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Immuno-electron microscopic localization of fibronectin on rat mast cells*

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Summary. Rabbit anti-rat plasma fibronectin (pFN) causes histamine release from rat mast cells in the presence of complement. Fibronectin (FN) on rat mast cells, as shown by immuno-electron microscopy, is principally localized on cell folds, so they may play a role of attachment in the matrix of connective tissue.

Cell surface FN is distributed on many cell types and one of its main functions is to mediate cellular adhesion to the substratum (see Pearlstein et al.[1] for a review). Rat mast cells also have FN molecules on their surfaces. It has been shown[2] that: a) anti-rat pFN serum releases histamine from rat mast cells, b) mast cells adhere to collagen-coated dishes and some of them flatten and elongate on the dishes, c) this adhesion is inhibited by anti-pFN serum treated at 56 °C for 30 min, and d) immunofluorescence microscopic study shows the presence of FN on mast cells. In the present study, complement-dependent histamine release from rat mast cells by rabbit anti-rat pFN serum is described and localization of FN is shown by immuno-electron microscopy.

Materials and methods. Purification of rat pFN and preparation of rabbit anti-rat pFN serum have been described previously[2]. Purified pFN showed a single band by SDS-polyacrylamide gel electrophoresis, and the antiserum formed a single precipitation arc against purified pFN and rat plasma by immunoelectrophoresis. The antiserum preabsorbed by the purified pFN lost histamine releasing activity from mast cells as well as a binding activity on these cells. Rat peritoneal cells were collected after the injection of physiological solution (PS: NaCl 154 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM and 10% Sörensen phosphate buffer, pH 7.2). Peritoneal cells from a few rats were pooled for each experiment and the isolation procedure for mast cells[2] was omitted in the experiment using immuno-electron microscopy. Isolated mast cells were suspended in 0.9 ml Hanks' solution containing 0.05% bovine serum albumin and incubated with 0.1 ml rabbit anti-pFN serum at 37 °C for 15 min. These cells were centrifuged at 2000 × g for 15 min and histamine in the supernatant and the precipitate was determined[3]. Histamine release was expressed as a percentage of total histamine content. For immuno-electron microscopy, collected peritoneal cells were washed and fixed with 0.125% glutaraldehyde in PS for 2 h in an ice bath. They were washed with PS 3 times for a total of more than 30 min, then incubated with rabbit anti-rat pFN serum diluted 1:40 for 30 min at room temperature. After washing, ferritin-conjugated goat anti-rabbit IgG (Miles-Yeda, Ltd) diluted 1:100–200 was used for detection of bound antibodies. The cells were refixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed with 1% OsO_4 in 0.05 M phosphate buffer (pH 7.4) in suspension, collected in agar[4], dehydrated with an acetone series and embedded in Epon. As a control, normal rabbit serum was used instead of antiserum. PS was used as a washing solution throughout the experiment unless otherwise stated. Unstained thin sections (60 nm thick) were viewed with a JEOL 100-CX electron microscope.

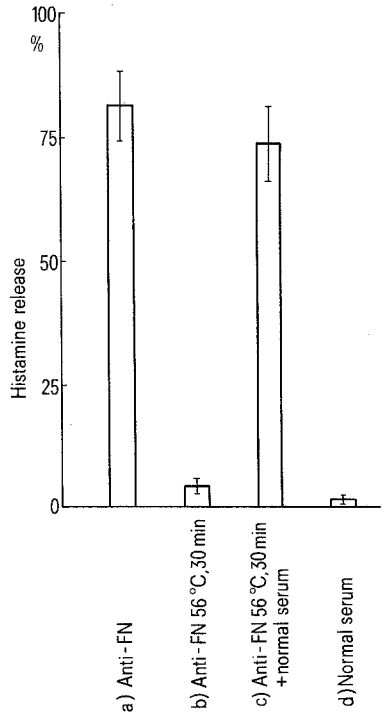

Figure 1. Histamine release (%, mean value ± SE) from isolated rat mast cells by *a* rabbit anti-rat pFN serum, *b* rabbit anti-rat pFN serum treated at 56 °C for 30 min, *c* rabbit anti-rat pFN serum treated at 56 °C for 30 min and normal rabbit serum and *d* normal rabbit serum.

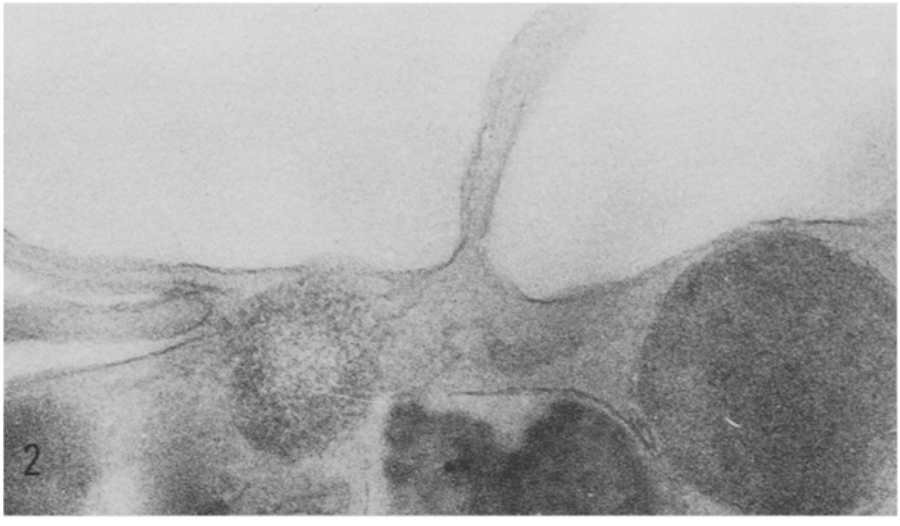

Figure 2. Unstained section of rat mast cell treated with normal rabbit serum followed by ferritin-conjugated goat anti-rabbit IgG. Ferritin grains are not observed. × 57,000.

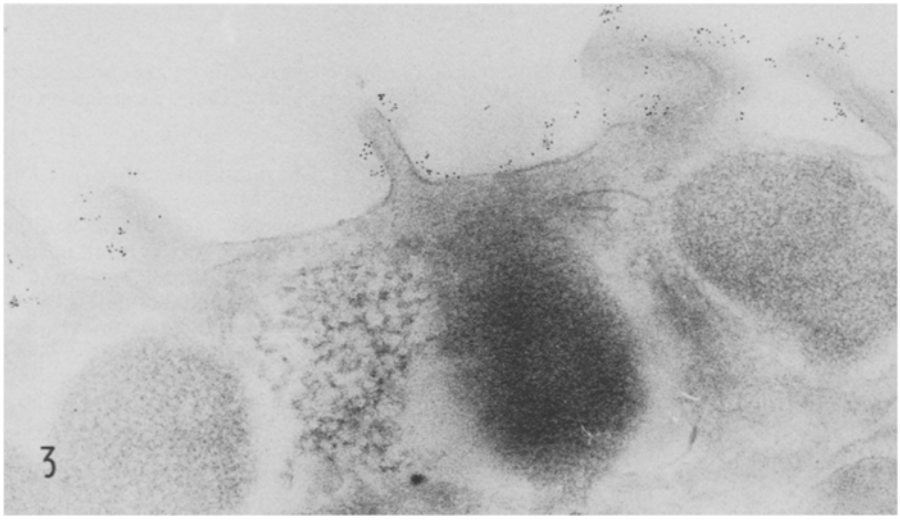

Figure 3. Unstained section of rat mast cell treated with rabbit anti-rat pFN serum followed by ferritin-conjugated goat anti-rabbit IgG. Ferritin grains are found on the cell surface. Concentration of grains on cell folds is evident. × 57,000.

Results and discussion. Rabbit anti-rat pFN serum induces histamine release from isolated rat mast cells concomitantly with their degranulation. This release is a complement-dependent process as summarized in figure 1. Rabbit anti-rat pFN releases 81.8 ± 7.0% (mean, % ± SE) histamine from mast cells (fig. 1a) while normal rabbit serum only releases 1.3 ± 0.8% histamine (fig. 1d). When antiserum, treated at 56 °C for 30 min, is used, histamine release reduces to 4.3 ± 1.5% (fig. 1b), but it recovers to 74.0 ± 7.8% by the addition of fresh normal rabbit serum (fig. 1c).

Specific binding of anti-rat pFN antibodies to the mast cell surface is confirmed. No ferritin grains are seen after fixed mast cells are treated with normal rabbit serum followed by ferritin-conjugated goat anti-rabbit IgG (fig. 2). When normal rabbit serum is substituted with anti-rat pFN serum, ferritin grains are found on mast cell surface (fig. 3). The distribution of grains is not uniform, but clusters consisting of more than 2 grains are localized at intervals. Mast cells have cell folds or ridges which appear as microvilli in section, on their surface[5,6]. Concentration of ferritin grain clusters on these cell folds is clearly shown, although grains are found elsewhere on the cell surface. Burwen et al.[6] reported that a definite correlation existed between membrane fold formation and secretion. Present results indicate another possible role of cell folds, namely adhesion to the intercellular matrix in vivo. In the cytoplasm, altered granules and wide clefts among granules are seen but ferritin grains are not observed. The amount of FN on mast cells is less than that on fibroblast[7]. The possibility that the antigenic site of FN was lost with glutaraldehyde fixation has to be considered. The concentration of glutaraldehyde may be low enough to keep the molecules intact[8], although the cytoplasm is not fixed well for observation of ultrastructure. Nonspecific binding of rabbit serum to mast cell is almost negligible, for only a few clusters of ferritin grains are sometimes seen around one cell in a thin section when normal rabbit serum is used.

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