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ACTION OF ANTI-MOUSE IgE ON MOUSE AND RAT MAST CELLS

By

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It has been established that both mouse and rat produce reaginic antibodies (2, 3, 17, 18, 19, 25, 28). Recently, these antibodies have been identified immunochemically as pertaining to distinct immunoglobulin class and designated IgE (24, 29), similar to IgE identified in man (8), monkey (9) and rabbit (7). It was also shown, that IgE antibodies of mouse and rat bind firmly to the homologous mast cells and such binding is responsible for sensitization of the cells (5, 18, 23, 32). Reaction of either antigen or anti-IgE with cell-bound IgE initiates activation of enzyme systems of mast cell which finally leads to the release of histamine (4, 12, 30, 32). It has been recently shown on human leukocytes that the number of cell-bound IgE may have some relationship to the sensitivity of these cells to anti-IgE for histamine release (5).

Mouse IgE is also able to sensitize rat skin (21) and rat peritoneal mast cells (24, 27). It was recently observed that anti-mouse IgE is able to induce degranulation of rat mast cells when they were previously sensitized with mouse reaginic antibodies (24, 27). Antigenic similarities between the IgE from these two species have been suggested (27).

The present experiments were undertaken to compare the action of anti-mouse IgE on mouse and rat mast cells, normal or sensitized with reaginic antibodies.

In these studies we used as the model the technique involving *in vitro* histamine release from mouse and rat peritoneal

mast cells. The action of anti-mouse IgE on the reaginic activity of the sera of sensitized animals was also tested.

MATERIAL AND METHODS

Animals: Random-bred Swiss female mice 6–8 weeks old were used for sensitization and mast cell experiments. Male Wistar rats weighing 150–300 g were used for sensitization and for mast cell experiments. Rats weighing 200–250 g were used for PCA reaction.

Sensitization procedures: Active sensitization of mouse peritoneal mast cells was obtained by subcutaneous injection of 100 μ g ovalbumin (5 times crystallised, Schwarz/Mann, Orangeburg, USA) mixed with 20 mg AL(OH)₃ gel (26). The rats were actively sensitized by a single dose of 500 μ g ovalbumin mixed with 60 mg AL(OH)₃ gel given subcutaneously into the back and in the foot-pads. Mouse reaginic sera for *in vitro* sensitization of rat peritoneal mast cells were produced by several i.p. injections of mice with 1 μ g ovalbumin mixed with 1 mg AL(OH)₃ gel, given 2 to 4 weeks apart (15). The antisera were obtained on the 7th day after the last injection of antigen, pooled and stored at -20° C.

Anti-mouse IgE antiserum: Antiserum against mouse IgE was prepared in Laboratoire de Médecine Expérimentale Collège de France (Paris): mouse reaginic serum was fractionated by precipitation at half saturation with ammonium sulphate followed by DEAE cellulose chromatography (24). Fractions obtained by elution at 0.02 M, 0.05 M and 0.1 M phosphate buffers were called respectively F₁, F₂ and F₃. Reaginic activity was found in fraction F₂, but not in F₁ or F₃. Rat antiserum against mouse IgE was obtained by immunisation of rats with homologous peritoneal mast cells previously passively sensitized with fraction F₂ of mouse reaginic serum and thoroughly washed (24). The antiserum collected was absorbed with fraction F₃ of mouse serum (24). The antiserum thus contains antibodies against both anti-ovalbumin IgE antibodies as well as unspecific IgE.

Passive cutaneous anaphylaxis (PCA): The assay of IgE antibodies in rat and mouse sera was carried out in rats (2, 18, 21, 22). One tenth milliliter of serial 2-fold dilutions of test samples was injected intracutaneously into the back. The samples to be compared were injected into the same rat. After 48 hours the sensitization animals received an intravenous injection of 1 ml 0.4 per cent Evans Blue including 1 mg ovalbumin. The reaction was evaluated from the inside of the skin. A blue spot with an average diameter of 5 mm or more was regarded as a positive reaction and PCA titre was expressed by the maximum dilution giving a positive reaction.

In vitro sensitization of mast cells and anaphylactic histamine release: *In vitro* experiments were performed using peritoneal mast cells collected from mice (25) or rats (31). Barron-modified Ringer medium (1) and buffered salt solution (31) were used respectively for mouse and rat peritoneal mast cells. Mouse peritoneal cells were washed 3 times by centrifugation at $200 \times g$ and resus-

pended in the medium. Rat mast cells were isolated by differential centrifugation through 37 per cent solution of bovine serum albumin (fraction V, Biomed, Poland) according to the method of Johnson & Moran (11). The mast cells were counted after staining with toluidine blue (20) and distributed into several equal samples. Sensitization of rat mast cells was performed by incubation with appropriate dilutions of mouse reaginic serum at 30° C for 2 h and subsequent thorough washing (3 times). The normal or sensitized cells were incubated at 37° C for 10 min, the specific antigen or anti-mouse IgE was added and incubation was continued for a further 6 min. At the end of the reaction the samples were cooled in ice-bath and then separated by centrifugation into cell and supernatant fraction. Mast cell disruption was performed by addition of distilled water, freezing and thawing (31). Both supernatant and cell fraction were assayed for histamine by bioassay on atropinized guinea pig ileum. Mepyramine was used for testing a specificity of histamine contractions.

Histamine release was expressed as a percentage of the supernatant histamine to the total histamine after deduction of the spontaneous release found in controls.

RESULTS

At 37° C anti-mouse IgE induced histamine release from normal mouse peritoneal mast cells and this effect was greatly enhanced after active sensitization of the mice mast cell donors (Table 1). Table 2 shows the histamine releasing activity of anti-mouse IgE in rat mast cells; anti-mouse IgE had a

TABLE 1

Antigen and Anti-Mouse IgE-Induced Histamine Release from Peritoneal Mast Cells of Normal and Actively Sensitized Mice.

Challenge	Per cent of histamine release Mouse mast cells	
	Normal	Sensitized
Antigen (ovalbumin, 100 µg/ml)	0	45.5 ± 4.2
Anti-mouse IgE		
dilution 1:1000	50.0 ± 5.6	57.1 ± 8.5
1:5000	10.3 ± 4.2	35.1 ± 5.9
1:10,000	6.9 ± 2.0	21.7 ± 3.2
1:20,000	4.7 ± 1.5	10.0 ± 2.4

Mice 7 weeks old; sensitization 14 days before an *in vitro* experiment. PCA titre of the sera of mice on 8th day of immunization = 200, and on 14th day of immunization = 50. The means ± S.D.

TABLE 2

Action of Anti-Mouse IgE on Isolated Rat Peritoneal Mast Cells, Normal or Sensitized by Mouse Reaginic Antibody.

Mast cells	Anti-mouse IgE dilution	Per cent of histamine release
Normal	1:20	11.7 $\pm$ 3.7
	1:40	0
	1:80	0
Sensitized*	1:20	37.7 $\pm$ 3.0
	1:40	30.2 $\pm$ 3.7
	1:80	29.1 $\pm$ 5.8
	1:160	23.7 $\pm$ 7.9
	1:320	18.3 $\pm$ 1.5
Sensitized§	1:20	22.2 $\pm$ 5.9
	1:40	14.9 $\pm$ 6.5
	1:80	7.3 $\pm$ 0.9
	1:160	5.0 $\pm$ 2.2

Sensitization of rat mast cells: incubation with appropriate dilution of mouse reaginic serum (PCA titre 2000) for 2 h at 30° C, followed by washing (3-fold). Incubation at 4° C as well as incubation at 37° C for 30 and 60 min were insufficient for sensitization of the cells.

* Sensitization with mouse reaginic serum in dilution 1:20. Antigen (ovalbumin 1 μ g/ml) induced 22.1 $\pm$ 3.7 per cent histamine release.

§ Sensitization with mouse reaginic serum in dilution 1:40. Antigen induced 14.6 $\pm$ 2.8 per cent histamine release. The means $\pm$ S.D.

slight effect on normal rat mast cells, but the anti-mouse IgE-induced histamine release was enhanced after sensitization of these cells with mouse reaginic antibodies. However, the dilutions of anti-mouse IgE necessary for histamine release from rat mast cells were less compared with those inducing histamine release from mouse mast cells (Table 1). In order to study the possibility of some antigenic similarities between rat and mouse IgE the experiments have been performed to see the effect of anti-mouse IgE on actively sensitized rat mast cells. Table 3 shows that anti-mouse IgE had higher histamine releasing activity in sensitized mast cells compared with non-sensitized mast cells. On the other hand, the percent-

TABLE 3

Action of Antigen and Anti-Mouse IgE on Rat Peritoneal Mast Cells, Normal or Actively Sensitized with Ovalbumin.

Challenge	Per cent of histamine release Rat mast cells	
	Normal	Sensitized*
Antigen (ovalbumin)		
concentration 0.1 μ g/ml	0	16.3 $\pm$ 0.6
1 μ g/ml	0	35.1 $\pm$ 1.2
10 μ g/ml	0	33.4 $\pm$ 4.2
100 μ g/ml	0	21.0 $\pm$ 1.0
Anti-mouse IgE		
dilution 1:20	7.0 $\pm$ 1.2	22.6 $\pm$ 2.4
1:40	0	19.6 $\pm$ 1.0
1:80	0	9.0 $\pm$ 2.4

* Rat sensitized 27 days before an *in vitro* experiment. PCA titre of the rat sera on 8th day of immunization = 160 and on 27th day of immunization = 80. The means $\pm$ S.D.

age of histamine release by anti-mouse IgE in all dilutions tested was less than those induced by optimal concentrations of antigen.

The neutralisation by anti-mouse IgE of the reagenic activity of rat serum has been also observed. Table 4 shows that absorption of both mouse and rat reagenic sera with anti-

TABLE 4

Action of Anti-Mouse IgE on Reagenic Activity of Mouse and Rat Antisera.

	Absorption with anti-mouse IgE*	PCA titre
Mouse anti-ovalbumin	no	200
	yes	<10
Rat anti-ovalbumin	no	160
	yes	<20

* Absorption by incubation of antisera with 2:1 vol. anti-mouse IgE for 30 min at 37° C and then overnight at 4° C. The mean results from six rats.

mouse IgE considerably reduced their ability to provoke the PCA reaction.

DISCUSSION

The data presented in this paper show that anti-mouse IgE is able to react with mouse and rat mast cell-bound IgE in so-called reversed anaphylactic reaction. In homologous system, anti-mouse IgE in high dilutions induced histamine release from normal mouse peritoneal mast cells and this effect was enhanced when mice were actively sensitized. Similar results have been obtained with human leukocytes and anti-human IgE (5, 6, 10, 16), and with rat mast cells and anti-rat IgE (27). Since in reversed anaphylactic reaction the specific IgE antibodies as well as non-specific IgE fixed on mast cells may be involved (27) the results suggest the presence of IgE molecules on the surface of both normal and sensitized mouse peritoneal mast cells. The histamine releasing activity of anti-mouse IgE in actively sensitized mouse mast cells may be due to the higher concentration of IgE on the cell membrane of these cells (5). Similar results have been obtained with the anti-mouse IgE-induced degranulation of mouse peritoneal mast cells (24). The present results confirm earlier findings that mouse IgE are able to bind specifically to rat peritoneal mast cells (13, 14, 24, 27). In our experiments the sensitization of rat peritoneal mast cells was obtained with unfractionated mouse reaginic serum and was both time-consuming and temperature-dependent. Anti-mouse IgE induced histamine release from sensitized mast cells, while its effect on normal mast cells was only slight. This confirms that heterologous sensitization is possible between mouse IgE antibodies and rat mast cells.

It seems probable that also non-specific IgE of mouse reaginic serum may compete for receptors on rat mast cells (13, 14). Whether other immunoglobulins of mouse reaginic serum interfere in the sensitization of rat mast cells with mouse IgE must be further investigated. It was previously

observed that mouse IgG₁ is unable to bind to rat peritoneal mast cells (14, 27), however, IgG₂ may have some activity (27).

The results showing that anti-mouse IgE is able to induce histamine release from actively sensitized rat peritoneal mast cells (Table 3) and to neutralise the PCA activity of rat IgE antibodies (Table 4) as well as the PCA activity of homologous IgE antibodies suggest that there may be some antigenic similarities between mouse and rat IgE molecules. Such similarities have already been demonstrated between IgE of man and monkey (9).

The data show that biological activity of mouse IgE can be tested not only by the use of the homologous mast cell system; the rat mast cell system may be useful for this purpose as well, and this could be of interest in laboratory investigations.

SUMMARY

The action of anti-mouse IgE on normal or sensitized mouse and rat peritoneal mast cells as well as on the reaginic activity of mouse and rat antisera was studied. Anti-mouse IgE induced histamine release from normal mouse peritoneal mast cells *in vitro* and this effect was greatly enhanced when mast cells were actively sensitized. The action of anti-mouse IgE on normal rat peritoneal mast cells was slight; it was enhanced after sensitization of these cells with mouse reaginic antibodies as well as after active sensitization. Moreover, anti-mouse IgE is able to neutralise the reaginic activity of mouse and rat antisera. The results suggest that there exist some functional and structural similarities between mouse and rat IgE.

ACKNOWLEDGEMENTS

We are grateful to Dr Annie Prouvost-Danon for the gift of anti-mouse IgE. We wish to thank Miss Teresa Marciniak and Mrs Zdzisława Wojtalik for technical assistance and Miss Bożena Leśniak for histamine determinations.

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