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Picryl Chloride-Induced Contact Dermatitis in IQI/Jic Male Mice

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Abstract: Ear skin responses to picryl chloride (PCL) were examined in IQI/Jic male mice. Following the sensitization with PCL to the shaved abdominal skin, the left ear was topically applied with PCL at 4, 11, 18 and 25 days later. The ear swelling responses tended to shift from delayed type to immediate one after the 4th application. The peak time of histopathological changes characterized by edema and inflammatory cell infiltration corresponded well with that of ear swelling responses, and marked elevation of total serum IgE levels and prominent increase in number of mast cells were observed after the 4th application. The numbers of CD4⁺ cells and Mac-1⁺ cells began to increase after the 1st application and much more increased after the 4th application while CD8⁺ cells increased after the 4th application. MHC class II⁺ cells increased mainly in the dermis after the 4th application, but CD19⁺ cells, i.e. B lymphocytes, were negligible throughout the experimental period. The present results suggest that not only CD4⁺ cells but also CD8⁺ cells may participate in the induction of PCL-induced contact dermatitis in IQI/Jic male mice and that the shift from Th1-dominant response to Th2-dominant one may occur following the repeated application. (*J Toxicol Pathol* 2001; **14**: 231–236)

Key words: picryl chloride, IQI/Jic male mice, contact dermatitis

Introduction

Contact dermatitis is a cell-mediated immune reaction, which occurs after the exposure of susceptible individuals to sensitizing chemicals. The number of patients with immune-mediated dermatitis such as contact dermatitis is increasing year by year, and many chemicals have been implicated in causing skin sensitization, suggesting a necessity for an accurate hazard evaluation. However the exact etiology and pathogenesis of such dermatitis have not yet been clarified. To find a clue to solve these problems, comparative studies using various animal models are necessary.

IQI/Jic (IQI) mice are an ICR-derived inbred strain, which had been developed in Japan. It is well known that IQI mice induce a high level of antinuclear autoantibody following mercuric chloride treatment¹, have thymic B cells², and show an age-related infiltration of lymphocytes with parenchymal destruction in both salivary and lacrimal glands³. Moreover, the prevalence of skin lesions of

spontaneous nature was noticed in IQI female mice of aged group. The authors suggest that certain abnormalities in the immune system might underlie the above-mentioned phenomena obtained in IQI mice.

Recently our research group reported that IQI female mice have high susceptibility to topical application of contact sensitizing agents^{4,5}, and therefore IQI female mice are considered to be a useful laboratory animal for the investigation of allergic dermatitis.

The purpose of this study was to examine the usefulness of IQI male mice as a laboratory animal for the investigation of contact dermatitis using the same experimental protocol with that used in IQI female mice⁵.

Materials and Methods

Animals

Fifty-six 8-week-old IQI male mice (National Institute of Industrial Health, Kawasaki, Japan) were used for examination at 10 weeks old after acclimation for 2 weeks. The animals were individually kept in polycarbonate cages and fed standard laboratory pellets (MF, Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum throughout the experimental period. The animal room was air-conditioned (temperature: 23 ± 2°C; relative humidity: 55 ± 5%) with a

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14-hr light and 10-hr dark cycle.

Treatments

The animals were sensitized with 150 μ l of 5% picryl chloride (PCL) (Nakalai Tesque Inc., Kyoto, Japan) to the shaved skin of abdomen. At 4 (1st), 11 (2nd), 18 (3rd) and 25 days (4th) after the sensitization, the left ear was topically applied with 20 μ l of 0.8% PCL. PCL was dissolved in acetone and olive oil (4:1). The right ear of each animal was applied with vehicle alone in the same way.

Measurement of ear swelling response

Ear thickness of each mouse was measured at 0, 1, 3, 6, 9, 12, 24 and 48 hr after each application with PCL or vehicle, respectively. The ear swelling response was judged from the difference between the ear thickness of the left and right ears of each animal at each point of measuring.

Measurement of total serum IgE levels

Total serum IgE levels were measured at 0, 9 and 24 hr after the 1st and 4th application with PCL, respectively, by sandwich ELISA method using mouse IgE measuring kit "YAMASA" EIA (Yamasa Shoyu Co., Ltd., Choshi, Japan).

Histopathology

At 0, 3, 6, 9, 12 and 24 hr after the 1st and 4th application, 4 mice were killed by heart puncture under ether anesthesia, respectively. For histopathological examination, the ears applied with PCL or vehicle were obtained from each mouse and fixed in 10% neutral buffered formalin. Paraffin sections (5 μ m) were stained with hematoxylin and eosin (HE) or toluidine blue (TB). The number of mast cells was calculated on TB-stained sections (5 areas/section/animal) under light microscope ($\times 200$), and the mean value was calculated for each animal.

Immunohistochemistry

Pieces of the ear samples obtained at 0, 9 and 24 hr after the 1st and 4th application were embedded in O.T.C. compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan), rapidly frozen in dry ice-acetone, and then stored at -80°C before used. Cryosections (6 μ m) were fixed in acetone for 10 min before immunohistochemical staining according to the avidin-biotin-peroxidase complex (ABC) method using Vectastain ABC kit (Vector Laboratories, California, USA). As the primary antibodies, rat monoclonal antibodies against mouse CD4 (clone RM4-5), CD8 (clone 53-6.7), Mac-1 (clone M1/70), CD19 (clone 1D3), and MHC class II (I-A^d/I-E^d, clone 2G9) were purchased from Pharmingen (California, USA). After ABC reaction, sections were visualized in 3,3'-deaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis MO, USA) solution. Sections were counterstained with methyl green. The number of positive cells for each antibody was calculated on immunostained sections (5 areas/section/animal) under light microscope ($\times 400$), and the mean value was calculated for each animal.

Statistical analysis

The data of ear swelling responses, total serum IgE levels, and the numbers of mast cells, and CD4⁺, CD8⁺, Mac-1⁺, CD19⁺ and MHC class II⁺ cells were expressed as mean $\pm$ standard deviation (SD) of 4 animals at each point of measurement. The data obtained at 0 hr after the 1st application were used as controls. Statistical analysis was done using the Student's t-test.

Results

Ear swelling response

The ear thickness increased with the number of times of application (Fig. 1). The peak time of ear swelling response was 24 hr after the 1st, 2nd and 3rd application, and it was 6 hr after the 4th application (Fig. 1).

Total serum IgE levels

There were no significant changes in the total serum IgE levels after the 1st application, but the levels were greatly elevated after the 4th application (Fig. 2).

Histopathological findings

There were no histopathological changes in the ear skin topically applied with vehicle alone.

In the dermis of the ear topically applied with PCL, edema slightly occurred at 3 hr after the 1st application and progressed towards 24 hr. Infiltration of inflammatory cells mainly composed of neutrophils started at 6 hr and progressed thereafter. Neutrophil infiltration was also observed in the epidermis (Fig. 3a).

After the 4th application, edema with inflammatory cell infiltration was most conspicuous at 6 and 9 hr, and the infiltrated cells were composed of mast cells, mononuclear

Fig. 1. Ear swelling responses in IQI male mice topically applied with PCL at 4 (□), 11 (●), 18 (△) and 25 days (○) following the sensitization with PCL to the abdominal skin. Data are shown as mean $\pm$ SD of 4 mice.

Fig. 2. Total serum IgE levels in IQI male mice after the 1st and 4th application with PCL following the sensitization with PCL. Data are shown as mean $\pm$ SD of 4 mice.

* $p < 0.05$, Significantly different from the data at 0 hr after the 1st application.

cells and eosinophils as well as neutrophils (Fig. 3b). In addition, epidermal hyperplasia, intraepidermal inflammatory cell infiltration and formation of immature granulation tissues in the superficial dermis were also observed (Fig. 3b). Edema and inflammatory cell infiltration decreased towards 24 hr.

The number of mast cells did not show a significant increase after the 1st application. After the 4th application, the number increased to the levels about 2 to 3 times higher than that of controls (Figs. 3c and 4).

Immunohistochemical findings

Table 1 shows the outline of immunohistochemical findings. The number of CD4⁺ cells moderately increased at 9 and 24 hr after the 1st application, and it increased markedly after the 4th application (Fig. 5a). The number of CD8⁺ cells did not show significant changes after the 1st application, but it increased moderately after the 4th application (Fig. 5b). Mac-1⁺ cells clearly increased at 9 and 24 hr after 1st application. After the 4th application, moderate numbers of Mac-1⁺ cells were observed at 0 hr, and the number increased thereafter (Fig. 5c). CD19⁺ cells were rarely seen at any time point examined. The number of MHC class II⁺ cells significantly increased after the 4th application (Fig. 5d).

Discussion

In the present study, the ear skin responses of IQI male mice were histopathologically and immunohistochemically examined after the repeated topical application with PCL following the sensitization with PCL to the abdominal skin.

The ear swelling response in IQI male mice increased with the time of application, but its degree was somewhat less severe than that in IQI female mice^{4,5}. The ear swelling

Fig. 3. Ear skin of IQI male mice at 24 hr after the 1st application (a) and at 9 hr after the 4th application with PCL (b, c) following the sensitization with PCL. (a) Intradermal edema with prominent inflammatory cell infiltration. HE, $\times 100$. (b) In addition to prominent edema with inflammatory cell infiltration, connective tissue proliferation is seen beneath the hyperplastic epidermis. HE, $\times 100$. (c) Many mast cells (arrowheads) are seen. TB, $\times 200$.

Fig. 4. The number of mast cells in the ear skin of IQI male mice after the 1st and 4th application with PCL following the sensitization with PCL. Data are shown as mean $\pm$ SD of 4 mice. * $p < 0.05$, Significantly different from the data at 0 hr after the 1st application.

response tended to shift from the delayed type to the acute one after the 4th application as already observed in IQI female mice⁵. Such shift of ear swelling responses corresponded well with the shift of the peak time of histopathological responses characterized by edema and inflammatory cell infiltration.

In addition, after the 4th application, the number of mast cells increased clearly, and the total serum IgE level were also elevated markedly, and these findings seem to have a close relation with the above-mentioned shift of ear swelling response after the 4th application.

At 9 hr and especially at 24 hr after the 1st application, the numbers of CD4⁺ and Mac-1⁺ cells significantly increased. After the 4th application, the numbers of these cells much more increased, and the numbers of CD8⁺ cells and MHC class II⁺ cells also showed significant increase. CD19⁺ cells, i.e. B lymphocytes, were however almost negligible. These immunohistochemical findings were similar to those in IQI female mice^{4,5}, though somewhat less prominent.

There are a few reports of the T cell subsets infiltrating

Fig. 5. Ear skin of IQI male mice at 9 hr after the 4th application with PCL following the sensitization with PCL. Immunostaining, $\times 240$. (a) CD4⁺ cells (arrowheads). (b) CD8⁺ cells (arrowheads). (c) Mac-1⁺ cells (arrowheads). (d) MHC class II⁺ cells (arrowheads).

Table 1. Immunohistochemistry of Component Cells in the Ear of IQI Male Mice after Repeated PCL-Application Following the Sensitization with PCL

Hours	1st application			4th application		
	0	9	24	0	9	24
CD4	1.2 ± 1.3	7.3 ± 2.6*	11.7 ± 3.1*	40.3 ± 4.2*	57.7 ± 14.1*	25.1 ± 7.4*
CD8	1.3 ± 0.6	2.3 ± 0.7	2.7 ± 0.9	9.0 ± 1.6*	8.4 ± 2.1*	18.3 ± 4.2*
Mac-1	0.4 ± 0.6	21.0 ± 2.9*	75.2 ± 7.2*	34.0 ± 5.8*	100.8 ± 12.9*	111.0 ± 20.7*
MHC class II	23.3 ± 2.1	31.3 ± 3.1*	25.3 ± 4.6	53.7 ± 4.9*	56.0 ± 8.1*	62.3 ± 3.6*

Data are expressed as mean ± SD of 4 mice.

* P<0.05, Significantly different from the data at 0 hr after 1st application.

in the skin lesion of contact dermatitis and the results are conflicting. Namely, some studies indicate that contact dermatitis is a CD4⁺ lymphocyte-mediated response^{6,7}, and others conclude that CD8⁺ lymphocytes are the effectors and CD4⁺ lymphocytes function as negative regulators^{8,9}.

As mentioned above, CD4⁺ cells were dominant among T lymphocytes after both the 1st and the 4th application, and a significant increase in the number of CD8⁺ cells was also detected after the 4th application. This suggests that, although CD4⁺ lymphocytes may play a main role, CD8⁺ lymphocytes may also participate in the induction of PCL-related contact dermatitis at least after the 4th application.

Up to the present time, there are several reports suggesting an important role of keratinocytes in the induction of contact dermatitis through expression of MHC class II^{10,11}. However, in the present study, only a few keratinocytes were positive for MHC class II, and the majority of MHC class II⁺ cells were considered to be Mac-1⁺ cells in the dermis.

There are several reports of allergic contact dermatitis induced in mice by topical exposure to chemicals. The characteristics of dermatitis differ by sensitizing agents used. For example, trimellitic anhydride (TMA) and diphenylmethane-4,4'-diisocyanate (MDI) induced dermatitis with increase in total serum IgE after a single application, but dermatitis induced by 2,4-dinitrochlorobenzene (DNCB), dicyclohexymethane-4,4'-diisocyanate (HMDI) or isophorone diisocyanate (IPDI) did not accompany the production of IgE^{12,13}. Therefore, they discussed that TMA and MDI selectively activated Th2 cells, and DNCB, HMDI and IPDI activated Th1 cells. Moreover, Kitagaki *et al.*¹⁴ reported that immediate-type hypersensitivity response with increase of antigen-specific IgE levels was induced by repeated epicutaneous application of PCL but it had never been observed after a single application of PCL in BALB/c mice. Nagai *et al.*¹⁵ also reported the similar findings in mice treated with 2,4-dinitrofluorobenzene (DNFB). They suggested that the shift from Th1-dominant response to Th2-dominant one might occur following the repeated application. Recent *in vivo* studies have also demonstrated that many responses started as Th1-dominant type and then shifted to Th2-dominant one¹⁶⁻¹⁸. The above-mentioned results of the ear swelling

response suggest that similar shift might occur in IQI male mice after the 4th application. In order to clarify this point, the analysis of subsets of not only CD4⁺ lymphocytes but also CD8⁺ ones and the measurement of Th1- and Th2-related cytokines are now in progress. In conclusion, in addition to IQI female mice, IQI male mice are also considered to be a useful animal for the investigation of contact dermatitis.

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