A NOVEL METHOD FOR INDUCTION AND DETECTION OF ANAPHYLACTIC REACTION USING THE MOUSE ABDOMINAL WALL (AW METHOD)

Hiromi KATAOKA,* Akiko TSUDA, Yoshimi TSUDA, Akiko BABA, Harumi YOSHIDA, Reiko HIRASAWA, Yoshiie TOBIMATSU, Minori NISHIGUCHI, Masanori SEMMA, and Yoshio ITO

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663, Japan

We found that an antigen-specific anaphylaxis was induced by antigen challenge to the abdominal wall, ear auricle, or subcutaneous tissue in mice sensitized 9 days previously with antigen and adjuvant. The anaphylactic reaction was detected by vascular permeability at the injected site 7 minutes after challenge, which was the best time for estimation. A novel method (AW method) for induction and detection of the anaphylactic reaction in mice was established using the abdominal wall as the challenge site. This method could detect the anaphylactic response in mice 1 to 3 weeks after sensitization. The increase in vascular permeability was completely inhibited by administration of diphenhydramine.

KEY WORDS mouse abdominal wall; antigen-specific anaphylactic model; detection; induction; immediate allergy

Previously, Osada et al.1) and Ishiguro et al.2) developed a mouse model of anaphylactic response that reflected the fall in blood pressure. In this paper, we describe a novel method (AW method) for the induction and estimation of antigen-specific anaphylaxis in mice using the abdominal wall as a challenge site. The principle of this AW method is the same as that of the skin prick test, the most simple clinical test for allergies (e.g., food allergy, atopic eczema, and asthma). In sensitized mice, chemical mediators such as histamine and leukotrienes are released from mast cells upon allergen challenge, followed by an increase in the vascular permeability of the abdominal wall.

Five-week-old male ddY mice (Japan SLC Co.) were sensitized intraperitoneally with hen egg-white lysozyme (HEL) and Freund's incomplete adjuvant (FIA) (DIFCO) as previously described.3) Nine days later, 0.1 ml of Evans blue dye (1%) was injected i.v., followed by anesthetization of the mice with ether. Within 5 minutes, the skin of the abdomen of the mice was detached without injury to the abdominal wall. Five minutes after injection of the dye, HEL solution was administered on the exposed abdominal wall (5 μg / 50 μl / site). Increased vascular permeability was observed on the abdominal wall. The mice were killed by cervical dislocation 7 minutes after challenge. It was observed that the best time to estimate the anaphylactic permeability was 7 minutes after injection.

When 50 μl of solution (e.g., saline, HEL, or ovalbumin [OVA]) was injected in the abdominal wall of normal mice, the injected site swelled to form a vesicle about 4 mm diameter, and the vesicle gradually disappeared within about 5 minutes of injection. In the case of the antigen-specific anaphylaxis, the disappearance of the vesicle and the permeation of the dye were visually observed at the same time.

* To whom correspondence should be addressed. © 1997 Pharmaceutical Society of Japan
Fig. 1. Time Course of the Induction of the Anaphylactic Reaction Using the AW Method

Mice of the ddY strain were sensitized i.p. (50 μg / mouse) with hen egg-white lysozyme (HEL) in Freund's incomplete adjuvant (FIA) on day 0. Every day after sensitization, the mice were challenged with HEL on the abdominal wall (5 μg / 50 μl / site). □=control group, ■=experimental group. Mice in the control group were not sensitized, but were maintained and challenged under the same conditions as the experimental group. Each vascular permeability value (VPV) represents the mean ± S.E. for 8 mice. Statistical analyses were performed by analysis of variance, followed by Fisher's PLSD test. Asterisks indicate significant differences from the control group (***p<0.001, **p<0.005).

Table 1. Antigen-Specific Anaphylactic Reaction Using the AW Method

<table>
<thead>
<tr>
<th>No.</th>
<th>Sens. a)</th>
<th>Chall. b)</th>
<th>VPV c)</th>
<th>p d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0±0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>HEL</td>
<td>3±3</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>HEL</td>
<td>72±7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>HEL</td>
<td>OVA</td>
<td>9±7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>OVA</td>
<td>0±0</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>OVA</td>
<td>OVA</td>
<td>75±7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>7 e)</td>
<td>HEL</td>
<td>HEL</td>
<td>3±3</td>
<td></td>
</tr>
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</table>

a) Mice of the ddY strain were not sensitized (1, 2, or 5) or were sensitized i.p. (50 μg / mouse) with hen egg-white lysozyme (HEL) (3, 4, or 7) or with ovalbumin (OVA) (6) in Freund's incomplete adjuvant (FIA) on day 0. b) 9 days after sensitization, the mice were challenged on the abdominal wall with either HEL or OVA (5 μg / 50 μl / site). c) Each vascular permeability value (VPV) represents the mean ± S.E. for 8 mice. d) Statistical analyses were performed by analysis of variance, followed by Fisher's PLSD test. e) Diphenhydramine hydrochloride (10mg/kg, i.v.) was administrated 1 h before the challenge.
After removal of the abdominal wall, the diameter of the blue area was measured and assigned a vascular permeability value (VPV). VPVs were defined on the following 5-degree scale: VPV=0: the blue area on the abdominal wall was less than 1 mm diameter; VPV=25: the diameter of the area was in the range of 2-4 mm; VPV=50: the diameter of the area was in the range of 5-7 mm; VPV=75: the diameter was in the range of 8-12 mm; and VPV=100: the diameter was greater than 13 mm. The boundary values of the diameter of the blue area were rounded off. The average of VPVs obtained with saline, HEL, or OVA in normal mice were 0-9 in all cases. The dyed area of the experimental group was compared with that of the nonsensitized control group.

Figure 1 shows the time course of the induction of the anaphylactic reaction using the AW method. This method could detect the antigen-specific anaphylactic response 1 to 3 weeks after sensitization. Fatal shock was not observed in any experiment. The results in Table 1 clearly indicate that: (1) the permeation of dye did not occur due to detachment of the skin (#1); (2) no increase in vascular permeability in normal mice was observed upon challenge with HEL or OVA (#2 and #5); (3) the anaphylactic response was antigen specific (#3, #4, and #6); and (4) the permeation of dye was inhibited by the preadministration of the anti-histamine H1 blocker diphenhydramine hydrochloride (10 mg/kg) (#7).

In preliminary investigations, anti-mouse IgE antibody instead of HEL challenge 9 days after sensitization resulted in increased vascular permeability. Although the result indicated that AW method could detect the IgE-dependent response, the possibility of the participation of other immunoglobulin classes (e.g., IgG1) remains. Further details of AW method mechanisms will be reported soon.

Furthermore, it was confirmed that the permeation of dye was observed upon the administration (50 μl/site) of a histamine dihydrochloride solution to the abdominal wall of normal mice. This phenomenon occurred when histamine solution had a concentration greater than 3 μg/ml but not less than 1 μg/ml. The increase in vascular permeability was dependent on the histamine concentration.

The results suggest that the novel AW method models a typical immediate allergic response. That is, anti-HEL IgE is produced by sensitization with HEL, and IgE binds to Fc ε receptors on mast cells located in the abdominal wall (the site of administration). The mast cells bound by anti-HEL IgE degranulate upon cross-linking with HEL, resulting in the induction of the release of a variety of chemical mediators, such as histamine and leukotrienes, which cause allergic reactions.

The AW method is useful as an immediate allergic model and is applicable to a wide range of research for allergic and/or anti-allergic components, without the need for expensive instruments such as blood pressure monitors. Furthermore, the use of other locations in mice, such as the ear auricle or subcutaneous tissue, in which anaphylactic reactions are also observed, requires the extraction of dye from the tissue and quantification of the amount of dye. Therefore, the AW method is a convenient technique to judge anaphylactic potency. The AW method is superior in terms of high sensitivity, reliability, and simplicity.

REFERENCES

(Received March 25, 1997; accepted April 18, 1997)