

A Murine Model of Toluene Diisocyanate-induced Contact Hypersensitivity

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ABSTRACT

Background: Toluene diisocyanate (TDI) can cause contact allergy and occupational asthma, but the mechanism underlying sensitization to this chemical compound remains controversial. Also the correlation of mast cell with contact hypersensitivity (CHS) and the role of mast cell in the TDI-induced CHS is unknown. This issue was investigated by administrating TDI on the skin of genetically mast cell-deficient WBB6F1/J-*Ki*^{W^v}/*Ki*^{W^v} (W/W^v) and congenic normal WBB6F1/J-*Ki*+/+ (+/+) mice. **Methods:** To development of animal model of TDI-induced CHS and to investigate the correlation of mast cell with CHS and the role of mast cell in the TDI-induced CHS, W/W^v and +/+ mice were sensitized with TDI on the back skin at day 1 and day 8, and then challenged with 1% TDI on the ear at day 15. At 1, 2, 4, 8, and 24 hours after 1% TDI challenge, the ear thicknesses were measured. It was investigated the histologic changes of dermis in the ear of W/W^v and +/+ mice at 24 hours after 1% TDI challenge. **Results:** TDI induced a significant ear swelling response in W/W^v and +/+ mice. TDI induced the significant infiltrations of polymorphonuclear leukocytes and eosinophils in W/W^v and +/+ mice, but not of mast cells in normal mice. And TDI increased a characteristic extent of mast cell degranulation in normal mice. There were no significant differences in the ear swelling and the infiltrations of polymorphonuclear leukocytes and eosinophils of normal versus W/W^v mice, either at baseline or after TDI-induced CHS. **Conclusion:** From the above results, TDI can be used as a murine CHS model, and the mast cells may not be essential in TDI-induced CHS. (*Immune Network* 2002;2(3):158-165)

Key Words: Mast cell, contact hypersensitivity, toluene diisocyanate, polymorphonuclear leukocyte, eosinophil

Introduction

As the outer barrier of the body, the skin represents the first line of defense against microorganisms and harmful substances. As a consequence of industrialization, the human population is exposed to ever increasing new chemicals and microparticles. Against this background, it is not surprising that skin diseases, including allergic reactions, are on the increase. The clinically most important allergic reactions

of the skin are atopic dermatitis and contact hypersensitivity (CHS). CHS, an example of a delayed-type (type IV) hypersensitivity (DTH), is a specific T cell mediated reaction towards chemically reactive compounds so-called happens (1,2). Typical for type IV hypersensitivities like CHS is a time delay of approximately 24 h between allergen provocation (of sensitized individuals) and clinical symptoms, as the response requires cell activation and migration.

A potential for human exposure to toluene diisocyanate (TDI), a low molecular weight, exists on account of its various industrial applications including the manufacture of polyurethane foams, paints, coatings, and elastomers (3-5). Exposure to TDI can cause irritation of the mucous membranes, progressive impairment of pulmonary function, and asthma

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This work was supported by the grants of KOSEF (1999-1-205-003-3).

(3,6,7). Morphologic studies in workers with asthma induced by TDI have shown the characteristic features of allergic inflammation in the bronchial mucosa, i.e., infiltration of eosinophils and mast cells.

For several decades previous studies have shown that cutaneous mast cells are involved in the elicitation of DTH in mice (8-10). And the inability of mast cell-deficient mice to express DTH was overcome when sensitized T cells and specific antigen were placed in the extravascular tissues by local passive transfer (8,9). However, in the recently studies, when the immune responses of mast cell-deficient (W/W^V) and mast cell-sufficient littermates (LM) were compared, CHS and DTH reactions of W/W^V and LM mice were similar. No evidence of immune deficiency of W/W^V mice was found (11-13). Teixeira et al (14) reported eosinophils recruitment into sites of DTH reaction in mice. So this study investigated whether TDI could elicit DTH reactions by administering TDI on the skin of genetically mast cell-deficient $WBB6F1/J-Kit^W/Kit^{W^v}$ and congenic normal $WBB6F1/J-Kit+/+$ mice sensitized by TDI or not and the role of mast cell in the TDI-induced CHS.

Materials and Methods

Animals. Mast cell-deficient mice and the normal littermates (($WB/ReJ-W/+C57BL/6J-W^V+$) $F1-(W/W^V, +/+)$), designated here $WBB6F1/J-Kit^W/Kit^{W^v}$, $WBB6F1/J-Kit+/+$ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The number of mast cells present in skin of adult mast cell deficient the W/W^V mice contains only under the 1.0% compare to that of the congenic normal $+/+$ mice. All mice were only healthy male and the 10~12 weeks of age. They were housed the experiments in a laminar flow cabinet and article lighting conditions with 12-h day/night cycle and had access to food and water *ad libitum*.

Chemicals. Toluene diisocyanate (TDI) was purchased from Sigma (MA, USA) in the form of a mixture containing 80% 2,4-TDI and 20% 2,6-TDI. Acetone (Junsei): olive oil (Filippo berio, Italy) (4 : 1) was used as vehicle.

Sensitization and challenge. An approximately 4 cm² area

shaved, cellophane tape-stripped three times and dosed topically with 100µl of 3% TDI dissolved in acetone:olive oil (4 : 1) under light anesthesia according to the method of Lauerma et al (15) (Fig. 1). Animals were kept in a supine position for 10 min after each sensitization. After 7 days, these animals were further sensitized with 50µl of 1.5% TDI dissolved in acetone:olive oil (4 : 1) at the same sites. A control group was treated similarly with vehicle, acetone:olive oil (4 : 1). On 7 days after the second sensitization (Day 15), animal groups were challenged topically with 25µl of vehicle, acetone:olive oil (4 : 1) on the right ear and 25µl of 1% TDI dissolved in acetone : olive oil (4 : 1) on the left ear.

Ear swelling measurement. Ear thickness was measured with a Mitutoyo engineers micrometer (Model 7326, Tokyo, Japan) (16). Ear thickness was measured three times before vehicle, acetone:olive oil (4 : 1) or TDI application, and ear was then measured at 1, 2, 4, 8 and 24 h following vehicle or TDI application.

Increment of ear thickness was calculated as the following formula.

$$\text{Increment} = [\text{thickness of ear after TDI challenge} - \text{thickness of ear before TDI challenge}]$$

Histologic examination. Twenty-four hours after challenge, animals were sacrificed and both ears of animals were excised. All specimens were fixed for two hours in 2.5% glutaraldehyde and rinsed twice in 0.1 M cacodylate buffer solution for 10 minutes. After that some specimens were postfixed for two hours in 1% osmium tetroxide (OsO_4) and rinsed twice in 0.1 M cacodylate buffer solution for 10 minutes. They were dehydrated in graded ethanol, clear in propylene oxide and embedded in epoxy resin. Sections 1µm thick were cut from epoxy resin-embedded tissue with glass knives, placed on glass slides, and stained with alkaline-Giemsa solution for mast cells and polymorphonuclear leukocytes. Other specimens were rinsed in 0.1 M cacodylate buffer solution, and embedded in the cryo tissue-tek. Five micrometer-frozen sections were prepared with cryomicrotome (Leica CM 1510, Leica, Germany) and

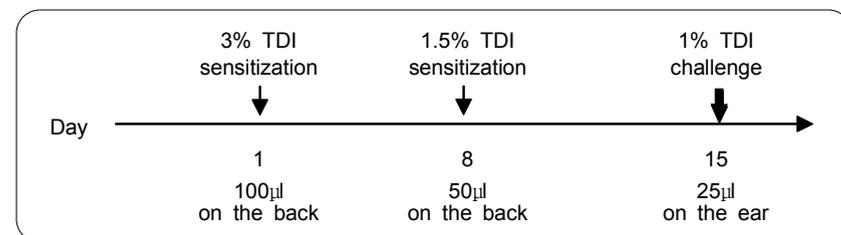


Figure 1. Schematic diagram of the experimental protocol. Mice were sensitized topically with 3% TDI on the back skin, and repeated with 1.5% TDI at the same sites 7 days later. Seven days after the second sensitization, mice were challenged with 1% TDI on the dorsums of ears.

on the back of the animals, 10~12 weeks of age, was

stained with Congo red solution for eosinophils (17).

In the alkaline-Giemsa method, the slides were stained in Giemsa solution (pH 8.2) for 1 h at 60°C and rinsed in tap water, and then destained with 50% ethanol for three dips, washed in distilled water. The slides were dried in 60°C oven for two hours and cleared in xylene, the cover slipped. In the Congo red method, the slides were stained in Hematoxylin solution for 1 min and rinsed in alkaline sodium chloride solution for 20 minutes. The slides were stained in Congo red solution for 20 minutes, washed in distilled water, dehydrated in graded ethanol and cleared in xylene, the cover slipped.

Statistical analysis. Data were presented as mean standard error of mean (SEM). Students unpaired *t*-test was applied to reveal significant differences between corresponding treated groups and control groups. Finally, a value of $P < 0.05$ was considered statistically significant.

Results

Toluene diisocyanate (TDI) induces a significant ear swelling response in mast cell-deficient WBB6F1/J-Kit^W/Kit^{W-v} and normal WBB6F1/J-Kit^{+/+} mice. In preliminary experiments, the negative controls, i.e., mice not sensitized with TDI but merely ear challenged, showed the ear swelling response (Fig. 2A). The ear swelling responses induced by single application of 0.5% or 1% TDI dissolved acetone:olive oil (4 : 1) were similar to that induced by vehicle, acetone : olive oil (4 : 1) application. But single application of 3% TDI induced the significant ear swelling response in normal mice. Therefore 1% concentration of TDI was chosen in the subsequent experiments to ensure

the ear swelling response in sensitized mice.

Fig. 2B showed the ear swelling responses on time course following 1% TDI challenge or vehicle application on the ear of mast cell-deficient and normal mice. Mice were sensitized with TDI on the back skin at d 1 and d 8, and then challenged with 1% TDI on the ear at d 15. At 1, 2, 4, 8, and 24 h after challenge, the ear thicknesses were measured. At twenty hour post-challenge, there was a significant increment of ear swelling responses in both mast cell-deficient and normal mice that sensitized to TDI. There was no significant difference in the ear swelling responses of normal versus mast cell-deficient mice, either at baseline or after TDI-induced contact hypersensitivity.

Table I. The number of eosinophils (Eos) and polymorphonuclear leukocytes (PMNLs) per unit area on 24 h after vehicle, 0.5%, 1% or 3% toluene diisocyanate (TDI) application in the non-sensitized normal mice (n=4~5)

TDI application		Number of cells/mm ²	
Sensitization	Challenge	Eos	PMNLs
-	0.0%	3.99±0.12	5.59±0.42
-	0.5%	4.09±0.19	7.05±0.79
-	1.0%	4.12±0.21	7.16±0.84
-	3.0%	38.60±3.23*	66.19±4.94**

*; $P < 0.01$, **; $P < 0.001$, 3.0% challenge *vs* 0.0% challenge

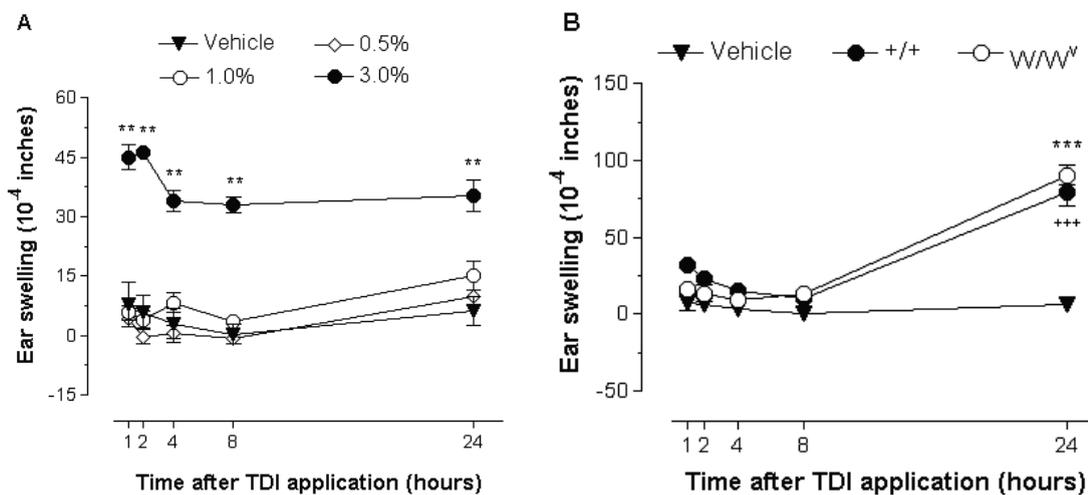


Figure 2. The ear swelling responses on time course following (A) TDI single application on the ear of non-sensitized negative controls, (B) 1% TDI challenge on the ear of mast cell-deficient WBB6F1/J-Kit^W/Kit^{W-v} (W/W^v) and normal WBB6F1/J-Kit^{+/+} (+/+) mice sensitized on the back skin with TDI. **: $P < 0.01$, 3.0% challenge *vs* non-sensitized negative control, ***: $P < 0.001$, +++: $P < 0.001$, sensitization & 1% challenge *vs* only sensitized negative control.

TDI induces the significant infiltration of eosinophils and polymorphonuclear leukocytes (PMNLs) into the dermis of the ear in mast cell-deficient *WBB6F1/J-Kit^W/Kit^{W-v}* and normal *WBB6F1/J-Kit+/+* mice. The infiltration of eosinophils and PMNLs was measured as a cellular mechanism underlying the ear swelling. Fig. 3 showed that, in the negative control which were received only ear challenge without TDI sensitization, a marked in-

filtration of eosinophils and PMNLs was observed only in the group challenged with 3% TDI (Table I).

Table II showed the infiltrations of eosinophils and PMNLs into the dermis of the ear at 24 h after 1% TDI challenge or vehicle application on the ear of mast cell-deficient and normal mice which sensitized with TDI on the back skin at d 1 and d 8. TDI challenge induced the significant infiltrations of

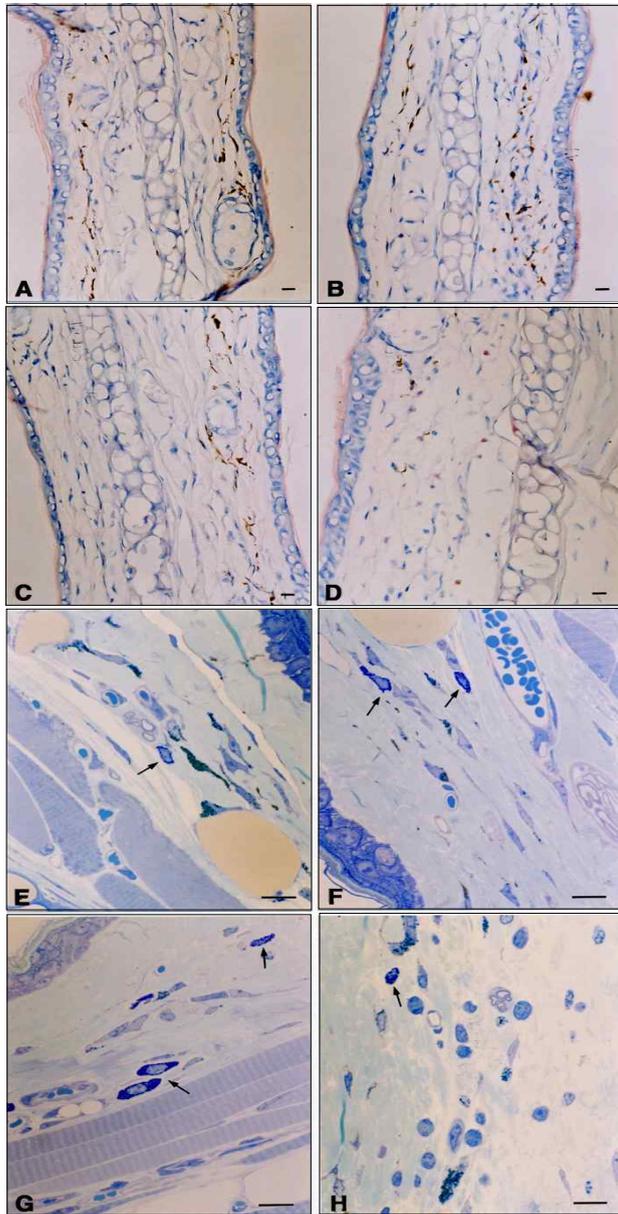


Figure 3. Micrographs of infiltration of eosinophil and PMNL, and the mast cell (arrow) on 24 hours after vehicle (A, E), 0.5% (B, F), 1% (C, G), or 3% (D, H) TDI application in the negative controls, i.e., mice not sensitized with TDI but merely ear challenged. Congo red stain (A-D); Giemsa stain (E-H); Bar=10 μ m.

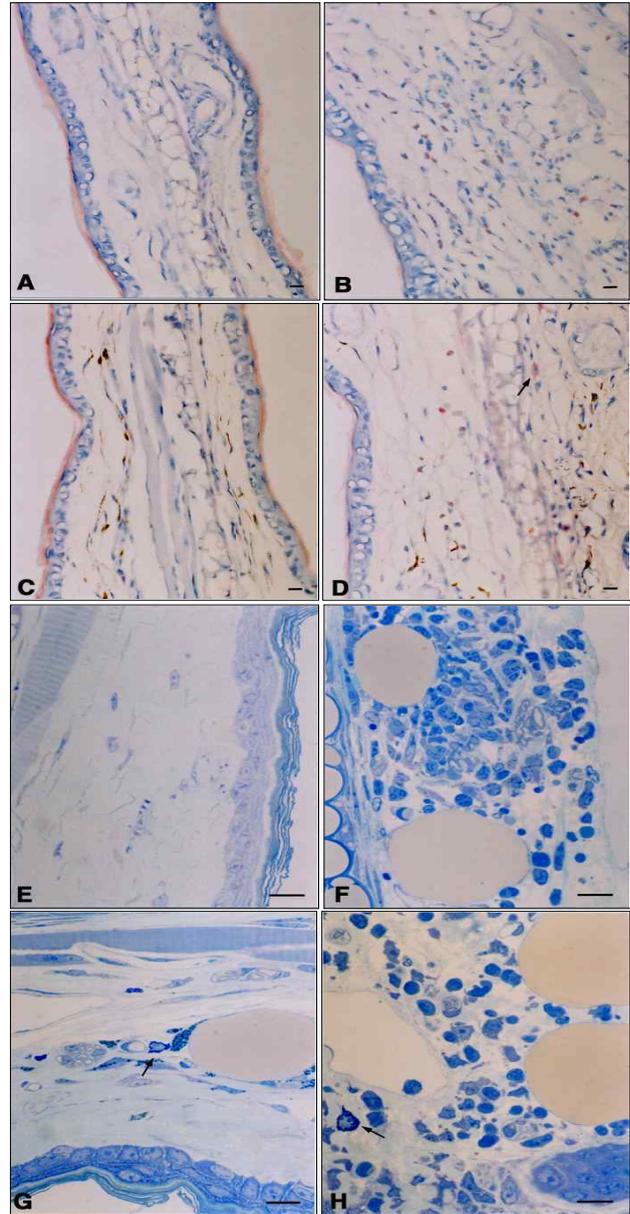


Figure 4. Micrographs of infiltration of eosinophil and PMNL, and the mast cell on 24 hours after vehicle application (A, C, E, G) or 1% TDI challenge (B, D, F, H) on the ear of mast cell-deficient *WBB6F1/J-Kit^W/Kit^{W-v}* (A, B, E, F) and normal *WBB6F1/J-Kit+/+* (C, D, G, H) mice sensitized on the back skin with TDI. Arrow: Mast cell; Congo red stain (A-D); Giemsa stain (E-H); Bar =10 μ m.

Table II. The number of eosinophils (Eos) and polymorphonuclear leukocytes (PMNLs) per unit area on 24 h following 1% toluene diisocyanate (TDI) challenge or vehicle application in TDI sensitized mast cell-deficient (W/W^c) and normal (+/+) mice (n=4~5)

TDI application		Eos/mm ²		PMNLs/mm ²	
Sensitization	Challenge	W/W^c	+/+	W/W^c	+/+
+	0.0%	2.00±0.14	1.80±0.14	2.64±0.24	2.07±0.17
+	1.0%	78.55±1.28*	73.67±1.94*	176.81±9.99*	163.56±7.82*

*, $P < 0.001$, 1.0% challenge vs 0.0% challenge

Table III. The number of mast cells (MCs) per unit area and the extent of mast cell degranulation (MCD) on 24 h after vehicle or toluene diisocyanate (TDI) in normal (+/+) mice (n=4~5)

TDI application		+/+	
Sensitization	Challenge	MCs/mm ²	MCD (%)
-	0.0%	63.18±2.32	2.97±0.27
-	0.5%	70.31±17.71	5.94±0.60
-	1.0%	78.85±13.34	10.69±1.70**
-	3.0%	54.43±1.27*	14.37±2.80**
+	0.0%	62.26±1.80	3.15±0.11
+	1.0%	37.73±2.39***	38.19±2.40***

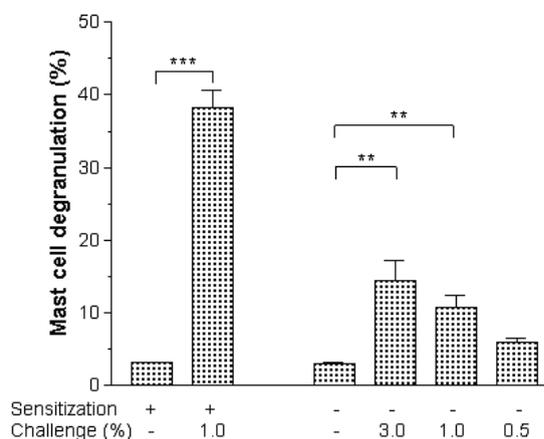
*, $P < 0.05$, **: $P < 0.01$, 1.0% or 3.0% challenge vs 0.0% challenge, ***: $P < 0.001$, sensitization & 1.0% challenge vs sensitization & 0.0% challenge

eosinophils and PMNLs into the dermis of the ear in mast cell-deficient and normal mice. Dilation of blood vessels in the dermis of skin and accumulation of PMNLs in the vessels occurred at peak time when contact hypersensitivity in mast cell-deficient and normal mice was observed (Fig. 4).

Collectively these data indicated that there was no significant difference in the infiltrations of eosinophils and PMNLs into the dermis of ear in normal versus mast cell-deficient mice, either after vehicle or TDI application.

TDI increases the extent of mast cell degranulation, but not the number of mast cells in normal WBB6F1/J-Kit+/+ mice. When the skin tissues were histologically examined, the extent of mast cell degranulation in the dermis of skin challenged with 1% or 0.5% TDI was similar to that of challenged with vehicle application in normal mice. But the number of mast cells per unit area in the dermis of skin in mice induced by 0.5%, 1%, or 3% TDI single application without TDI sensitization was similar to that induced by vehicle application (Table III).

Fig. 5 showed the extent of mast cell degranulation at 24 h after 1% TDI challenge or vehicle application

**Figure 5.** The extent of mast cell degranulation on 24 hours after TDI application in the non-sensitized negative control and normal WBB6F1/J-Kit+/+ mice sensitized on the back skin with TDI. **: $P < 0.01$, 1.0% or 3.0% challenge vs non-sensitized negative control, ***: $P < 0.001$, sensitization & 1% challenge vs only sensitized negative control.

on the ear of normal mice that sensitized with TDI on the back skin at d 1 and d 8, and then challenged with 1% TDI on the ear at d 15. The extent of mast cell degranulation in the ear of normal mice induced by 1% TDI challenge increased, that compared with vehicle application. The number of mast cells per unit area in the ear of normal mice induced by 1% TDI challenge decreased because of ear swelling (Table III).

Discussion

In 1935, Landsteiner and Jacobs (18) showed that epicutaneous application of small reactive compounds resulted in the induction of contact hypersensitivity (CHS). The first treatment (sensitization) had no visible effect, but when the same happen was applied after a week for a second time (elicitation), a local inflammation at the site of application occurred with a delay of 24-48 h. This local CHS reaction was characterized by infiltrations of lymphocytes and polymorphonuclear leukocytes (PMNLs), especial neutrophils.

Toluene diisocyanate (TDI), low molecular weight compound, is the most prevalent cause of occupational asthma in Korea (19,20), as well as in westernized countries (21,22). The pathogenetic mechanism is still unclear, but it has been suggested that TDI may act as a hapten, combining with protein-carrier molecules to provoke an immune response (19,20,22). Elevated serum-specific IgE antibodies have been detected in a part of subjects with TDI-induced asthma, which may reflect the involvement of other immunologic and/or non-immunologic mechanisms. Sensitized subjects exhibited specific airway hyperresponsiveness and a marked inflammation of the airways characterized by influx of neutrophils and eosinophils (23,24).

For skin reactivity in animals sensitized with TDI, guinea pigs were treated daily with a 10% TDI solution by intranasal application for 5 consecutive days. And then, local application of TDI onto the flank skin of these TDI-sensitized animals resulted in an immediate development of the flare-and-wheal reaction at the site of application (25). The extent of the skin response was dependent on the concentration of TDI used for provocation.

In this study, the negative controls, i.e., mice not sensitized with TDI but merely ear challenged, showed the ear swelling response. Only 3% TDI single application induced the significant ear swelling response, but the ear swelling responses induced by 1% and 0.5% TDI single application were similar to that induced by vehicle, acetone : olive oil (4 : 1) application. The extent of the ear swelling response was dependent on the dose of TDI used for application. Also, when TDI-sensitized mice were challenged with 1% TDI dissolved in acetone : olive oil (4 : 1), ear thickness marked and significant increased 24 h after topical challenge when compared to the non-sensitized mice. There was no significant difference in the ear swelling responses of normal versus mast cell-deficient mice, either at baseline or after TDI-induced CHS.

Histological examination revealed the infiltration of basophils and eosinophils in the superficial dermis as the characteristics of the animal skin reaction (25). In morphologic study of allergic contact dermatitis and DHS in man skin reactions (26-28), a number of important features were found to characterize these reactions, including infiltration and piecemeal degranulation of basophils; degranulation and replication of fixed tissue mast cells; infiltration of eosinophil and neutrophils; increased vascular permeability leading to dermal and epidermal edema, vascular compaction, and erythrocyte extravasation; microvascular alterations affecting endothelial cells and pericytes, with compromise of vessel lumina and basement mem-

brane thickening.

In TDI-exposed animal, there were dramatic signs of airway mucosal damage associated with the bronchial hypersensitivity that included substantial decreases in epithelial cilia, mucin content, and mast cells, as well as squamous metaplasia, numerous mitotic figures, and a prominent PMNL infiltration (29). Some reports have shown that exposing guinea pigs to TDI causes a rapid increase in airway responsiveness in association with morphologic evidence of an acute inflammatory response (30,31). Exposing guinea pigs to TDI also causes airway edema, as demonstrated by marked increase in extravasation of Evans blue dye into the tracheal wall (32) and increment of PMNL in the intravascular and extravascular tracheal lamina propria (30).

Mast cells and, in some cases, mast cell-derived cytokines can have a critical role in the expression of the acute, late-phase and chronic components of IgE-dependent allergic inflammation (33). And these can influence the development of an important function consequence of such reaction; airways hypersensitivity. However, mast cells can also perform important beneficial roles in host defense, both in IgE-dependent immune responses to certain parasites and in natural immunity to bacterial infection.

In the previous studies, using mast cell-deficient mice, DTH and CHS found to be either intact (34) or only partially decreased (8), in comparison with non-mast cell-deficient +/+ controls. Importantly, in the studies showing decreased DTH in mast cell-deficient mice (8) and decreased in cellular infiltration in platelet-depleted +/+ mice (11), the remaining DTH was also shown to depend on vasoactive amine serotonin (5-HT), suggesting other sources of 5-HT. CHS in mast cell-deficient mice was probably more dependent on platelet 5-HT, because mast cell 5-HT was virtually absent (11). When the immune responses of mast cell-deficient (W/W^V) and mast cell-sufficient littermates (LM) were compared, CHS and DTH reactions of W/W^V and LM mice were similar. No evidence of immune deficiency of W/W^V mice was found (12). In the experiment about the ability of mast cell-deficient W^f/W^f and W/W^V mice to produced DTH responses, mice of both genotypes produced large DTH responses to the contact sensitizers oxazolone and picryl cholride (13).

In these experiments, the infiltration of eosinophils and PMNLs and the extent of mast cell degranulation were measured as a cellular mechanism underlying the ear swelling. No significant histological changes were observed in the dermis of skin when the vehicle, 1% and 0.5% TDI single application examined using unsensitized animals. The TDI-sensitized mice were topically challenged with 1% TDI, the significant

infiltrations of eosinophils and PMNLs into the dermis of the ear was induced in mast cell-deficient and normal mice compared to the non-sensitized mice. And the extent of mast cell degranulation in the ear of normal mice induced by 1% TDI challenge increased, that compared with vehicle application. But the number of mast cells per unit area in the ear of normal mice induced by 1% TDI challenge decreased, compared with the mice was similar to that induced by vehicle application. These results were similar, as had been reported by some worker (12-14,34).

In summary, TDI induced a characteristic increment of mast cell degranulation in normal mice. And TDI induced a significant ear swelling response and increment of PMNLs and eosinophils in TDI-sensitized mast cell-deficient and normal mice, but not of mast cell in normal mice. There were no significant differences in the ear swelling and the number of PMNLs and eosinophils of normal versus mast cell-deficient mice, either at baseline or after TDI-induced CHS. In Conclusion, TDI can be used as a murine CHS model, and the mast cells may not be essential in TDI-induced CHS.

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