ANOVA). If a difference was significant, the individual group difference was determined by Bonferroni's multiple test. A probability value (*P*) below 0.05 was considered statistically significant.

Results

Repeated application of TDI-induced scratching and edema in sensitized HR-1 mice

Time-course changes in scratching frequency induced by up to 10 TDI challenges in the sensitized HR-1 mice are shown in Fig. 1. A 24–25 h after the first challenge with 0.1% TDI (Group A), the mice showed an increased scratching frequency of approximately 40 times/h. The response peaked at 48–49 h, followed by gradual decrease of the frequency until 96–97 h later. At challenges 2–6, a delayed scratching response appeared that peaked at 24–25 or 48–49 h, but the degrees of the response were similar or somewhat decreased compared with that at the first challenge. The delayed response, however, was not obvious at challenges 7–10. On the other hand, at the first and second challenges with 1% TDI (Group B), scratching of over 60 times/h was immediately observed (0–1 h) after the challenges, and the delayed scratching response was also persistent with a peak at 24–25 h. However, the degree of the delayed response tended to decrease with repeated TDI challenge while substantially retaining the magnitude of the immediate response.

Figure 2 shows time-course changes in edema volume after the first to tenth challenges with TDI in the sensitized mice. After all challenges in Group A, edema formed with a peak at 5–25 h, and then the volume decreased until 97 h. Furthermore, initiation of the edema at 1 h became obvious between challenges 6 and 10. After all challenges in Group B, more prominent edema peaked at 5–25 h later. Edema was obviously initiated even 1 h after the first challenge.

When 0.1% TDI was epicutaneously applied to non-sensitized mice, scratching frequency did not increase and edema did not develop (Fig. 3). However, scratching frequency was slightly elevated at 0–1, 24–25, and 48–49 h; and weak edema was induced at 1–49 h after applying 1% TDI to the non-sensitized mice (Fig. 3).

Influence of mepyramine, methysergide, and naloxone on TDI-induced scratching and edema in sensitized HR-1 mice

We evaluated the influence of the H₁-receptor antagonist mepyramine, the 5-HT_{1/2}-receptor antagonist methysergide, and the opioid receptor antagonist naloxone on the occurrence of scratching behavior and edema at the respective first, second, and third challenges with 0.1% TDI. At these times, the delayed type scratching response was relatively prominent compared with later times (Fig. 1). Mepyramine (Fig. 4) and methysergide (Fig. 5) administered 0.5 h before and at 23.5 and 47.5 h after the respective first and second challenges did not inhibit either the induction of scratching at 24–25 and 48–49 h (Figs. 4a and 5a) or edema at 25 and 49 h (Figs. 4b and 5b). On the other hand, when naloxone was administered before and after the third challenge, delayed type scratching response was significantly suppressed (Fig. 6a), whereas the edema formation was not influenced (Fig. 6b).

Comparison of scratching responsiveness to histamine and serotonin in HR-1, ICR, and ddY mice

Figure 7 shows time-course changes in scratching frequencies induced by intradermal injection of histamine and serotonin in HR-1, ICR, and ddY mice. Although ddY mice scratched less than 10 times/10 min at 0–20 min after the histamine injection, histamine induced obvious scratching behavior in both HR-1 and ICR mice at 0–20 min (30–50 times/10 min, Fig. 7a). On the other hand, HR-1 mice scratched the most in response to serotonin among the 3 strains, with a frequency of approximately 110 and 50 times/10 min at 0–10 and 10–20 min, respectively. The response of ICR mice to serotonin was moderate at 0–10 min, but that of ddY was quite weak (Fig. 7b). Both histamine- and serotonin-induced scratching in HR-1 and ICR mice ceased within 30 min after the respective injections (Figs. 7: a and b).

Scratching frequencies within 60 min after injection of vehicle (saline) were less than 10 times/10 min in all three strains (data not shown).

Discussion

There has been no dermatitis model of experimental animals showing persistent scratching that is based on the allergic response. Thus, we attempted to develop one by repeatedly painting TDI onto the skin of HR-1 mice and observing the induction of itch-associated scratching behavior. When HR-1 mice were sensitized by an epicutaneous application of 1% TDI on the abdomen once every 2 days for 5 days and then chal-

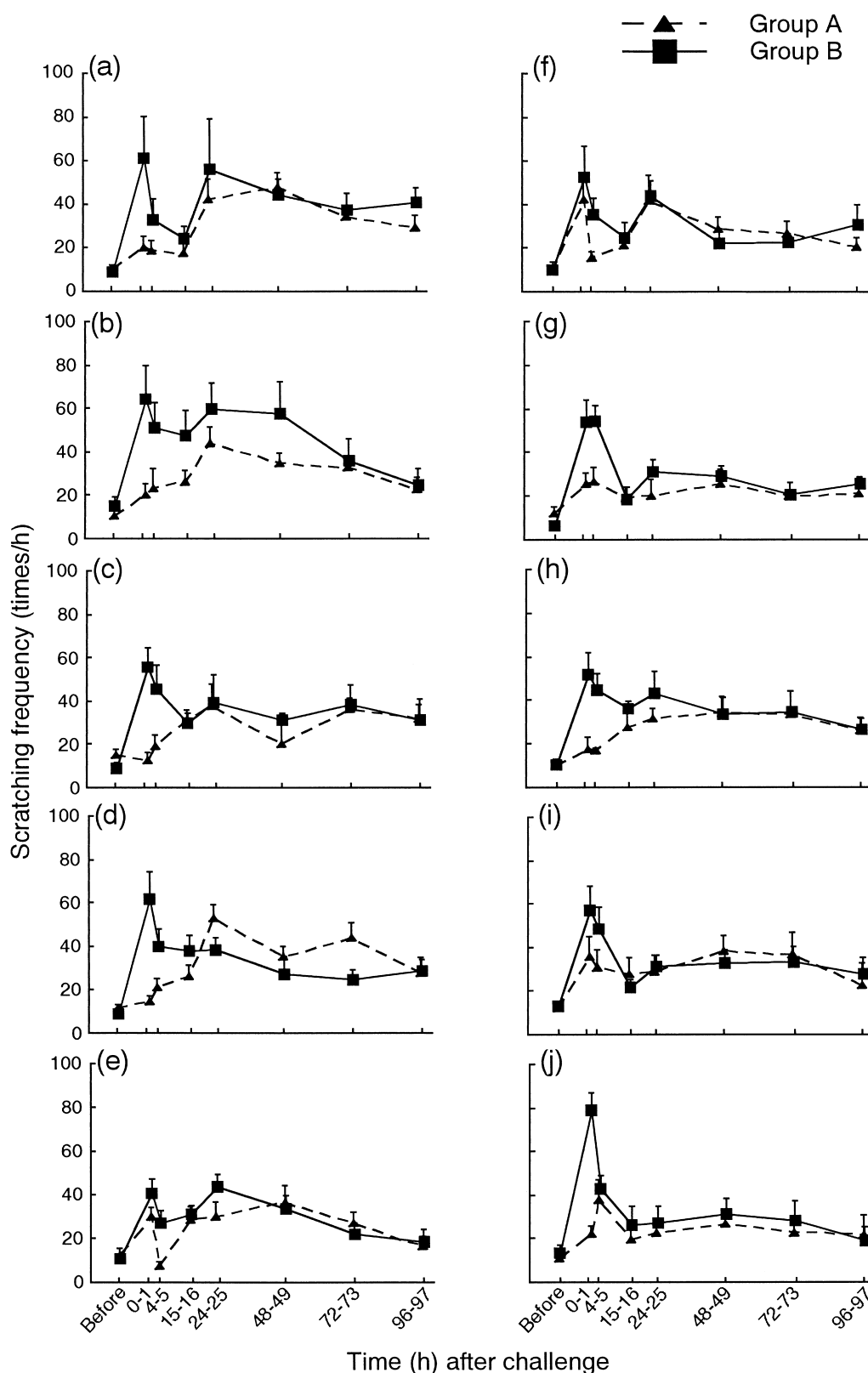


Fig. 1. Time-course changes in scratching frequency induced by 1 (a), 2 (b), 3 (c), 4 (d), 5 (e), 6 (f), 7 (g), 8 (h), 9 (i), and 10 (j) challenges with 0.1% (Group A) or 1% (Group B) toluene-2,4-diisocyanate (TDI) in sensitized HR-1 mice. Twenty microliters/site of 1% TDI was painted on the abdominal skin of HR-1 mice once every 2 days for 5 days. From 14 days after the first sensitization, 0.1% (Group A) or 1% (Group B) TDI was epicutaneously applied to the cervicodorsum of the mice at a volume of 10 μ l/site once every 10 days. Each point represents the mean \pm S.E.M. of 5 or 6 animals.

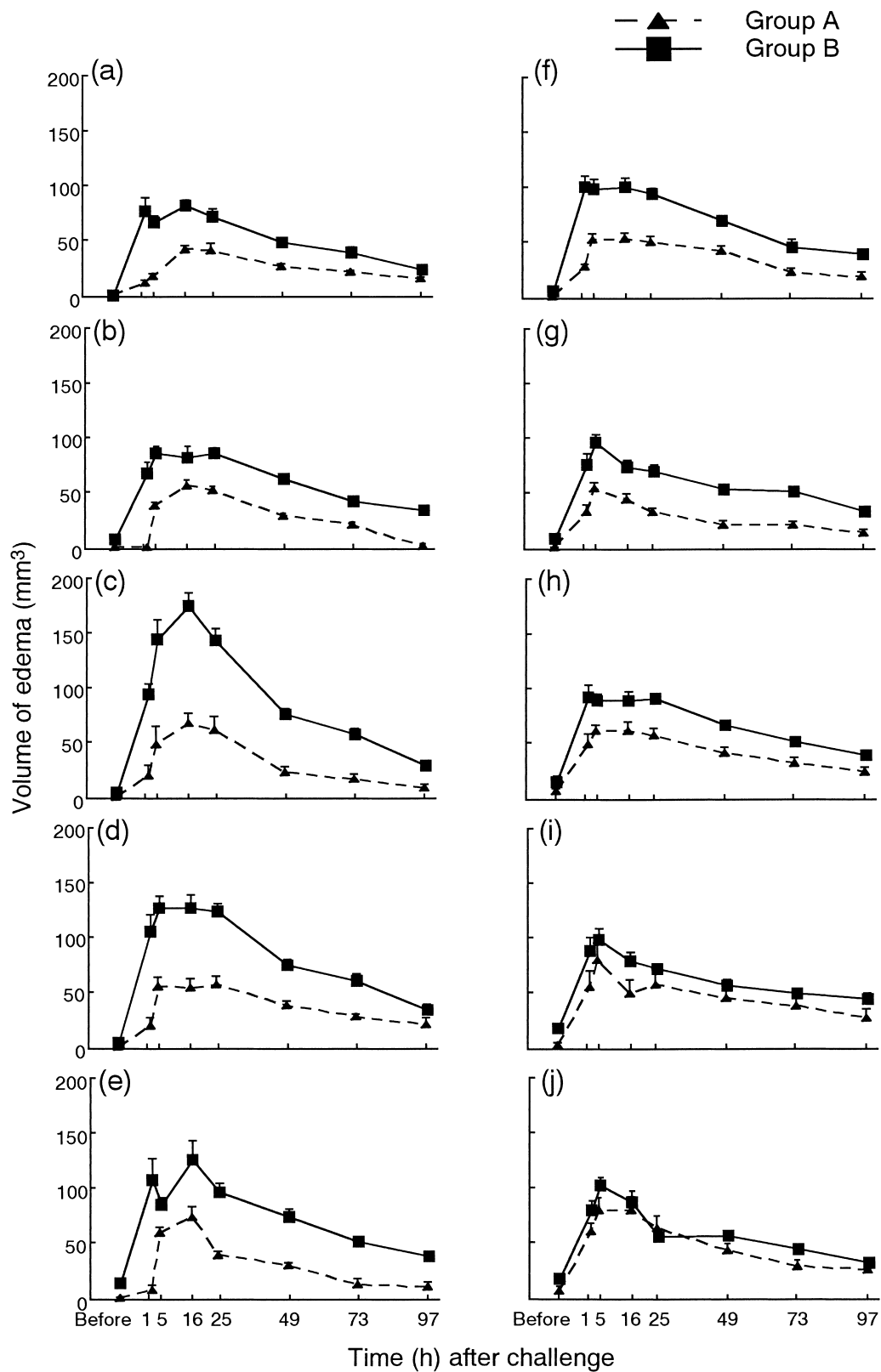


Fig. 2. Time-course changes in volume of edema induced by 1 (a), 2 (b), 3 (c), 4 (d), 5 (e), 6 (f), 7 (g), 8 (h), 9 (i), and 10 (j) challenges with 0.1% (Group A) or 1% (Group B) toluene-2,4-diisocyanate (TDI) in sensitized HR-1 mice. Twenty microliters/site of 1% TDI was painted on the abdominal skin of HR-1 mice once every 2 days for 5 days. From 14 days after the first sensitization, 0.1% (Group A) or 1% (Group B) TDI was epicutaneously applied to the cervicodorsum of the mice at a volume of 10 μ l/site once every 10 days. Each point represents the mean \pm S.E.M. of 5 or 6 animals.

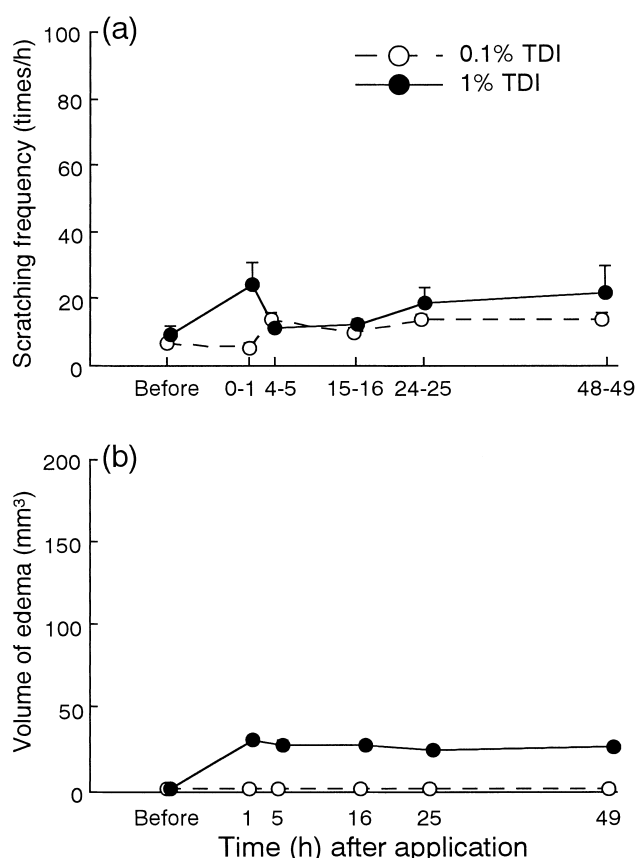


Fig. 3. Time-course changes in scratching frequency (a) and volume of edema (b) induced by painting non-sensitized HR-1 mice with 0.1% and 1% toluene-2,4-diisocyanate (TDI). Each point represents the mean \pm S.E.M. of 3 or 16 animals.

lenged by repeated painting with 0.1% TDI onto the cervicodorsal skin once every 10 days, a delayed type scratching response was observed by the 6th challenge. However, the frequency of delayed scratching tended to be decreased in response to repeated challenge with 0.1% TDI (Group A). On the other hand, challenge with 1% TDI induced not only a delayed but also an immediate type scratching response, although the delayed response was also decreased by repeated challenge (Group B).

Because the epicutaneous application of TDI produces TDI-specific IgE antibody in the serum of mice in a specific sensitization/challenge protocol (11), part of the immediate response observed in Group B may be due to an anaphylactic reaction mediated by mast cell activation. In addition, because we found that 1% TDI has a primarily irritative action on the skin of HR-1 mice, at least part of the immediate response could be induced by non-acquired immunity. On the other hand, a delayed type scratching response like that observed in both Groups A and B has never been reported. Because

0.1% TDI has no irritative action on the skin of non-sensitized HR-1 mice, the delayed type scratching response might be mediated by the activation of memory T lymphocytes. Although edema was also caused in a delayed manner, the time-courses of the scratching response and edema formation differed. Additionally, the induction of delayed type scratching but not of edema formation was potently inhibited by naloxone that has been reported to inhibit itch but not pain (12, 13). Thus, the scratching response should be mediated by itch sensation, and cannot be closely associated with edema formation.

Because the 1% TDI-induced scratching response (Group B) may include non-allergic mechanisms, we used Group A to elucidate the involvement of histamine and serotonin in the induction of the delayed type allergic scratching response and edema formation at the first and second challenges. At these times, the delayed scratching response was relatively obvious compared with later challenges. Because histamine (6) and serotonin (12) induce a scratching response via stimulating H_1 and $5-HT_2$ receptors, respectively, we applied the classical and selective H_1 -receptor antagonist mepyramine, and the non-selective $5-HT_{1/2}$ -receptor antagonist methysergide. Consequently, neither mepyramine nor methysergide affected the delayed type scratching response and edema formation. These results indicate that the delayed type response is not mediated by histamine and serotonin. Thus, mechanisms underlying this scratching response are considerably different from other acute models of allergic itch in mice such as the scratching response induced by passive cutaneous anaphylaxis (6), but might be similar to those of chronic dermatitis such as atopic dermatitis in the clinical setting.

On the other hand, the scratching response to stimuli, especially to histamine, considerably differs among strains of mice (3, 14). However, responsiveness of HR-1 mice to stimuli has not been reported. Thus, we evaluated whether intradermal administration of not only histamine but also serotonin induces the scratching response in HR-1 mice and compared their responsiveness with those of ddY and ICR mice. Responsiveness to histamine was comparable in HR-1 and ICR mice, the latter of which are the most potent responders to histamine among the various strains (14). Serotonin induces moderate and severe scratching in ddY and ICR mice, respectively (14). The scratching response to serotonin was more obvious in HR-1 mice than in ICR mice. These results indicate that HR-1 mice are sensitive to stimuli, suggesting that this strain would be an appropriate dermatitis model due to the itch-associated scratching response.

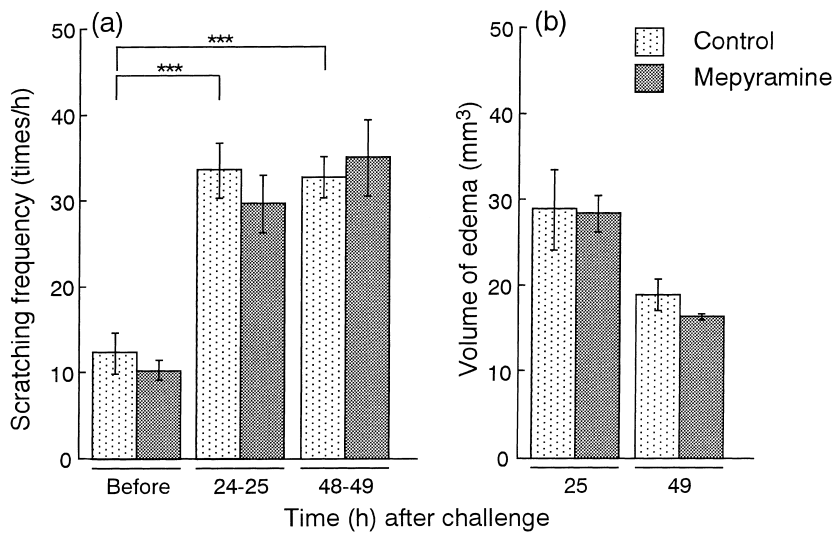


Fig. 4. Effect of mepyramine on toluene-2,4-diisocyanate-induced increases of scratching frequency (a) and edema volume (b) at the first challenge in sensitized HR-1 mice (Group A). Mepyramine (10 mg/kg) or saline was administered i.p. 30 min before and at 23.5 and 47.5 h after challenge. Each column represents the mean \pm S.E.M. of 8 or 9 animals. *** P <0.001.

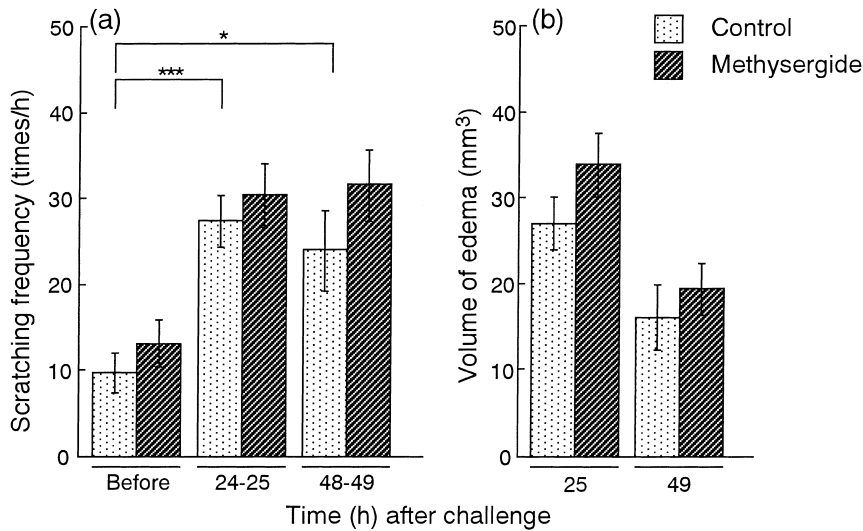


Fig. 5. Effect of methysergide on toluene-2,4-diisocyanate-induced increases of scratching frequency (a) and edema volume (b) at the second challenge in sensitized HR-1 mice (Group A). Methysergide (1 mg/kg) or saline was administered i.p. 30 min before and at 23.5 and 47.5 h after challenge. Each column represents the mean \pm S.E.M. of 8 or 9 animals. * P <0.05 and *** P <0.001.

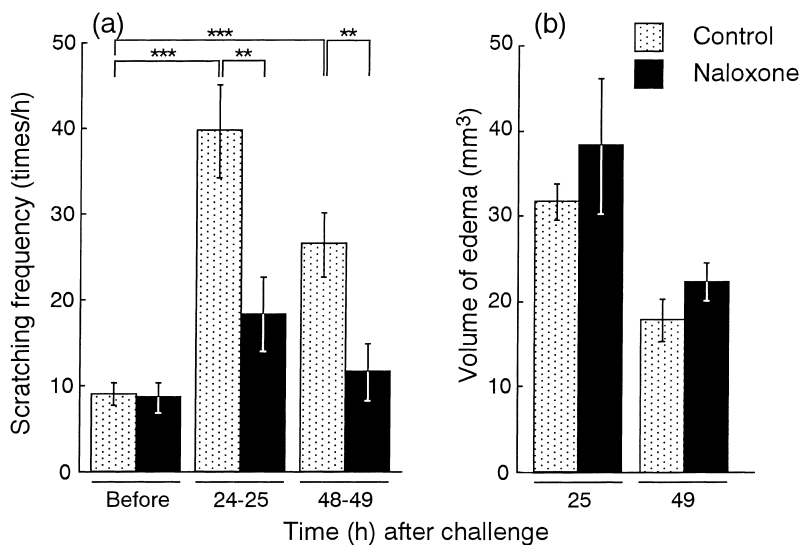


Fig. 6. Effect of naloxone on toluene-2,4-diisocyanate-induced increases of scratching frequency (a) and edema volume (b) at the third challenge in sensitized HR-1 mice (Group A). Naloxone (1 mg/kg) or saline was administered s.c. 30 min before and at 23.5 and 47.5 h after challenge. Each column represents the mean \pm S.E.M. of 8 or 9 animals. ** P <0.01 and *** P <0.001.

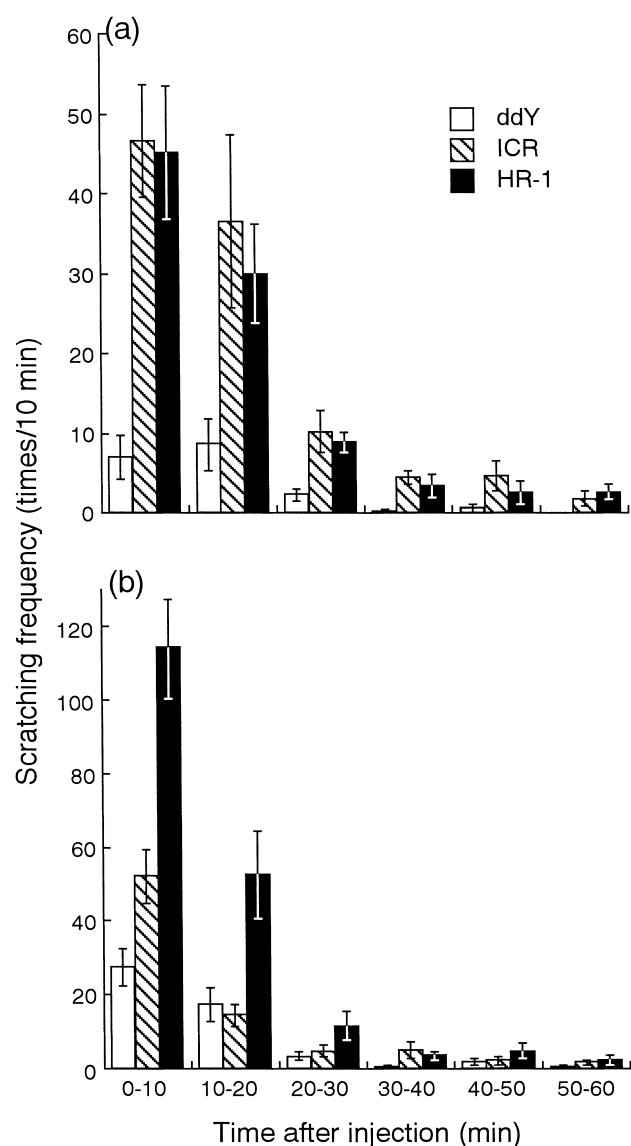


Fig. 7. Time-course changes in scratching frequency after intradermal injections of histamine (a) and serotonin (b) in ddY, ICR, and HR-1 mice. Histamine (100 nmol/site per animal) or serotonin (100 nmol/site per animal) was intradermally administered to the cervicodorsum. Each column represents the mean \pm S.E.M. of 7 or 8 animals.

In conclusion, HR-1 mice are high responders to both histamine and serotonin in the itch-associated scratching response. Repeated application of 0.1% TDI can induce a delayed type scratching response that is based on an allergic reaction, although the induction of the response depends on neither histamine nor serotonin. This delayed type response may be useful for analyzing at least part of the mechanisms involved in allergic pruritus. However, the magnitude of the scratching response could not be aggravated during repetitive challenges

using the present sensitization/challenge protocols of Groups A and B. As chronic dermatitis in the clinical setting must include various complicating factors other than the allergic reaction, pro-inflammatory factors such as dry skin and infection must be included to establish a useful pruritus model.

Acknowledgments

This study was supported in part by the Bioventure Developing Program of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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