Comparative Study on Picryl Chloride (PCL)-Induced Contact Dermatitis in Female IQI/Jic and BALB/c Mice

Ji-Youn JUNG1), Junzo SAEGUSA2), Hiroyuki NAKAYAMA1), and Kunio DOI1)

1) Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113-8657, and 2) Department of Human Engineering, National Institute of Industrial Health, 6–21–1 Nagao, Tama-ku, Kawasaki 214, Japan

Abstract: Ear skin responses to picryl chloride (PCL)-induced contact dermatitis were compared in detail between IQI/Jic mice developed in Japan and BALB/c mice often used for the investigation of contact dermatitis. PCL was applied to the left ear of each mouse 4 (1st), 11 (2nd), 18 (3rd) and 25 days (4th) after sensitization of the abdominal skin with PCL. Time course examinations were carried out on the ear swelling responses, total IgE levels, skin histology and immunohistochemistry for infiltrated cells after the 1st and 4th application. In IQI mice, the peak time of the ear swelling responses tended to shift from 24 h to 9 h with marked elevation of total IgE levels and marked increase of mast cells showing degranulation after the 4th application when CD8+ cells as well as CD4+ cells also prominently increased. In BALB/c mice, except for the total IgE levels and the number of mast cells, the degrees of ear swelling responses, histological changes and increase of CD4+ and CD8+ cells were much less severe. Female IQI mice are considered to be a useful mouse strain for further investigations on the role of CD4+ and CD8+ T cells in the pathogenesis of contact dermatitis.

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

Introduction

Contact dermatitis is a common and important occupational health problem. It develops in susceptible individuals, who have been sensitized with a certain haptenic chemical, after epicutaneous exposure to the same chemical. The number of patients suffering from contact dermatitis is increasing year by year, and many chemicals are thought to participate in skin sensitization. This suggests the necessity of an accurate hazard evaluation, but the exact etiology and pathogenesis of contact dermatitis have not fully been clarified yet. To find clues to solve these problems, comparative studies using various animal models are necessary.

IQI/Jic (IQI) mice are an ICR-derived inbred strain developed in Japan [10], and accumulated evidence suggests the existence of certain abnormalities in their immune system. Namely, they induce a high level of antinuclear auto-antibody following mercuric chloride treatment [21], have thymic B cells [19], and show an age-related development of Sjögren’s syndrome-like sialadenitis [20]. In addition, the prevalence of allergic

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Address corresponding: J.-Y. Jung, Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
skin lesions of a spontaneous nature has been noticed in aged female IQI mice kept in an animal room of the National Institute of Industrial Health, Japan, suggesting that female IQI mice would be useful animals for the induction of contact dermatitis [8].

The present study was carried out to compare the ear skin responses to picryl chloride (PCL)-induced contact dermatitis in detail between female IQI/Jic mice and BALB/c mice, which are most often used for the induction of contact dermatitis [12, 13]. The present study was approved by the Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo.

**Materials and Methods**

**Mice:** Fifty-six 8-week-old female IQI/Jic (IQI) mice (National Institute of Industrial Health, Kawasaki, Japan) and 56 age-matched female BALB/c CrSlc (BALB/c) mice (Japan SLC Co., Hamamatsu, Japan) were used for examination at 10 weeks of age after acclimatization for 2 weeks. The animals were individually kept in polycarbonate cages in an air-conditioned animal room (temperature: 23 ± 2°C; relative humidity: 55 ± 5%; light/dark cycle: 12/12 h). They were fed standard laboratory pellets (MF, Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum throughout the experimental period.

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

**Ear swelling response:** Ear thickness of each mouse was measured at 0, 1, 3, 6, 9, 12, 24 and 48 h after each application. The ear swelling response was judged by the difference between the ear thickness of the left and right ears of each animal at each time point.

**Total serum IgE levels:** Total serum IgE levels were measured at 0, 9 and 24 h after the 1st and 4th applications by the sandwich ELISA method using a mouse IgE measuring kit “YAMASA” EIA (Yamasa Shoyu Co., Ltd., Choshi, Japan).

**Histopathology:** At 0, 3, 6, 9, 12 and 24 h after the 1st and 4th applications, 4 mice of each strain were sacrificed by heart puncture under deep CO₂/O₂ anesthesia. The ears to which PCL or vehicle had been applied were taken from each mouse and fixed in 10% neutral buffered formalin. Paraffin sections (5 µm) 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) obtained from each animal at 0, 9 and 24 h after the 1st and 4th application under a light microscope (× 200), and the mean value was calculated for each animal.

**Immunohistochemistry:** Pieces of the left ear sample obtained from each animal at 0, 9 and 24 h after the 1st and 4th applications were embedded in O.T.C. compound (Sakura Fine Technical Co., Ltd., Tokyo, Japan), rapidly frozen in dry ice-acetone, and then stored at –80°C until used. Cryosections (6 µm) were fixed in acetone for 10 min before immunohistochemical staining by 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 ID3), and MHC class II (I-A<sup>+</sup>/I-E<sup>+</sup>, clone 2G9) (Pharmingen, California, USA) were used. After ABC reaction, sections were visualized in 3,3’-diaminobenzidine 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 a light microscope (× 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<sup>+</sup>, CD8<sup>+</sup>, Mac-1<sup>+</sup>, CD19<sup>+</sup> and MHC class II<sup>+</sup> cells were expressed as mean ± standard deviation (SD) of 4 animals at each point of measurement. The data obtained at 0 h after the 1st application, i.e. just before application, were used as controls. Statistical analysis was done using Student’s t-test.

**Results**

**Ear swelling response:** The severity of ear swelling responses was much stronger in IQI mice than in BALB/
c mice, and the ear thickness increased with the number of applications in both strains (Fig. 1). In IQI mice, although not significant, the peak time of ear swelling response tended to shift from 24 h to 9 h after the 4th application (Fig. 1a). On the other hand, BALB/c mice showed an acute-type response, and the peak time did not show such a shift even after the 4th application (Fig. 1b).

**Total serum IgE levels:** There were no significant increases in the total serum IgE levels after the 1st application in both strains. After the 4th application, the levels were greatly elevated in both strains without strain difference (Fig. 2).

**Histopathological findings:** There were no histopathological changes in the ear skin which was topically applied with vehicle alone.

In the dermis of the left ear of IQI mice, edema slightly occurred at 3 h after the 1st application and progressed towards 24 h (Fig. 3a). Infiltration of inflammatory cells mainly composed of neutrophils started at 6 h and progressed thereafter (Fig. 3a). Neutrophil infiltration was also observed in the epidermis. After the 4th application, edema with inflammatory cell infiltration was most conspicuous at 9 h (Fig. 3b), and the infiltrated cells included mast cells, mononuclear cells and eosinophils as well as neutrophils. In addition, epidermal hyperplasia, intra-epidermal 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 h.

In BALB/c mice, as compared with IQI mice, neutrophil infiltration was almost similar, but edema was less severe and epidermal hyperplasia after the 4th application was apparently weaker (Figs. 3c and 3d). The changes were most prominent at 9 h after both the 1st and the 4th application.

The number of mast cells showed no significant increase after the 1st application in both strains. After the 4th application, the number increased to levels about 2 to 3 times higher than those after the 1st application in both strains (Fig. 4), and, as compared with after the 1st application, degranulation of mast cells was frequently observed (Fig. 5).

**Immunohistochemical findings:** Table 1 shows the outline of immunohistochemical findings. CD19+ cells were rarely seen in both strains throughout the experience.
At 0 h after the 1st application, the number of MHC class II+ cells was significantly larger in IQI mice than in BALB/c mice, although the numbers of CD4+, CD8+ and Mac-1+ cells were similar between the two strains.

After the 1st application, in IQI mice, the numbers of CD4+ (Fig. 6a) and Mac-1+ cells were significantly increased at 9 and 24 h, while the numbers of CD8+ and MHC class II+ cells did not show any significant changes (Table 1). BALB/c mice also showed similar changes except for a significant increase in the number of MHC class II+ cells at 24 h (Table 1). In addition, the number of CD4+ cells at 24 h was significantly larger in BALB/c mice than in IQI mice.

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Mental period. At 0 h after the 1st application, the number of MHC class II+ cells was significantly larger in IQI mice than in BALB/c mice, although the numbers of CD4+, CD8+ and Mac-1+ cells were similar between the two strains.

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After the 4th application, in both strains, the numbers of CD4+, CD8+, Mac-1+ and MHC class II+ cells had markedly increased at 0 h (Table 1), and their numbers except for Mac-1+ cells were maintained at almost the same levels thereafter. From the comparative view-
Table 1. Immunohistochemistry of component cells in the ear of IQI/Jic and BALB/c female mice at 0, 9 and 24 h after the 1st and 4th applications of PCL following sensitization with PCL

<table>
<thead>
<tr>
<th>Strain</th>
<th>CD4</th>
<th>CD8</th>
<th>Mac-1</th>
<th>MHC II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>9 h</td>
<td>24 h</td>
<td>0 h</td>
</tr>
<tr>
<td>IQI/Jic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>1.3 ± 1.5</td>
<td>10.1 ± 3.2*</td>
<td>10.9 ± 1.6**</td>
<td>40.5 ± 6.3**</td>
</tr>
<tr>
<td>CD8</td>
<td>0.7 ± 0.6</td>
<td>1.3 ± 0.8</td>
<td>1.7 ± 0.5</td>
<td>14.7 ± 1.8**</td>
</tr>
<tr>
<td>Mac-1</td>
<td>0.3 ± 0.4</td>
<td>22.0 ± 4.4**</td>
<td>74.8 ± 5.3*</td>
<td>34.0 ± 5.5*</td>
</tr>
<tr>
<td>MHC II</td>
<td>29.3 ± 3.5*</td>
<td>35.7 ± 3.3*</td>
<td>35.3 ± 4.7</td>
<td>59.7 ± 2.5**</td>
</tr>
<tr>
<td>BALB/c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>1.1 ± 1.4</td>
<td>8.3 ± 2.6*</td>
<td>24.4 ± 3.1*</td>
<td>25.5 ± 4.8*</td>
</tr>
<tr>
<td>CD8</td>
<td>0.7 ± 0.5</td>
<td>1.6 ± 0.6</td>
<td>1.2 ± 0.7</td>
<td>7.3 ± 1.8*</td>
</tr>
<tr>
<td>Mac-1</td>
<td>0.4 ± 0.5</td>
<td>38.1 ± 6.3*</td>
<td>78.6 ± 6.2*</td>
<td>31.0 ± 6.1*</td>
</tr>
<tr>
<td>MHC II</td>
<td>12.3 ± 2.3</td>
<td>17.5 ± 5.3</td>
<td>27.7 ± 7.5*</td>
<td>45.7 ± 5.7*</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD of 4 mice per group. * P<0.05, Significantly different from the value at 0 h after 1st application. ‡ P<0.05, Significantly different from the value at 0 h after 4th application. † P<0.05, Significantly different from the value in BALB/c mice.

Discussion

Contact dermatitis results from epicutaneous sensitization and challenge with haptens [2, 12, 13, 16]. In the present study, the ear skin responses to PCL-induced contact dermatitis were examined in detail between female IQI mice and BALB/c mice, which have been often used for the induction of contact dermatitis.

The degree of the ear swelling response increased with the number of applications of PCL following sensitization with the same chemical in both strains, and it was much more severer in IQI mice than in BALB/c mice. In this study, the peak time of the ear swelling response tended to shift from 24 h to 9 h after the 4th application in IQI mice, whereas the ear swelling response in BALB/c mice was generally acute-type (peak time: 6–12 h). Kitagaki et al. [13] reported that immediate-type hypersensitivity response with increase of antigen-specific IgE levels followed by a late reaction was induced by repeated epicutaneous application of 2,4,6-trinitro-1-chlorobenzene or PCL following the sensitization with the same chemical in BALB/c mice [12, 13]. Nagai et al. [16] also reported similar findings in
mice treated with 2,4-dinitrofluorobenzene. They suggested that a shift from a Th1-dominant response to a Th2-dominant one might occur following repeated applications. Recent studies have also demonstrated that, in addition to allergic dermatitis, many immunological responses start as Th1-dominant type and then shift to Th2-dominant ones [2, 5, 18]. Further studies on cytokine profiles in PCL-induced contact dermatitis are now in progress in IQI and BALB/c mice.

The peak time of histopathological response characterized by edema and inflammatory cell infiltration corresponded well to that of the above-mentioned ear swelling response. After the 4th application, the number of mast cells increased prominently, and the total serum IgE level was also elevated markedly in both strains with no strain difference. IgE response differs with the sensitizing agents used. For example, trimellitic anhydride and diphenylmethane-4,4′-diisocyanate induced dermatitis with increase in total serum IgE while dermatitis induced by 2,4-dinitrochlorobenzene, dicyclohexylmethane-4,4′-diisocyanate and isophorone diisocyanate was not accompanied by the production of IgE [3, 4]. Degranulation of mast cells was conspicuous after the 4th application. Degranulation of mast cells was also confirmed by electron microscopic examination [9]. These findings suggest a relationship

Fig. 6. Immunohistochemical staining of the ear at 24 h after the 1st application (a) and at 9 h after the 4th application of PCL (b-f) following the sensitization with PCL in female IQI/Jic (a-d, f) and BALB/c (e) mice. × 240. (a) Moderate numbers of CD4+ cells (arrowheads). (b) Many CD4+ cells (arrowheads). (c) Moderate numbers of CD8+ cells (arrowheads). (d) Many Mac-1+ cells (arrowheads). (e) Many CD4+ cells (arrowheads). (f) Moderate numbers of MHC class II+ cells (arrowheads).
between the elevation of IgE levels and the degranulation of mast cells. Mast cells are well known to produce a wide variety of mediators and cytokines [11] which may be important in the pathogenesis of contact dermatitis.

The immunohistochemical examinations showed that IQI mice had more MHC class II⁺ cells in the ear dermis than BALB/c mice by nature while the numbers of CD4⁺, CD8⁺ and Mac-1⁺ cells were similar between the two strains. There are several reports suggesting an important role of keratinocytes in the induction of contact dermatitis through expression of MHC class II [7, 17]. In the present study, the majority of MHC class II⁺ cells were however considered to be dermal cells in both strains throughout the experimental period.

After the 1st application, the numbers of CD4⁺ and Mac-1⁺ cells significantly increased in both strains at 9 and 24 h. After the 4th application, the numbers of CD4⁺, CD8⁺ and Mac-1⁺ cells as well as MHC class II⁺ cells were much more increased in both strains already at 0 h, and, except for that of Mac-1⁺ cells, they maintained almost the same levels thereafter. The absolute numbers of CD4⁺, CD8⁺ and MHC class II⁺ cells were significantly larger in IQI mice than in BALB/c mice, and this may be related to the difference in the severity of contact dermatitis between IQI and BALB/c mice. On the other hand, CD19⁺ cells, i.e. B lymphocytes, were however almost negligible throughout the experimental period.

Reports of T cell subsets infiltrating the skin lesion of contact dermatitis are conflicting. For example, some studies indicated that contact dermatitis is a CD4⁺ cell-mediated response [6, 15], and others concluded that CD8⁺ cells are the effectors and CD4⁺ cells function as negative regulators [1, 14, 22]. As mentioned above, although CD4⁺ cells were a predominant T cell subset after both the 1st and the 4th applications, a significant increase in the number of CD8⁺ cells was detected after the 4th application in both strains. This suggests that, although CD4⁺ cells play a main role, CD8⁺ cells may also participate in the induction of contact dermatitis at least after the 4th application with PCL.

In conclusion, PCL-induced contact dermatitis was markedly more prominent in female IQI mice than in female BALB/c mice, and the peak time of the ear swelling responses tended to shift from 24 h to 9 h in IQI mice while it was 6–12 h throughout the observation period in BALB/c mice. We considered female IQI mice will be a very useful animal for further investigation of the roles of CD4⁺ and CD8⁺ cells in the pathogenesis of contact dermatitis.

References


