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Anti-granulocyte antibody suppression of active and passive anaphylactic shock in WBB6F1-W/W^v mice

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Abstract. Our previous study revealed that anaphylactic shock can be produced in WBB6F1-W/W^v (abbreviated as W/W^v) mice. Because they are congenitally deficient in mast cells, we are certain that some other cell types are involved as mediator sources. In the present study, with the aim of examining the role of circulatory granulocytes, the effect of monoclonal antibody to a mouse granulocyte antigen, Gr-1, upon active and passive anaphylactic shock was tested in W/W^v mice using several mast cell-bearing strains as references. An intravenous injection of 40 μg of anti-granulocyte antibody one day before the antigen challenge produced a marked decrease in neutrophils and nearly complete abolition of lethal shock in W/W^v mice regardless of the sensitizing method. Both prevention of shock and reduction of neutrophils lasted for three days after the treatment with the antibody but not for six days. From these results, a reasonable conclusion would be that circulating Gr-1$^+$ cells (predominantly composed of neutrophils) are the major mediator source. Reference experiments using mast cell-bearing strains revealed that the suppressive effect of anti-granulocyte antibody was also observed against active anaphylactic shock in C3H/HeN mice but not against active and passive anaphylactic shock in the other mice.

Key words. PAF; anaphylactic shock; WBB6F1-W/W^v; anti-mouse granulocyte antibody.

Type I hypersensitivity such as anaphylactic shock and passive cutaneous anaphylaxis (PCA) can be produced in the WBB6F1-W/W^v (abbreviated below as W/W^v) mouse, a congenitally mast cell-deficient strain [1–3], although it is generally accepted that Type I hypersensitivity in the mouse is provoked by antigen-triggered mediator release from mast cells [4]. Therefore, it is obvious that mediators derived from some cells other than mast cells play a crucial role in this strain. Our recent study showed that active anaphylactic shock in W/W^v mice is extremely susceptible to an antagonist of platelet-activating factor (PAF) [5], CV-6209, but not to an antagonist of histamine, cyproheptadine [6], which suggests the role of PAF. Although PAF can be released from a variety of cells, one possible major source is circulating granulocytes [7]. In the present study, therefore, to examine the possibility that granulocytes are involved in anaphylactic shock, the effect of monoclonal antibody to a mouse granulocyte antigen, Gr-1, which could selectively impair and reduce granulocytes, was studied against active and passive anaphylactic shock in this strain. Reference tests were also performed using some mast cell-bearing strains to examine the generality of the suppressive action of the antibody.

Materials and methods

Young (approximately 3 months old) female W/W^v mice were purchased from Japan SLC, Inc. As reference animals, age-matched female C3H/HeN and C57BL/6N mice purchased from Charles River Japan, Inc and

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Table 1. Effect of anti-granulocyte antibody on active and passive anaphylactic shock in W/W^v and reference mice.

Strain	(Sex)	Sensitization		Shock potentiator	Treatment		Incidence of lethal shock
W/W^v	(F)	active[1)]		none	saline	day(−1)	5/10
					anti-Gr. Ab[3)]	day(−1)	1/6
		passive	IgG1	DL-prop.	saline	day(−1)	4/5
					anti-Gr. Ab	day(−1)	0/5*
						day(−3)	0/5*
			antiserum	none	saline	day(−1)	12/14
					anti-Gr. Ab	day(−1)	0/8**
				DL-prop.	saline	day(−1)	18/20
					anti-Gr. Ab	day(−1)	0/10**
						day(−3)	0/9**
						day(−6)	11/14
C3H/He	(F)	active[1)]		none	saline	day(−1)	10/10
					anti-Gr. Ab	day(−1)	0/11**
C57BL/6N	(F)	active[1)]		none	saline	day(−1)	5/5
					anti-Gr. Ab	day(−1)	5/5
B6D2F1	(F)	active[2)]		none	Saline	day(−1)	5/5
					anti-Gr. Ab	day(−1)	5/5
CTS	(F)	passive	IgG1	none	saline	day(−1)	5/5
					anti-Gr. Ab	day(−1)	5/5
	(M)	passive	antiserum	none	saline	day(−1)	4/5
					anti-Gr. Ab	day(−1)	5/5
DS	(F)	passive	antiserum	DL-prop.	saline	day(−1)	4/4
					anti-Gr. Ab	day(−1)	4/4

[1)]Sensitized for 2 weeks. [2)]Sensitized for 1 week. [3)]Abbreviation: Anti-granulocyte antibody. *Statistically significant ($p < 0.05$). **Statistically significant ($p < 0.01$).

female and/or male CTS, DS and B6D2F1 mice bred at Shionogi Aburahi Laboratories were used. The CTS (cataract Shionogi) mouse, an inbred strain closely related to the NOD (nonobese diabetic) mouse which is widely used as a model of human autoimmune insulin-dependent diabetes mellitus, is characterized by deficiency in peripheral T lymphocytes [8] and high sensitivity to anaphylactic shock [9]. The DS mouse, an inbred strain established from a closed colony of dd mice, is characterized by high sensitivity to IgE antibody-mediated passive cutaneous anaphylaxis [10].

Active sensitization was done by a single intraperitoneal injection of 0.2 ml of a mixture of bovine serum albumin (BSA, Armour, 10 mg/ml in physiological saline solution) and an equal volume of a suspension of killed *Bordetella pertussis* organisms (Nacalai Tesque, Inc, 20 billion/ml). One or two weeks later, anaphylactic shock was elicited by an intravenous injection of 1 mg (0.2 ml) of BSA. Shock intensity was expressed in terms of incidence of lethal shock within 1 hour of the antigen injection. Passive sensitization was produced by an intravenous injection of 0.2 ml of anti-benzylpenicilloyl (BPO) IgG1 monoclonal antibody ascites (BIG-3N, described elsewhere [11]) or pooled anti-BSA hyperimmune serum of the B6D2F1 mouse origin. The anti-BPO IgG1 antibody titre of the ascites was 1:1024, when assessed by 1-hour PCA in DS mice. The IgE and IgG1 antibody titres of the anti-BSA serum were 1:512 (assessed by 1-day PCA in rats) and 1:4096 (assessed by 1-hour PCA in DS mice), respectively. After sensitization for 3 hours, shock was provoked by intravenously injecting 1 mg of the homologous elicitor, i.e. BPO_{16}-GSA (BPO haptens conjugated with guinea pig serum albumin (GSA, Sigma)) or BSA. The preparation of the BPO-GSA conjugate and determination of its hapten content were performed according to Levine et al. [12] and Ebata et al. [13], respectively. When active or passive sensitization alone was not sufficient for producing lethal shock, DL-propranolol (10 mg/kg), a β-blocker, was intraperitoneally injected as a shock potentiator 1 hour before the shock provocation.

Lyophilized rat monoclonal antibody to a mouse granulocyte antigen, Gr-1 (clone: RB6-8C5 PharMingen) was restored by adding pure water with the concentration adjusted to 200 μg/ml, and then dialysed against physiological saline solution for 1 day at 4° C with the external fluid stirred and exchanged three times in order to remove sodium azide. A 0.2 ml portion of this preparation containing 40 μg of the antibody was intravenously injected 1 day before the antigen challenge unless otherwise described. In both active and passive anaphylactic shock tests, statistical significance of the difference in lethality between a control (saline-treated) and an anti-granulocyte antibody-treated groups was examined by a 2×2 contigency chi-square test with Yate's correction: a difference with a p value less than 0.05 was taken as significant.

Effects of anti-granulocyte antibody on leukocyte components were assessed in W/W^v, C3H/HeN, and C57BL/6N mice by means of laser flow cytometry using

Technicon THMS H-1 system (Bayer). A portion of 0.49 ml of blood was removed by heart puncture using a syringe with 0.01 ml of an anticoagulant solution (Angrot/ET®, Nihon Shouji) and transferred to a small tube. Neutrophils, eosinophils, and basophils were counted by the WBC peroxidase channel according to Groner et al. [14] using the cell size and peroxidase activity as the indices. Statistical significance of the differences between the control and anti-granulocyte antibody-treated groups was examined by Student's *t*-test.

Results

As shown in table 1, the incidence of lethal anaphylactic shock was markedly reduced by the anti-granulocyte antibody treatment in W/W^v mice sensitized by active means, although not statistically significant because of the relatively low lethality in the control group. Suppression of lethal shock was significant in C3H/HeN mice sensitized in the same way, while no suppression was observed in C57BL/6N and B6D2F1 mice. Prevention of lethal shock also occurred in W/W^v mice passively sensitized with either anti-BPO monoclonal IgG1 antibody or anti-BSA hyperimmune serum regardless of whether or not DL-propranolol treatment had been given. The suppressive effect of anti-granulocyte antibody in W/W^v mice lasted for at least 3 days: the treatment 3 days before the antigen challenge was suppressive but that 6 days before was not. In contrast, lethal passive anaphylactic shock was not prevented by anti-granulocyte antibody in DL-propranolol-treated DS mice sensitized with anti-BSA serum and in CTS mice sensitized with either anti-BPO IgG1 antibody or anti-BSA serum without DL-propranolol.

Laser flow cytometry revealed that the treatment with anti-granulocyte antibody 1 day before caused significant decrease in the number of neutrophils in blood of W/W^v mice, the number (mean $\pm$ SE) of the treated group ($n = 8$) being $101 \pm 46/\mu l$ in comparison with $549 \pm 195/\mu l$ of the control group ($n = 14$) ($p < 0.05$). The treatment 3 days before also produced definite decrease ($173 \pm 66/\mu l$, $n = 4$) but that 6 days before did not at all. Eosinophils and basophils were so small in number even in the control mice (eosinophils $33 \pm 6/\mu l$, basophils $10 \pm 2/\mu l$) that reduction of these cells was ambiguous and not necessarily parallel with shock prevention. Conspicuous reduction of neutrophils was also observed in the two reference strains, C3H/HeN and C57BL/6N, 1 day after the treatment with anti-granulocyte antibody. Number of neutrophils and degree of their reduction were nearly comparable in C3H/HeN mice with those in W/W^v mice, the numbers ($n = 5$) before and after the treatment being $651 \pm 62/\mu l$ and $45 \pm 16/\mu l$, respectively ($p < 0.05$). In C57BL/6N mice, neutrophils were much smaller in number ($197 \pm 19/\mu l$, $n = 5$) than those in W/W^v and C3H/HeN mice before the treatment, but their reduction was definite ($28 \pm 4/\mu l$, $n = 5$) ($p < 0.01$).

Discussion

The present results revealed that both active and passive anaphylactic shock in W/W^v mice are highly susceptible to monoclonal anti-granulocyte antibody. Because intravenous injections of an unrelated antigen and antibody to it (e.g. ovalbumin and anti-ovalbumin hyperimmune serum) 1 day before did not protect the mice from BSA-induced anaphylactic shock (unpublished data), prevention of anaphylactic shock by pretreatment with anti-granulocyte antibody cannot be attributed to a non-specific effect caused by a preceding antigen-antibody interaction. Suppression of shock is presumably due to the anti-granulocyte antibody-mediated removal of the cells expressing Gr-1 antigen via complement-dependent cytotoxicity and/or antibody-dependent cell cytotoxicity (ADCC), because this monoclonal antibody is of rat IgG2b isotype having these biological activities. The finding that the time course of shock prevention ran parallel with that of reduction of granulocytes (especially neutrophils) supports this speculation. Putting the present result together with our previous finding that active anaphylactic shock in this strain is very sensitive to CV-6209 [6], it is highly probable that PAF mainly released from circulating granulocytes plays a major role. Although identification of the cell type is impossible at present, neutrophils are presumed to be the main PAF source. This is because neutrophils are the predominant component, constituting 18.3% (mean, $n = 14$) of white blood cells in W/W^v mouse blood. Even though the possibility of involvement of eosinophils and basophils cannot be ruled out, their role would be only minor, if any, since they are much smaller in number than neutrophils (eosinophils, 1.4% and basophils, 0.3%) and their reduction after the treatment with anti-granulocyte antibody was not so striking as that of neutrophils and did not necessarily run parallel with shock prevention.

The same mechanism as that in W/W^v mice seems to operate for producing active anaphylactic shock in C3H/HeN mice in view of the findings that the shock is highly susceptible to CV-6209 [6] and anti-granulocyte antibody, and that neutrophil number and its reduction by this antibody were nearly comparable with those in W/W^v mice. In contrast, no prevention of shock was produced by the antibody in C57BL/6N mice. This seems to correlate with our previous results that shock was suppressed by cyproheptadine but not by CV-6209 in this strain [6]. Presumably, mast cell-derived histamine rather than neutrophil-derived PAF would play

a crucial role. The present finding that only a relatively small number of neutrophils were counted in blood supports this speculation. A similar explanation could be offered for the lack of shock prevention by anti-granulocyte antibody in the other reference strains tested, because shock in these mice was also insensitive to CV-6209 alone [6].

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