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Comparison of Mouse Strain Skin Sensitivities to Anaphylactic Mediators and Susceptibility to Passive Cutaneous Anaphylactic Reactions

D. E. JUSTUS AND CATHARINE SAELINGER[1]

Department of Microbiology and Immunology, University of Louisville School of Medicine, Health Sciences Center, Louisville, Kentucky 40201

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CFW, ICR, and C57Bl/6J mouse strains were examined and compared for their levels of skin sensitivity to histamine and mellitin (a potent mast cell degranulator) and for their susceptibilities to immunoglobulin E-induced passive cutaneous anaphylactic (PCA) reactions. ICR mice were found to exhibit the highest level of sensitivity to histamine and mellitin, whereas C57Bl/6J exhibited the least. CFW mice proved to be the best PCA recipients, whereas ICR mice were the poorest. On the basis of this evidence, no direct correlation is indicated between level of sensitivity to anaphylactic mediators and degree of susceptibility to immunoglobulin E-induced PCA reactions.

Considerable evidence has accumulated indicating that mice are capable of forming two distinct species of anaphylactic antibodies (9, 11, 13, 14). One of these, designated immunoglobulin (Ig) G_1 or gamma-one, is a non-complement-fixing and heat-stable globulin. The second type has properties similar to human IgE in that it is non-complement fixing and heat labile (16). Both of these antibodies are able to sensitize mouse mast cells for release of histamine after contact with specific antigen (18), but they can be differentiated in passive cutaneous anaphylactic (PCA) test on the basis of their persistence at intradermal sites of deposition. Thus, IgG_1 antibodies persist at such sites for relatively short time periods and can be detected by 2- or 4-h PCA reactions. In contrast, IgE antibodies remain at injection sites for long time periods and can be demonstrated by 48- or 72-h PCA reactions.

Although mouse anaphylactic antibodies are detectable by PCA, studies by Clausen et al. (1) and more recently by DeSouza et al. (2) have shown that mouse strains vary to a considerable degree in their susceptibilities to both IgG_1- and IgE-induced PCA reactions. Since mouse strains also vary in their sensitivity levels to histamine (6), it has been suggested that the PCA variation observed could possibly be related to differences in sensitivity levels to anaphylactic mediators. It was therefore of interest to determine whether the level of local skin sensitivity to anaphylactic mediators could be correlated with the degree of susceptibility to PCA reactions.

MATERIALS AND METHODS

Mice. Female CFW, ICR, and C57Bl/6J mice, 22 to 25 g, were used as serum donors and PCA recipients. Animals were maintained in temperature- and humidity-controlled quarters.

Antigen. A large number of *Trichinella spiralis* larvae (1 to 2 ml, packed volume) were collected from previously infected mice according to the method of Larsh and Kent (8). The larvae were washed, sonicated, extracted, and lyophilized by procedures previously described (7). Lyophilized extracts were reconstituted in pyrogen-free saline just before use.

Antisera. Groups of ICR, CFW, and C57Bl/6J mice were immunized by the oral administration of 100 viable *T. spiralis* larvae. Second and third inoculations, consisting of 50 larvae each, were given 7 and 12 weeks later. One week after the last inoculation, animals were bled by cardiac puncture, and the serum collected from three mice was pooled and stored at −5 C.

PCA testing. The method of Ovary (10) was used to test serum for presence of 48-h PCA antibodies. Briefly, recipients were given an intravenous injection of 0.25 ml of saline containing 500 μg (dry weight) of larval antigen and 0.4% Evans blue dye 48 h after receiving intradermal injections of serum. Each serum dilution was tested in six different recipients of each mouse strain. Undiluted normal mouse serum was used as a control. Animals were sacrificed 30 min later, and dye-infiltrated lesions were measured.

Histamine and mellitin. Skin sensitivities to histamine and mellitin were evaluated as follows. Twenty-four hours after removal of hair from the backs of mice (by clippers), intravenous injections of

[1] Present address: Department of Microbiology, University of Cincinnati, Cincinnati, Ohio 45221.

0.25 ml of 0.4% Evans blue dye-saline solution were given. Immediately afterwards, intradermal injections of 0.05 ml of saline containing one-half log graded doses of either histamine dihydrochloride (National Biochemical Corp.) or mellitin (Nutritional Biochemicals Corp.) were given. Doses ranged from 0.1 to 1.0 μg. Saline alone served as a control. Thirty minutes after injection, animals were sacrificed, the dorsal skin was reversed, and diameters of dye-infiltrated lesions were determined.

RESULTS

Experiments were first done to examine and compare mouse strains for their local sensitivity levels to anaphylactic mediators. For this purpose, groups of normal ICR, CFW, and C57Bl/6J mice were prepared as described above and skin tested with histamine and mellitin. Mellitin is a potent mast cell degranulator and a good releaser of histamine (3). To quantitate these responses, animals were tested with one of a series of one-half log graded doses of the two agents, and each dose level was tested in five to seven different mice of each strain. A mean lesion diameter of 0.22 cm (±0.05) was obtained from injection sites of saline (control) alone. A dose-response effect that was linear in nature and readily measurable in the range of 0.1 to 1.0 μg of histamine and mellitin was obtained (Fig. 1). For comparative purposes, the amounts of histamine and mellitin required to induce dye-infiltrated lesions 0.6 cm (arbitrarily selected) in diameter were determined from these curves. ICR in comparison to CFW and C57Bl/6J mice exhibited a higher level of skin sensitivity to both histamine and mellitin in that smaller doses were required to induce the given effect in these animals (Table 1). It is also interesting to note that on a molar basis less mellitin versus histamine was needed to induce this reaction (lesion 0.6 cm in diameter) in all three strains. Mention should be made that connective tissue samples were obtained (using the method of Higginbotham and Jee [4]) from a limited number of mice of each strain that had received 1 μg of mellitin and were examined for mast cell degranulation. In all cases a significant number of well-degranulated cells (38 to 48%) were observed. Also noted was that the general overall distribution of mast cells was similar for all three strains. This was indicated by the number of microscopic fields required to count 200 cells.

To evaluate the relationship between skin sensitivity to anaphylactic mediators and susceptibility to IgE-induced PCA reactions, groups of normal ICR, CFW, and C57Bl/6J mice were prepared as previously described and examined for their PCA responses, using antisera raised in these same strains by infection with *T. spiralis*. Each serum dilution was tested in six different recipients of each strain. This approach was taken to provide some indication as to the presence or absence of blocking substances in the test sera. Previous studies by DeSouza et al. (2) had shown that normal se-

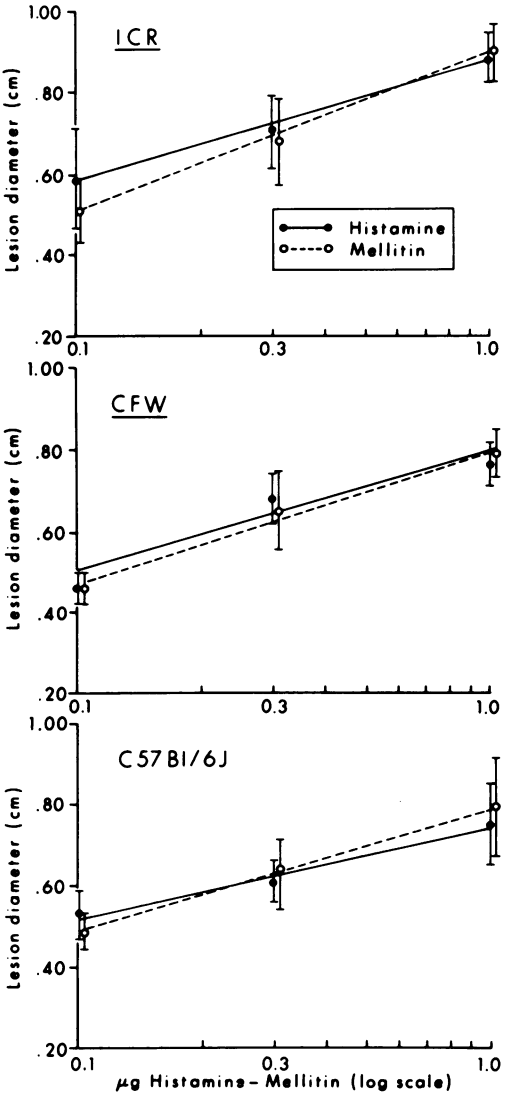

FIG. 1. *Comparison of skin dose-response effects of histamine and mellitin in ICR, CFW, and C57Bl/6J mouse strains. Animals were given an intravenous injection of 0.4% Evans blue dye followed immediately by intradermal injections of 0.05 ml of saline containing one of a series of half-log graded doses of test material. Mean values with standard deviations are presented.*

TABLE 1. *Comparison of skin responses to histamine and mellitin*[a]

Mouse strain	Histamine (nmol)	Mellitin (nmol)
ICR	0.8[b]	0.06
CFW	1.3	0.09
C57Bl/6J	1.6	0.08

[a] After an intravenous injection of saline containing 0.4% Evans blue dye, mice received intradermal injections of saline containing one of a series of half-log graded doses of histamine or mellitin.

[b] Values indicated were obtained from dose-response curves (Fig. 1) and represent that amount of agent (nanomoles) required to induce a lesion 0.6 cm in diameter.

TABLE 2. *Strain differences in susceptibility to 48-h PCA reactions*

Serum donor strain	Reciprocal of serum dilution	Recipient[a] strain		
		CFW	ICR	C57Bl/6J
CFW	20	6/6[b]	3/6	6/6
	40	6/6	2/6	6/6
	80	6/6	2/6	0/6
	160	6/6	0/6	0/6
	320	6/6	0/6	0/6
C57Bl/6J	20	6/6	5/6	6/6
	40	6/6	5/6	6/6
	80	5/6	5/6	6/6
	160	5/6	5/6	6/6
	320	6/6	2/6	2/6
ICR	20	6/6	1/6	2/6
	40	5/6	1/6	2/6
	80	4/6	1/6	1/6
	160	2/6	2/6	2/6
	320	0/6	0/6	0/6

[a] Recipients were challenged intravenously with saline containing 0.4% Evans blue dye and 0.5 mg of *T. spiralis* larval antigen 48 h after intradermal injection of 0.05 ml of serum. Injections of normal serum produced no reactions.

[b] Number of responding animals per six recipients.

rum of mouse strains less susceptible to passive sensitization contained such substances. Undiluted normal mouse serum used as a control produced no visible reactions. Intense bluing at the site of serum injection 30 min after antigenic challenge was taken as a positive reaction. CFW in comparison to ICR and C57Bl/6J recipients displayed a much higher degree of susceptibility to PCA reactions induced with the antisera obtained from CFW infected animals (Table 2). This is indicated by the fact that higher titers as well as more uniform and consistent responses were obtained in these animals. CFW mice also proved to be slightly more susceptible or responsive to the C57 antisera in that 93% of these animals produced positive reactions, whereas 86 and 73% of the C57 and ICR recipients, respectively, did so. These figures are based upon the number of positive responders recorded for all serum dilutions.

When the three strains were tested with ICR antiserum, CFW mice again exhibited a greater susceptibility to 48-h PCA reactions. However, in comparison to the results of the previous experiments, the degree of susceptibility was not as great. Although extremely poor responses were obtained in ICR and C57 mice with this antiserum, the same antibody titer (1:160) was obtained in all three mouse strains. These results indicate that of the three strains, CFW mice were considerably more susceptible to IgE-induced PCA reactions. Also indicated is that serum components capable of blocking or interfering with reactions were most likely not responsible for the negative PCA responders.

DISCUSSION

The results of this study clearly indicate that there is no direct correlation between levels of skin sensitivity to anaphylactic mediators and degree of susceptibility to PCA reactions. Thus, of the three mouse strains examined, ICR mice proved to be more sensitive to histamine and mellitin (Fig. 1 and Table 1) but the least susceptible to passive sensitization by IgE antibodies (Table 2). CFW mice, on the other hand, were highly susceptible to PCA reactions and only moderately sensitive to anaphylactic mediators. In contrast, C57Bl/6J mice exhibited both low levels of skin sensitivity and PCA susceptibility.

The suggestion has been made (2) that the degree of PCA strain susceptibility might be influenced by the numbers of target mast cells present in the connective tissue. Thus, animals having a large number of mast cells should be very responsive or more responsive than those having fewer cells. From connective tissue samples that we examined for mast cell degranulation, this does not appear to be true in that the distribution of cells was found to be similar for all three mouse strains.

Evidence has been presented indicating that nonsensitizing antibodies such as mouse IgG_{2a} and IgG_{2b} (12, 15) as well as nonspecific IgE antibodies (2) can interfere with binding of specific IgE antibodies. Although to some extent interference by these antibodies might account for some of the PCA variation observed here, this would not seem to explain the difficulty

encountered in obtaining PCA reactions in ICR recipients with ICR antiserum. Had such immunoglobulins been present in this test serum, they should have also inhibited to the same degree PCA reactions in CFW recipients. This was not the case (Table 2). There is the remote possibility that some of the ICR recipients contained sufficient extravascular as opposed to circulating levels of nonspecific blocking immunoglobulins to effectively inhibit reactions. However, PCA reactions were difficult to induce in ICR as well as C57 mice with ICR antiserum, but they were relatively easy to obtain in these animals with C57 antiserum. Consequently, this would suggest that a factor(s) other than or in addition to nonspecific blocking antibodies, present either in the circulation or tissue spaces, is involved in this phenomenon. It could be argued that the reactions or lack of reactions observed in ICR recipients with the ICR and C57 antisera were simply due to a difference in antibody concentration. If this were true, then one would expect to see a greater percentage of recipients given the higher concentrations of test serum responding. This did not occur (Table 2). The number of responding ICR and C57 recipients was little influenced by the concentration of antibody given.

On the basis of the observations made here, it is quite clear that more definitive studies are needed to explain the phenomenon of animal variation to passive sensitization by anaphylactic antibodies. Such studies will probably reveal that several factors are involved in this phenomenon. Perhaps one such factor is related to differences in mast cell membrane receptor sites. In this regard, in vitro studies by Vaz and Ovary (17) have shown that mast cells of a particular mouse strain can be passively sensitized with IgG antibodies whereas cells from other strains cannot.

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