

## Oral and epicutaneous desensitization in urushiol contact dermatitis in guinea pigs sensitized by 2 methods of different sensitizing potency

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This investigation was conducted to study the relationship between the different intensities of sensitization to urushiol induced by the maximization test method (MT) or by the ear-flank method (EF) in guinea pigs, and hyposensitization induced by repeated oral and/or epicutaneous administration of the allergen, and to compare the immune tolerance achieved between these routes of dosing. During the observation period of 10 weeks, both oral and epicutaneous administration produced hyposensitization to urushiol after weak sensitization by EF; however, only combined administrations via the oral and epicutaneous routes achieved hyposensitization after potent sensitization, while inadequate tolerance was induced by oral dosing and none by epicutaneous dosing. Thus, it is indicated that difficulty in establishing immune tolerance may increase with increasing intensity of primary sensitization, and that hyposensitizing potency tends to increase in the order epicutaneous, oral and combined (e.p.+p.o.) administrations of urushiol.

**Key words:** contact sensitivity; hyposensitization; immune tolerance; urushiol allergy; guinea pig study; sensitization intensity; maximization test; ear-flank test. © Munksgaard, 1998.

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The mainstay of management of contact dermatitis has been symptomatic treatment and avoidance of exposure to causative allergens. However, there are situations when allergen avoidance is not possible, e.g., for occupational reasons. It is said that sensitivity to Japanese lacquer, which causes eruptions on the skin, will decrease if craftsmen continue to use the lacquer. A tradition among craftsmen has been that repeated licking of trace amounts of lacquer may facilitate the induction of hyposensitization. Kawai et al. (1) in a questionnaire survey of lacquer craftsmen, reported that 189 of 232 craftsmen experienced lacquer dermatitis and that 158 of the 189 who had lacquer dermatitis experienced gradual, spontaneous relief during continued occupational exposure to the allergen without taking specific measures, including licking of the lacquer (1). They also conducted serial patch tests on 8 college students of fine arts who used lacquer. 5 of the 8 students had a positive result 1 month after initial contact with lacquer, and the degree of reaction to the skin test decreased as the dermatitis improved during 9 to

10 months of repeated exposure. Thus, they suggested that spontaneous hyposensitization is likely in lacquer craftsmen through continuous exposure to the allergen (2).

Other reports state that repeated topical application of an allergen induces immunological tolerance. Boerringer et al. succeeded in experimentally inducing hyposensitization in guinea pigs sensitized to dinitrochlorobenzene (DNCB) or 4-ethoxymethylene-2-phenyloxazolone (OX) by repeated exposure of the skin to DNCB or OX (3). In guinea pigs sensitized to urushiol by the ear-flank method (EF) (4), we noted hyposensitization induced by repeated epicutaneous application of the compound and determined that this hyposensitization was systemic because reduced responsiveness was observed at skin sites other than those to which the allergen was applied (5). However, regarding repeated epicutaneous application of allergens, it has also been reported that immune reactions were potentiated in mice sensitized to dinitrofluorobenzene (DNFB) by repeated topical applications of DNFB (6). Similarly, our study

using DNFB as the antigen did not produce hyp sensitization in the guinea pigs (7). Recently, it has been reported that a shift from the delayed type to the immediate type of hypersensitivity was observed in mice following repeated epicutaneous application of contact sensitizing agents (8). Thus, varied results have been obtained depending on the experimental conditions.

It is common for dermatologists to observe such reactions as redness and pruritus to subside when alopecia areata is treated with repeated topical applications of DNCB. Observation of 66 alopecia areata patients treated with DNCB or squaric acid dibutylester (SADBE) revealed that the outcome was favorable in patients whose initial response lasted through the treatment period, whereas the outcome was poor in patients whose initial response was either marginal or declined with time (9). In contrast to the finding that hyposensitization develops with repeated exposure to lacquer in craftsmen and art students using lacquer (1, 2), spontaneous hyposensitization occurs less frequently in chrysanthemum growers despite repeated contact with the flower. Haneda et al. (10) reported that oral intake of chrysanthemum juice was effective in relieving symptoms in patients with contact dermatitis.

Because inconclusive results have been reported, we investigated the feasibility of inducing hyposensitization by repeated epicutaneous administrations of an antigen in an attempt to apply the phenomenon of epicutaneous immune tolerance to the treatment of contact dermatitis. As mentioned above, we succeeded in inducing antigen-specific hyposensitization in the guinea pigs by repeated epicutaneous applications of urushiol as the antigen (5), but not when DNFB was used as the antigen (7). Thus, the present study was designed to test the hypothesis that intensity of sensitization is related to difficulty in achieving subsequent hyposensitization. This was formulated based on the finding that reactions associated with DNFB sensitization were more marked than those with urushiol sensitization (Fig. 1).

This investigation was intended to clarify the relationship between intensity of sensitization and degree of difficulty in inducing hyposensitization, by repeated epicutaneous applications of urushiol to guinea pigs sensitized to urushiol by EF or by the maximization test method (MT), the latter of which is thought to be of a higher sensitizing potency. The results of this study could provide those in occupations requiring constant exposure to active allergenic substances that frequently cause contact dermatitis, such as Japanese lacquer, with suggestions on how to handle allergens better, especially in the early stages of occupational con-

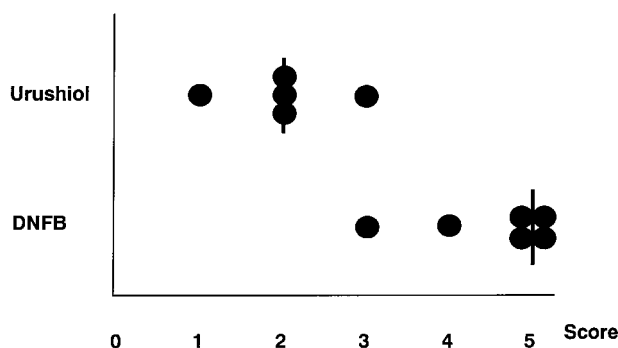


Fig. 1. Comparison of intensity of sensitization; 2,4-dinitrofluoroben (DNFB) and urushiol. Groups of 5 guinea pigs were sensitized either with urushiol or with DNFB by the ear-flank method. 1 week after the sensitization, challenge doses of the hapten were applied to the skin of the left flank. Skin reactions were read at 24 h after application and were rated according to the scoring system of Draize. Data were shown as median readings.

tact with the allergen, in order to minimize subsequent allergic symptoms.

Oral hyposensitization, as well as epicutaneous hyposensitization, have been tried for the treatment of contact dermatitis. Oral hyposensitization, induced by oral dosing of the potent allergenic compound, suppresses systemic immune reactions against the specific antigen. Although depletion of T-cells, anergy of T-cells and induction of suppressor cytokines have been suggested to be involved in the phenomenon, the exact mechanism of oral desensitization and hyposensitization has not been completely clarified. However, this phenomenon has been known for a long time, and clinical studies have been conducted to investigate the feasibility of inducing hyposensitization by oral administration of urushiol or structurally similar compounds for the treatment of lacquer-induced contact dermatitis. Although hyposensitization and desensitization were shown to be induced in guinea pigs by oral administration of urushiol acetate (11), its clinical application in humans is difficult because of adverse reactions observed with the high doses required to achieve hyposensitization by oral dosing (12). There are significant differences among studies in clinical data on the efficacy and safety of oral hyposensitization. However, there has been no published report of a comparison of oral versus epicutaneous hyposensitization.

This study, using 3 types of repeated administrations (epicutaneous (e.p.), oral (p.o.), and e.p.+p.o. dosing) of urushiol, examined the relationship between epicutaneous and oral hyposensitization in an experimental model of contact dermatitis in guinea pigs.

## Materials and Methods

### Animals

Female Hartley strain guinea pigs, weighing 300–350 g, were obtained from Seibun Sangyou, Kamiisizu, Yourou County, Gifu Prefecture, Japan.

### Hapten

Urushiol was purified as follows: sap collected from Japanese lacquer trees (*Rhus verniciflua*) was filtrated to obtain raw urushi. Raw urushi was dissolved in 10 parts of acetone to 1 part of raw urushi. Urushiol was extracted from the sap by deacetylation.

### Assessment of primary irritating dose

Prior to initiating the study, we treated a group of naive animals with varying amounts of urushiol ( $15 \mu\text{g}/4 \text{ cm}^2$  to  $300 \mu\text{g}/4 \text{ cm}^2$ ) and DNFB ( $30 \mu\text{g}/4 \text{ cm}^2$  to  $150 \mu\text{g}/4 \text{ cm}^2$ ; Wako Chemical Co., Japan) to determine the primary irritating dose and the appropriate dose range for assessing induction of hypersensitivity. Skin readings at 24 and 48 h after urushiol application established that 2 out of 3 animals showed mild skin irritation after exposure to  $30 \mu\text{g}/4 \text{ cm}^2$  urushiol. No skin reaction could be observed with administration of  $15 \mu\text{g}/4 \text{ cm}^2$  urushiol. No skin reaction could either be observed with  $60 \mu\text{g}/4 \text{ cm}^2$  DNFB. Therefore, we used a 15

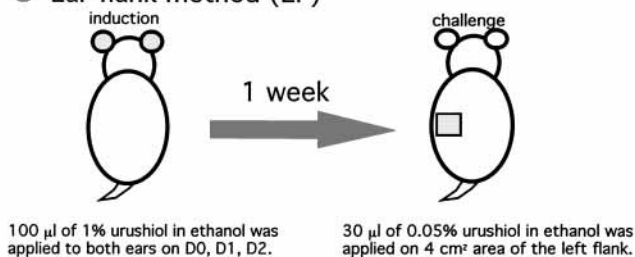
$\mu\text{g}$  dose of urushiol and a  $60 \mu\text{g}$  dose of DNFB as the non-irritating application doses in our studies.

### Sensitization

**Ear-flank method (EF).** Guinea pigs were sensitized to urushiol by epicutaneous application of  $100 \mu\text{l}$  of a 1% (w/v) solution of urushiol in ethanol on the lateral sides of the ear lobes bilaterally  $1 \times$  a day for 3 days (D0, D1, D2). 1 week later, the animals were first challenged on a  $4 \text{ cm}^2$  area of the clipped and shaved left flank with non-irritating concentrations of urushiol ( $30 \mu\text{l}$  of a 0.05% (w/v) solution) in ethanol (Fig. 2). Skin reactions were read 24 and 48 h after application of the challenge dose and were rated according to the scoring system of Draize (13).

**Maximization test (MT).** The induction procedure involved both intradermal and topical applications. 3 duplicate intradermal injections consisting of (i) 0.1 ml of Freund's complete adjuvant (FCA), (ii) 0.1 ml of 0.1% urushiol in liquid paraffin, and (iii) 0.1 ml of 0.1% urushiol emulsified in the adjuvant were injected intradermally into the shaved shoulder region, on either side of the midline. This was followed 7 days later by the application to the same region of an occlusive patch ( $2 \times 4 \text{ cm}$ ) of 1% urushiol in petrolatum, which was left in place for 48 h. The shaved shoulder region was treated with 10% sodium lauryl sulfate the day before topical application. 21 days after the initial

#### ● Ear-flank method (EF)



#### ● Maximization test (MT)

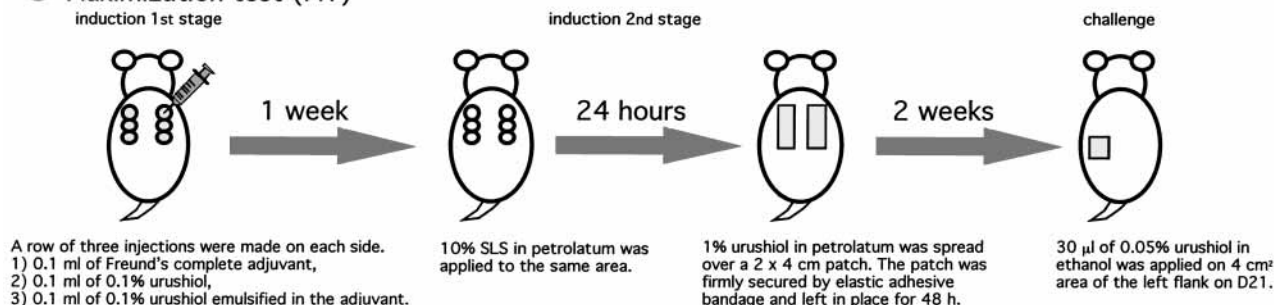


Fig. 2. The method of sensitization.

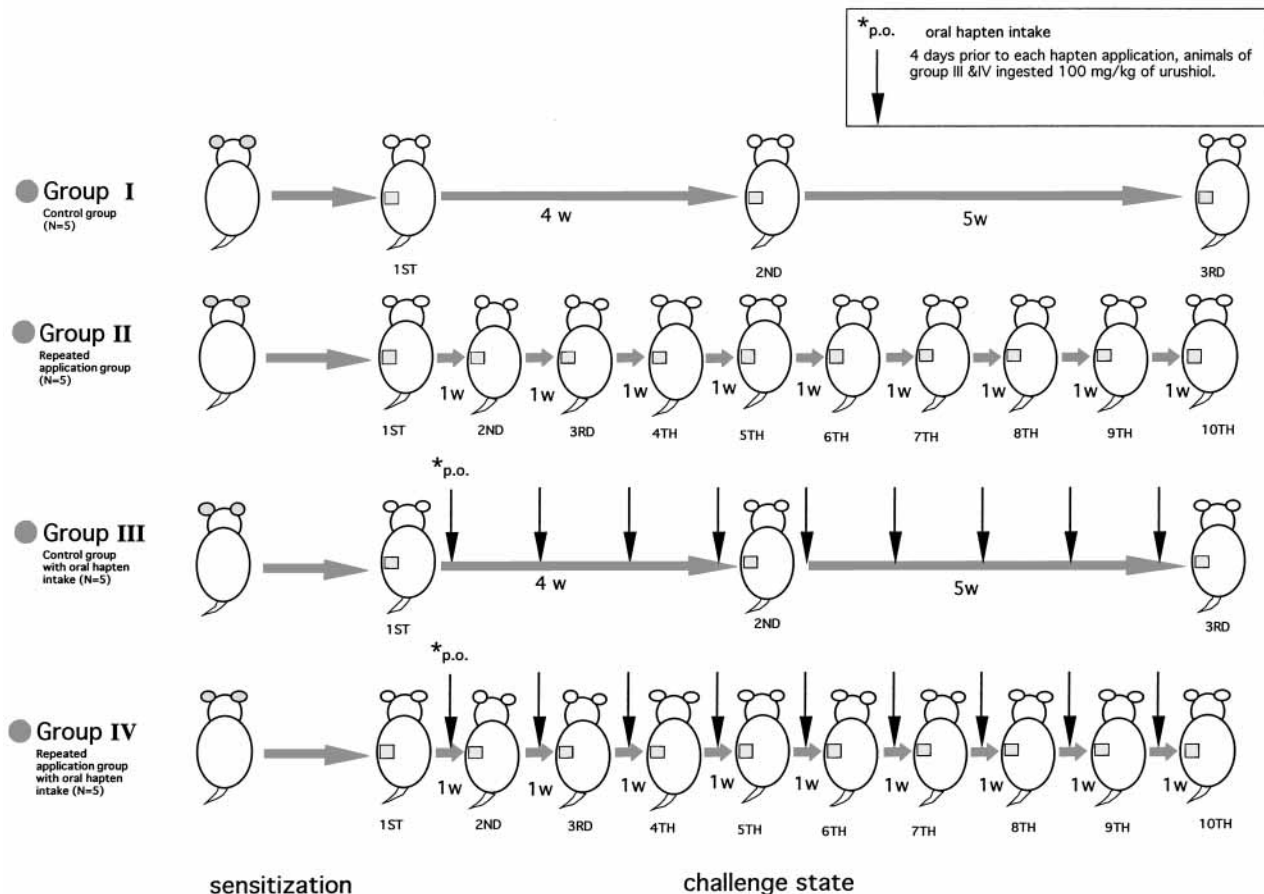


Fig. 3. The application and administration procedure. Animals were sensitized either by the ear-flank method or by the maximization test. After each sensitization, animals were divided into 4 groups, respectively, depending on the application procedures shown in the figure. Each group consisted of 5 guinea pigs.

induction phase, 30  $\mu$ l of 0.05% urushiol in ethanol was applied on a 4 cm<sup>2</sup> area of the clipped and shaved left flank (Fig. 2). Skin reactions were read in the same manner as with EF.

#### Application and administration procedure

The application and administration procedure is shown in Fig. 3. Animals were sensitized either by EF or MT. After each sensitization, animals were divided into 4 groups, depending on the application procedures. Each group consisted of 5 guinea pigs.

Group 1 guinea pigs were exposed to non-irritating concentrations of urushiol (30  $\mu$ l of a 0.05% (w/v) solution) on a 4 cm<sup>2</sup> area of the clipped and shaved left flank 1, 5 and 10 weeks after the sensitization. Group 2 guinea pigs were repeatedly exposed to the same dose of urushiol applied to the same area of the skin 1  $\times$  a week for 10 weeks. Group 3 animals had the same dose of urushiol applied to the same area of the skin 1, 5 and 10

weeks after the sensitization and, in addition, 100 mg/kg urushiol was administered orally 1  $\times$  a week during the same 10 weeks. Group 4 guinea pigs were repeatedly exposed to the same dose of uru-

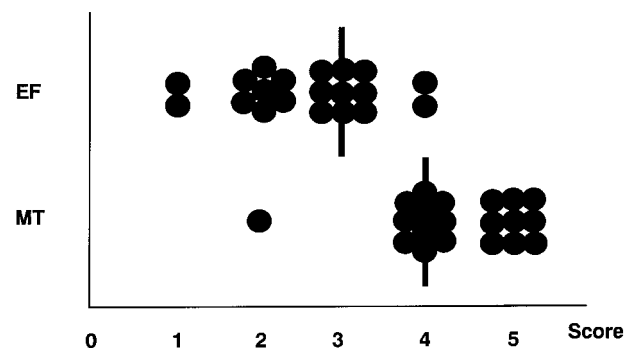


Fig. 4. Comparison of initial reactions; the ear-flank method (EF) and the maximization test method (MT). Initial reactions at the 1st challenge. Skin reactions were read at 24 h after application and were rated according to the scoring system of Draize. Data were shown as median readings.

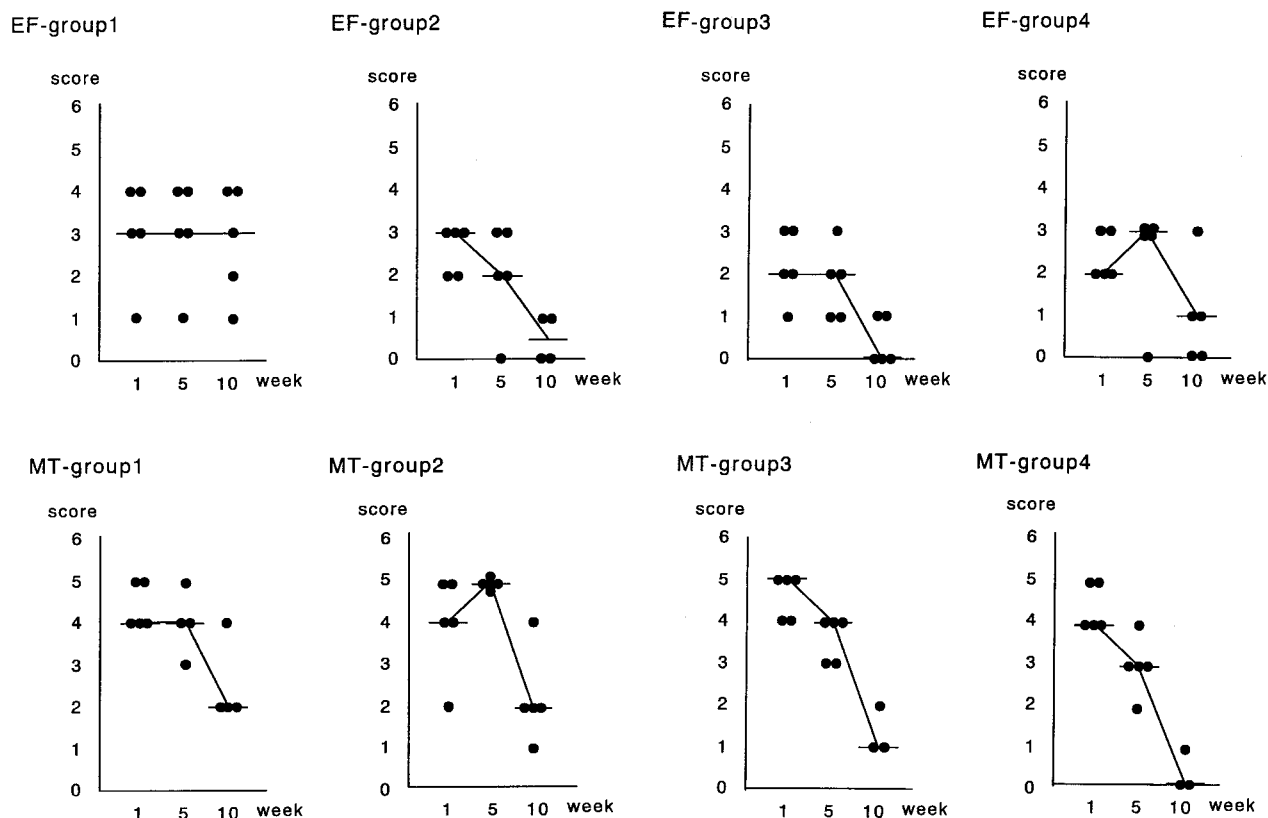


Fig. 5. Hyposensitization by repeated hapten application or administration. Repeated epicutaneous hapten applications ( $1 \times$  a week for 10 weeks) induced hypsensitization in the animals sensitized by EF, but not in the animals sensitized by MT. Oral hapten ingestions induced hypsensitization in the animals sensitized by both EF and MT. In MT-sensitized animals, repeated epicutaneous applications of hapten had no significant hypsensitization, while oral applications resulted in moderate hypsensitization and combined oral and epicutaneous applications resulted in marked hypsensitization, respectively, at 10 weeks. Data were shown as median readings, and the medians were linked.

shiol to the same area of the skin  $1 \times$  a week and, in addition, were administered 100 mg/kg urushiol orally  $1 \times$  a week, for 10 weeks after the sensitization. The earlier work (11) was helpful to the choice of dose for peroral administration, and in our preliminary experiments, we had found that 100 mg/kg urushiol orally produced a higher degree of hypsensitization than a lower dose of 10 mg/kg. The skin reactions were rated according to the scoring system of Draize (12).

## Results

### *Hypsensitization by repeated hapten application or administration*

Animals sensitized by MT showed stronger reactions to urushiol at the 1st challenge than animals sensitized by EF (Fig. 4). Repeated epicutaneous hapten applications ( $1 \times$  a week for 10 weeks) induced hypsensitization in the animals sensitized by EF, but not in the animals sensitized by MT. Oral hapten ingestions induced hypsensitization

in the animals sensitized by both EF and MT. In MT-sensitized animals, repeated epicutaneous applications of hapten gave no significant hypsensitization, while oral applications resulted in moderate hypsensitization and combined oral and epicutaneous applications resulted in marked hypsensitization, respectively, at 10 weeks (Fig. 5).

## Discussion

In the present study, both oral and epicutaneous administrations induced hypsensitization to the allergen after weak sensitization by EF during the observation period of 10 weeks. However, after the higher level of sensitization produced by MT, the degree of immune tolerance induced depended on the method of dosing of the antigen. The degree of immune tolerance induced increased in the order epicutaneous, oral and e.p.+p.o. dosing of the antigen. These findings indicate that the intensity of sensitization is a factor related to the difficulty in inducing hypsensitization. The hypsensitizing

potency of repeated oral dosing is greater than that of repeated epicutaneous dosing; the potency of combined dosing is superior to that of either oral or epicutaneous dosing alone. The mechanism generating the difference in hyposensitizing potency between the 2 routes of dosing is not known. In immunofluorescent distribution studies of DNCB in guinea pigs, Nakagawa et al. (14–16) found that after epicutaneous dosing of DNCB, the 2,4-dinitrophenyl (DNP) group of the DNCB molecule was present in epidermal cells and regional lymph nodes at the site of topical application, thoracic duct, spleen and peripheral blood lymphocytic cells. Takei (17) reported that after oral dosing of DNCB, the DNP group was observed in the epithelial cells of the gastrointestinal mucosa, mesenteric lymph nodes, Peyer's patches, spleen and peripheral blood lymphocytic cells, a finding common to that obtained after epicutaneous dosing showing the presence of the DNP group at the site of application and in the regional lymph nodes, spleen and peripheral blood lymphocytic cells. Scanning immunoelectron microscopic studies of the distribution of epicutaneous DNCB in guinea pigs revealed that the DNP group was present on the surface of epidermal and lymphocytic cells, indicating that DNCB binds with components of the plasma membrane of these cells in vitro (18, 19).

The "2 signal theory" of T lymphocyte activation, proposed by Schwartz et al. (20), is now widely accepted as a valid theory which holds that in addition to the signal from T cell receptors (TCR), a 2nd signal, or "co-stimulatory signal", is required for activation of T lymphocytes. It has been shown that the signal from TCR alone induces unresponsiveness (immunological tolerance) or cell death due to apoptosis. In allergic contact dermatitis, although keratinocytes in the skin express MHC molecules (21), they are not capable of transmitting the co-stimulatory signal (22). The possible involvement of keratinocytes in tolerance has been suggested (23, 24). In the intestinal tract, allergen-presenting cells, such as macrophages, melanocytes and B cells, are present in Peyer's patches (25, 26). However, no evidence has been published to date that show that these cells are involved in the development of tolerance by oral dosing. Intestinal epithelial cells, which are capable of expressing MHC molecules (27) but unable to transmit the co-stimulatory signal, have also been shown to have allergen-presenting potential and may play a role in generating hyposensitization (28). Like oral hyposensitization, epicutaneous hyposensitization is systemic, as described earlier. If the lack of the co-stimulatory signal which occurs in intestinal epithelial cells when allergens are

given orally, and in the epidermal keratinocytes when allergens are given epicutaneously, plays a role in the induction of tolerance, the enhanced induction of hyposensitization achieved by combined dosing may be accounted for.

In the management of contact dermatitis in lacquer craftsmen, weak sensitization, if induced in the early stage of professional contact with the substance, may facilitate the subsequent induction of epicutaneous hyposensitization. As traditionally discussed, continuous oral intake of a trace amount of lacquer may have some effect in inducing tolerance to the allergen. It may be worthwhile to investigate the clinical use of urushiol glycosides in an attempt to induce hyposensitization by oral dosing, as with the management of contact dermatitis in chrysanthemum growers. The mechanism of the induction of hyposensitization is said to involve T-cell depletion, T-cell anergy, or induction of suppressor cytokines. Histological examinations in the present study (data not shown) show an infiltration of mononuclear cells and eosinophils even at sites on the skin that do not have apparent gross changes after induction of sensitization. This suggests the induction of hyposensitization may be better explained by the T-cell anergy hypothesis than by the T-cell depletion hypothesis.

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