

From the Health Protection Branch, Health and Welfare Canada,
Ottawa, Ontario, Canada

AN ELECTRON MICROSCOPE STUDY OF RECEPTOR SITES ON RAT MAST CELLS FOR HUMAN IMMUNOGLOBULIN E

By

R. PONTEFRACI & L. PERELMUTTER

This study is concerned with the distribution of receptor sites on rat mast cells for human IgE immunoglobulin using ferritin-labelled antisera. Binding of IgE immunoglobulin to rat mast cells has been previously shown using immunofluorescence and radioautography (1). Sullivan et al. (4), using ferritin-labelled antisera, showed receptor sites for human immunoglobulin E by visually identifying ferritin particles in electron micrographs of thin sections of human basophils. However, mast cells and basophils contain intragranular particles which appear similar to ferritin particles in conventionally stained thin sections when examined under the EM.

In this paper, a method is described by the use of which a direct measure of the iron (Fe) in the ferritin molecule is obtained through an energy dispersive X-ray analysis unit attached to a transmission electron microscope (TEM). With the use of horse anti-human IgE and ferritin-labelled anti-horse sera, receptor sites on rat mast cells (RMC) were confirmed by measuring the relative amount of Fe present on the surface of the cells.

MATERIALS AND METHODS

Preparation of Cell Suspension

Mast cell suspensions were removed from the peritoneal cavity of rats as described previously (2). An aliquot of 0.3 ml of cell suspension was mixed with 100 μ g of human IgE myeloma protein PS (kindly supplied by Dr. K. Ishizaka of Baltimore, Md.) or 0.5 ml of allergic serum containing 1.25 μ g/ml of IgE. After incubation for 30 min at 37° C, the cells were spun down at 200 g and washed three times in M 199 containing 1 per cent normal rat serum. The cells were then fixed in 3 per cent glutaraldehyde for 20 min at 5° C, and washed two times with phosphate buffered saline (PBS), pH 7.4. An aliquot of 0.1 ml of horse anti-human IgE serum (Kallsted Laboratories Incorporated, Chaska, Minn., U.S.A.) was added to the cells. After incubation for 20 min at 37° C, the cells were centrifuged at 400 g, then washed three times with PBS. Ferritin-labelled rabbit anti-horse serum (0.1 ml) (Cappell Laboratories, Inc., Downingtown, Pa.) was added to the cells for 20 min at 37° C. The cells were again centrifuged at 400 g at 5° C, washed twice in PBS and then a drop of the concentrated cell suspension was placed on carbon-formvar-coated EM grids. In control experiments, the peritoneal mast cell preparation was treated in the same way as the experimental preparation except the IgE immunoglobulin material was replaced by PBS. To render the surface of the grids hydrophilic, they were treated for a short time (circa 15 sec) on a plasma unit (Tegal Corporation, Tegal Industries, Burlingame, California). This treatment aids in spreading the cells evenly over the grid. Before examination under the EM, the mast cells on the grids were identified using phase contrast Nomarski optics. The granular appearance of the mast cells is enhanced under these optical conditions and they can be readily distinguished from eosinophils and neutrophils.

A map, marking the position of the mast cells on the grid squares in relation to the centre mark, was prepared to help in locating these cells under the EM.

Electron Microscopy and X-ray Analysis

The control and test specimens were examined in a Siemens Elmiskop 101 with an attached Siemens energy dispersion silicon lithium (Si Li) detector for the analysis of X-rays. Both 80 kv and 60 kv accelerating voltages were employed for specimen examination. Analysis was made of the X-ray spectrum for the presence or absence of Fe (from ferritin) in the specimens examined. The results were expressed either as total counts in the Fe region of the spectrum or a scan was made of the area and recorded on a Kompensograph III strip chart recorder using a specially designed spectrum scanner integrated in the analysis equipment (3). Each cell was examined in whole or in part by adjusting the size of the electron beam spot accordingly. X-ray analyses were carried out for various periods of time depending on the level of Fe present in the specimen.

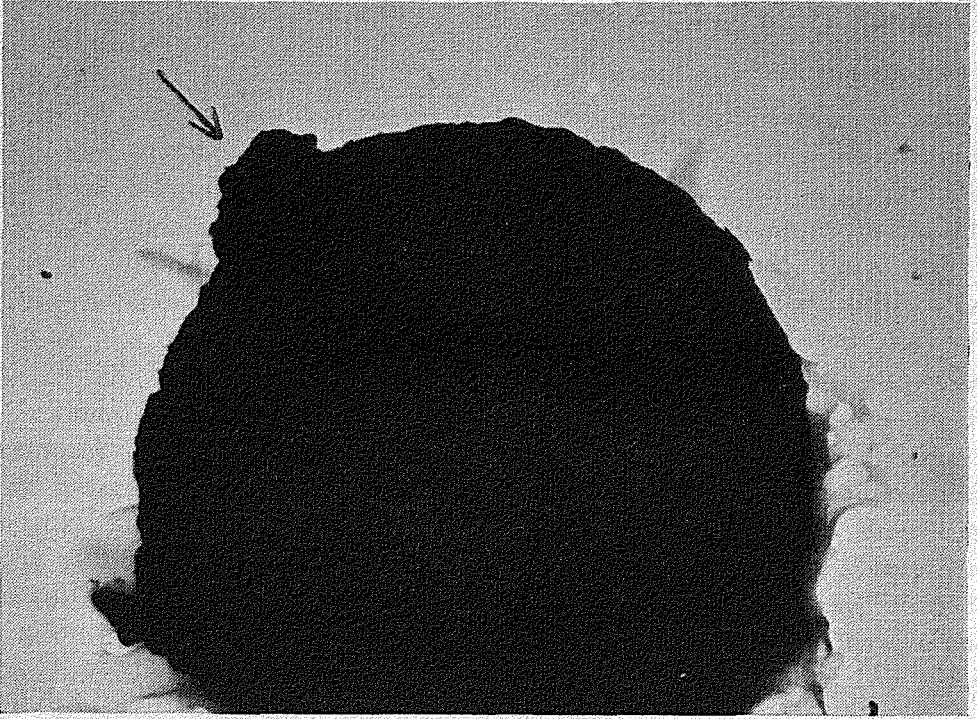


Fig. 1.

Electron micrograph of rat mast cell treated with IgE, horse anti-IgE and ferritin-labelled rabbit anti-horse serum. The arrow denotes location of "cap" on cell.

RESULTS

The RMC, shown in Fig. 1, has been treated with human IgE and the appropriate ferritin-labelled antisera. The sharply defined protuberance indicated by the arrow appeared darker than the rest of the cell and, upon X-ray analysis, contained the bulk of the Fe. Such areas on the mast cells have been designated as "caps" (4) and are considered to be caused by a concentration of receptor sites on the cell (2). The shape and size of these caps varied. Fig. 2 represents the recording of an X-ray scan of the "cap" region and a distinct Fe peak can be easily seen at 6.4 kv. Fig. 3 shows a scan of a region of the same cell remote from the "cap". No Fe peak was observed, even though a more intense beam of electron was used as evidenced by the size of the Cu peak.

Table 1 summarizes the X-ray analyses of several mast cell

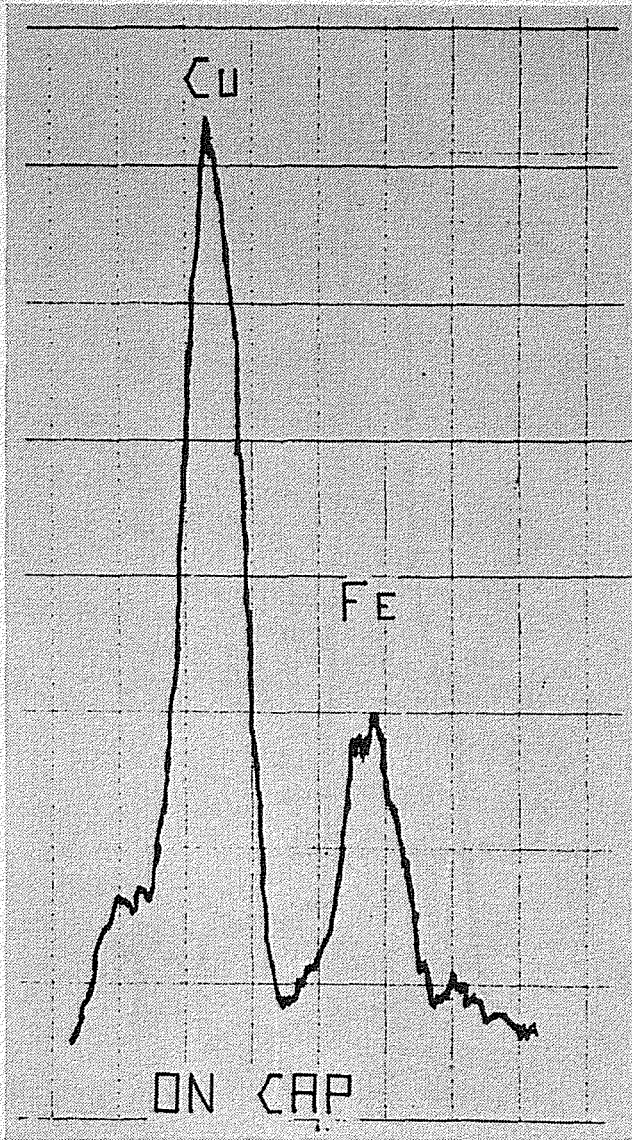


Fig. 2.

X-ray scan of "cap" region of treated rat mast cells. The X axis represents the relative concentration of metals. The Y axis represents the energy peaks of the metals. Cu peak is at 8.1 KEV and Fe peak at 6.4 KEV.

preparations treated with IgE myeloma protein PS. Of the 47 mast cells examined, 12 (25.5 per cent) showed a dense area of ferritin on specific areas of the cells. Most cells showed one "cap" and a few showed two. Each mast cell was examined over several areas to arrive at the baseline level of Fe for that cell.

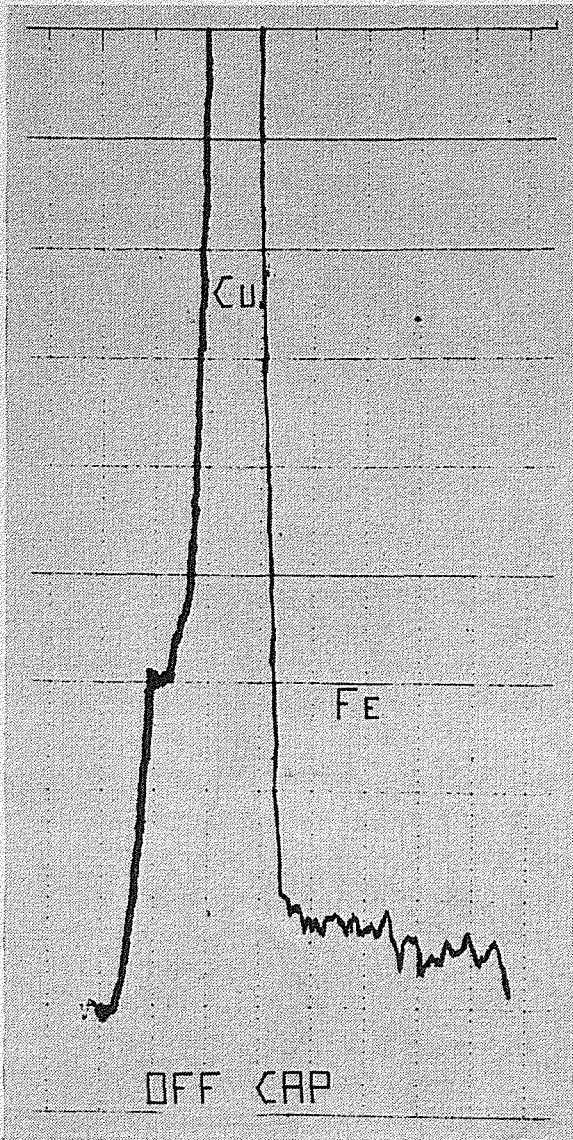


Fig. 3.

X-ray scan of "cap" region remote from "cap" as in same cell of Fig. 1. The X axis represents the relative concentration of metals. The Y axis represents the energy peaks of the metals. Cu peak is at 8.1 KEV and Fe peak at 6.4 KEV.

The figures shown in Table 1 represent the Fe peak to background ratio (P/B) upon analysis of the "cap" areas on the cell. It can be seen that the P/B ratio ranged from a low of 1.4 to a high of 16.4 on the "cap", whereas areas remote from the "cap" (but on the same cell) gave P/B ratios between 0.96 and 1.16, indicating that in effect, no Fe could be

TABLE 1
Iron Counts on Mast Cells with "Caps"

Cell no.	"Cap" P/B ratio	Other part(s) of cell P/B ratio
1	2.78*	1.04* 1.16*
2	1.90	—
3	1.4	1.01
4	1.76	—
5	4.80	1.20 1.26
6	6.68	1.12 1.10
7	16.40	1.15*
8	2.28*	—
9	1.88*	1.01*
10	4.25*	1.11* 1.23* ** 1.26**
11	4.14	—
12	12.34	1.09

* Corrected for Compton escape.

** Close to "cap".

— Not done.

detected. It is of interest to note that measurements taken from areas adjacent to the cap showed small but significant ($>2\sigma\sqrt{B}$) amounts of Fe present.

Control cell preparations were analyzed in parallel and no Fe could be detected with this host system. Neutrophils and eosinophils from samples treated with IgE were also examined and Fe could not be demonstrated on these cells.

In an added set of experiments, allergic serum L.C. [containing 1.25 $\mu\text{g/ml}$ IgE protein as determined by the use of a RIST kit (Pharmacia, Uppsala, Sweden)] was employed in treating RMC for 3 h at 37° C, then the same procedure was followed as described in Materials and Methods. As for controls, the same allergic serum was passed through two im-

TABLE 2

Effect of Absorption of Allergic Serum L.C. on "Cap" Formation

Allergic serum L.C.	No. of mast cells showing "caps"	Total no. of mast cells examined
Unabsorbed	6	22
Absorbed with anti-IgE	0	18
Absorbed with control immunosorbent	5	25

munosorbents: one containing horse anti-human IgE and the other normal horse serum. It can be seen from Table 2 that 6 out of 22 (27.7 per cent) of the mast cells contained "caps" when treated with unabsorbed allergic serum L.C. whereas no "cap" formation was detectable after absorption with anti-IgE immunosorbent. The abolishment of "cap" formation appeared to be specifically due to lack of IgE in the absorbed allergic serum, since the passage of allergic serum L.C. through the control immunosorbent did not cause it to lose its ability to induce "cap" formation.

DISCUSSION

In the present study, the method of XMA combined with TEM was successful in locating receptor sites on RMC for human IgE. Thus, by the use of ferritin-labelled antisera, specific binding of ferritin was readily detected on the mast cells. This technique has obvious advantages over the standard EM procedures performed with ferritin-labelled antisera on ultra-thin sections of fixed embedded cells. With the use of whole cells mounted directly on EM grids, time-consuming dehydrating and embedding procedures can be avoided. Staining of thin sections also results in a similarity in appearance between ferritin particles and some intragranular material found commonly in mast cells, which may cause some confusion in differentiating between the two. This risk is enhanced when degranulation occurs leading to migration of

these intragranular particles outward to the periphery of the cells.

"Cap" formation on target cells has been described previously (4). This phenomenon has been observed also in the present study. The technique employed in this work facilitates the examination of large numbers of cells for the detection of "caps". Coupled with the semi-quantitative measurement of ferritin (Fe) concentration, the location of the mass of receptor sites can be observed. The incubation of RMC and human IgE at 37° C with the resulting "cap" formation is similar to that observed with human basophils at the same temperature (4). By the use of this combined technique, studies are under way to elucidate the temperature dependence of the possible migration of receptors.

The results of the present study confirm earlier findings (1) on RMC receptor sites for human IgE. In addition, no significant uptake of IgE by other types of cells was observed. Although the autoradiography technique (1) was more sensitive in detecting receptors on RMC for human IgE, the results obtained in the present study by the use of XMA combined with TEM was advantageous in locating the receptor sites.

SUMMARY

Previous studies using immunofluorescence and autoradiography demonstrated the presence of specific immunoglobulin E (IgE) receptors on rat mast cells (RMC). This finding was confirmed by the use of transmission electron microscopy (TEM) combined with X-ray microanalysis (XMA) employing ferritin-labelled antisera. XMA was used to detect ferritin as Fe in the target cells. On examination by TEM and XMA, 25 per cent of RMC showed a dense area of Fe deposits on the surface of the cells. No Fe could be detected on neutrophils or eosinophils or in the control cell preparations without human IgE. The Fe was concentrated in well-defined areas of the treated mast cells and this distinct concentration

of receptor sites was similar to that observed by other investigators who have termed this phenomenon, "cap" formation.

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Authors' address:

L. Perelmutter, Ph. D.,
Laboratory Centre for Disease Control,
Ottawa, Ontario,
K1A-OL2 Canada.

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