

Active Cutaneous Anaphylaxis (ACA) in the Mouse Ear

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ABSTRACT—Active cutaneous anaphylaxis (ACA) was studied in the ear of female BALB/c mice. Mice were immunized with ovalbumin in the presence of aluminium hydroxide gel or complete Freund's adjuvant (CFA). Two weeks after the immunization, ACA was elicited in the mouse ear by injecting 10 μ l of antigen solution intradermally into the ear lobe. ACA was assessed by the amount of extravasated dye, which was given intravenously just after the antigen injection. Antiallergic drugs (tranilast, ketotifen and azelastine), antihistamines (chlorpheniramine, diphenhydramine and mequitazine), β -stimulants (isoproterenol and salbutamol), theophylline and glucocorticoids (hydrocortisone, prednisolone and dexamethasone) inhibited the reaction significantly. These drugs inhibited both ACA in mice immunized with alum-precipitated antigen and ACA in mice injected with CFA-emulsified antigen similarly. ACA in the mouse ear might be a useful tool for studying drugs for allergy.

Keywords: Active cutaneous anaphylaxis, Ear (mouse), Antiallergic drug, IgE antibody, IgG1 antibody

Passive cutaneous anaphylaxis (PCA) (1, 2) is a useful experimental model for studying drugs for allergy (3–6). The rat has been the most useful species for this purpose and the reaction is assessed by measuring the amount of extravasated dye (7, 8). On the other hand, the mouse is the most established experimental animal and there are many strains of mice with unique immunological characteristics (9–12). Furthermore, Feinberg (13) reported the suitability of mouse ear for induction of PCA, and Prouvost-Danon and Binaghi (14) demonstrated that mouse ear had higher reactivity for PCA than mouse dorsal skin. According to these reports, we established a method for PCA in the mouse ear by assessing the amount of extravasated dye (15) and characterized it pharmacologically and immunologically (16–23). However, although passive sensitization provides a simple model that is suitable for analyzing the mechanisms involved in both allergic reactions and drug action, active sensitization is also important for studying allergic diseases, because allergic diseases are caused by active sensitization. In the present study, therefore, we applied the technique of mouse ear PCA to active cutaneous anaphylaxis (ACA) elicited in female BALB/c mice, and examined the effect of drugs which are used for allergic diseases on the ACA.

MATERIALS AND METHODS

Mice

Female BALB/c mice were used for ACA and male ddY mice were used for titration of serum antibodies. Both mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan).

Antigen and adjuvants

Ovalbumin (OA, 5 times crystalized) was purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). As adjuvants, aluminium hydroxide gel (alum) and complete Freund's adjuvant (CFA) were used. Alum was prepared according to the method of Levine and Vaz (24), and CFA was obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

ACA

Ten-weeks-old female BALB/c mice were immunized with OA in the presence of an adjuvant. To induce IgE antibody mainly, 1 μ g of OA mixed with 1 mg of alum was injected intraperitoneally. To induce IgG1 antibody mainly, 100 μ g of OA emulsified in CFA was given intraperitoneally. Four or five weeks later, in some experiments, booster immunization was performed in the same manner. Two weeks after the first immunization or 1 week after the booster immunization, ACA was

elicited in the ear of mice by injecting 10 μ l of antigen saline solution followed by an intravenous injection of 0.25 ml of 0.5% Evans blue saline solution. Thirty minutes after the challenge, mice were sacrificed by cervical dislocation, and ears were removed for measurement of extravasated dye. To measure the amount of dye detected non-specifically, non-immunized mice received a similar antigenic challenge.

Extraction and quantification of extravasated dye was performed using a previously described method (15). Briefly, a pair of ears from a mouse 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 phosphoric acid and acetone (5:13) was added. After vigorous shaking, precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nm.

Mediator skin reaction

The mediator skin reaction was examined in immunized BALB/c mice as previously described (17). One week after the booster immunization, the skin reaction was elicited by injecting 10 μ l of mediator solution into the ear. The reaction was evaluated similarly to ACA by assessing the amount of extravasated dye which was given intravenously just after the mediator injection. As mediators, 2×10^{-4} g/ml histamine (hydrochloride, Nacalai Tesque), 2×10^{-6} g/ml serotonin (creatininsulfate, Merck), 2×10^{-6} g/ml platelet activating factor (PAF, Bachem), and 5×10^{-6} g/ml leukotriene C₄ (LTC₄, Wako) were used.

Serum antibody titer

Serum IgE and IgG1 antibody titers were examined by means of ear PCA in male ddY mice (15). Serum samples were obtained from BALB/c mice one day before ACA and pooled within a group. Sera were two-fold serially diluted with saline and injected in a volume of 10 μ l into ddY mouse ear. When assaying IgG1 antibody titer, sample sera were heated at 56°C for 2 hours before dilution (19, 25). For detecting IgG1 antibody, mice were challenged by intravenous injection of 0.25 ml of 0.5% Evans blue saline solution containing 0.25 mg of antigen 1.5 hours after the sensitization. For detecting IgE antibody, similar antigenic challenge was performed 48 hours after the sensitization. Thirty minutes later, mice were sacrificed by cervical dislocation, and the bluing reaction in the ear was evaluated.

Drugs

Tranilast (Kissei), ketotifen (fumarate, Sandoz), azelastine (hydrochloride, Eisai), chlorpheniramine (maleate, Sigma), diphenhydramine (hydrochloride, Sigma),

mequitazine (Nippon Shoji), isoproterenol (hydrochloride, Sigma), salbutamol (sulfate, Fujikawa), theophylline (Sigma), hydrocortisone (acetate, aqueous suspension, Nippon Merck-Banyu), prednisolone (acetate, aqueous suspension, Shionogi) and dexamethasone (acetate, aqueous suspension, Banyu) were used. Tranilast, mequitazine, and theophylline were suspended in 0.2% sodium carboxymethyl cellulose solution, and aqueous suspensions of glucocorticoids were diluted with 0.2% sodium carboxymethyl cellulose saline solution. The others were dissolved in water. Drugs except for glucocorticoids, which were administered intraperitoneally 8 hours prior to ACA (22), were given to mice orally one hour prior to ACA. When drugs were given orally, mice were fasted for 6 hours before administration.

Statistics

Statistical analyses were performed by Student's *t*-test.

RESULTS

Challenging antigen dose

Mice were immunized 2 times with OA in the presence of alum. One week after the booster immunization, ACA was elicited by injecting 0.01, 0.1 and 1 μ g of the antigen into the ear. As shown in Fig. 1, 0.01–1 μ g of the antigen caused potent reactions in a dose-dependent manner, and the amount of extravasated dye was 20.5–43.6 μ g. When assessing the extravasated dye in the mouse ear, it is important for an accurate determination that the bluing reaction is limited within the ear lobe. We, therefore, chose a moderate dose of 0.1 μ g for ACA elicitation and used this dose hereafter.

Relationships between ACA and serum antibody titers

Mice were immunized with OA in the presence of alum or CFA, and ACA was elicited with 0.1 μ g of the antigen 2 weeks after the primary immunization and 1 week after the booster immunization. Serum samples were collected 1 day before ACA, and antibody titers were estimated by means of mouse ear PCA. Results are shown in Fig. 2. Two weeks after the primary immunization, IgG1 and IgE titers in the alum group were 1:2⁶ and 1:2⁹, respectively, and in the CFA group 1:2⁹ and 1:2⁴, respectively. In both groups, however, potent ACA with a similar intensity was elicited. One week after the booster immunization, antibody titers were elevated significantly as compared to those observed in the primary response, and the differences among antibody titers in the primary response disappeared. Although potent ACA was elicited in both the alum and CFA

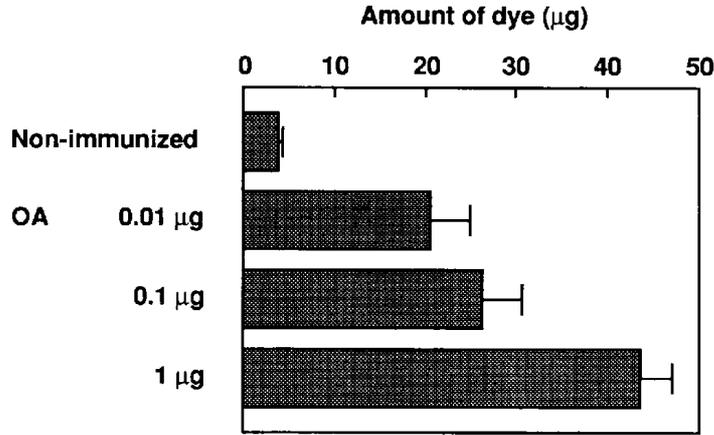


Fig. 1. Relationship between ACA and antigen dose. Mice were immunized with OA in the presence of alum 2 times at an interval of 4 weeks. ACA was elicited 1 week after the secondary immunization by injecting 0.01 – 1 µg of OA into the ear. Each value represents the mean and the standard error of 5 mice.

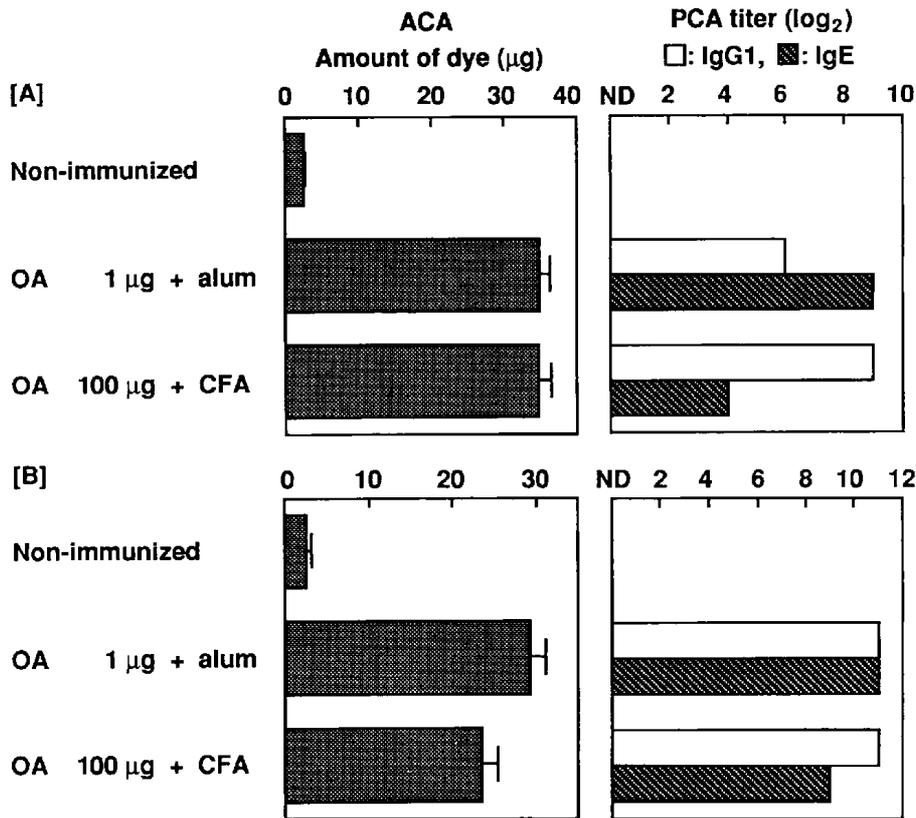


Fig. 2. Relationships between ACA and serum antibody titers. Mice were immunized with OA 2 times at an interval of 5 weeks. ACA was elicited 2 weeks after the primary immunization [A] and 1 week after the secondary immunization [B] by injecting 0.1 µg of OA into the ear. Serum samples were obtained 1 day before ACA. Results of ACA are the mean value and the standard error of 9 or 10 mice, except for the non-immunized groups of 3 mice. PCA titers were estimated using pooled sera. ND: not detected.

groups, the reaction was not as potent as that observed after the primary immunization. Based on these results, we decided to examine the effects of drugs on ACA elicited 2 weeks after the primary immunization.

Effect of immunization on sensitivity of mouse ear against chemical mediators

Effect of immunization on mediator skin reaction in the mouse ear was examined. Mice were immunized 2 times with OA in the presence of alum or CFA, and skin reactions by histamine, serotonin, PAF and LTC₄

were elicited 1 week after the booster immunization. As shown in Fig. 3, although all mediators caused a significant vascular permeability increase, no significant difference was observed among non-immunized, alum and CFA groups.

Effects of drugs on ACA

Effects of antiallergic drugs on ACA in mice immunized with OA in the presence of alum or CFA were examined. Drugs were administered orally 1 hour prior to challenge. Results are shown in Fig. 4. Fasting for 6 hours resulted in depressed ACA in the control groups in comparison to those indicated in Fig. 2. Tranilast at doses of 100 and 200 mg/kg and ketotifen and azelastine at doses of 0.5 and 1 mg/kg significantly inhibited both ACA dose-dependently.

Results of antihistamines are shown in Fig. 5. Drugs were administered orally 1 hour prior to challenge. Chlorpheniramine at doses of 50 and 100 mg/kg, diphenhydramine at doses of 20 and 50 mg/kg, and mequitazine at doses of 2 and 5 mg/kg significantly inhibited both ACA dose-dependently.

Results of β -stimulants and theophylline are shown in Fig. 6. Drugs were administered orally 1 hour prior to challenge. Salbutamol at doses of 10 and 20 mg/kg and theophylline at doses of 50 and 100 mg/kg significantly inhibited both ACA dose-dependently. Although isoproterenol at the dose of 20 mg/kg significantly inhibited ACA in mice immunized in the presence of alum, it failed to inhibit ACA in mice immunized in the presence of CFA. In a separate experiment, however, it was confirmed that isoproterenol inhibited both ACA, and that the potency was slightly less than that of salbu-

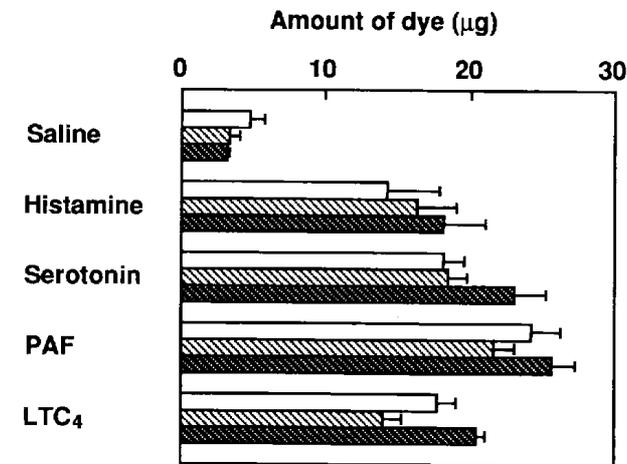


Fig. 3. Effect of immunization on mediator skin reaction in the mouse ear. Mice were immunized with OA 2 times at an interval of 5 weeks. One week after the secondary immunization, skin reactions were elicited by histamine, serotonin, PAF and LTC₄. Each value represents the mean and the standard error of 5 mice. □: non-immunized, ▨: immunized with OA and alum, ▩: immunized with OA and CFA.

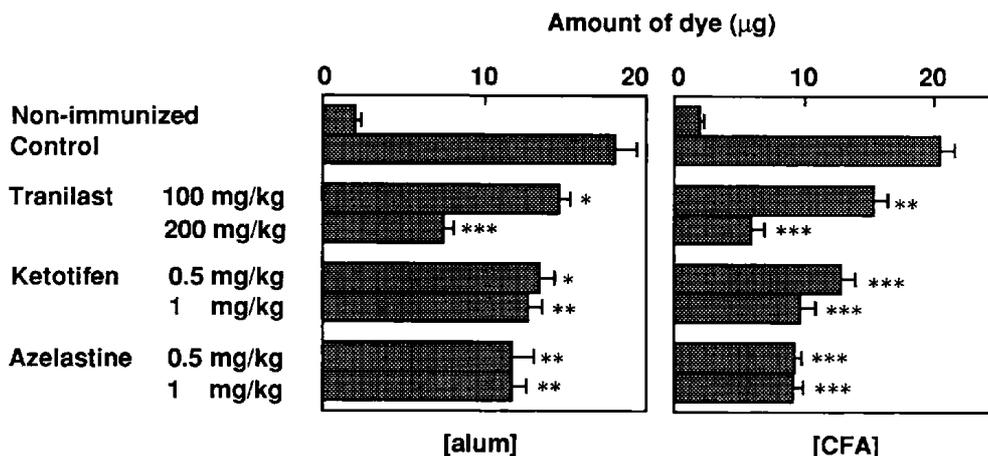


Fig. 4. Effects of tranilast, ketotifen and azelastine on ACA in the mouse ear. Mice were immunized with OA in the presence of alum or CFA. ACA was elicited 2 weeks after the immunization. Drugs were administered orally 1 hour prior to challenge. Each value represents the mean and the standard error of 6 or 7 mice, except for the non-immunized groups of 3 mice. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (vs. control).

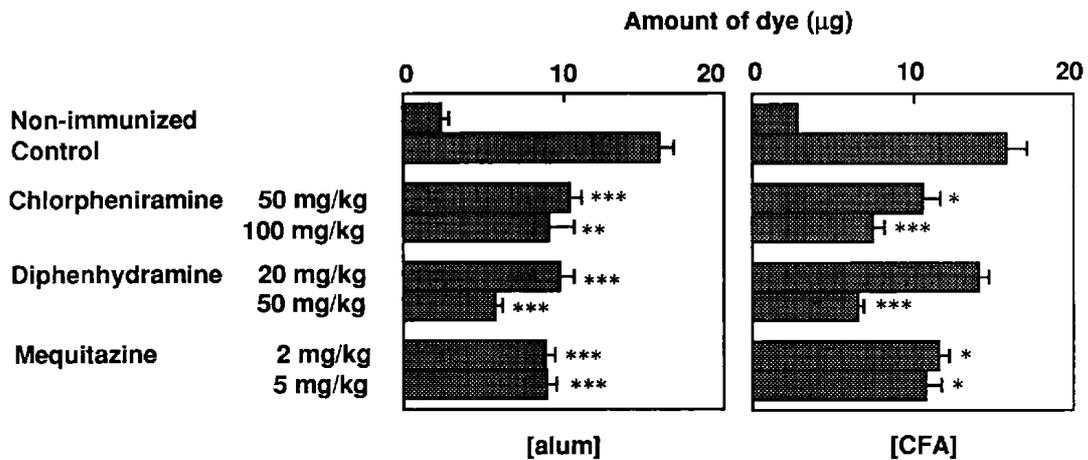


Fig. 5. Effects of chlorpheniramine, diphenhydramine and mequitazine on ACA in the mouse ear. Mice were immunized with OA in the presence of alum or CFA. ACA was elicited 2 weeks after the immunization. Drugs were administered orally 1 hour prior to challenge. Each value represents the mean and the standard error of 6 or 7 mice, except for the non-immunized groups of 2 or 3 mice. *: P < 0.05, **: P < 0.01, ***: P < 0.001 (vs. control).

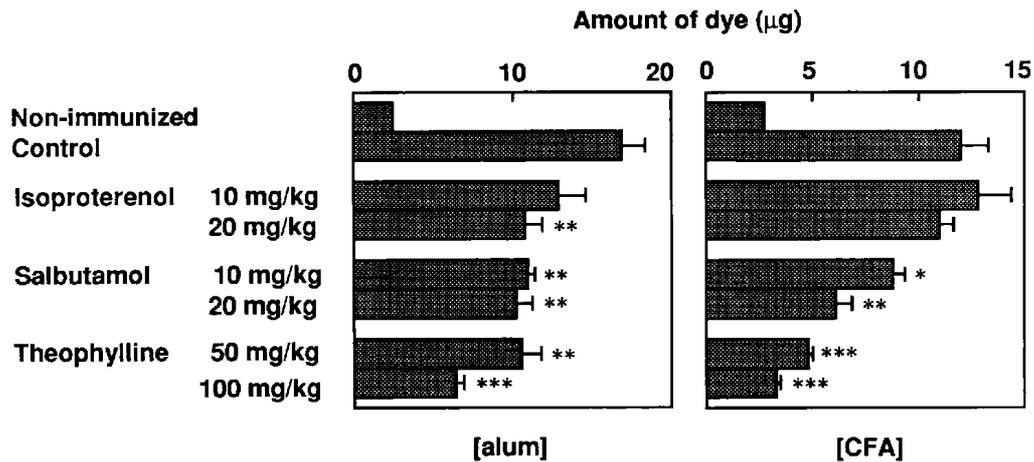


Fig. 6. Effects of isoproterenol, salbutamol and theophylline on ACA in the mouse ear. Mice were immunized with OA in the presence of alum or CFA. ACA was elicited 2 weeks after the immunization. Drugs were administered orally 1 hour prior to challenge. Each value represents the mean and the standard error of 4 to 7 mice, except for the non-immunized groups of 2 mice. *: P < 0.05, **: P < 0.01, ***: P < 0.001 (vs. control).

tamol under the present experimental conditions (data not shown).

Results of glucocorticoids are shown in Fig. 7. Drugs were administered intraperitoneally 8 hours prior to challenge. Hydrocortisone at doses of 10 and 20 mg/kg and dexamethasone at doses of 0.5 and 1 mg/kg significantly inhibited both ACA dose-dependently. Although prednisolone in doses of 2 and 5 mg/kg inhibited ACA in mice immunized with CFA-emulsified antigen, it only showed a tendency to inhibit the ACA in mice immunized with alum-precipitated antigen.

DISCUSSION

In the present study, we established a method for ear ACA in female BALB/c mice and demonstrated that several drugs for allergic diseases effectively inhibited the ACA. It is noteworthy that the ACA was highly reproducible in comparison with the mouse ear PCA reported previously (15), in spite of the active immunization.

Mice were immunized with an antigen, OA, in the presence of an adjuvant, alum or CFA. We used alum to induce IgE antibody effectively and CFA to induce IgG1 antibody predominantly (24, 26). Two weeks after

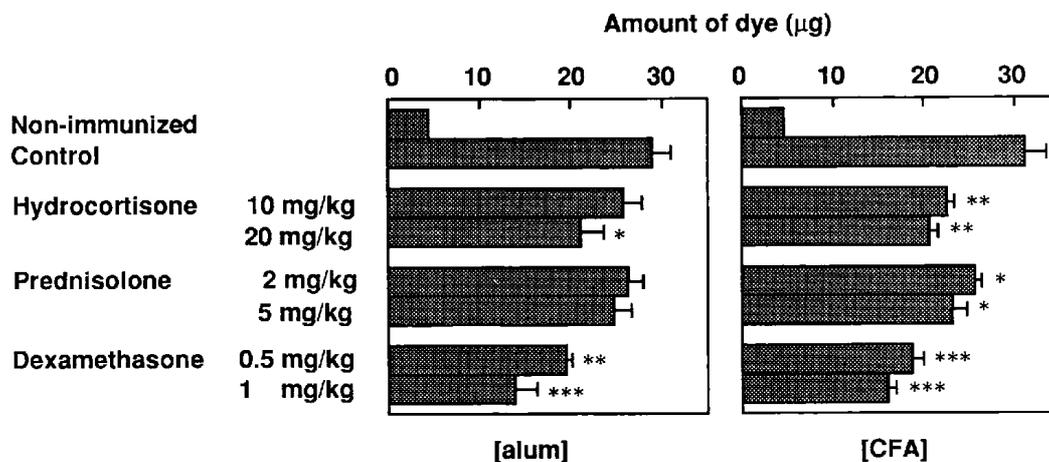


Fig. 7. Effects of hydrocortisone, prednisolone and dexamethasone on ACA in the mouse ear. Mice were immunized with OA in the presence of alum or CFA. ACA was elicited 2 weeks after the immunization. Drugs were administered intraperitoneally 8 hours prior to challenge. Each value represents the mean and the standard error of 6 or 7 mice, except for the non-immunized groups of 2 mice. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ (vs. control).

the primary immunization, potent and comparable ACA was observed in both groups of mice. Although both the IgE antibody titer in the alum group and the IgG1 antibody titer in the CFA group were high, 1:2⁹, IgG1 antibody in the alum group and the IgE antibody in the CFA group were also detected, and their titers were not negligible. We considered, therefore, that both types of antibodies could be responsible for eliciting the ACA in both alum and CFA groups. One week after the secondary immunization, antibody titers were elevated significantly, and both IgE and IgG1 antibody titers in the alum group were 1:2¹¹, and those in the CFA group were 1:2⁹ and 1:2¹¹, respectively. In spite of the elevated serum antibody titers, the ACA was not so potent as that observed 2 weeks after the primary immunization. These results suggest that the occurrence of ACA does not parallel the elevation in IgE or IgG1 antibody titer. On the other hand, as increased reactivity against various stimuli was observed in allergic patients (27, 28), we examined the effect of immunization on the mediator skin reaction in the mouse ear 1 week after the secondary immunization. No significant difference was observed among non-immunized, alum and CFA groups in skin reactions caused by histamine, serotonin, PAF and LTC₄, suggesting that the immunizing procedure employed here does not alter the reactivity in mice against chemical mediators. Repeated challenge with antigen at a shorter interval may be effective for causing increased reactivity.

Antiallergic drugs, tranilast, ketotifen and azelastine, inhibited the ACA significantly. Inhibition of mediator release from mast cells may be involved in the inhibition of ACA by tranilast (4, 29), and the antihistaminic

properties of ketotifen and azelastine may play important roles in their inhibition (5, 6). Antihistamines, chlorpheniramine, diphenhydramine and mequitazine also inhibited the ACA significantly. Mequitazine is a potent antihistamine with an antiasthma property (30, 31). Bronchodilating drugs, isoproterenol, salbutamol and theophylline, and glucocorticoids, hydrocortisone, prednisolone and dexamethasone, inhibited the ACA significantly. Although these drugs are reported to inhibit antigen-induced mediator release from mast cells (32–34), they also inhibit vascular permeability increase non-specifically (35, 36). These drugs inhibit PCA and skin reactions caused by chemical mediators significantly in rats and mice (22, 37). In rats, PCA is inhibited more potently than the mediator skin reaction by these drugs (37). On the contrary, in mice, glucocorticoids only inhibit PCA to an extent similar to inhibition of the mediator skin reaction (22). These results suggest that the inhibition of both mediator release from mast cells and vascular permeability increase by chemical mediators are involved in the inhibition of PCA in rats, and that the inhibition of vascular permeability increase is important in the case of mouse PCA. It is also suggested that inhibition of vascular permeability increase may play important roles in the inhibition of ACA by β -stimulants, theophylline and glucocorticoids observed in the present study. All drugs studied here inhibited the ACA in mice immunized with alum-precipitated antigen and CFA-emulsified antigen similarly. This may be related to the fact that both immunizing procedures resulted in the production of both IgE and IgG1 antibodies responsible for ACA. We reported previously, however, that mouse ear PCA

mediated by both IgE and IgG1 antibodies are inhibited similarly by these drugs (16, 20).

PCA is a reaction that involves mast cell activation and a subsequent increase in vascular permeability. Since a process of lymphocyte-antigen interaction leading to antibody production is also involved in ACA, drugs affecting antibody production should also be evaluated as anti-allergic drugs. Mice are handled easily because of their small size and many interesting strains of mice are reported (9–12). Mice are the best experimental animals for inducing IgE antibody production. Furthermore, the ACA reported here is highly reproducible. Mouse ear ACA may become a useful tool for studying drugs for allergy.

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