Comparative Study of 1.5-Hour and 48-Hour Homologous Passive Cutaneous Anaphylaxis in the Mouse Ear

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To characterize passive cutaneous anaphylaxis (PCA) in the mouse ear, reactions caused by non-heated and heated antiserum were compared to those caused by monoclonal immunoglobulin E antibody (mc-IgE) and monoclonal immunoglobulin G₁ antibody (mc-IgG₁). Heat treatment at 56 °C did not alter the activity of antiserum or mc-IgG₁ to elicit the 1.5-h PCA. The 1.5-h PCA mediated by mc-IgE and 48-h PCA's mediated by both antiserum and mc-IgE were abrogated almost completely by heating at 56 °C for 2 h. The 48-h PCA was not elicited by mc-IgG₁. The sensitized state persisted at least for 7 d when mice were sensitized with non-heated antiserum or mc-IgE. In contrast, the sensitized state disappeared rapidly in cases of heated antiserum and mc-IgG₁. In 48-h PCA's mediated by antiserum and mc-IgE, extravasated dye in the ear was detected 5 min after challenge and the reaction was terminated in 15 min. In contrast, in the 1.5-h PCA mediated by heated antiserum, the accumulation of dye in the ear was delayed slightly when compared to the 48-h PCA. In mc-IgG₁-mediated 1.5-h PCA, the delay was significant. Both 1.5-h PCA's mediated by heated antiserum and mc-IgG₁, and the 48-h PCA's mediated by antiserum and mc-IgE were not observed in WBB6 F₁-W/W^v mice, which lack mast cells. All these PCA's were inhibited equally by tranilast, an inhibitor of mediator release from mast cells. The 1.5-h PCA was abrogated completely when heated antiserum was absorbed with a rabbit anti-mouse IgG₁ serum coupled-Sepharose 4B. On the other hand, the 48-h PCA was abrogated completely by absorption of the antiserum with goat anti-mouse IgE serum coupled-Sepharose 4B. These results indicated that the 48-h PCA in mice sensitized with antiserum is mediated by IgE antibody. In the 1.5-h PCA in mice sensitized with heated antiserum, it is considered that IgG₁ antibody plays a major role to elicit PCA. However, it is also considered that the reaction is modified by other serum components.

Keywords — mouse ear passive cutaneous anaphylaxis; IgE antibody; IgG₁ antibody; monoclonal IgE antibody; monoclonal IgG₁ antibody; heat-treatment

Introduction

Experimental animals produce 2 types of homocytotropic antibodies which are detectable by passive cutaneous anaphylaxis (PCA).^{1–7)} One is immunoglobulin E (IgE) antibody and the other belongs to the immunoglobulin G (IgG) class and these antibodies are characterized by their thermostability and causing a persistence of the sensitized state of the skin. The IgE antibody is important in type I allergy-related diseases in man and the mechanism of the allergic reaction caused by IgE antibody has studied in detail.^{8–10)} IgG homocytotropic antibody in man belongs to the IgG₄ subclass and the blocking activity is attributed to this subclass.¹¹⁾ However, the mechanism of IgG homocytotropic antibody-mediated reaction is not

fully elucidated. In mice, the IgG₁ subclass corresponds to human IgG₄ subclass and acts as homocytotropic antibody.

Mouse ear is a useful site to study PCA^{12–14)} and we have used the ear in a method for quantitative evaluation of homologous PCA using sensitizing period of 48 h (48-h PCA).^{14–16)} In these reports, we confirmed that the 48-h PCA elicited in the mouse ear is highly responsive and reproducible. On the other hand, mouse IgG₁ antibody is detected using sensitizing period of 1.5 h in the homologous PCA (1.5-h PCA).¹⁷⁾ In the present study, a comparison of 1.5-h and 48-h PCA's caused by antiserum, monoclonal IgE antibody (mc-IgE) and monoclonal IgG₁ antibody (mc-IgG₁) in the mouse ear was undertaken.

Materials and Methods

Mice — Female BALB/c mice which were 8 weeks old were used for the preparation of antiserum. Male ddY, WBB6 F₁-+/+ and WBB6 F₁-W/W^v mice of 7 weeks old were used as recipients for PCA. All mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan).

Antigens — Dinitrophenylated *Ascaris suum* extract (DNP-As) and bovine serum albumin (DNP-BSA) were prepared according to the method of Eisen *et al.*¹⁸⁾ Human IgG (HIG) was purchased from Miles Laboratories, Inc. (Naperville, USA).

Antiserum and Monoclonal Antibodies — Antiserum was prepared as follows. BALB/c mice were immunized twice or thrice with 10 µg of DNP-As and 1 mg of alum intraperitoneally at intervals of 30 d. Serum was obtained 7–10 d after the last immunization and pooled. The antiserum was lyophilized and stored at –20 °C. The 48-h PCA titer of the antiserum, estimated in the mouse ear (mouse ear PCA, see below), was 1 : 512. The 1.5-h PCA titer of heated (at 56 °C for 2 h) antiserum in the mouse ear was 1 : 1024. The PCA titer estimated in the rat (Wistar, sensitizing period: 24 h, challenging antigen: 1 mg of DNP-BSA) was 1 : 2048.

Anti-DNP monoclonal IgE antibody (mc-IgE), prepared in ascites fluid of BALB/c × C57BL/6 F₁ mice, was purchased from Serotec, Ltd. (Bicester, UK). The 48-h PCA titer of mc-IgE estimated in the mouse ear was 1 : 2000. The PCA titer estimated in the rat (Wistar, sensitizing period: 24 h, challenging antigen: 1 mg of DNP-BSA) was 1 : 5000–8000.

Anti-HIG monoclonal IgG₁ antibody (mc-IgG₁), prepared in ascites fluid of BALB/c mice, was obtained from Miles Laboratories, Inc. (Naperville, USA). The 1.5-h PCA titer of heated (at 56 °C for 2 h) mc-IgG₁ estimated in the mouse ear was 1:5 000. The 50-fold diluted mc-IgG₁ did not evoke PCA in rats (Wistar, sensitizing period: 24 h, challenging antigen: 1 mg of HIG).

To evaluate the effect of heating on PCA, antiserum and monoclonal antibodies were heated at 56 °C for 0.5–2 h before dilution.

Absorption of Antiserum — Rabbit anti-mouse IgG₁ serum (γ₁-specific, Litton Bionetics, Charleston, USA) or goat anti-mouse IgE serum (ε-specific, Miles Laboratories, Naperville, USA) was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Non-heated or heated antiserum was rotated gently with anti-mouse IgG₁ serum-coupled Sepharose 4B (anti-γ₁-Sepharose 4B) or anti-mouse IgE serum-coupled Sepharose 4B (anti-ε-Sepharose 4B) for 30 min at room temperature and filtered on a glass filter (G3). Each filtrate was concentrated and adjusted to an appropriate volume required for sensitization.

Mouse Ear PCA — Mouse ear PCA was carried out according to the method described previously.^{15,16)} Briefly, 10 µl of diluted antiserum or monoclonal antibody were injected into both sides of ears of ether anesthetized mice. After 1.5 or 48 h, PCA was elicited by an intravenous injection of 0.25 mg of antigen (DNP-BSA or HIG) dissolved in 0.5% Evans blue saline solution in a volume of 0.25 ml. Thirty min after the challenge, mice were sacrificed by cervical dislocation and ears were removed. To measure the amount of dye detected nonspecifically, non-sensitized mice received a similar antigenic challenge and results were indicated as PCA(–). In one experiment, the sensitizing period was varied from 1.5 h to 7 d. In another experiment, to examine the accumulation of dye in the ear, mice were sacrificed 2–40 min after the challenge.

The amount of dilutions used for antiserum (30- or 40-fold), mc-IgE (500-fold) and mc-IgG₁ (1 000-fold) depended upon the results of preliminary experiments. The amounts of dye leaked at these dilutions in the preliminary experiments were as follows: heated antiserum (×40), 19.24 ± 1.58 µg (*n* = 8, 1.5-h PCA); non-heated antiserum (×30), 19.87 ± 1.73 µg (*n* = 8, 48-h PCA); mc-IgE, 13.82 ± 1.64 µg (*n* = 5, 48-h PCA); mc-IgG₁, 18.24 ± 2.88 µg (*n* = 5, 1.5-h PCA).

Tranilast, an anti-allergic agent,^{19,20)} was obtained from Kissei Pharmaceutical Co., Ltd. (Matsumoto, Japan). Tranilast was suspended in distilled water containing 0.2% sodium carboxymethylcellulose and given orally 1 h prior

to antigenic challenge. In this experiment, mice were fasted for 6–8 h before the administration.

Measurement of Dye — Extraction and measurement of extravasated dye were performed according to the method described previously.^{15,16)} A pair of ears were dissolved with 0.7 ml of 1 N KOH solution in a stoppered tube at 37 °C overnight and 9.3 ml of a mixture of 0.6 N H₃PO₄ solution and acetone (5:13) was added. After vigorous shaking, precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

Statistical Analysis — Each value of the results is presented as the mean and the standard error. The difference between two groups was evaluated statistically by the Student's *t* test.

Results

Effect of Heat-Treatment

Effects of heat-treatment of antiserum or monoclonal antibodies on 1.5-h and 48-h PCA's were investigated. Antiserum, mc-IgE and mc-IgG₁ were heated at 56 °C for 0.5, 1 or 2 h. Results are shown in Fig. 1. In the case of antiserum, heating did not affect the 1.5-h PCA. However, the 48-h PCA was reduced in proportion to the heating time. On the other hand, both PCA's mediated by mc-IgE were reduced in proportion to heating time similar to the 48-h PCA mediated by antiserum. Although the 1.5-h PCA mediated by mc-IgG₁ was not affected by heating like that mediated by antiserum, the 48-h PCA was not elicited by mc-IgG₁.

Fig. 1. Effect of Heat-Treatment of Antiserum and Monoclonal Antibodies on 1.5-h and 48-h PCA's in the Mouse Ear
Antiserum and monoclonal antibodies were heated at 56 °C for 0.5, 1 or 2 h. Each value represents the mean and the standard error of 5 or 6 mice.

Fig. 2. Effect of Heat-Treatment of Antiserum and Monoclonal Antibodies on PCA Elicited at Various Time after Sensitization

Antiserum and monoclonal antibodies were heated at 56 °C for 2 h. Sensitized mice were challenged 1.5 h–7 d after the sensitization. Each value represents the mean and the standard error of 3–6 mice. ○, non-heated; ●, heated; ▲, PCA(–).

To examine the persistence of sensitized state, mice were sensitized with a non-heated or heated sample and the PCA's were elicited 1.5 h–7 d after sensitization. Heat-treatment at 56 °C was performed for 2 h. The results are shown in Fig. 2. The PCA's mediated by non-heated antiserum were almost comparable to those mediated by heated antiserum at 1.5 and 6 h, which gradually decreased thereafter. However, a potent reaction was elicited even at 7 d, and the amount of extravasated dye was $16.57 \pm 2.64 \mu\text{g}$ ($n=5$). On the contrary, the PCA mediated by heated antiserum reduced rapidly with the sensitizing period and the level reached were almost negligible at 48 h. The unheated mc-IgE caused strong reactions at all sensitizing periods and the levels did not reduce even at 7 d. In contrast, heated mc-IgE did not elicit PCA and the level was comparable to that

Fig. 3. Accumulation of Dye in the Mouse Ear Caused by PCA

Mice were sacrificed 2–40 min after challenge. Each value represents the mean and the standard error of 3–6 mice. Closed circle indicates PCA(–).

for each PCA(–) group. Although mc-IgG₁, in both non-heated and heated forms, elicited potent PCA's at 1.5 h, the levels reduced rapidly and completely disappeared at 48 h. Heating of mc-IgG₁ did not affect PCA.

Accumulation of Dye in the Ear

To compare the accumulating processes of dye in 1.5-h and 48-h PCA's, mice were sacrificed 2–40 min after the challenge. In the 1.5-h PCA, antiserum and mc-IgG₁ were heated at 56 °C for 2 h. The results are shown in Fig. 3. Dye leakage in the 1.5-h PCA mediated by antiserum occurred at 10 min and to 20 min after the challenge. In contrast, in the case of the 48-h PCA mediated by antiserum, leaked dye was detected at 5 min and the leakage terminated 15 min after

Fig. 4. The 1.5-h and 48-h PCA's in WBB6 F₁-+/+ and W/W^v Mice

Dotted column indicates PCA(-). Each value represents the mean and the standard error of 3 (in PCA(-) group) or 5 (in PCA group) mice.

the challenge. A similar pattern was observed in the case of the 48-h PCA mediated by mc-IgE. On the contrary, dye leakage in the 1.5-h PCA mediated by mc-IgG₁ was delayed significantly. Leaked dye was detected at 20 to 30 min after the challenge.

PCA in WBB6 F₁ Mice

The 1.5-h and 48-h PCA's were investigated in WBB6 F₁ mice. Antiserum and mc-IgG₁ were heated at 56 °C for 2 h for 1.5-h PCA. Results are shown in Fig. 4. In WBB6 F₁-+/+ mice, potent PCA's were evoked in all 4 cases. In contrast, the PCA was not elicited in any of the cases in WBB6 F₁-W/W^v mice.

Effect of Tranilast

Effect of tranilast on the 1.5-h and 48-h

Fig. 5. Effect of Tranilast on 1.5-h and 48-h PCA's in Mouse Ear

Tranilast was suspended in distilled water containing 0.2% sodium carboxymethylcellulose and given orally 1 h prior to challenge. Mice fasted for 6–8 h before the administration. Each value represents the mean and the standard error of 4–8 mice. a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$.

PCA's was investigated. In the 1.5-h PCA, antiserum and mc-IgG₁ were heated at 56 °C for 2 h. Tranilast was suspended in distilled water containing 0.2% sodium carboxymethylcellulose and given 50–200 mg/kg orally 1 h prior to challenge. As shown in Fig. 5, the PCA's mediated by both antiserum and monoclonal antibodies were inhibited in a dose-dependent manner and the degree of inhibition in each case was almost comparable.

Absorption of Antiserum

Antiserum was absorbed with anti-ε-Sepha-

Fig. 6. Effect of Absorption of Antiserum on PCA in the Mouse Ear

Antiserum was mixed with anti- ϵ -Sepharose 4B or anti- γ_1 -Sepharose 4B and rotated gently for 30 min at room temperature. The unabsorbed fraction was concentrated and examined for the activity to elicit 1.5-h or 48-h PCA. In the 1.5-h PCA, antiserum was heated at 56 °C for 2 h. Each value represents the mean and the standard error of 5–10 mice.

rose 4B or anti- γ_1 -Sepharose 4B and the activity of the unabsorbed fraction to elicit PCA was examined. In the 1.5-h PCA, antiserum was heated at 56 °C for 2 h. As shown in Fig. 6, although absorption of heated antiserum with anti- ϵ -Sepharose 4B did not affect the 1.5-h PCA, absorption with anti- γ_1 -Sepharose 4B significantly reduced the 1.5-h PCA. Repeated absorption abrogated 1.5-h PCA completely. Similarly, although absorption of non-heated antiserum with anti- γ_1 -Sepharose 4B did not affect the 48-h PCA, absorption with anti- ϵ -Sepharose 4B significantly reduced 48-h PCA. When the absorption was repeated, the 48-h PCA was abrogated completely.

Discussion

In the present study, PCA's were elicited in the mouse ear sensitized with antiserum, mc-IgE and mc-IgG₁ and investigated comparatively. In the mc-IgE-mediated PCA, sensitized state was achieved soon after the injection of the antibody and persisted at least for 7 d. Treatment at 56 °C for 2 h abrogated the activity of mc-IgE to elicit PCA. These properties of the IgE antibody are well established.^{1–3)} In the case of antiserum, although sensitized state persisted at least for 7 d, the PCA elicited 1.5–24 h after the sensitization was not abrogated by heating of antiserum at 56 °C for 2 h. On the other hand, the 48-h PCA was abrogated completely by the absorption of antiserum with anti- ϵ -Sepharose 4B. Furthermore, the accumulation pattern of dye in the 48-h PCA mediated by non-heated antiserum was in good agreement with that in the case of mc-IgE. These results indicated that the 48-h PCA in the mouse ear caused by antiserum is mediated by the IgE antibody. In contrast, the sensitized state disappeared rapidly in cases of heated antiserum and mc-IgG₁, and the 1.5-h PCA's mediated by antiserum and mc-IgG₁ were not affected by heating. Furthermore, the 1.5-h PCA mediated by heated antiserum was completely abrogated by absorption with anti- γ_1 -Sepharose 4B. These results suggested that the IgG₁ antibody mediated 1.5-h PCA in the mouse ear sensitized with heated antiserum. However, accumulation of dye observed in mc-IgG₁-mediated 1.5-h PCA delayed significantly when compared to that observed in heated antiserum-mediated 1.5-h PCA. In 1982, Ovary²¹⁾ reported that mouse IgG_{2a} and IgG_{2b} antibodies sensitize homologous skin for PCA. In fact, it must be considered that diluted antiserum for sensitization also contains many serum components, except for IgG₁ antibody, and that 1.5 h may be too short for these serum components to disappear from injection sites. Therefore, the difference observed in the accumulation of dye between the 1.5-h PCA's mediated by heated antiserum and mc-IgG₁ may be explained by participation of other serum components in antiserum-mediated PCA. However, the precise mechanism is not clear, and further

characterization of the 1.5-h PCA caused by the IgG₁ antibody must be undertaken using other monoclonal IgG₁ antibody preparations.

It is well established that mast cells have many receptors for the Fc portion of the IgE antibody (FcεR) on their surface and that the IgE antibody binds strongly to FcεR.²²⁾ When sensitized mast cells are challenged with a specific antigen, two or more IgE antibody molecules on the mast cell surface are linked together by an antigen molecule.^{10,23)} This crosslinkage is an essential process for IgE antibody-mediated mast cell activation, and degranulation and mediator release are evoked within a very short time. In PCA, vascular permeability is increased by released mediators as a result. In contrast, though the IgG homocytotropic antibody causes an anaphylactic reaction similar to that caused by the IgE antibody, the mechanism is not fully understood. Nakagawa and de Weck²⁴⁾ indicated the presence of membrane receptors for IgG₄ subclass on human basophils and mast cells. However, in mice, the affinity of the receptor to the IgG₁ antibody is considered to be low²⁵⁾ and its role in IgG₁ antibody-mediated reaction is not yet established.

In the present results with WBB6 F₁-W/W^v mice which lack mast cells²⁶⁾ and with tranilast which inhibits mediator release from mast cells,^{19,20)} it was indicated that the mast cell is essential not only for the 48-h PCA's mediated by antiserum and mc-IgE but also for 1.5-h PCA's caused by heated antiserum and mc-IgG₁. Tranilast dose not exhibit antagonistic effect against mediators from mast cells. On the other hand, the dye leakage was confirmed from 5 min to 15 min after challenge in the 48-h PCA's mediated by both antiserum and mc-IgE. On the contrary, the dye leakage in the 1.5-h PCA was delayed when compared to that of the 48-h PCA and the delay was about 5 min in antiserum-mediated PCA and about 15 min in mc-IgG₁-mediated PCA. It is considered that the rapid initiation and termination of dye leakage in the 48-h PCA was caused by the direct activation of mast cells *via* FcεR. The delay observed in the 1.5-h PCA may be explained by the involvement of indirect process in IgG₁ antibody-mediated mast cell activation. As Binaghi²⁵⁾ stressed the importance

of the formation of immune complex in IgG₁ antibody-mediated reaction *in vitro*, the mast cell may be activated secondarily in IgG₁ antibody-mediated 1.5-h PCA in the mouse ear.

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