

## Original article

## Nonimmediate reactions to systemic corticosteroids suggest an immunological mechanism

**Background:** Administration of corticosteroids (CS) by different routes may cause varying types of allergic reactions, thereby hampering their further use in affected patients. In order to verify an immunological involvement we evaluated a group of patients with symptoms compatible with nonimmediate allergic reactions to CS.

**Methods:** Studies included patch and intradermal tests, immunohistochemical studies and controlled administration to reproduce the response. The cytokines interleukin (IL)-4, interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  were quantified in peripheral blood during the response.

**Results:** Of 32 subjects evaluated presenting nonimmediate urticaria or exanthema, 21 were finally considered positive after re-exposure. The drugs most frequently involved were betamethasone and dexamethasone. Fewer than half the patients responded to prednisolone whilst some responded to three or more CS. Hydrocortisone and deflazacort were well tolerated by all the patients. Subjects with a positive intradermal or patch test had a perivascular mononuclear cell infiltrate with the presence of CD4 and CD8 lymphocytes positive for CD45RO+ (memory) and CD69 (activation marker) cells. Monitoring peripheral blood during the acute response showed expression of IFN- $\gamma$  and TNF- $\alpha$ , with downregulation of IL-4.

**Conclusion:** Adverse systemic responses to different CS are suggestive of a nonimmediate reaction. The symptoms elicited together with the immunological studies suggest a T-cell mediated response. The response to closely related CS was especially marked between betamethasone and dexamethasone, whereas hydrocortisone and deflazacort were well tolerated.

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Although corticosteroids (CS) are used to treat immunological disorders, including allergy, sensitization to these compounds can occur, which may hamper their further clinical use. Most reactions arise after topical administration of the drug, ranging in frequency from 2.9 to 4% (1, 2). These drugs represent the seventh most common agent inducing contact dermatitis, after nickel sulfate, cobalt chloride, colophony, fragrance mix, balsam of Peru and potassium dichromate (2). Allergic reactions to systemic or intralesional administration of CS, however, seem to be infrequent (3, 4). Reactions attributed to these drugs include anaphylaxis, bronchospasm, urticaria and/or angioedema, exanthema, purpura, and generalized erythroderma, often associated with bullae (5–10).

The symptoms induced by these drugs, their reproducibility after drug intake, and the appearance of positive immediate or delayed skin tests all suggest that at least two immunological mechanisms are involved: IgE

mediated reactions and T-cell effector dependent responses. Although evidence favors an IgE mechanism in immediate reactions, this is based on the skin test response in subjects with anaphylaxis, and specific IgE antibodies to CS have not yet been detected *in vitro* (5). No data are available concerning the underlying mechanisms in acute bronchospasm or other manifestations associated with a rapid release of histamine attributed to IgE antibodies.

Increasing evidence in allergy to different drugs now indicates that a T-cell-mediated immune response is involved in maculopapular exanthema or delayed urticaria (11–15). Specific T cells to haptens from epicutaneous biopsies have been isolated in exanthematic reactions to drugs (16). Whether this also occurs with CS has not yet been studied in detail, although some evidence supports T-cell involvement after systemic administration (17).

Independently of the type and mechanism of the reaction, patients allergic to one CS may respond to another (18). The chemistry of the drug and patch test results in sensitized subjects with contact dermatitis have

enabled determination of four major reactive groups of drugs: A, drugs which include hydrocortisone, methylprednisolone and tixocortol pivalate; B, drugs with a substitution at C9, such as triamcinolone acetonide, budesonide, fluocinolone and flunisolide; C, drugs with a double bond at C1–C2, including dexamethasone and betamethasone; and D, drugs with a long-chain ester at C17 and/or C21, such as beclomethasone dipropionate and fluticasone propionate (19). The most commonly involved CS are hydrocortisone and methylprednisolone, although reactions have also been reported with dexamethasone, prednisone, prednisolone and triamcinolone (19–21).

As CS are often essential and substitutes are not always available, plus the fact that the demand for allergological workup is increasing, we attempted to confirm nonimmediate allergic reactions (NIR) to CS in subjects referred to our clinic and to clarify the immunological mechanisms involved.

## Material and methods

### Patient selection

All patients referred to our clinic between July 2000 and December 2002 for a history of suspected nonimmediate allergic reaction to systemic CS were included in the study. A nonimmediate reaction was considered when exanthema or urticaria, with or without angioedema, appeared 2 h or more after drug intake.

### Skin test

**Intradermal tests.** These were carried out using different soluble, commercially available CS at the following concentrations: hydrocortisone at 100 and 50 mg/ml (Actocortina, Byk Elm, Madrid, Spain), methylprednisolone succinate at 20 and 10 mg/ml (Urbason, Aventis, Madrid, Spain), dexamethasone at 40 and 4 mg/ml (Fortecortin, Merck, Barcelona, Spain), and betamethasone at 4 and 0.4 mg/ml (Celestone, Schering-Plough, Madrid, Spain). Intradermal tests with preservatives (monosodium phosphate and disodium phosphate) included in these commercial preparations were performed at 10 mg/ml. All the reagents were tested on the volar skin of the forearm. Readings were made 15 min and 24, 48 and 96 h later. Results were considered positive when an infiltrated erythema > 5 mm was present. Positive controls were made with histamine at 1 mg/ml. Normal saline was used as a negative control. The concentrations used for CS and preservatives had proved to be nonirritant in a control group of 12 healthy subjects.

**Patch test.** This was performed with standardized CS patch testing formulations (Chemotechnique Diagnostics, Malmö, Sweden) which include: budesonide 0.1%, betamethasone 1%, triamcinolone acetonide 1%, tixocortol pivalate 1%, beclomethasone 0.5%, clobetasol 1%, dexamethasone 1% and hydrocortisone 1%. The vehicle in all cases was petrolatum, except for ethanol in hydrocortisone. Two commercial preparations were also tested: methylprednisolone succinate (Urbason) and paramethasone (Cortidene, Eclon, Madrid, Spain), both at 1% in petrolatum. The occlusion time was 48 h. The test reactions were read at 48 and 96 h and scored as recommended by the International Contact Dermatitis Research Group (21).

### Controlled administration test

Controlled administration with increasing doses of the relevant CS was carried out to confirm positivity and determine the type of reaction involved. Patients who developed a positive response were further studied to determine the existence of any possible cross-reactivity to CS with different chemical structures.

The drugs and the doses used, administered parenterally at 1-h intervals, were hydrocortisone (20,50,100 mg), methylprednisolone succinate (8, 20, 40 mg), dexamethasone (4,20,40 mg), betamethasone (2,4 mg), triamcinolone acetonide (10,20,40 mg) and paramethasone (10,20,40 mg). Additionally, prednisone and deflazacort, both at 5,15, and 30 mg, were administered orally. These concentrations were established arbitrarily and administration was carried out in a single-blind fashion together with placebo. Preservatives used in these commercial preparations were administered with a negative response. Subjects who responded to placebo or had no clear response were excluded from the study, as were patients with a history of chronic urticaria. The development of urticaria, whether or not accompanied by angioedema or exanthema, within an arbitrarily established interval of 2 h to 7 days after drug administration was considered as a positive response. The patients remained under observation for 8 h after the last dose, after which they were in telephone contact with the hospital. In the event of a positive response, this was confirmed by one of the investigators, the time to the response was recorded and serum samples were taken for the measurements described below. Once a positive response appeared, patients were followed at 8–24-h intervals until resolution.

### Immunopathological study

In those cases with a positive response after intradermal and patch testing, a 4 mm punch (Stiefel Laboratorios, Madrid, Spain) biopsy was obtained at 48 h. Samples were processed in formaldehyde and embedded in paraffin. Microtome sections (4 µm) were processed for hematoxylin–eosin and immunohistochemical staining with the following monoclonal antibodies: CD4, granzyme B (Novocastra Laboratories, Newcastle upon Tyne, England), CD8, CD45RO and perforin (Dako, Ely, Cambridgeshire, England), using the automated immunostaining system Techmate 500plus (Dako) with the Envision Plus visualization software.

### Sample collection from patients and controls

Patients who developed a positive response after controlled administration of CS gave two blood samples: the first at the moment the reaction started and the second 30 days later. In each sample 10 ml of peripheral blood was obtained and processed for RNA extraction and cDNA synthesis (22).

A control group of 10 subjects, with no history of drug allergy, were all administered betamethasone (4 mg) to assess whether it could induce any change in cytokine expression. Two samples were taken at 24 h and 30 days, and processed as above.

### Quantification of mRNA expression

The expression levels of interleukin (IL)-4, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α were detected by real time polymerase chain reaction (PCR) (LightCycler; Roche Diagnostics, Indianapolis, IN). cDNA from 1 µg of total RNA was used for PCR. Real-time PCR was performed with the Fast-Start DNA master SYBR Green system (Roche). The primer sequences were: IL-4, 5'-CATCGGCATTTTGAACGAGGTCA3', 5'-CTTATCGATGA

ATCCAGGCATCG3'; IFN- $\gamma$ , 5'CATTGAAAGCCTAGAAAGTCTG3', 5'CTCATGAATGCATCCTTTTCG3'. All results were normalized with respect to the expression of  $\beta$ 2-microglobulin.

The cDNA copy number for each gene of interest was determined using a seven-point standard curve. Standard curves were run with each set of samples. Correlation coefficients ( $r^2$ ) for standard curves were typically  $\geq 0.98$ , and the precision of target  $\beta$ 2-microglobulin from the same cDNA was similar. To confirm that each primer pair correctly amplified the sequence of interest, initial PCR products from peripheral blood mononuclear cells were run on agarose gel, stained with 0.5  $\mu$ g/ml ethidium bromide, and viewed by UV transillumination. To confirm the specificity of the reaction product during each run, the melting profile of each sample was analyzed using the LightCycler. The melting profile was determined by holding the reaction at 80°C for 10 s and then heating slowly to 95°C with a linear rate of 0.1°C/s while the fluorescence emitted was measured. The melting curve analysis demonstrated that each primer pair amplified a single product.

### Statistical analysis

The ratios of IL-4, IFN- $\gamma$  and TNF- $\alpha$  to  $\beta$ 2-microglobulin were determined in the patients at the start of the reaction following drug intake and 30 days later, and in the controls at the same time intervals after drug administration. Comparisons were made using the Mann-Whitney test. Comparisons of the cytokine ratio and transcription factors in the patients and the controls at the first determination and 30 days later were made with the Wilcoxon test.

All patients and controls gave written informed consent prior to testing, and the study was approved by the Clinical and Ethics Committee of La Paz Hospital.

## Results

### Patients

Study of the clinical history resulted in the selection of 38 subjects (35 women, 3 men). The mean age was 51.82 years (range 14–76). The mean time interval between the clinical episode and the evaluation in our clinic was 6 months (range 10 days–10 years). Most subjects (94.28%) had been prescribed CS for osteoarticular diseases and CS were administered intralesionally in 71% of the cases. The CS most frequently involved in the reaction were dexamethasone, betamethasone and triamcinolone acetonide. Based on the clinical evaluation, 62% of the subjects reported symptoms compatible with urticaria and angioedema and 38% exanthema, but with no other accompanying symptoms suggestive of an allergic response. The medical history showed that in 14 patients the reaction had occurred between 2 and 24 h after drug administration, in 16 after 24 h, and in eight at 48 h.

### Skin testing

This was performed in all the patients and no immediate response was observed to any of the drugs tested. However, there were two cases with positive delayed intradermal and patch tests to both dexamethasone and betamethasone. The skin biopsy specimens obtained from

these two patients with positive patch test reactions showed a perivascular mononuclear cell infiltrate without the presence of eosinophils or neutrophils. Immunohistochemical studies showed a T-cell infiltrate (CD3+) with the presence of CD4+ and, to a lesser degree, CD8+ cells. These cells were CD45RO+ with very few CD45RA+, with a moderate expression of the activation marker CD25. No perforin or granzyme B was detected in the infiltrate. Similar results were obtained in the intradermal samples.

### Controlled administration

Six of the 38 patients refused to be tested. Of the remaining 32 patients, 21 were finally diagnosed as being allergic to CS by controlled administration. In all instances the symptoms were compatible with a NIR. Of these 21, 14 responded to betamethasone, five to dexamethasone and two to triamcinolone acetonide. Table 1 shows the clinical symptoms and the time interval after drug administration. Most patients developed the reaction within 24 h of drug administration, 16 urticaria, accompanied by angioedema in two cases, and five exanthema. All 14 patients who responded to betamethasone also showed symptoms with dexamethasone, but only six responded to methylprednisolone. Two of the five subjects who responded to dexamethasone responded to methylprednisolone. Only one subject responded to betamethasone, dexamethasone, methylprednisolone and prednisone. All patients tolerated hydrocortisone, paramethasone and deflazacort.

Table 1. Drug involved, clinical symptoms and time interval in 21 subjects with an allergic reaction after controlled administration of corticosteroids

| Patient | Drug involved    | DPT              | Symptoms     | Time interval (h) |
|---------|------------------|------------------|--------------|-------------------|
| 1       | Triamcinolone Ac | Triamcinolone Ac | Urticaria    | 24                |
| 2       | Betamethasone    | Bet; Dex; MP-S   | Exanthema    | 24                |
| 3       | Dexamethasone    | Dex; MP-S        | Urticaria    | 16                |
| 4       | Betamethasone    | Bet; Dex         | Urticaria/AE | 24                |
| 5       | Betamethasone    | Bet; Dex; MP-S   | Urticaria    | 6                 |
| 6       | Dexamethasone    | Dex              | Exanthema    | 24                |
| 7       | Triamcinolone Ac | Triamcinolone Ac | Urticaria    | 6                 |
| 8       | Betamethasone    | Bet; Dex; MP-S;P | Urticaria/AE | 24                |
| 9       | Betamethasone    | Bet; Dex         | Exanthema    | 10                |
| 10      | Betamethasone    | Bet; Dex; MP-S   | Urticaria    | 24                |
| 11      | Betamethasone    | Bet; Dex; MP-S   | Urticaria    | 24                |
| 12      | Betamethasone    | Bet; Dex         | Urticaria    | 6                 |
| 13      | Betamethasone    | Bet; Dex         | Urticaria    | 24                |
| 14      | Betamethasone    | Bet; Dex         | Urticaria    | 6                 |
| 15      | Betamethasone    | Bet; Dex         | Urticaria    | 8                 |
| 16      | Dexamethasone    | Dex; MP-S        | Urticaria    | 24                |
| 17      | Dexamethasone    | Dex              | Urticaria    | 24                |
| 18      | Betamethasone    | Bet; Dex; MP-S   | Urticaria    | 24                |
| 19      | Dexamethasone    | Dex              | Urticaria    | 6                 |
| 20      | Betamethasone    | Bet; Dex         | Exanthema    | 24                |
| 21      | Betamethasone    | Bet; Dex         | Exanthema    | 12                |

DPT, drug provocation test; Bet, betamethasone; Dex, dexamethasone; MP-S, methylprednisolone succinate; P, prednisone; AE, angioedema.

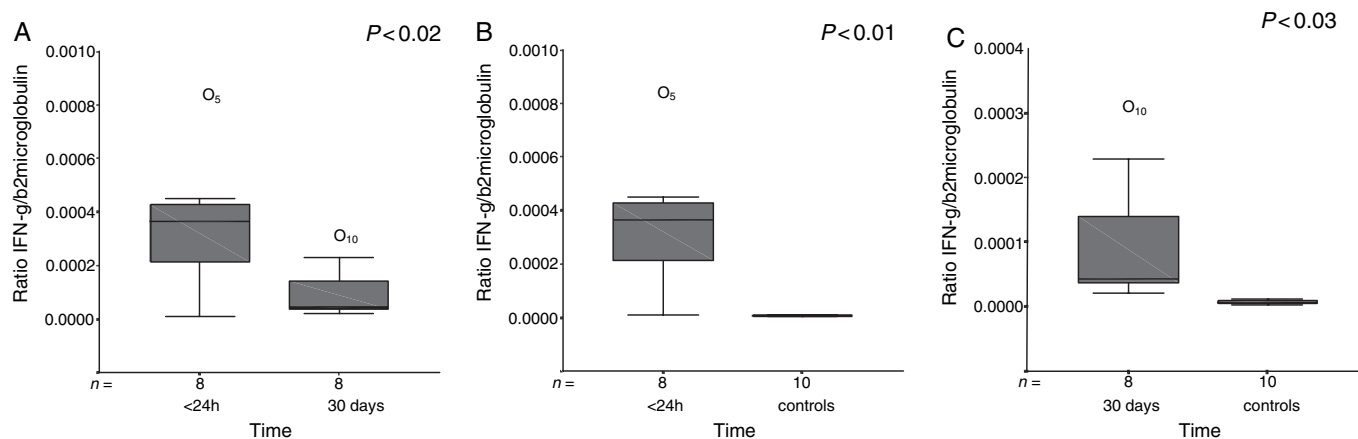


**Figure 1.** Comparison of the interferon- $\gamma$ / $\beta$ 2 microglobulin ratio between the acute episode and the resolution period in the patients (A), the acute episode and the controls after they had taken the drug (B), and the resolution period and controls 30 days after (C). Different scales are shown in each graph for the same value in order to show a better view.

#### Cytokine expression profile

The ratio of IFN- $\gamma$  to  $\beta$ 2-microglobulin was increased fourfold in the acute episode (median: 0.000375; IR: 0.00022, 0.00046) compared with the resolution phase (median: 0.0000960; IR: 0.000060, 0.00025) ( $P < 0.02$ ) (Fig. 1A). These differences were also observed comparing the acute phase of the patients with the samples from the controls after they had taken the drug (median: 0.0000060; IR: 0.0000015, 0.0000075) ( $P < 0.01$ ) (Fig. 1B). There were no differences for the 30-day samples between patients and controls (Fig. 1C).

The ratio of IL-4 to  $\beta$ 2-microglobulin in the patients between the acute episode (median: 0.00001300; IR: 0.0000042, 0.0000175) and the resolution phase (median: 0.00015371; IR: 0.000035, 0.00045) showed a 10-fold increase ( $P < 0.003$ ) (Fig. 2A). Comparison between the patients at the acute phase and the first sample from the controls (median: 0.00001373; IR: 0.0000012, 0.0000185) showed no differences (Fig. 2B). Comparison of the

30-day samples between patients and controls showed an increased ratio in the patients ( $P < 0.03$ ) (Fig. 2C).

The ratio of TNF- $\alpha$  to  $\beta$ 2-microglobulin showed a significant threefold increase at the 30-day sample in the patients (median: 0.002460; IR: 0.00125, 0.003245) compared with the controls (median: 0.001475; IR: 0.000745, 0.002275) ( $P < 0.02$ ).

#### Discussion

Controlled administration confirmed that 21 of the 32 patients evaluated thus were allergic to CS. The symptoms were of a similar nature and intensity to those reported by the patients. The two cases with a positive patch and intradermal test also received controlled administration of the drug, and the positive response confirmed the specificity of the reaction. These results were compatible with a T-cell response, with the expression of both memory and activation markers on

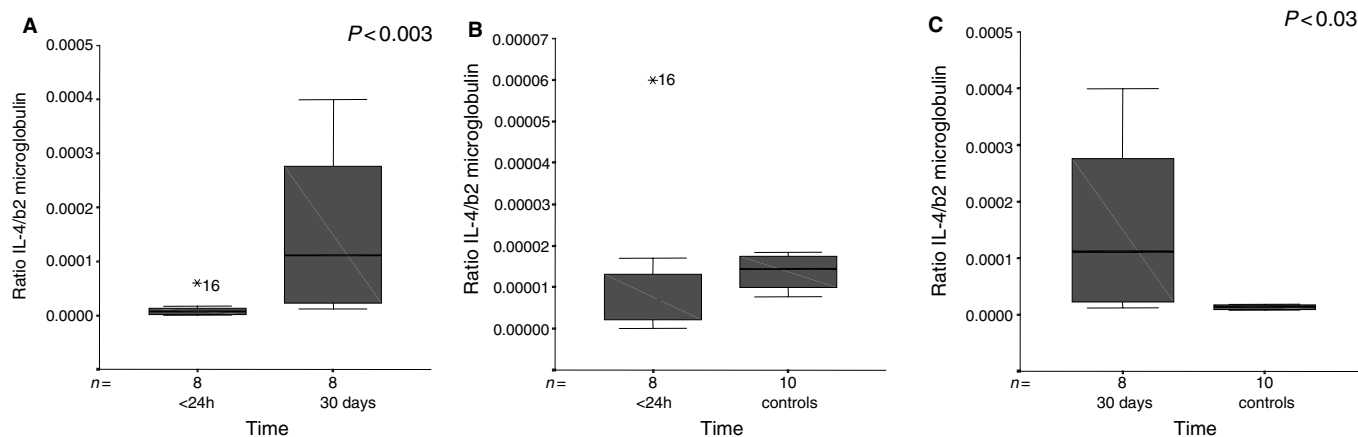


**Figure 2.** Comparison of the interleukin-4/ $\beta$ 2 microglobulin ratio between the acute episode and the resolution period in the patients (A), the acute episode and the controls after they had taken the drug (B), and the resolution period and controls 30 days after (C). Different scales are shown in each graph for the same value in order to show a better view.

CD4 cells, and to a lesser extent CD8 cells. Similar results have been reported for other drug reactions, including CS (17), but our data are more informative.

These results lead to much speculation. It is clear that in contact eczema an immunological mechanism is involved, but less information is available about reactions occurring after systemic administration.

Studies of allergy to drugs have provided variable results regarding the cytokine pattern produced by T lymphocytes. For example, in allergy to betalactams, penicillin G specific T cell clones produce IFN- $\gamma$  and a variable amount of IL-4 and IL-5, suggesting a TH2 response (14). Other studies of exanthematic reactions report a high expression of IL-4, IL-5 and IL-13, but no expression of IFN- $\gamma$  (12). T cell clones raised from patients with delayed reactions to lidocaine show a Th2 cytokine pattern (23). Thus, TH1/Tc1, Th2/Tc2 involvement remains a matter of debate (24).

An alternative, more realistic approach is to monitor the peripheral blood and skin involved during the two periods of acute response and resolution. CD4 and CD8 blood lymphocytes expressing the skin homing antigen CLA are activated and show a Th1 cytokine pattern, with expression of IL-2, IFN- $\gamma$ , and TNF- $\alpha$ , and downregulation of IL-4 during the acute phase (22, 25). Also, the parallel monitoring of peripheral blood, affected skin tissue and blister fluid has shown the same pattern initially, with CD4 and CD8 lymphocytes producing perforin, granzyme B and FAS-L (26). These findings are strongly consistent with an immunological phenomenon.

Unlike penicillin and other drugs, monitoring the acute response to CS may have drawbacks, because their physiological action may modify cytokine expression. Corticosteroids inhibit transcriptional activation of IL-1 by AP1 and NF $\kappa$ B by induction of the inhibitor I $\kappa$ B (27). They decrease synthesis of IFN- $\gamma$  and promote T cell differentiation towards a Th2 phenotype (28, 29). This effect was not observed in our study and the pattern found was similar to that for reactions to other drugs, with increased expression of IFN- $\gamma$  and downregulation of IL-4 (22, 25, 26).

In favor of an immunological mechanism we should consider that studies of delayed skin test responses and cross-reactivity in patients with contact dermatitis have distinguished four major groups of drugs, represented by hydrocortisone, triamcinolone, betamethasone and hydro-

cortisone butyrate (1, 4). Many subjects who were positive to hydrocortisone were also positive to prednisolone or other CS from the same group, but other patients were positive to drugs from more than one group, indicating that either the pattern of reactivity is complex or that coexisting sensitivity to different CS may occur (19–21). Betamethasone was the most frequent drug involved in our study, followed by dexamethasone and triamcinolone, but as not all the subjects in our study who were positive to betamethasone had taken dexamethasone or triamcinolone previously, this was probably an intra- or inter-group cross-reactivity. Cross reactivity between betamethasone and methylprednisolone could also exist, although we are unable to provide confirmation. In one case the subject responded to all four CS tested.

Based on the similarity with previous studies by our group and by others (17), our findings suggest an adverse reaction mediated by an immunological mechanism. Because just two cases were skin test positive but negative to the preservatives these CS could be considered responsible. The remaining cases were skin test negative despite having a positive challenge, which indicates that skin testing is not a sensitive method for diagnosing these cases. The good tolerance after administration to some of the corticoids and the pattern of cross-reactivity in those with a positive response also support an immunological mechanism.

In summary, we provide evidence from a number of subjects with urticarial or exanthematic reactions to CS with a pattern of skin response or cytokine expression in peripheral blood that suggests an immunological mechanism. Furthermore, patterns of cross-reactivity were similar to those reported for contact dermatitis. However, further studies to clarify the mechanism are now under way.

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