CMLS, Cell. Mol. Life Sci. 54 (1998) 456–460
1420-682X/98/050456-05 $ 1.50 + 0.20/0


CMLS Cellular and Molecular Life Sciences

# Research Article

# Drug susceptibility of PCA in WBB6F1-W/W$^v$ mice

**S. Kimura*, A. Watanabe, M. Takeuchi, M. Nagata, K. Nakamura and M. Harada**

Developmental Research Laboratories, Shionogi & Co. Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825 (Japan), Fax +81 6 332 6385

Received 10 December 1997; received after revision 2 February 1998; accepted 23 February 1998

**Abstract.** Our previous study revealed that passive cutaneous anaphylaxis (PCA) can be produced in congenitally mast cell-deficient WBB6F1-W/W$^v$ (abbreviated as W/W$^v$) mice on sensitization with undiluted or slightly diluted allogeneic and xenogeneic antisera but not on sensitization with allogeneic monoclonal immunoglobulin (Ig)E and IgG1 antibodies regardless of the antibody concentration [1]. In view of these findings, the present study was conducted to characterize PCA in this strain from its drug susceptibilities using mast cell-bearing WBB6F1-+/+ (abbreviated as +/+) and B6D2F1 mice as references. PCA in W/W$^v$ mice mediated by a low dilution (1:4) of hyperimmune serum to bovine serum albumin of the B6D2F1 mouse origin was markedly suppressed by CV-6209, an antagonist of platelet-activating factor (PAF), but not by antihistamines such as cyproheptadine and oxatomide. In contrast, PCA in +/+ and B6D2F1 mice mediated by a high dilution (1:128) of the anti-serum (virtually by IgG1 antibody) was nearly completely suppressed by antihistamines but not by CV-6209. A remarkable difference between PCA in W/W$^v$ and reference mice was also observed in the susceptibility to monoclonal anti-mouse granulocyte (Gr-1) antibody: PCA in W/W$^v$ mice was potently suppressed by the 1- to 3-day pretreatment with this antibody but that in references was not at all. Putting these present results together with the previous finding that anti-granulocyte antibody greatly reduces circulatory Gr-1$^+$ leukocytes, 1 to 3 days after the treatment [2], it is highly probable that PCA in W/W$^v$ mice mediated by some antibody isotypes other than IgE and IgG1 is produced by PAF mainly released from Gr-1$^+$ cells, while IgG1 antibody-mediated PCA in mast cell-bearing reference mice is evoked by histamine derived from mast cells. PCA homologous to that in W/W$^v$ mice could also be produced in the reference mice on sensitization with undiluted or slightly diluted antiserum, when generalized blueing due to excess IgG1 antibody was removed by the oxatomide treatment before the antigen challenge.

**Key words.** PAF; PCA; WBB6F1-W/W$^v$; anti-mouse granulocyte antibody.

Our previous studies demonstrated that type I hypersensitivities can be produced in WBB6F1-W/W$^v$ (abbreviated below as W/W$^v$) mice, a congenitally mast cell-deficient strain. Lethal anaphylactic shock can be induced when W/W$^v$ mice are sensitized by active means or passive means with allogeneic monoclonal immunoglobulin (Ig)G1 antibody or allogeneic and xenogeneic hyperimmune sera, although not when they are sensitized with monoclonal IgE antibody [3]. A more recent study has shown that anaphylactic shock in this strain can be prevented by an anti-platelet-activating factor (PAF) agent or anti-granulocyte (Gr-1) antibody regardless of the sensitizing method [2], suggesting that PAF released from Gr-1$^+$ granulocytes plays a crucial role in anaphylactic shock. On the other hand, cutaneous anaphylaxis can be produced in W/W$^v$ mice pas-

* Corresponding author.

sively sensitized with allogeneic or xenogeneic hyperimmune serum but not in those sensitized actively or passively with monoclonal IgE or IgG1 antibody preparation [1], which indicates that there is some difference between the manners of tissue sensitization for systemic and cutaneous anaphylactic reactions. Under such a circumstance, the present study was conducted to characterize PCA in W/W$^v$ mice from the immunopharmacological standpoint, comparing its susceptibilities to antihistamines, an anti-PAF agent, and anti-granulocyte antibody with those of PCA in mast cell-bearing reference mice.

## Materials and methods

**Experimental animals.** Female mice of the W/W$^v$ and mast cell-bearing WBB6F1-+/+ (abbreviated as +/+) strains approximately 3 months old were purchased from Japan SLC. These mice were tested for PCA after 1-week acclimatization. Female B6D2F1 mice 3 months old supplied from Shionogi Aburahi Laboratories were employed as the PCA recipients and antibody producers to bovine serum albumin (BSA). Female WS rats and DS mice about 2 months old supplied from Shionogi Aburahi Laboratories were used as the recipients in PCA tests for the titration of IgE and IgG1 antibodies of anti-BSA serum, respectively.

**Antigens and antibodies.** Hyperimmune serum to BSA was used as skin-sensitizing antibodies. For preparing anti-BSA serum, B6D2F1 mice were given two intraperitoneal injections of 0.2 ml of a CFA (complete Freund's adjuvant) emulsion containing 1 mg of BSA (Armor) at 2-week intervals. Two weeks after the 2nd injection, the mice were bled by heart puncture, and the sera were pooled. The IgE antibody titre of this pooled serum assessed by 1-day PCA in WS rats was 1:512, and the IgG1 antibody titre assessed by 1-h PCA in DS mice was 1:4096. Anti-BSA IgG2a antibody was assessed by ELISA (enzyme-linked immunosorbent assay) using peroxidase-linked goat anti-mouse IgG2a monoclonal antibody (Cappel) as the secondary antibody according to the technique described elsewhere [4], because mouse IgG2a antibody has been considered in general to be devoid of skin-sensitizing activity in mice and rats. The pooled antiserum had a very high IgG2a antibody titre of $1:10^6$.

**Production of PCA in W/W$^v$ and reference mice.** W/W$^v$, B6D2F1, and +/+ mice were shaved on the dorsal skin and intradermally injected with 0.05 ml of the anti-BSA serum appropriately diluted. After 1-h sensitization, they were intravenously injected with 1 mg of BSA mixed with 0.5 mg of Evans blue (0.2 ml in volume). Thirty minutes later, they were sacrificed, and the dye infiltrated into the skin was extracted and determined according to the technique previously reported [5]. Briefly, blue-stained areas were cut off and chopped into small pieces. The pieces were immersed in a mixture of acetone (7 vol) and a 0.5% aqueous solution of sodium sulphate (3 vol) and left to stand for 2 to 3 days with occasional shaking. After centrifugation, optical density of the supernatants was determined at 620 nm. The statistical significance of the difference in the amount of the infiltrated dye between the control and drug-treated groups was examined by Student's $t$ test.

**Drug treatments.** CV-6209 was used as an anti-PAF agent and cyproheptadine and oxatomide as antihistamines, and the dosages of these agents were set up based on the previous studies on their suppressive activities against anaphylactic shock [6] and/or IgE antibody-mediated ear PCA [7]. CV-6209 synthesized and supplied by Dr. T. Tsuri of Shionogi Research Laboratories was dissolved in physiological saline solution and given intravenously 5 min before the antigen challenge according to Terashita et al. [8] at the dose of 3.3 mg/kg (0.1 ml per 10 g of body weight). Cyproheptadine (Sigma) was suspended in the saline solution and intraperitoneally injected (10 mg/kg) 30 min before the antigen injection with attention paid to homogeneous sampling. Oxatomide purified from Celtect® tablets (Kyowa Hakko Kogyou) was suspended in a 0.2% solution of carboxymethylcellulose (CMC) with a mortar and administered orally (100 mg/kg) 30 min before elicitation of PCA. Lyophilized rat monoclonal antibody to a mouse granulocyte antigen, Gr-1 (clone: RB6-8C5, PharMingen), was reconstituted with distilled water and dialysed against physiological saline solution under stirring, with frequent exchange of external fluid to remove the sodium azide. This antibody preparation (0.2 ml containing 40 μg of the antibody) was intravenously injected 1 day before the test unless otherwise described. Physiological saline solution was intraperitoneally injected 30 min before the antigen challenge to control groups in all the experiments, because preliminary tests revealed little difference in PCA intensity among the animals injected with saline solution intraperitoneally or intravenously and those given CMC orally.

## Results

**PCA-suppressing effects of CV-6209 and antihistamines.** The suppressive effects of CV-6209 and the two antihistamines on PCA mediated by anti-BSA serum were compared between W/W$^v$ and mast cell-bearing reference (+/+ and B6D2F1) mice. As previously reported, a high concentration of antiserum is needed to sensitize the dorsal skin of W/W$^v$ mice for PCA in comparison with the concentration required in

Table 1. Effects of CV-6209, cyproheptadine and oxatomide on PCA in W/W$^v$, +/+ and B6D2F1 mice sensitized by allogeneic anti-BSA serum.

| Strain | Serum dilution | Exp. no. | Amount of infiltrated dye* (inhibition %) | | | |
|---|---|---|---|---|---|---|
| | | | saline | CV-6209† | cyproheptadine‡ | oxatomide§ |
| W/W$^v$ | 1:4 | 1 | 31 ± 3.3 | 10 ± 2.5 (68) ‖ | 26 ± 4.7 (24) | 28 ± 3.4 (9) |
| | | 2 | 27 ± 2.0 | 5 ± 0.2 (81) ‖ | 29 ± 2.9 (−7) | 33 ± 3.5 (−22) |
| +/+ | 1:128 | 1 | 39 ± 2.1 | 40 ± 2.5 (−3) | 3 ± 0.2 (92) ‖ | 9 ± 2.2 (77) ‖ |
| | | 2 | 23 ± 1.7 | 20 ± 3.4 (13) | 3 ± 0.4 (87) ‖ | 2 ± 0.3 (91) ‖ |
| B6D2F1 | 1:128 | 1 | 40 ± 6.0 | 38 ± 2.2 (6) | 0 ± 0 (100) ‖ | 0 ± 0 (100) ‖ |
| | | 2 | 33 ± 7.9 | 28 ± 4.2 (15) | | |
| | 1:256 | 1 | 16 ± 1.9 | 18 ± 2.9 (−13) | | 2 ± 1.0 (88) ‖ |

*μg (mean ± SE, $n = 5$). †3.3 mg/kg, iv, 5 min before the antigen challenge. ‡10 mg/kg, ip, 30 min before the antigen challenge. §100 mg/kg, po, 30 min before the antigen challenge. ‖ Statistically significant inhibition ($p < 0.01$).

mast cell-bearing strains [3]. Therefore, anti-BSA serum diluted 1:4 was intradermally injected to W/W$^v$ mice, while a higher dilution (1:128 or 1:256) was injected to +/+ and B6D2F1 mice. As shown in table 1, PCA in W/W$^v$ mice was markedly suppressed by CV-6209 but not by cyproheptadine and oxatomide. In contrast, PCA in +/+ and B6D2F1 mice was nearly completely suppressed by cyproheptadine and oxatomide but not by CV-6209.

**PCA-suppressing effect of anti-granulocyte antibody.** As shown in table 2, PCA in W/W$^v$ mice was greatly alleviated, when they had been given an intravenous injection of anti-granulocyte antibody 1 day before the test. Suppression was also obvious in case of 3-day pretreatment with the antibody but not in case of the 6-day pretreatment. On the other hand, suppression by antigranulocyte antibody was not observed against PCA in the reference mice mediated by the 1:128 or 1:258 dilution of the anti-BSA serum.

**Suppressive effects of CV-6209 and anti-granulocyte antibody on antihistamine-resistant PCA in +/+ and B6D2F1 mice.** The above description indicates a clear-cut difference in drug susceptibility between PCA in W/W$^v$ mice mediated by a low dilution of the anti-serum and that in +/+ and B6D2F1 mice mediated by its high dilution. Nevertheless, PCA homologous to that in W/W$^v$ mice could also be produced in the reference strains on sensitization with undiluted or slightly diluted anti-serum, although production of compact blue spots is unsuccessful under this condition because of blue staining of whole back skin mediated by an excess amount of IgG1 antibody. However, we found that blocking of this generalized blueing by pretreatment with antihistamines makes it possible to evoke compact PCA, which is estimated to be homologous to PCA in W/W$^v$ mice. Thus, the susceptibilities of this antihistamine-resistant PCA to CV-6209 and anti-granulocyte antibody were examined. As demonstrated in table 3, marked suppression was produced by both agents against PCA in +/+ and B6D2F1 mice treated with oxatomide 1 h before the antigen challenge.

## Discussion

In contrast with an early report [9], our previous study revealed that PCA can be produced in W/W$^v$ mice sensitized with hyperimmune serum of the allogeneic or xenogeneic origin [1]. However, because W/W$^v$ mice lack mast cells and cannot produce PCA on sensitization with monoclonal IgE and IgG1 antibodies [1], it is certain that the pathogenic mechanism of PCA in this strain differs from that mediated by these two homocytotropic antibodies in mast cell-bearing strains. In view of the evidence that IgG2a and/or IgG2b antibodies are capable of mediating PCA at high dosages [10–12], the possible antibodies responsible for PCA in W/W$^v$ mice are of the IgG2 subclass. Considering that the anti-BSA serum which produced PCA in W/W$^v$ mice had a very high IgG2a antibody titre, this speculation could prove correct. The present study dealing with the drug susceptibilities of this PCA provided further evidence of its peculiar feature. PCA in this strain was markedly suppressed by CV-6209 but not by antihistamines, suggesting that PAF plays a crucial role. On the other hand, PCA in mast cell-bearing reference mice sensitized by a highly diluted (1:128) immune serum (virtually by IgG1 antibody) was strongly suppressed by antihistamines but not at all by CV-6209, which suggests that histamine released from mast cells is the major mediator for IgG1 antibody-mediated PCA. This was further evidenced by nearly complete inhibition by cyproheptadine of PCA in +/+ mice mediated by monoclonal anti-benzylpenicilloyl IgG1 antibody regardless of the dilution of sensitizing ascites (unpublished data). Lack of suppression of IgG1 antibody-mediated PCA by CV-6209 appears to conflict with Inagaki et al. [13], who reported that CV-3988, another antagonist of PAF, inhibits PCA in ddY mouse ear mediated by

Table 2. Effects of anti-mouse granulocyte (Gr-1) antibody on PCA in W/W$^v$, +/+ and B6D2F1 mice mediated by allogeneic anti-BSA serum.

| Strain | Antiserum dilution | Exp. no. | Treatment | Amount of infiltrated dye* | inhibition (%) |
|---|---|---|---|---|---|
| W/W$^v$ | 1:4 | 1 | saline | $38 \pm 8.4$ | |
| | | | anti-Gr-1† Ab (day − 1) | $10 \pm 3.5$ | 74‡ |
| | | 2 | saline | $27 \pm 5.1$ | |
| | | | anti-Gr-1 Ab (day − 1) | $1 \pm 0.7$ | 96‡ |
| | | | (day − 3) | $12 \pm 3.0$ | 56‡ |
| | | | (day − 6) | $22 \pm 2.3$ | 26 |
| +/+ | 1:128 | 1 | saline | $41 \pm 2.8$ | |
| | | | anti-Gr-1 Ab (day − 1) | $38 \pm 3.5$ | 7 |
| | | 2 | saline | $26 \pm 3.3$ | |
| | | | anti-Gr-1 Ab (day − 1) | $33 \pm 3.2$ | −27 |
| B6D2F1 | 1:128 | 1 | saline | $40 \pm 2.7$ | |
| | | | anti-Gr-1 Ab (day − 1) | $33 \pm 1.2$ | 16 |

*μg (mean ± SE, $n = 5$). †40 μg, iv. ‡Statistically significant inhibition ($P < 0.01$).

Table 3. Effects of CV-6209 and anti-mouse granulocyte (Gr-1) antibody on oxatomide-resistant PCA in +/+ and B6D2F1 mice mediated by undiluted or slightly diluted anti-BSA serum.

| Strain† | Antiserum dilution | Exp. no. | Amount of infiltrated dye* Inhibition (%) | | |
|---|---|---|---|---|---|
| | | | saline | CV-6209‡ | Anti-Gr-1 Ab§ |
| +/+ | 1:4 | 1 | $30 \pm 1.3$ | $10 \pm 1.8$ (67) ‖ | $13 \pm 1.5$ (57) ‖ |
| | | 2 | $27 \pm 1.1$ | $9 \pm 1.8$ (67) ‖ | |
| B6D2F1 | 1:1 | 1 | $28 \pm 0.7$ | $10 \pm 0.4$ (65) ‖ | $0.6 \pm 0.6$ (98) ‖ |
| | | 2 | $35 \pm 1.4$ | $10 \pm 2.0$ (71) ‖ | |

*μg (mean ± SE, $n = 5$). †Oxatomide (100 mg/kg, po) was given 1 h before the antigen challenge. ‡3.3 mg/kg, iv, 5 min before the antigen challenge. §40 μg, iv, 1 day before the test. ‖ Statistically significant inhibition ($P < 0.01$).

heat-treated anti-serum and monoclonal anti-HGG (human gammaglobulin) IgG1 antibody. On examination of their results, however, inhibition by CV-3988 is controversial, because it is not dependent on the drug dose and is only partial, if it occurs at all. In addition, we could not confirm the inhibitory effect of CV-3988 against IgG1 antibody-mediated PCA in dorsal skin of +/+ and B6D2F1 mice at the doses ranging from 3 to 30 mg/kg (data not shown). The discrepancy might be due to the differences in the reaction sites and mouse strains. Thus, admitting their description, PAF does not seem to play a major role in IgG1 antibody-mediated PCA.

Marked differences were also found between PCA in W/W$^v$ and reference mice with regard to susceptibility to anti-granulocyte antibody. PCA was remarkably suppressed by the 1- to 3-day pretreatment with this antibody in W/W$^v$ mice but not at all in +/+ and B6D2F1 mice sensitized with highly diluted antiserum. The time course of PCA suppression in W/W$^v$ mice like this grossly coincides with that of reduction of circulatory granulocytes (predominately neutrophils) previously reported [2]. From these findings, it could be postulated that PCA in this strain is produced by PAF mainly released from Gr-1-expressing leukocytes sensitized by IgG2 antibodies, although precise identification of the cellular PAF source awaits further analysis. This type of PCA was also observed in antihistamine-pretreated reference mice on sensitization with undiluted or slightly diluted antiserum, although masked by histamine-induced dye leakage without the treatment with antihistamines.

According to our previous studies, systemic anaphylaxis can be produced, but cutaneous anaphylaxis cannot be, in W/W$^v$ mice, when sensitized by active means or passive means with monoclonal IgG1 antibody [1, 3]. The reason for such a discrepancy is unclear at present. However, putting the present results together with the preceding finding that anaphylactic shock in W/W$^v$ mice is highly susceptible to CV-6209 and anti-granulocyte antibody [7, 2], it is very likely from the immunopharmacological standpoint that a similar mechanism operates for both systemic and cutaneous type I hypersensitivities in this strain.

1 Arimura A., Nagata M., Takeuchi M., Watanabe A., Nakamura K. and Harada M. (1990) Active and passive cutaneous anaphylaxis in WBB6F1 mouse, a mast cell-deficient strain. Immunol. Invest. **19:** 227–233
2 Kimura S., Nagata M., Takeuchi M., Takano K. and Harada M. (1997) Anti-granulocyte antibody suppression of active and passive anaphylactic shock in WBB6F1-W/W$^{v}$ mice. Cell. Mol. Life Sci. **53:** 663–666
3 Arimura A., Nagata M., Watanabe A., Nakamura K., Takeuchi M. and Harada M. (1990) Production of active and passive anaphylactic shock in the WBB6F1 mouse, a mast cell-deficient strain. Experientia **46:** 739–742
4 Harada M., Nagata M. and Takeuchi M. (1989) Immunological properties of 7432-S, a new cephalosporin antibiotic agent for oral use. Chemotherapy **37(suppl. 1):** 1127–1139
5 Harada M., Takeuchi M., Fukao T. and Katagiri K. (1971) A simple method for the quantitative extraction of dye extravasated into the skin. J. Pharm. Pharmacol. **23:** 218–219
6 Kimura S., Watanabe A., Nagata M., Niinomi Y. and Harada M. (1996) Heterogeneity of drug susceptibility of mouse active anaphylactic shock. Immunol. Invest. **25:** 425–435
7 Inagaki N., Goto S., Nagai H. and Koda A. (1985) Pharmacological characterization of mouse ear PCA. Int. Arch. Allergy Appl. Immunol. **78:** 113–117
8 Terashita Z., Imura Y., Takatani M., Tsushima S. and Nishikawa K. (1987) CV-6209, a highly potent antagonist of platelet-activating factor in vitro and in vivo. J. Pharmacol. Exp. Ther. **242:** 263–268
9 Uber C. L., Roth R. L. and Levy D. A. (1980) Expulsion of *Nippostrongylus brasiliensis* by mice deficitent in mast cells. Nature **287:** 226–228
10 Hirayama N., Hirano T., Köhler G., Kurata A., Okumura K. and Ovary Z. (1982) Biological activities of antitrinitrophenyl and antidinitrophenyl mouse monoclonal antibodies. Proc. Natl. Acad. Sci. USA **79:** 613–615
11 Däeron M., Couderc J., Ventura M., Liacopoulos P. and Visin G. A. (1982) Anaphylactic properties of mouse monoclonal IgG2a antibodies. Cell. Immunol. **70:** 27–40
12 Ovary Z. (1982) Recent insight into class-specific properties of murine immunoglobulins obtained with the help of monoclonal antibodies. Int. Arch. Allergy Appl. Immunol. **69:** 385–392
13 Inagaki N., Miura T., Ohira K., Nagai H., Qiang X. U. and Koda A. (1990) Effect of CV-3988, a specific antagonist against platelet activating factor, on homologous passive cutaneous anaphylaxis in the mouse ear. J. Pharmacobio-Dyn. **13:** 272–277