THE TURKISH JOURNAL OF
PEDIATRICS
A HACETTEPE BULLETIN VOLUME 20/Nos. 1-2/JAN.-APRIL 1978

Comparison Between Rat Mast Cell Degranulation Test and Allergic Skin Tests in Children with Atopic Diseases

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Introduction

According to the current concept, human IgE antibodies fix to human or primate tissues through the Fc portion of the immunoglobulin molecule, leading to the sensitization of the mast cells and basophils and release of histamine and other mediators on challenge with an appropriate antigen.[1] Attempts to sensitize non-primate tissues with human IgE have yielded variable results. In 1962 the initial reports[2] of the rabbit basophil degranulation after sensitization with human reagins have been questioned in the subsequent studies. Direct immunofluorescence investigations using fluorescent IgE have demonstrated fixation of human myeloma IgE on heterologous target rat mast cells.[4] This was confirmed by Perelmutter[5] and Renoux[6] who used fluorescent anti-IgE on fixed cells, radioautography with I^{125} labeled IgE and mixed agglutination technique. These preceeding studies indicated that human immunoglobulin E can be cytotropic for the rat mast cell[4-8] and that rat peritoneal mast cells undergo specific morphological changes in the presence of human allergic sera and specific antigen.[9-11]

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The rat mast cell degranulation (RMCD) technique was developed by Schwartz[12] in 1965 and modified by Korotzer and Haddad[7] in 1971. Several authors reported reproducible results and a good correlation with clinical data with the use of the rat mast cell test.[9-11] On the other hand rat mast cell sensitization by human IgE is not accepted by others who obtained negative results.[13] Today the rat mast cell degranulation test is still to be considered a research procedure.

Our study was designed to check the binding of human allergic sera on rat mast cells and the value of this test in clinical practice. Only a few in vivo tests have been found useful for comparison with in vitro tests. Among these the most widely used is the skin test. We have checked the correlation between the allergy skin tests and the results of the rat mast cell degranulation test in the allergic patients. In our work, in addition to antigens investigated by others, we have selected some other antigens such as Verbascum, Monilia, Rhizopus, and Pinus which were not used by other workers.

Material and Methods

Selection of Patients: 42 children, 22 girls and 20 boys, who were shown to have an atopic disease according to their history and physical and laboratory examination, and who were evaluated using the Rappaport's[14] allergic index, were selected for this study. The diagnoses of these cases are shown in Table I. The ages of the patients ranged from 5 to 20 years (mean 12 years).

TABLE I

DIAGNOSES OF 42 ATOPİC CHILDREN STUDIED FOR COMPARISON BETWEEN RAT MAST CELL DEGRANULATION TEST AND ALLERGIC SKIN TESTS

Diagnosis	No
Asthma and Perennial Rhinitis	26
Perennial Rhinitis	7
Asthma	5
Perennial Rhinitis Asthma and eczema	4
Total	42

Skin Testing: Intradermal tests were performed with 10 common allergens on the arm of each patient. Skin reactions were read as follows:

— No reaction or same as control with normal saline

1+ Wheal which is a little larger than in the controls; no pseudopods; no erythema.

2+ Erythema with raised wheal in the center.

3+ Wheal is less than 1 cm in diameter and small pseudopods.

4+ Wheal is more than 1 cm in diameter and is surrounded by pseudopods and an area of erythema.

We used the same allergens both for the skin tests and the RMCD test. The concentrations of the allergens were 1/500 for the intradermal skin testing and 1/1000 for RMCD. The allergens used were:

Pollens: Cynodon dactylon, Lolium, Poa pratensis, Salsola kali, Verbascum, and Pinus.

Environmentals: Monilia, Rhizopus, cat dander, house dust.

The water soluble extracts of these allergens were prepared in our laboratory as previously reported.[15] The solutions of the allergens for RMCD test were prepared in Tris-ACM buffer (consisting of Tris-A plus Ca^{++} and Mg^{++} ions) and stored at 4 °C.

Rat Mast Cell Degranulation Technique: The RMCD technique modified by Perelmutter and Millard [16] was used with slight alterations and is illustrated in Figure 1.

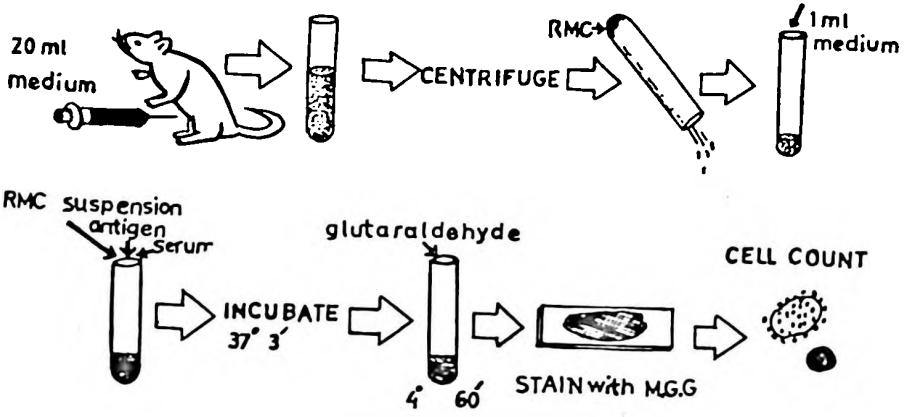


Figure 1
Rat mast cell degranulation procedure.

Animals: Male Wistar strain of albino rats weighing 200 grams were used.

Glassware: All glassware which came into contact with cells were siliconized with 1 % solution of silicone; alternately polycarbonate tubes were used.

Serum Samples: Serum samples were obtained from the patients when they were subjected to the skin testing. In addition, non-allergic sera from 8 neonates were included as controls. Sera were stored in 1 ml aliquots at –20 °C. Generally fresh sera were used in our experiments. All sera were filtered through 2 millipore membranes (HAWP 0.45 μm)[2] (Millipore Corporation Bedford, Massachusetts).

Collecting and Suspending Media: A solution was made under sterile conditions by mixing 1 part glutamine enriched Medium 199, 10x (BDH Chemicals Ltd, England), 9 parts distilled water and $NaHCO_3$ at a final concentration of 0.35 g/l. From this solution the collecting and suspending media were made by further addition of EDTA (ethylene-diamine-tetra-acetic acid) at a final concentration of 0.5 mg/ml and enough 10 %$NaHCO_3$ to adjust the pH to 6.8. For testing human sera, the suspending medium was further enriched with a final concentration of 1 mg/ml of bovine serum albumin (General Biochemical Co., Changrin, Ohio).

Isolation of Mast Cells: Rats were anesthetized with ether, 20 ml of collecting media was injected into the peritoneal cavity. After two minutes, the rats were killed by exsanguination. A large incision was made in the abdomen. The fluid in the abdominal cavity was drained using a pipette into a polypropylen tube and this was then centrifuged at 150 g for 8 minutes at 4 °C. Supernatant was discarded and the precipitated pellet was resuspended in 1 ml of fresh suspending media. Mast cells obtained this way were used immediately.

Experimental Design: Equal aliquots (0.05 ml) of subjects' sera, rat mast cell suspension and an allergen were mixed in a tube and incubated for 3 minutes at 37 °C. Subsequently, the mixture was fixed with 2 % glutaraldehyde solution in 0.2 M sodium phosphate buffer, pH 7.3. After a 60 minute incubation at 4 °C, a drop of this mixture was removed and placed onto a slide. This smear was allowed to air-dry and stained with May-Grunwald Giemsa.

One hundred mast cells over several randomly distributed microscopic fields were counted under high magnification to determine the percent showing degranulation. Mast cells which were not degranulated are easily distinguishable from degranulated ones. (Figure 2. A-B)

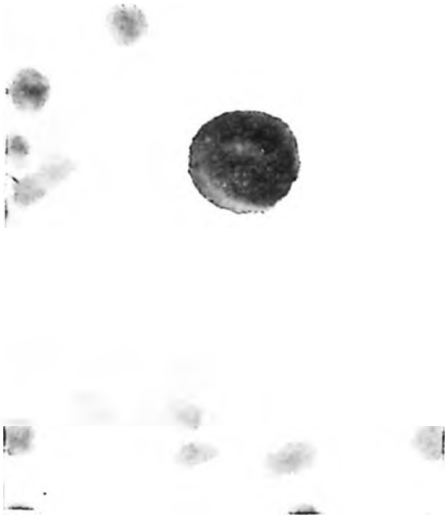


Figure 2-A
Intact rat mast cell.

Figure 2-B
Degranulated rat mast cell.

The basic design we have used in the RMCD experiments is as follows:

1. Rat mast cells (RMC) + allergic serum (AS) + allergen (Ag)
2. RMC + AS
3. RMC + Ag
4. RMC + non-allergic serum + Ag

In order to establish reproducibility of this procedure, the sera of 12 patients were tested 2 times with all 10 antigens used, in another day.

Statistical Analysis: Correlation coefficients (r) between the skin test reactions and RMCD were calculated for each antigen.

Results

The control values of RMCD for all 42 allergic sera without antigens were as follows: The lowest value was 9 %, the highest 22 % and the mean 13.2 %.

The control values of RMCD for antigens without sera were from 2 % to 13 %, the mean was 7.6 %.

Non-specific degranulation obtained with 8 non-allergic sera plus antigens ranged from 6 % to 26 %, the mean was 15.4 %.

The RMCD values associated with the 104 negative skin tests showed a level resembling the control values. The lowest value was 7 % and the highest 27 %, the mean was 14 %.

By considering all the control values we accepted a value higher than 25 % RMCD to be significant for this work.

Table II shows the mean values of RMCD percentage for the 10 allergens in each skin test group. In negative test column, mean values of RMCD varied from 10.8 % to 15.8 %. Mean values of RMCD vary from 14.1 % to 31.7 % in 1+ skin test group, from 21.6 % to 43.7 % in 2+ skin test group, from 17.5 % to 68 % in 3+ skin test group, and from 40 % to 90 % in 4+ skin test group. As this table shows, when the grade of the skin tests increase, mean values of RMCD rise for each antigen.

TABLE II

MEAN VALUES OF RMCD PER SKIN TEST GRADE FOR 10 ALLERGENS ON 42 ATOPIC CHILDREN AND CONTROLS

	Mean values of RMCD in percent				
Skin Test Reaction	—	1+	2+	3+	4+
Allergens					
House Dust	12.7	23	37.7	65.4	63.2
Cat Dander	14.9	15.4	29	52	40
Monilia	14	27.2	35.3	53.8	88.2
Rhizopus	15.8	28.3	35.9	57.8	64
Lolium	13	24.6	34.2	50.7	90
Poa Pratensis	14.9	22.2	35.3	49	74.5
Cynodon Dactylon	15	30.2	41.5	68	55
Pinus	10.8	14.1	21.6	17.5	–
Salsola Kali	15.7	29.2	41.1	46.4	69.2
Verbascum	13.5	31.7	43.7	62.3	51
Non-Specific Degranulation with					
Allergic Sera	9 – 22 mean 13.2 ∓ 3.4				
Antigens	2 – 13 mean 7.6 ∓ 2.1				
Non-Allergic Sera+Antigens	6 – 26 mean 15.4 ∓ 4.4				

The coefficients of correlation (r) between skin tests and RMCD values for each of the 10 allergens showed significant correlation. P was <0.01 in all cases. The highest r value (0.90) was obtained by Lolium, then in descending order, by Verbascum (0.85), Poa (0.80), Cynadon (0.78), Monilia (0.76), house dust (0.73), Salsola (0.73), cat dander (0.69), Rhizopus (0.68). The lowest r value (0.57) was shown by Pinus.

In Figure 3 we have shown RMCD percentage produced by each patient's serum compared with the result of skin tests performed with all 10 allergens. The vertical columns indicate from negative to 4+ skin test reactions and vertical scale shows the percent of RMCD. As illustrated in Figure 3 although almost half of the sera in the 1+ and 2+ skin test columns produced degranulation in control range, the values in the 3+ and 4+ columns were entirely above control range, going as high as 96 %, with the exception of Pinus.

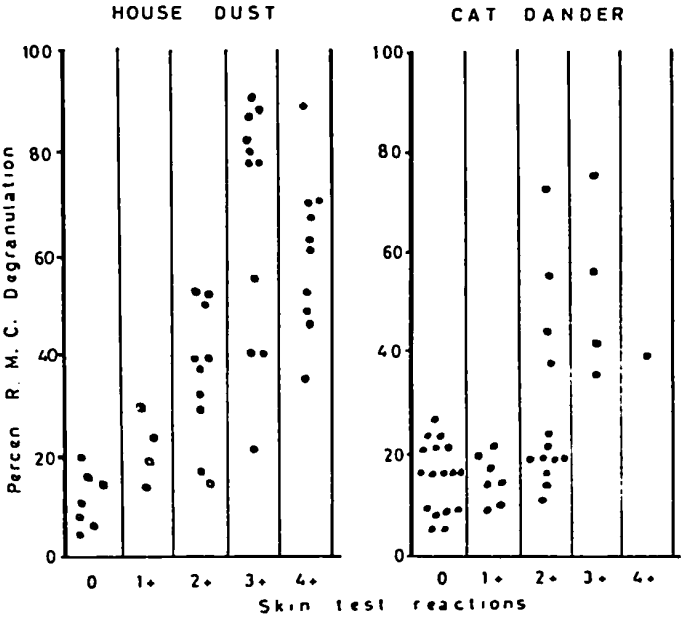
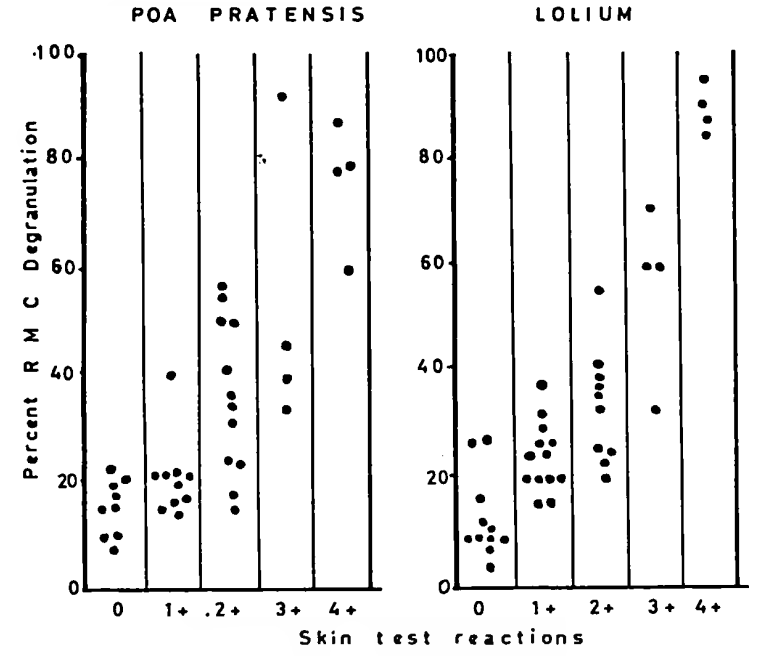
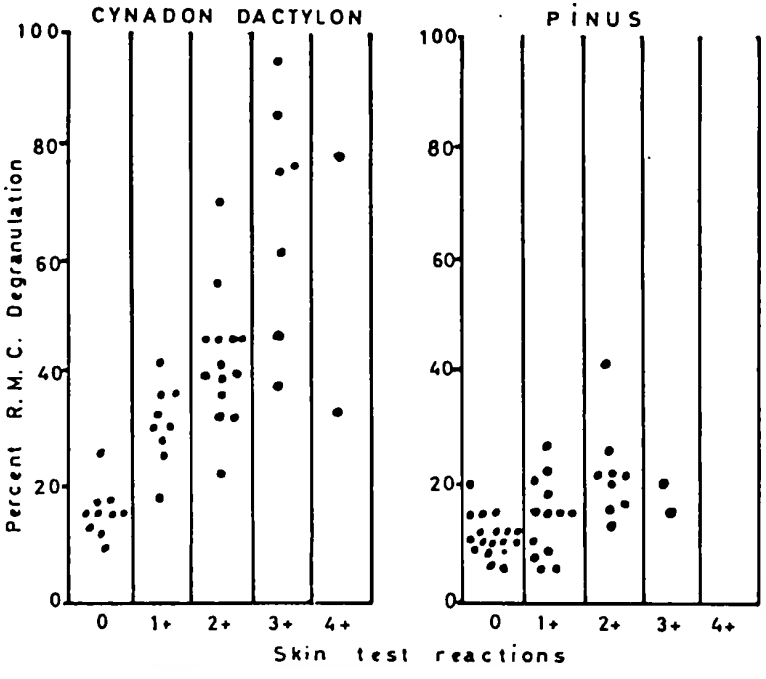


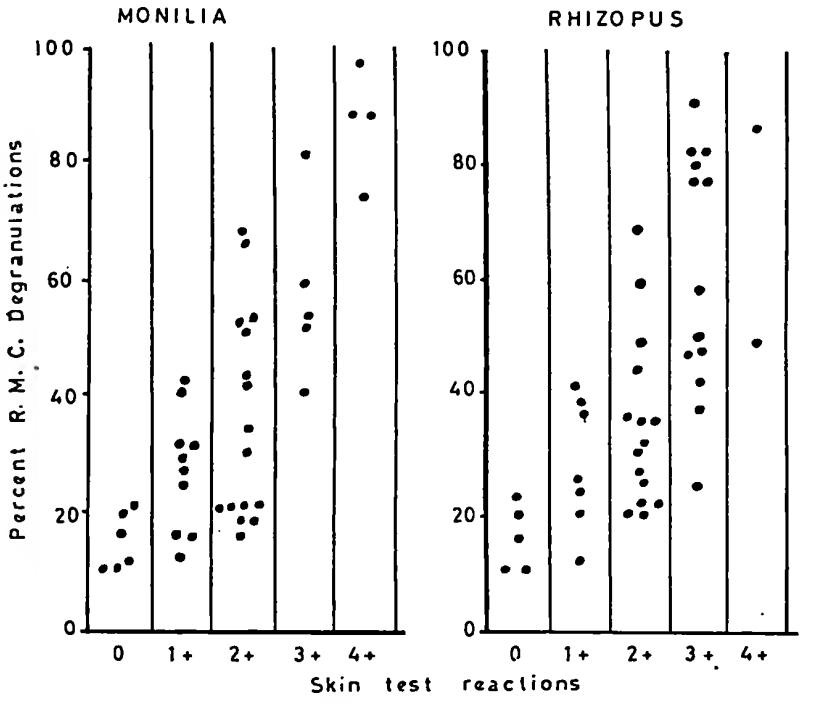
Figure 3

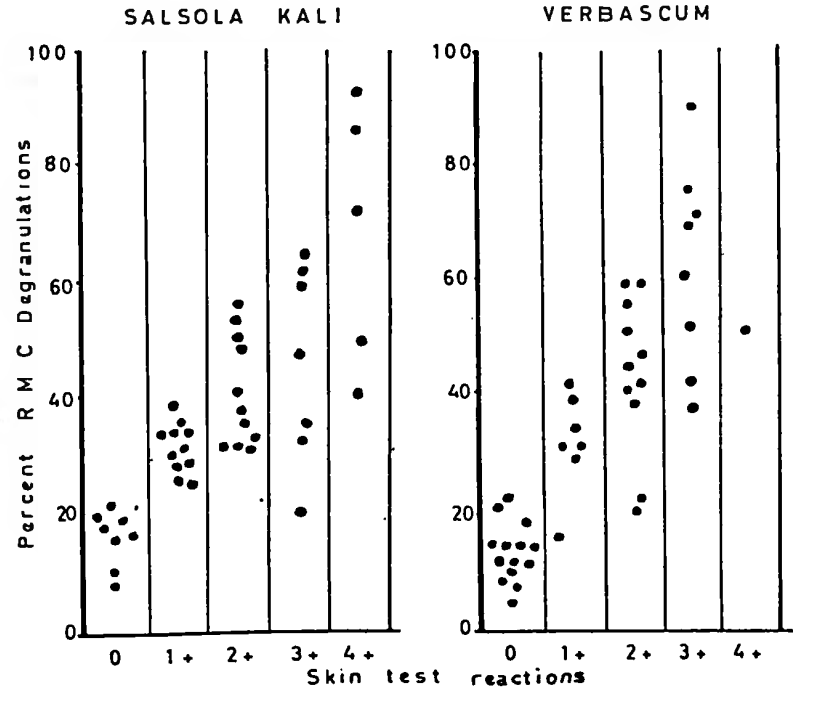
RMCD percentage produced by each patients serum compared with the result of skin tests performed with all 10 allergens.

Duplicate Measurements of RMCD: The difference between the first and second RMCD values, using the sera of 12 patients was found statistically insignificant ($p > 0.05$).


POA PRATENSIS
LOLIUM
Percent R M C Degranulation
100
80
60
40
20
0
0 1+ .2+ 3+ 4+
0 1+ 2+ 3+ 4+
Skin test reactions


CYNADON DACTYLON
PİNUS
Percent R.M.C. Degranulation
100
80
60
40
20
0
0 1+ 2+ 3+ 4+
0 1+ 2+ 3+ 4+
Skin test reactions


MONILIA
RHIZOPUS
Percent R. M. C. Degranulations
100
80
60
40
20
0
0
1+
2+
3+
4+
Skin test reactions


SALSOLA KALI
VERBASCUM
Percent R M C Degranulations
100
80
60
40
20
0
0
1+
2+
3+
4+
Skin test reactions

Discussion

Conventional allergologic diagnostic procedures such as skin testing and especially provocation tests are not entirely risk-free and may sometimes be difficult to perform for practical and psychological reasons. It seems clear that an in vitro method capable of solving even a part of the problems involved in the diagnosis of allergy would be most valuable, provided that the method is reliable and relatively simple. At present, histamine release from human basophil leukocytes or sensitive radioimmunoassays (e.g. R.A.S.T.) capable of detecting minimal amounts of specific IgE seem to be promising and reliable. Though such methods are of great scientific values, they are not suitable for any routine use in the clinical practice of allergy because of the tedious steps included. After the RMCD technique was developed by Schwartz[12] in 1965 and modified by Korotzer and Haddad[7], several studies have been carried out on this subject and it has been compared with the other in vitro and in vivo methods.

Perelmutter and Liakopoulou[17], using sera from birch-ragweed and penicillin allergic patients, found that histamine release and the morphological changes of rat mast cells correlated well for all sera studied. In addition, they obtained good correlation between the results of the RMCD tests and R.A.S.T. on coded sera from patients allergic to several pollens.[17]

Haddad and Korotzer[10], using sera from 25 subjects with clear-cut clinical histories of immediate-type hypersensitivity, reactions to foods demonstrated that the rat mast cell results correlated well with the clinical histories and the skin tests. They proposed this test as a useful adjunct in the diagnosis of food allergy. Similarly, Maclaren et al[11] reported that the RMCD test correlated well with skin tests and clinical sensitivity in a patient anaphylactically sensitive to foods.

The results of our study clearly demonstrate that rat mast cells can undergo specific morphological changes in the presence of allergic sera and specific allergen. Such changes were not attributed to either the allergic sera or allergens alone in spite of the fact that the cell preparation may contain some degranulated cells. In addition, cytologic response in a mixture of mast cells and nonallergic neonate sera remained unaffected by the addition of antigen.

In our study, the correlation between allergic skin tests and RMCD is very high ($p<0.01$). Similar results have been obtained by McLaren et al[18] with the RMCD test, using a wide variety of allergens (pollens,

molds and inhalents). They found that 50 patients with large, medium, small or negative direct skin tests could be separated out according to morphological response of their sera with specific antigen. In our study, a significant RMCD was associated with strong 3+ or 4+ skin test. On other hand, sera of some patients with 1+ and 2+ skin test produced degranulation below the significant level. According to these results, we convey the impression that the RMCD test should not be considered a replacement for standard skin testing. However, it appears reliable, safe, supplemental method in determining severe allergy.

Summary

The correlation between the allergic skin tests and the results of the rat mast cell degranulation test was measured in a series of 42 allergic patients, using six pollens and four environmental allergens. Correlation coefficients between skin tests and RMCD tests varied from 0.57 to 0.90 depending on the allergen used. This result indicates a good correlation between the two methods.

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