

From the Drug Research Laboratory, Health Protection Branch, National Health and Welfare, Ottawa, Ont., Canada.

COMPARISON BETWEEN MORPHOLOGICAL CHANGES AND HISTAMINE RELEASE INDUCED IN RAT MAST CELLS BY HUMAN ALLERGIC SERA AND SPECIFIC ALLERGEN

By

L. PERELMUTTER & A. LIAKOPOULOU

There has been considerable evidence that the rat mast cell test (RMCT) specifically detects IgE antibodies. Studies have been done using either morphological criteria (1, 4, 6, 7, 9-14, 18), or histamine release from rat mast cells (2, 3, 15, 19, 20). In one study, serotonin release was also employed (2). In the present study, a comparison was made between morphological alterations of rat mast cells in the presence of allergic serum and specific antigen and histamine release from rat mast cells under similar conditions. As a further comparison, the radioallergosorbent test (RAST) was also employed for some sera to determine the levels of IgE antibodies. For this purpose, sera from birch-, ragweed- and penicillin-allergic individuals were used. It was found that, in general, there was a good correlation between the cellular morphological and histamine release techniques in as much as sera giving altered morphology gave histamine release; and those which were negative by one method were also negative by the other method. Part of this study has been previously presented (15).

MATERIALS AND METHODS

Human Allergic Sera

Sera from seven birch-, five ragweed- and two penicillin-sensitive individuals were used in this study. Sera from six non-allergic volunteers were employed as

controls. The birch allergic sera were kindly supplied by Dr. S. G. O. Johansson of Uppsala, Sweden.

Antigens

A birch pollen antigen preparation, vitrum AB, was used for both the RAST and RMCT. Ragweed antigen D and penicillin G, sodium salt (Sigma Chem. Co., St. Louis, Mo.) were employed in the RMCT. The concentrations used for all the antigens are indicated in the text.

RMCT (Morphological Test)

The cellular morphological test of the RMCT used has been described previously (12-14). Hereafter RMCT refers to the morphological changes of the rat mast cell test.

Histamine Release from Rat Mast Cells

The technique of histamine release from rat mast cells, as previously used in our laboratory, has been described (15). Briefly, equal aliquots of 0.2 ml of allergic serum, antigen, normal rat serum and rat mast cell suspension were incubated at 37° C for 3 min. Appropriate serum and antigen controls were employed by replacing the human allergic serum and antigen by suspending media. After the incubation period, the cells were spun down at 250 g for 5 min at 5° C. The supernatants were deproteinized by boiling for 5 min, centrifuged again at 750 g, and the supernatants adjusted to pH 6.8 with 0.1 HCl. Histamine standards were run for each experiment in order to standardize the Schultz-Dale technique.

Radioallergosorbent Test (RAST) (5, 21)

The RAST was performed by Dr. S. G. O. Johansson, Uppsala, Sweden using sera from birch-allergic individuals. One ml of birch allergen extract, vitrum AB was coupled to 100 mg of CNBr-activated cellulose particles. Half a mg of the allergen polymer complex (APC) was incubated with 50 μ l of undiluted serum. The results were expressed as U/ml of a reference reaginic serum, as previously described.

RESULTS

Allergic sera were analyzed for the presence of IgE antibody using the morphological changes involved in the RMCT with birch, ragweed and penicillin antigens. In addition, the birch allergic sera were analyzed for IgE antibody levels with the RAST. Fig. 1 shows typical dose response curves as a function

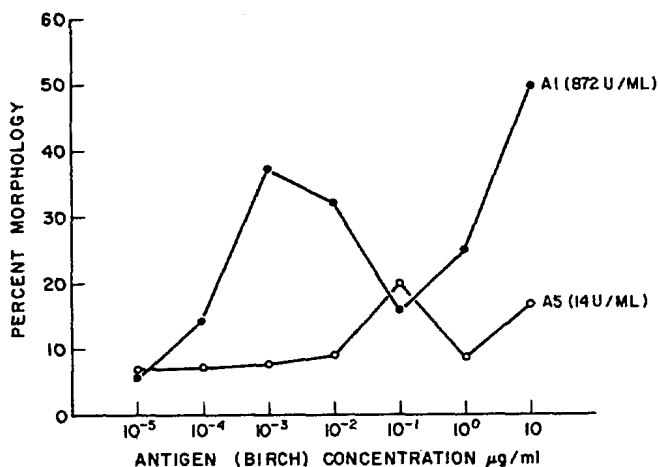


Fig. 1.

Analyses of two sera from birch-allergic individuals (A₁ and A₅) using the RMCT. The figures in brackets refer to the IgE antibody levels as determined by the RAST.

of antigen concentration with the RMCT using two sera from birch-sensitive individuals. Although multiple peaks of response were obtained, serum A₁ with an IgE antibody level of 872 U/ml produced a significant response (39 per cent) at 10⁻³ µg/ml of birch antigen; whereas serum A₅, with an antibody level of 14 U/ml, produced a much less pronounced response with respect to morphology (19 per cent) at 10⁻¹ µg/ml of birch antigen. Fig. 2 compares the minimum concentration of antigen that produced a response in the RMCT with the concentration of IgE antibody determined by RAST for six birch allergic sera. It was found that the higher the antibody level in the sera, the lower the concentration of antigen needed to produce a significant response in the RMCT. Four additional birch allergic sera were analyzed by the RMCT against birch antigen, and all ten sera were examined for their ability to release histamine from rat mast cells in the presence

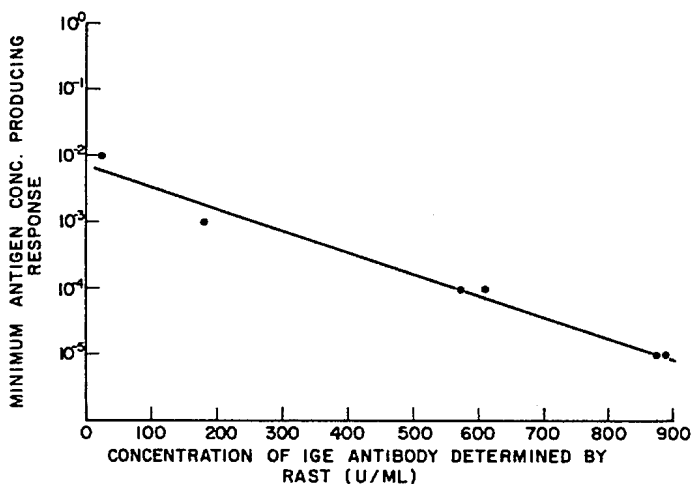


Fig. 2.

Analyses of sera from six birch-allergic individuals using the RMCT and RAST. The abscissa refers to the minimum concentration of antigen that produced a morphological response in the RMCT. The ordinate refers to concentration of IgE antibody as determined by RAST.

of birch antigen. Figs. 3 and 4 show histamine release from birch allergic sera A₄ and A₆ respectively. Significant histamine release could be observed over the range of antigen concentrations tested. For serum A₄, the percentage release was from 6.8 to 30.4, compared to controls of 4 to 6 per cent. With serum A₆, responses ranged from 10.4 to 45.6 per cent, compared to control values of 2 to 3 per cent. Table 1 summarizes the results of the morphological technique, the histamine release method and the RAST. The percentage morphology and histamine release represent the peak responses in each case. In general, there is good agreement for all sera tested with each technique used. Thus, sera numbers 1, 2, 4 and 6, with relatively high levels of IgE antibody when tested with RAST gave strong reactions in the RMCT both by morphological changes in, and histamine release from, rat

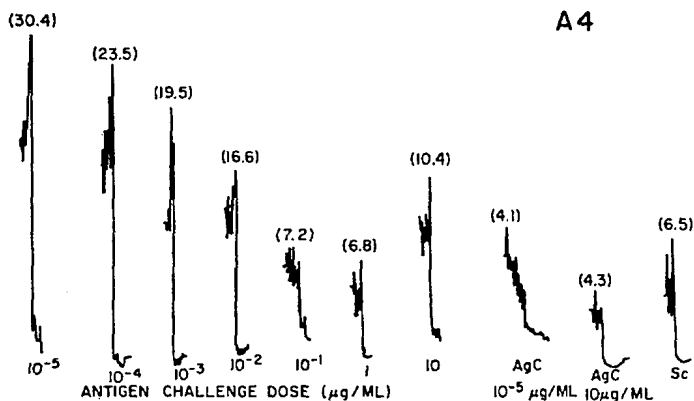


Fig. 3.

Release of histamine from rat mast cells with birch allergic serum A₄ and birch antigen. Histamine was determined by the Schultz-Dale technique. Sc and Agc refer to serum and antigen controls respectively. The numbers in brackets represent the percentage histamine release from rat mast cells.

mast cells. Sera 8 to 10 contained no IgE antibody according to RAST, and produced little or no response in the RMCT. Differences in sensitivities between the two techniques may account for the fact that serum no. 3, with a relatively low level of antibody as measured by RAST, was found to be negative by the RMCT.

Sera from five ragweed- and two penicillin-allergic individuals, as well as five sera from non-allergic volunteers, were analyzed by both the RMCT and histamine release procedures. The ragweed allergic sera were tested with ragweed fraction DWSR from 10^{-2} to $10 \mu\text{g/ml}$, and the penicillin allergic sera from 1 to $10,000 \text{ U/ml}$ penicillin G. Table 2 summarizes these results and indicates that there is a good correlation between these two procedures.

Although a good correlation existed between the morphology and histamine release techniques for all sera studied, an interesting observation was made with two of the sera tested. Thus, analysis of these two sera using the antigen titration

TABLE 1

Analysis of Birch-Allergic Sera using (1) Morphological Changes and Histamine Release from Rat Mast Cells and (2) RAST.

Serum No.	RMCT		RAST U/ml
	% Morphology	% Histamine release	
1	50	46	872
2	33	26	607
3	7	4	18
4	52	30	882
5	27	22	182
6	37	24	568
7	18	12	14
8	10	4	< 2
9	6	3	< 2
10	8	3	< 2

TABLE 2

Analysis of Ragweed- and Penicillin-Allergic Sera using the Morphology and Histamine Release Procedures of the RMCT.

		RMCT	
	Antigen	% Morphology	% Histamine release
<i>Ragweed-allergic sera</i>			
1	Ragweed	45	32
2	"	27	21
3	"	69	37
4	"	53	32
5	"	21	14
<i>Penicillin-allergic sera</i>			
1	Penicillin G	27	18
2	"	23	13
<i>Non-allergic sera</i>			
1	Ragweed	6	3
2	"	3	2
3	"	2	1
4	Penicillin G	3	2
5	"	2	2

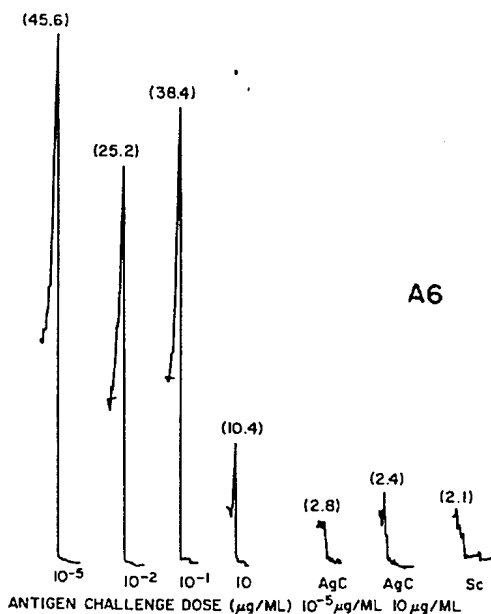


Fig. 4.

Release of histamine from rat mast cells with birch-allergic serum A6 and birch antigen. Histamine was determined by the Schultz-Dale technique. Sc and Agc refer to serum and antigen control respectively. The numbers in brackets represent the percentage histamine release from rat mast cells.

procedure indicated that, at lower antigen concentrations, histamine was specifically released with negative morphology, whereas both histamine release and morphology were found at the higher antigen concentrations; i.e. there appeared to be two peaks of response for histamine release and only one for morphology (at the higher antigen concentration level) for both sera (Table 3).

DISCUSSION

Rat mast cells have been shown specifically to bind human IgE immunoglobulin, and hence antibodies belonging to this

TABLE 3

Analysis of Allergic Sera using the Antigen Titration Method of the RMCT.

Ragweed-allergic serum	Antigen concentration		
	$\mu\text{g/ml}$ (DWSR)	% Morphology	% Histamine release
	10^{-6}	6	14
	10^{-4}	8	16
	10^{-3}	6	11
	10^{-2}	18	7
	10^{-1}	21	10
	10^0	27	22
	10	18	13

Ragweed-allergic serum	(Birch Vitrum AB)		
	$\mu\text{g/ml}$	% Morphology	% Histamine release
	10^{-6}	4	11
	10^{-4}	3	12
	10^{-3}	5	7
	10^{-2}	9	4
	10^{-1}	21	14
	10^0	16	12
	10	11	9

class of immunoglobulin. Exposure of rat mast cells sensitized with IgE antibodies to specific antigen has resulted in morphological changes of rat mast cells (1, 4, 6, 7, 9-14, 18), or histamine and/or serotonin release from the same cells (2, 3, 15, 19, 20). In the present study, using sera from birch-, ragweed- and penicillin-allergic patients, it was found that the morphological changes and histamine release correlated well for all sera studied. Furthermore, examination of 10 sera (seven birch allergic and three normal) with birch antigen using the RAST as well as RMCT (morphological criteria and histamine release) resulted in a good correlation between these two *in vitro* methods. It is clear from this study, as well

as from previous reports (16), that the higher the IgE antibody level (as determined by RAST) in the sera, the lower the concentration of antigen required to give a significant response using the morphological criteria of the RMCT. Further analysis of the allergic sera by the antigen titration method of the RMCT revealed that, for two allergic sera, histamine release was obtained without morphological changes at the lower antigen concentrations; whereas both morphological changes and histamine release were found at the higher antigen concentrations. The finding of histamine release from rat mast cells without accompanying alterations of these cells (palisading of granules and vacuolization of cytoplasm) has been reported previously in experiments using toluidine blue dye and calcium salts (8). However, the interesting feature of the experiments in our study is the fact that differences in histamine release and morphological changes appeared to be antigen concentration dependent. More studies should be done in order to determine the nature of the antigen-antibody complexes involved in eliciting these phenomena.

The results of the present study, using either the morphological criteria or histamine release technique, should not convey the impression that these methods be performed routinely using the antigen titration procedure. In more recent studies by McLaren et al. (10) with the RMCT, using a wide variety of allergens (pollens, molds and inhalents), it was found that 50 patients with large, medium, small or negative direct skin tests could be separated out according to the morphological response of their sera with specific antigen. In their study (10), a limited range of antigen concentration was found sufficient to detect responses. Similar results have been obtained in some preliminary experiments performed in our own laboratory with sera from patients tested with pollens, molds, inhalents and some drug allergens. The results of the present study, as well as those of McLaren et al. (10), suggest that the RMCT has a potential use as a routine test for analyses of allergic sera, compared to the more tedious procedure of histamine release from rat mast cells.

SUMMARY

In the present study it was found that morphological changes and histamine release from rat mast cells in the presence of human allergic sera (seven birch, five ragweed and two penicillin) and appropriate allergen gave comparable results. In addition, measurements of levels of IgE antibodies of ten sera (seven allergic and three normal) to birch antigens with the RAST were in good agreement with the results obtained using the morphological criteria of the RMCT or histamine release from rat mast cells. It was interesting to find that with two allergic sera (one ragweed and the other birch), histamine was released from rat mast cells without accompanying morphology at lower antigen concentrations, whereas both histamine and positive morphological changes were found at higher antigen concentrations.

REFERENCES

1. Bernstein, I. L. & Safferman, R. (1972): Hypersensitivity to algae: correlation between provocation tests, leukocyte histamine release and rat mast cells. *J. Allergy Clin. Immunol.* 49, 113.
2. Frick, O. L. & Ashton, F. (1973): Serotonin and histamine release from rat mast cells sensitized with allergic serum. *J. Allergy Clin. Immunol.* 51, 82.
3. Gillman, S. A. & Haddad, Z. H. (1972): Histamine release from rat peritoneal mast cells after passive sensitization with human reaginic serum or E myeloma protein. *J. Allergy Clin. Immunol.* 50, 131.
4. Haddad, Z. H. & Korotzer, J. L. (1972): 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, 219.
5. Johansson, S. G. O., Bennich, H. & Berg, T. (1971): In vitro diagnosis of atopic allergy. *Int. Arch. Allergy* 41, 443.
6. Kimura, Y., Inoue, Y. & Honda, H. (1973): Inhibitory effect of CAMP related compounds upon IgE mediated mast cell degranulation and PCA in rat. *Excerpta med. (Amst.)*, Sect. 5, 300, 20.
7. Korotzer, J., Haddad, Z. H. & Lopapa, A. (1971): Detection of human IgE antibody by a modified rat mast cell degranulation technique. *Immunology* 20, 545.
8. Krüger, P. G. & Bloom, G. D. (1973): Granule alteration and vacuole formation, two primary structures features of the histamine release process in mast cells. *Experimentia* 29, 329.

9. MacLaren, W. R., Ho, F. C. & Lopapa, A. (1972): The rat mast cell degranulation tests as applied to a case of severe food allergy. *Ann. Allergy* 30, 41.
10. MacLaren, W. R., Ho, F. C. & Lopapa, A. (1972): Rat mast cell degranulation and routine skin tests in allergic patients. *Ann. Allergy* 30, 579.
11. Miyashita, H., Ishikama, T., Shimada, T. & Kitamura, T. (1973): Evaluation of antigen antibody complexes consisting of IgE and ragweed antigen in nasal secretion by rat mast cell degranulation technique. *Excerpta med. (Amst.)*, Sect. 5, 300, 15.
12. Perelmutter, L. & Khera, K. (1970): A study on the detection of human reagins with rat peritoneal mast cells. *Int. Arch. Allergy* 39, 27.
13. Perelmutter, L. & Liakopoulou, A. (1971): Detection of IgE mediated immediate hypersensitivity reactions in the sera of ragweed sensitive individuals using rat mast cells. *Int. Arch. Allergy* 40, 481.
14. Perelmutter, L. & Millard, E. (1971): A modified rat mast cell technique for the detection of human IgE-type reagins. *Int. Arch. Allergy* 40, 675.
15. Perelmutter, L., Liakopoulou, A. & Phills, J. A. (1973): Human IgE—Rat mast cell reactions. *Mechanisms in Allergy* 1, 235.
16. Perelmutter, L. & Liakopoulou, A. (1974): Interrelationship of human IgE antibody and allergen in the rat mast cell technique. *Int. Arch. Allergy* 46, 280.
17. Perelmutter, L. & Ricketts, S. (In preparation): The use of the rat mast cell technique to detect drug allergic reactions.
18. Phills, J. A., Katona, A., Everett, M. & Perelmutter, L. (1973): An immunological study of allergic type reactions to aspirin. *Excerpta med. (Amst.)*, Sect. 14, 300, 59.
19. Reneaux, M., De Montis, G., Marcelli, D. & Roche, A. (1973): A study of the fixation of human IgE on rat mast cells. *Excerpta med. (Amst.)*, Sect. 5, 300, 20.
20. Vijayaneger, H. M., Attallah, N. A., David, M. F. & Schon, A. H. (1973): In vitro detection of human reagins with rat mast cells. *Mechanisms in Allergy* 1, 255.
21. Wide, L., Bennich, H. & Johansson, S. G. O. (1967): Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* ii, 1105.

Author's address:

Dr. L. Perelmutter,
Head, Immunology Section,
Tunney's Pasture,
Ottawa,
Canada.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.