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Histamine release from rat peritoneal mast cells after passive sensitization with human reaginic serum or E myeloma protein

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Significant histamine release (HR) (averaging 15 per cent) was obtained from rat peritoneal cells upon incubation with human reaginic sera and a specific antigen (grass pollen) or a monospecific anti-IgE antiserum. Similar histamine values were obtained from rat mast cells (RMC) incubated with E myeloma protein and challenged with anti-IgE. Washing the cells after incubation with reaginic serum or E myeloma protein did not result in a decrease in HR upon challenge with grass pollen antigen or anti-IgE. No significant HR was observed in control situations. There was also no significant antigen-induced HR from RMC by nonreaginic (newborn) serum or by reaginic serum that was heated for 4 hours at 56° C. Furthermore, anti-IgG, -IgA, and -IgM failed to induce HR from RMC incubated with reaginic serum. These observations support the role of human IgE in passive sensitization of heterologous cells (RMC) for the release of a chemical mediator (histamine).

It has been shown previously that rat peritoneal mast cells could be used to detect immediate hypersensitivity-type reactions mediated by human reaginic (IgE) antibodies to a gamut of allergens: pollens,¹⁻³ penicillin,^{4, 5} and foods.⁶

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TABLE I. Procedure for histamine release from rat mast cells

Order	Procedure
1	Collect rat peritoneal cells in 10 ml. Tris ACM, pH 7.6
2	Centrifuge 250 RCF at 4° C.
3	Suspend in 25 ml. Tris ACM
4	Place 1 ml. aliquots of cell suspension in polycarbonate tubes
5	A. Incubate cells at 37° C. for 10 minutes with 0.1 ml. of reagenic serum or 0.1 ml. of IgE (ND) (1 µg/ml.) and then wash cells and resuspend in 1.1 ml. Tris ACM or B. Add 0.1 ml. of reagenic serum or 0.1 ml. of IgE (ND) (1 µg/ml.)
6	Add 0.1 ml. of antigen or monospecific anti-IgE, -IgA, -IgM, or -IgG
7	Incubate at 37° C. for 3 minutes
8	Centrifuge 250 RCF at 4° C.
9	Extract supernatant and determine histamine fluorometrically

This was based on the morphologic alteration that mast cells undergo in the presence of reagenic sera and antigen, or antihuman IgE. We proposed that the degranulation of the rat mast cells occurred as a result of either antigen-antibody complex formation between IgE and antigen or anti-IgE, or by prior fixation of IgE on rat mast cells followed by the reaction with antigen or anti-IgE on the surface of the cell.²

Subsequently, direct immunofluorescence studies in our laboratory using fluorescent IgE have demonstrated fixation of human myeloma IgE on heterologous target rat mast cells.⁷ This was confirmed by Perelmutter⁸ using fluorescent anti-IgE on fixed cells and radioautography using I¹²⁵-labeled IgE. The preceding studies, although confined to morphologic criteria, did indicate that human immunoglobulin E can be cytotropic for the rat mast cell. The logical sequel to these observations was to explore if measurable histamine release could be detected using this system.

We report here, reproducible histamine release from sensitized rat peritoneal mast cells as determined from multiple experiments using reagenic sera from grass pollen-sensitive individuals or a purified preparation of E myeloma (ND) in the presence of grass pollen antigen or a monospecific anti-IgE.

METHODS

Mast cells were harvested in Tris ACM buffer solution (pH 7.6), in a manner described previously, from the peritoneal cavity of male Wistar albino rats weighing 300 to 400 grams.² After centrifugation at 250 relative centrifugal force (RCF) in a refrigerated centrifuge at 4° C., and washing in Tris ACM buffer, a 1 ml. volume of cell suspension (containing approximately 275,000 mast cells) was incubated at 37° C. for 3 minutes with 0.1 ml. of serum from a grass-sensitive individual* or a purified preparation of myeloma ND† and 0.1 ml. of various concentrations of grass pollen antigen‡ (2.5 to 1,000 PNU), or monospecific antiserum

*Four individuals with a clinical history of grass-pollen induced hay fever, a marked immediate wheal and flare skin reaction to grass pollen antigens, and whose sera have been repeatedly shown to cause significant degranulation of rat mast cells in presence of grass antigen or anti-IgE.

†Kindly provided by Dr. S. G. O. Johansson.

‡Hollister-Stier Labs., Spokane, Wash.

TABLE II. Histamine release from rat mast cells

Order	Content	RFI*	% histamine released†
1	Reagent blank	15‡	
2	Spontaneous: cells	30	
3	Total: cells + 8% HClO ₄	230	100
4	Cells + serum	33	0
5	Cells + heated serum (56° C. for 4 hr.)	31	
6	Cells + newborn serum	25	
7	Cells + IgE (ND) (1,000 ng./ml.)	17	
8	Cells + grass pollen extract (75 PNU/ml.)	22	
9	Cells + anti-IgE (300 ng. AbN/ml.)	27	
10	Cells + anti-IgG	23	
11	Cells + anti-IgA	21	
12	Cells + anti-IgM	25	
13	Cells + heated serum (4 hr.) + grass pollen extract	38	2.5
14	Cells + heated serum (30 min.) + grass pollen extract	61	13.6
15	Cells + serum + grass pollen extract (75 PNU/ml.)	65	16.0
16	Cells + heated serum (4 hr.) + anti-IgE	37	2.0
17	Cells + serum + anti-IgE (300 mg. AbN/ml.)	60	13.5
18	Cells + serum + anti-IgG	22	
19	Cells + serum + anti-IgA	20	
20	Cells + serum + anti-IgM	23	
21	Cells + IgE (ND) (1,000 ng./ml.) + anti-IgE (300 ng. AbN/ml.)	55	12
22	Cells + newborn serum + anti-IgE	26	0
23	Cells + serum (10') and washing in Tris ACM + grass pollen	61	14
24	Cells + serum (10') and washing in Tris ACM + anti-IgE	55	11
25	40 µg histamine/L.	40	
26	20 µg histamine/L.	19	
27	10 µg histamine/L.	9	

*Relative fluorescence index at slit No. 2 and meter multiplier 0.3 in Aminco Bowman fluorometer Model No. 4-8202.

†Reaction - highest control.

‡ $\frac{\text{Total} - \text{spontaneous}}{\text{Total} - \text{spontaneous}}$.

‡Average of duplicates.

to IgE* (100 to 3,000 ng. antibody nitrogen [AbN] per milliliter). In some experiments cells were incubated with reaginic serum for 10 minutes at 37° C. and then washed with Tris ACM buffer at pH 7.6 before the addition of antigen or antiserum, as shown in Table I.

After incubation the cells were centrifuged and the supernatant was assayed for histamine content spectrophotofluorometrically following the procedures of Lichtenstein and Osler,⁹ and May and associates.¹⁰ As indicated in Table II, suitable controls were simultaneously processed and included cells alone for spontaneous histamine release, cells and serum, cells and antigen, and cells and anti-IgE, as well as a reagent blank. Histamine release, 100 per cent, was taken as that obtained by treating the cells with 0.2 ml. of 8 per cent perchloric acid. In addition, other experiments were conducted using the same human reaginic serum after heating at 56° C. for 30 minutes, 45 minutes, and 4 hours. A cord serum from a premature infant was used as a nonsensitive, nonreaginic control. Monospecific anti-IgG, -IgA, -IgM, and -IgE absorbed with kappa and lambda Bence Jones protein to remove any anti-light chain activity were used to identify further the immunoglobulin classes involved.

*The anti-IgE was obtained by immunization of rabbits with IgE myeloma protein (PS). The antibody content was measured by precipitin reaction with the Fc fragments of the myeloma protein. This antiserum was absorbed with 1 mg. per milliliter of kappa and lambda Bence Jones proteins to remove any anti-light chain activity. (Courtesy Dr. Kimishige Ishizaka.)

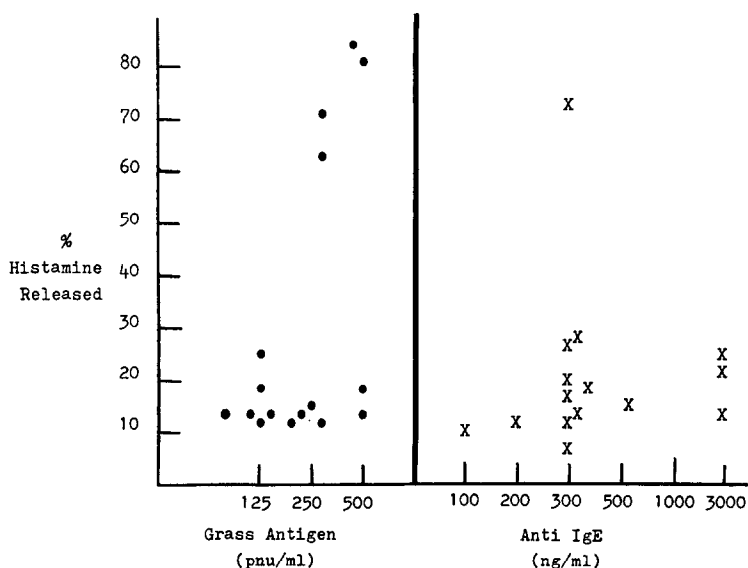


FIG. 1. Maximum histamine release obtained from human reaginic sera and varying concentrations of grass antigen, or anti-IgE.

RESULTS

Significant histamine release was obtained only in the reaction tubes containing cells, grass-sensitive serum, and antigen or anti-IgE, as well as in those containing cells, E myeloma protein, and anti-IgE. Washing the cells after incubation with reaginic sera or E myeloma protein did not decrease the histamine released. A representative experiment is illustrated in Table II. Duplicate samples always agreed within 5 per cent. Additional evidence for the role of IgE in this system is shown by the absence of histamine release from rat mast cells with grass-sensitive serum heated at 56° C. for 4 hours by antigen or anti-IgE and the observation that anti-IgE but *not* anti-IgG, -IgA, or -IgM could cause significant histamine release from sensitized cells. However, after heating the reaginic serum at 56° C. for 30 or 45 minutes, there was still significant histamine release. There was no measurable histamine release from rat mast cells incubated with newborn serum (nonreaginic control) and challenged with antigen or anti-IgE.

Fig. 1 summarizes the results we have obtained in multiple experiments using grass-sensitive sera with grass pollen antigen or anti-IgE in varying concentrations. The *maximum* histamine release ranged from 5 to 80 per cent but was usually around 15 per cent. Histamine release in function of the concentration of grass pollen antigen is illustrated in Fig. 2, and in function of the concentration of anti-IgE in Fig. 3.

DISCUSSION

It has been shown by Humphrey and associates,¹¹ using homologous rat antibody in the rat mast cell system, that as much as 60 to 87 per cent histamine

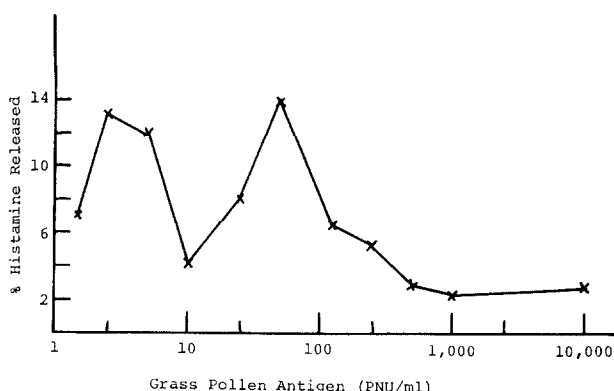


FIG. 2. Histamine release in function of the concentration of grass pollen antigen.

could be released by anti-rat immunoglobulin. Studies employing human basophil leukocytes from ragweed-sensitive individuals also have yielded similar levels of histamine release.^{9, 10} However, Johnson and Moran¹² reported 20 per cent histamine release from rat mast cells using rat homocytotropic antibody and antigen. This is the percentage of histamine we have observed more frequently in our system using human reaginic serum with antigen or anti-IgE and IgE myeloma protein with monospecific anti-IgE antiserum (Fig. 1).

The bimodal dose response curve obtained in the rat mast cell system (Fig. 2) was found to be reproducible in several experiments and is unlike the dose response curve in the human basophil leukocyte system.^{9, 10} Most of the latter data have been obtained with fairly pure antigen, that is, antigen E of ragweed, while we are working with commercial-grade relatively crude extracts of a mixture of grass pollens. Thus our response curve with grass pollen may reflect antigen heterogeneity.

Two mechanisms are possible to initiate the release of histamine. One involves the reaction of antigen or anti-IgE with cell-fixed human IgE; the second involves the reaction of preformed immune complexes with the rat mast cells. Experiments consisting of incubation of rat mast cells with reaginic serum and washing followed by antigen or anti-IgE challenge clearly demonstrate fixation of human IgE on rat mast cells. Further evidence for fixation of IgE antibody on rat mast cells is furnished by the absence of histamine release after heating the reaginic serum at 56° C. for 4 hours, which is known to abolish the cell-fixing property of IgE. However, when heating of the reaginic serum was carried out at 56° C. for only 30 minutes, there was still significant histamine release. This suggests that complement is not indispensable in histamine release in this *in vitro* system. Serologic evidence of homology between human and rat IgE has been reported recently by Kanyerezi and associates¹³ and Liakopoulou and Perelmutter.¹⁴ These observations strongly support the first mechanism, that is, the sensitization of rat mast cells by fixation of human IgE. To extend and corroborate our findings, a study of the reaction employing isolated rat mast cells and the Fc fragment of the IgE myeloma protein is contemplated.

Evidence of histamine release from rat peritoneal mast cells by human

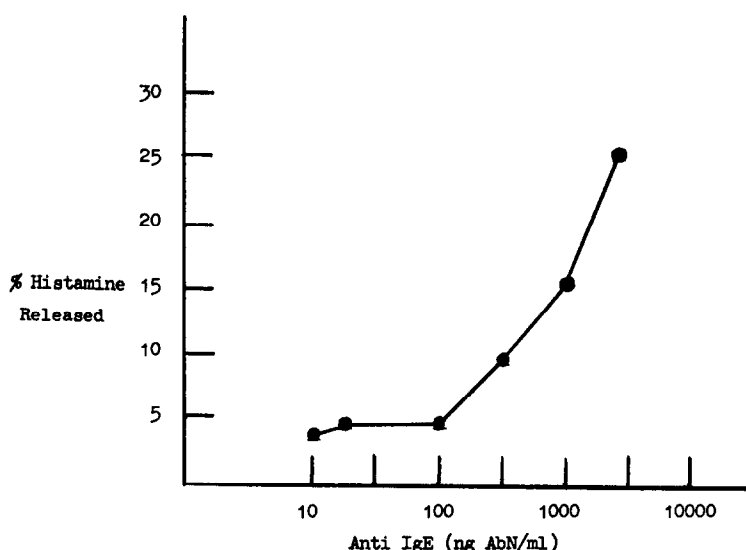


FIG. 3. Histamine release in function of the concentration of anti-IgE.

reaginic serum was recently presented by Vijayanagar and associates.¹⁵ Their report confirmed our earlier report,¹⁶ which was the first account of histamine release from rat mast cells by a heterologous antibody, human IgE.

REFERENCES

- 1 Korotzer, J., and Haddad, Z. H.: In vitro detection of human IgE-mediated immediate hypersensitivity reactions to pollens and penicillin(s) by a modified rat mast cell degranulation technique, *J. ALLERGY* 45: 126, 1970. (Abst.)
- 2 Korotzer, J., Haddad, Z. H., and Lopapa, A.: Detection of human IgE antibody by a modified rat mast cell degranulation technique, *Immunology* 20: 545, 1971.
- 3 Perelmutter, L., and Liakopoulou, A.: Detection of IgE mediated hypersensitivity reactions in the sera of ragweed sensitive individuals using rat mast cells, *Int. Arch. Allergy Appl. Immunol.* 40: 481, 1971.
- 4 Perelmutter, L., and Khera, K.: Rat mast cells in human reagin detection, *Lancet* 1: 1269, 1969.
- 5 Haddad, Z. H., and Korotzer, J. L.: In vitro studies on the mechanism of penicillin and ampicillin drug reactions, *Int. Arch. Allergy Appl. Immunol.* 41: 72, 1971.
- 6 Haddad, Z. H., and Korotzer, J. L.: Immediate hypersensitivity reactions to food antigens: detection of human IgE antibodies in vitro and exploration of immunologic mechanisms using the rat mast cell system, *J. ALLERGY CLIN. IMMUNOL.* 49: 210, 1972.
- 7 Haddad, Z. H., Lopapa, A., and Korotzer, J.: Direct demonstration of human immunoglobulin E (IgE) on rat mast cells, after passive sensitization in vitro, *J. ALLERGY* 47: 88, 1971. (Abst.)
- 8 Perelmutter, L., Liakopoulou, A., Larose, C., and Phills, J. A.: A study on the sensitization of rat mast cells with human IgE-type reagents, *J. ALLERGY* 47: 88, 1971. (Abst.)
- 9 Lichtenstein, L. M., and Osler, A. G.: Histamine release from human leukocytes by ragweed pollen antigen, *J. Exp. Med.* 120: 507, 1964.
- 10 May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, *J. ALLERGY* 46: 12, 1970.
- 11 Humphrey, J. H., Austen, K. P., and Rapp, H. J.: In vitro studies of reversed anaphylaxis with rat cells, *Immunology* 6: 226, 1963.
- 12 Johnson, A. R., and Moran, N. C.: Selective release of histamine from rat mast cells by compound 48/80 and antigen, *Am. J. Physiol.* 216: 453, 1969.

- 13 Kanyerezi, B., Jaton, J. C., and Bloch, K. J.: Human and rat γ E: serological evidence of homology, *J. Immunol.* **106**: 1411, 1971.
- 14 Liakopoulou, A., and Perelmutter, L.: Antigenic relationship between human IgE immunoglobulin and rat homocytotropic antibody, *J. Immunol.* **107**: 131, 1971.
- 15 Vijayanagar, H. M., Attallah, N. A., David, M. F., and Schon, A. H.: In vitro detection of human reagins, *J. ALLERGY CLIN. IMMUNO.* **49**: 119, 1972. (Abst.)
- 16 Haddad, Z. H., and Gillman, S. A.: Workshop on homocytotropic antibodies, First International Congress of Immunology, Washington, D. C., Aug. 3, 1971, *in* Amos, B.: *Proceedings in progress in immunology*, New York, 1971, Academic Press, Inc., p. 1301.