

Phase-contrast microscopic studies using cinematographic techniques and scanning electron microscopy on IgE-mediated degranulation of cultured human mast cells

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Summary

Background Isolating human mast cells is a laborious procedure. Recently, cultured human mast cells raised from umbilical cord blood cells have become available. It is necessary to investigate whether IgE-mediated activation of these cells is mediated by exocytosis.

Objective To verify IgE-mediated activation of these cultured human mast cells morphologically.

Methods The mast cells were raised from human umbilical cord blood cells in the presence of stem cell factor and interleukin-6. IgE-sensitized cultured human mast cells were activated by anti-IgE, and morphological changes of the cells were examined under phase-contrast microscopy using cinematographic techniques and scanning electron microscopy. Histamine release from the cells was measured with high-performance liquid chromatography.

Results Under the condition in which a significant histamine release was observed from the mast cells, phase-contrast microscopy showed that the cultured human mast cells became swollen and extruded granules. Scanning electron microscopy disclosed the extrusion of smooth and round bodies from pores formed on the activated mast cell surface.

Conclusion IgE-mediated histamine release from cultured human mast cells is accompanied by exocytosis morphologically, indicating that cultured human mast cells will help in studying the functional properties of human mast cells.

Keywords: cultured human mast cell, histamine release, IgE, exocytosis, phase-contrast microscopy, scanning electron microscopy

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Introduction

Investigations of mast cell functions have been conducted using mainly rat peritoneal mast cells because they are relatively easy to obtain. The morphology of mast cell degranulation has been studied extensively, and exocytosis

is accepted as the degranulation mechanism in rat mast cells [1,2]. The problem is that mast cells are heterogeneous [3,4] and there may be functional differences in mast cells between different species.

Isolated human mast cells from several organ sources, such as lung [5] and skin [6], have been used to examine a regulated secretion by transmission electron microscopy. Dissociated human foreskin mast cells have been shown to degranulate in response to anti-IgE and substance P [7]. However, complicated processes are required to obtain human mast cells, usually from surgical specimens, and

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cultured human mast cells can now be obtained from umbilical cord blood cells [8–10]. Their advantages are that relatively large numbers of the cells are accessible, and that alterations which might occur during the purification process can be avoided. Although the work from our laboratory showed that the cultured human mast cells resemble lung mast cells in their ability to release histamine [9], it is necessary to examine whether these cells are useful in investigating the role of mast cells in allergic human diseases. Therefore, in the present study, IgE-sensitized cultured human mast cells were activated by anti-IgE, and we examined for the first time the morphological changes of the activated-cultured human mast cells under phase-contrast microscopy using cinematographic techniques and scanning electron microscopy. Our results indicate that IgE-mediated histamine release from the cultured human mast cells is accompanied by exocytosis morphologically, indicating that they may contribute a great deal to mast cell research, and could be a useful model of human mast cells in allergic diseases.

Materials and methods

Human mast cell culture

Mast cells were raised from human umbilical cord blood cells by the method as described [9,10]. Briefly, freshly obtained heparin-treated umbilical cord blood (about 20 mL) was layered over Ficoll-paque (Pharmacia & Upjohn, Uppsala, Sweden), and centrifuged at $400\times g$ for 20 min. The mononuclear cell layer was collected and suspended at 1×10^6 cells/mL in Media I, which was a mixture of Dulbecco's modified Eagle medium component plus 10 μ g/mL insulin, 10 μ g/mL transferrin, 5×10^{-5} M 2-mercaptoethanol, 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid, and 2.6 ng/mL NaSeO₃ (IBL Men-eki Seibutsu Kenkyujo, Gunma, Japan), supplemented by 5% fetal bovine serum (Cytosystems, Castle Hill, Australia), 50 ng/mL human recombinant interleukin-6 and 80 ng/mL human recombinant stem cell factor (kindly provided by Kirin Brewery, Tokyo, Japan) in a 37 °C CO₂ (5%) humidified incubator. The cells were passaged every week and maintained in the same media. Cell counting was performed by toluidine blue staining. Mast cells were then fixed with Carnoy's fixative for 15 min at room temperature and stained for mast cell tryptase and chymase using monoclonal murine antitryptase and antichymase antibodies, which were originally reported by Irani *et al.* [11]. This study complied with the Review Board of Gunma University School of Medicine.

Assay for histamine release from cultured human mast cells

The cultured human mast cells were incubated with 1 μ g/mL

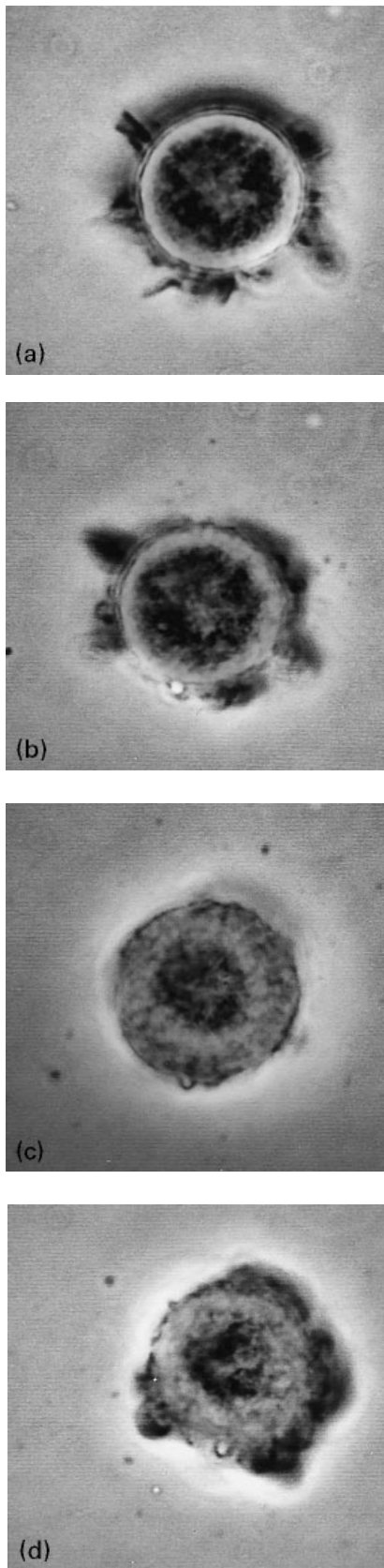
human myeloma IgE (Chemicon, Temecula, CA, USA) for 1 h at 37 °C. After washing the cells with a cold buffer as described [9], the cells were preincubated for 1 min at 37 °C, and incubated for the indicated time at 37 °C with anti-human IgE (Cappel, Durham, NC, USA). After stopping the reaction by adding ice-cold buffer to the cell suspension, histamine in both the supernatant and the cell pellet was measured by high-performance liquid chromatography as described [9]. The percentage histamine release was calculated by the amount of histamine in the cell supernatant divided by that both in the supernatant and in the cell pellet. Experiments were performed in triplicate, and the data represent the mean and standard error of the mean (SEM) of four independent experiments under the same conditions. Significance was determined by employing the analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. *P*-values less than 0.05 were considered statistically significant.

Phase-contrast microscopy using cinematographic techniques

The IgE-sensitized mast cells were first mounted on a cover slip coated with poly D-lysine and left stand for 5–10 min in a moisture box, and the cover slip was mounted on a perfusion chamber. The chamber was sealed with a cover slip and a gasket, and was continuously perfused with a solution through syringe needles inserted through the gasket. Stimulation of the cells with anti-IgE was performed by perfusion. Mast cells were examined under phase contrast microscopy ($\times 400$) using an inverted microscope (Nikon TMD: Nikon, Tokyo, Japan) equipped with a colour video camera (Sony pxc-930 3CCD: Sony, Tokyo, Japan), and a video recorder (Sony LVR-3000N: Sony, Tokyo, Japan). For each sample five micrographs were taken. Additional ones were taken as warranted.

Scanning electron microscopy

The IgE-sensitized mast cells were exposed to anti-IgE in the same manner as in the case of phase-contrast microscopy. The mast cells were fixed at a predetermined time by exchanging the culture solution with 0.1 M cacodylate-buffered paraformaldehyde-glutaraldehyde solution, pH 7.4, perfused through the syringe needle inserted into the chamber gasket. The mast cells were left standing in the fixation solution for 2 h at room temperature. The fixed mast cells, still mounted on the cover slip, were washed three times with 0.1 M cacodylate-buffered osmium tetroxide solution for 2 h at 4 °C and washed again with 0.1 M cacodylate buffer. After postfixation, the mast cells on the cover slip were dehydrated in an ascending series of ethanols, processed through isoamyl acetate, critical-point-dried by



carbon dioxide, and coated with gold. The prepared samples were examined under a scanning electron microscope (JSM-T330 A: JEOL, Tokyo, Japan). For each sample five micrographs were taken at $\times 3500$ and five micrographs at $\times 10\,000$ primary magnification.

Results

The purity of mast cells reached 90% after 10 weeks as described [8], and in the present study we used cells more than 120 days old. At the time of the study, 98.5 ± 0.7 (mean $\pm$ SEM, $n = 6$)% of the cells were trypan blue negative and $95.8 \pm 1.7\%$ toluidine blue positive ($n = 6$). When the cells were immunocytochemically stained for tryptase, $94.8 \pm 1.7\%$ were positively stained ($n = 6$). Among tryptase-positive cells, $20.6 \pm 4.0\%$ contained chymase ($n = 6$). Six different cultures of mast cells were used in this study.

Preliminary studies showed that histamine release from the cells was in manners of a dose-dependent and a time-dependent with anti-IgE [8]. At various times afterward, aliquots of the cells were removed for histamine assay, and phase-contrast microscopic studies. Significant histamine release ($40.5 \pm 7.3\%$, $n = 4$) from the IgE-sensitized cultured human mast cells was observed at 5 min by the stimulation with $10\ \mu\text{g/mL}$ anti-IgE.

Phase-contrast microscopy using cinematographic techniques showed that the mast cells were generally spherical or nearly so, with microvilli on the cell surface, filled with fine granules surrounding a prominent nucleus, and highly refractile (Fig. 1a). After the stimulation with $10\ \mu\text{g/mL}$ anti-IgE for 15 s, microvilli on the cell surface became to be shortened and the cells extruded granules (Fig. 1b). At 80 s the microvilli disappeared and the cells began to bubble (Fig. 1c), and at 210 s the cells became swollen, and extruded granules clearly evident near the cell surface and in the surrounding medium (Fig. 1d). In parallel a significant histamine release was observed by the stimulation and the amounts of histamine release from the cells with $10\ \mu\text{g/mL}$ anti-IgE for 15, 80 and 210 s were 6.8 ± 1.6 , 19.4 ± 3.0 and $42.4 \pm 6.8\%$, respectively.

Scanning electron microscopy disclosed that the mast cells sensitized passively with IgE antibody were generally globular, and the entire visible surface of each cell was covered with low round elevations, presumably due to the presence of granules just beneath the cell membrane. There

Fig. 1. Phase-contrast micrographs using cinematographic techniques of IgE-sensitized cultured human mast cells before anti-IgE challenge (a), 15 s (b), 80 s (c) and 210 s (d) after $10\ \mu\text{g/mL}$ anti-IgE challenge. Very similar results were obtained in five micrographs at each time under the same conditions.

were also irregularly distributed membranous ridges and microvilli on the cell surface (Fig. 2 a,b). In the mast cells exposed to 10 $\mu\text{g/mL}$ anti-IgE for 80 s, pores between 0.6 and 0.8 μm in diameter were found on the cell surface

and smooth round bodies, probably granules of about 0.6 μm in diameter, were observed within the pores and on the cell surface near the pores (Fig. 2 c,d). The mast cells exposed to the anti-IgE for 210 s showed much irregularly

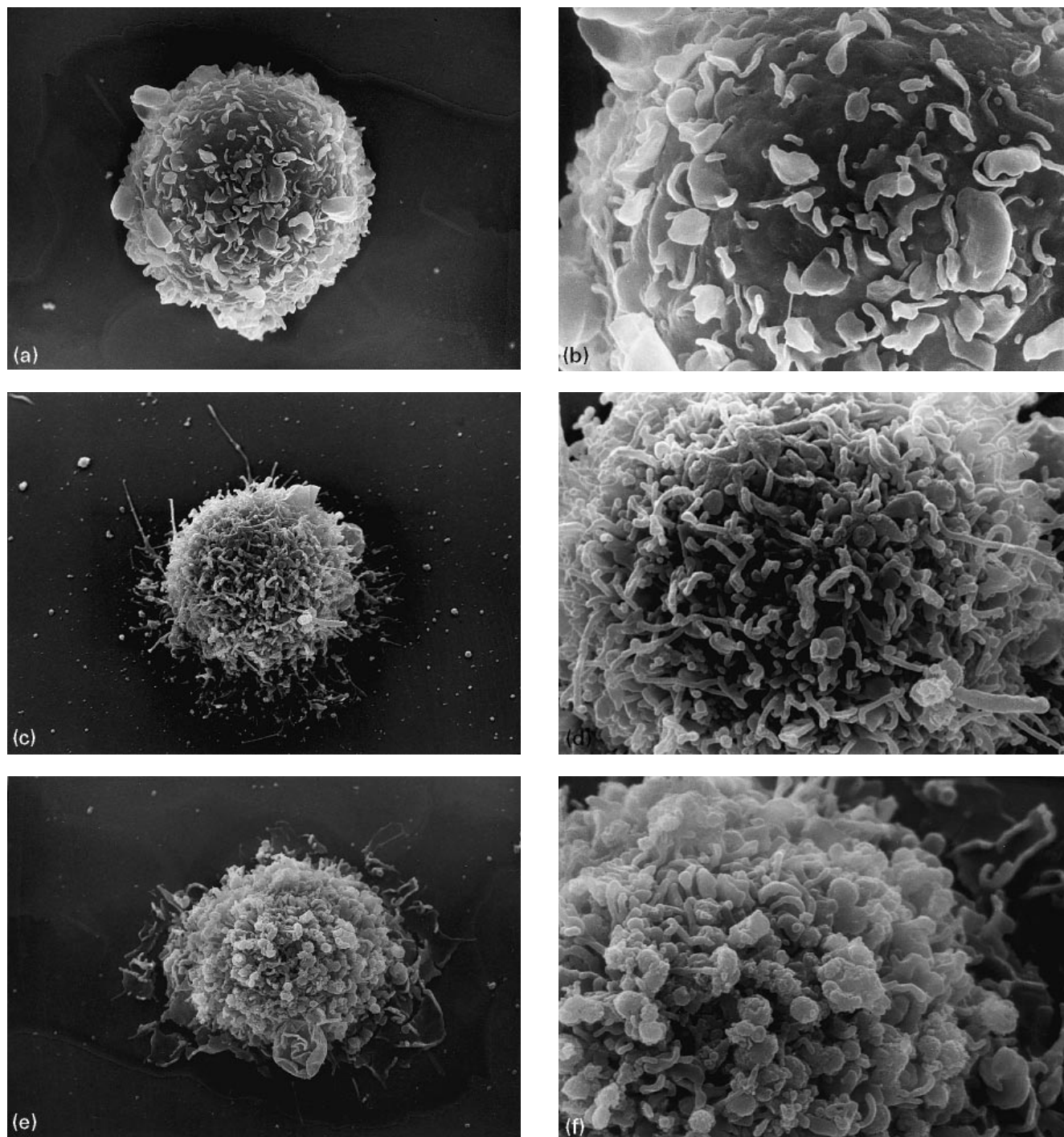


Fig. 2. Scanning electron micrographs of IgE-sensitized cultured human mast cells before anti-IgE challenge (a, b), 80 s (c, d) and 210 s (e, f) after 10 $\mu\text{g/mL}$ anti-IgE challenge. a, c, e, $\times 3500$. b, d, f, $\times 10\,000$. Very similar results were obtained in five micrographs at each time under the same conditions.

distributed membranous ridges and had larger pores, from which the extrusion of many bodies could be seen (Fig. 2 e,f).

Discussion

The process of mast cell degranulation by exocytosis has been studied in detail in rat peritoneal mast cells [1,2]. Tizard and Holmes [12] studied degranulation of rat mast cells by scanning electron microscopy, and concluded that both exocytosis and pore formation on the cell surface were significant mechanisms for the degranulation. These observations were confirmed by the studies at other laboratories [13,14]. On the other hand, isolated human mast cells from several organ sources, such as lung [5] and skin [6], have been used to examine a regulated secretion by transmission electron microscopy, and anaphylactic degranulation of these isolated human mast cells characteristically showed extrusion of altered, membrane-free granules through multiple pores in the plasma membrane to the exterior of the cell [5,6]. Dissociated human foreskin mast cells have been shown to degranulate in response to receptor-mediated stimuli [7]. These studies [5–7] show little artifact that might be related to the isolation procedure and the mast cells seem to be structurally and functionally intact, however, complicated processes are required to obtain human mast cells.

Recent advances of science led us to obtain cultured human mast cells raised from umbilical cord blood cells [8–10]. Their advantages are that relatively large numbers of the cells are accessible, and that alterations which might occur during the purification process can be avoided. However, cultured human mast cells are not just freshly isolated cells and are maintained in the presence of interleukin-6 and stem cell factor. So, it is necessary to examine whether these cells are useful in investigating the role of mast cells in human diseases. In fact, these cells are histamine-containing, IgE receptor-positive and metachromatic cells [8–10] and can be activated through IgE involvement [9,10]. Ultrastructural analysis of the cells revealed completely granulated, well-preserved mature human mast cells [8,10]. However, there have been no reports to examine the morphological changes of the activated-cultured human mast cells under phase-contrast microscopy using cinematographic techniques and scanning electron microscopy.

The present study was intended to clarify the morphological appearances of the degranulation process in the cultured human mast cells using an IgE-sensitized cell model. The IgE-sensitized cultured human mast cells released a significant amount of histamine by anti-IgE with morphologic changes in phase-contrast microscopy and scanning electron microscopy. Although we did not examine the effect of specific antigen, this reaction was

presumably mediated by IgE because no histamine release was observed without the preincubation with IgE (data not shown). On the basis of the published light and electron microscopic studies on rat mast cell activation [1,2,12], it seems likely that similar granule extrusions observed in the present phase-contrast microscopy studies using cinematographic techniques is indeed exocytosis. Scanning electron microscopic studies have confirmed this phenomenon and shown that IgE-mediated process we used in the present study yielded human mast cells with abundant exocytotic figures resembling those obtained from activated-rat mast cells [1,2,12–14], indicating that the cultured human mast cells may contribute a great deal to mast cell research, such as signal transduction and regulation of mediator release, and these studies would require sequential biochemical and detailed ultrastructural analysis.

In conclusion, we have found for the first time morphologically that IgE-sensitized cultured human mast cell are degranulated in the manner of exocytosis to release histamine by anti-IgE.

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