

IgE-mediated mast cell degranulation and recovery monitored by time-lapse photography

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Background: Mast cells are long-lived resident cells that are of great importance in an allergic reaction. It has previously been suggested that after IgE-mediated degranulation mast cells can undergo regranulation. Such a process is probably of great importance with respect to the severity and perpetuation of the allergic response.

Objective: Our purpose was to investigate whether mast cells recover from degranulation and whether they still have the potential to release a granule-associated mediator and upregulate certain cytokine genes.

Methods: Mouse mast cells were repeatedly activated by IgE and specific antigen with a 24-hour or 48-hour interval. During each of the 2 activation stages, release of β -hexosaminidase was measured by means of enzymatic colorimetric analysis, and IL-13 and IL-6 mRNA was detected by ribonuclease protection assay. Both scanning electron microscopy and time-lapse photography were used to reveal the process of mast cell recovery.

Results: We found that re-activation of degranulated mast cells in response to high-affinity IgE-receptor cross-linkage triggers β -hexosaminidase release and upregulation of IL-13 and IL-6 gene expression levels similar to what is seen in the initial activation. Scanning electron microscopy documented cells at various stages during the recovery process 30 minutes after the activation. With time-lapse photography, a single cell that had undergone degranulation could be visualized consecutively during its recovery process.

Conclusion: Mast cells can recover after an IgE-mediated activation and can repeatedly release β -hexosaminidase and express IL-6 and IL-13 mRNA after re-activation. (J Allergy Clin Immunol 2001;108:116-21.)

Key words: Allergy, cross-linkage, degranulation, Fc ϵ RI, IgE, mast cells, regranulation, time-lapse photography

Abbreviations used

BMCMC: Bone marrow-derived cultured mouse mast cell
Fc ϵ RI: High-affinity IgE-receptor
TNP: Trinitrophenyl

Mast cells synthesize and store inflammatory mediators in granules that are the source of anaphylactic degranulation, by which process mast cells function as major effector cells in IgE-mediated immune responses to allergen challenge. As a result of a typical degranulation process, mast cells release large numbers of secretory granules, which contain a variety of proinflammatory mediators, chemotactic factors, and immunoregulatory cytokines, within a couple of minutes. Extrusion of whole granules and smooth and round bodies, intracellular dissolution of the granule material,^{1,2} and shedding of large amounts of membranes, surface processes, and cytoplasmic fragments³ can be observed through ultrastructural studies during this process.

Mast cells are unique among hematopoietic cells in that they can undergo repeated rounds of degranulation and regranulation.^{1,3-5} For example, in cultured human lung mast cells, IgE-mediated activation triggers a dramatic anaphylactic degranulation process, which results in some small and immature mast cells.³ These small cells undergo morphologic changes accompanied by nuclear and cytoplasmic expansions, with the rapid development of synthetic structures such as Golgi apparatus and ribosomes. Eventually, these immature granule-containing cells further expand into mature mast cells.³

Although observations regarding mast cell degranulation and regranulation were reported decades ago, the actual recovering processes were mainly investigated ultrastructurally by means of transmission electron microscopy on isolated individual cells. The consecutive morphologic changes of the individual cell, as well as the cytokine transcript expression during degranulation and recovery processes, remain largely unclear. In this study, we reveal that after recovery, the upregulation kinetics of cytokines IL-13 and IL-6 by mast cells after a second anaphylactic activation is similar to that seen during the initial stimulation, thus suggesting recovery of the cell. We also show by means of time-lapse photography the consecutive morphologic events after IgE-mediated degranulation; the recovery of an individual cell is clearly indicated.

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METHODS

Mast cell cultures

Bone marrow-derived cultured mouse mast cells (BMCMCs) were obtained by culturing mouse (C57BL/6, 3 to 4 months old, Bomimice, Ry, Denmark) bone marrow cells for 6 to 8 weeks in 10% WEHI-3-enriched conditioned RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum (Gibco-BRL, Eggenstein, Germany), 4 mmol/L L-glutamine, 50 μ mol/L 2-mercaptoethanol, 1 mmol/L sodium pyruvate, 0.1 mmol/L non-essential amino acids, 10 mmol/L Hepes, and 100 μ g/mL penicillin/streptomycin (Sigma Chemical Co, St Louis, Mo). The growth factor-dependent mouse mast cell line MCP5/L⁶ was maintained in the same medium.

Activation of mast cells in vitro

MCP5/L cells or BMCMCs were washed and resuspended at 1×10^6 cells/mL and sensitized through use of a monoclonal murine IgE anti-trinitrophenyl (TNP) antibody (IgE1-b4, ATCC, Manassas, Va) (15% hybridoma supernatant) for 90 minutes. The cells were washed twice and then challenged with TNP-BSA at 1 μ g/mL (coupling ratio, 4.8 [kindly provided by Dr Birgitta Heyman, Uppsala]) or at 100 ng/mL (coupling ratio, 9 [Biosearch Technologies, Novato, Calif]) for periods as indicated. The antibody sensitization and antigen challenge were carried out in medium supplemented with 0.5% BSA (Sigma) at 37°C. Thirty minutes after the addition of TNP-BSA, samples were centrifuged at 4°C and supernatants were collected for further analysis of β -hexosaminidase release from activated mast cells.⁷ To measure total cellular β -hexosaminidase, cells were centrifuged and 10% of the supernatant was replaced with an equal volume of 1.2% Triton X-100 (Sigma) and mixed by pipetting. To investigate release after repeated activation, the cells were washed twice with PBS 6 hours after the initial IgE cross-linkage and maintained in normal culture medium, as described above. The previously stimulated cells were then split into control and activation groups, and the cells were treated in the same manner as they had been during the initial activation. The second activation was approximately 24 hours (for BMCMCs) and 48 hours (for MCP5/L) after the initial IgE-mediated activation.

Measurement of mediators

The granular enzyme β -hexosaminidase was detected by an enzymatic colorimetric assay, as described.⁸

Isolation of RNA and ribonuclease protection assay

Total cellular RNA was isolated through use of the TriPure isolation reagent (Boehringer Mannheim, Mannheim, Germany). Ribonuclease protection assay was performed with the mCK-1 multiprobe set from the RiboQuant System (PharMingen, San Diego, Calif) according to the protocol recommended by the supplier, as described.⁹

Scanning electron microscopy

Mast cells, resting or activated, were attached to cover glasses and fixed for 3 hours in 2.5% glutaraldehyde in 100-mmol/L phosphate buffer at pH 7.4. The specimens were rinsed 3 times by dipping the glasses in 100-mmol/L phosphate buffer. The specimens were dehydrated in a gradient series of ethanol and then critical-point dried. After drying, the specimens were mounted on stubs and coated with gold-palladium in a sputtercoater. The specimens were examined by means of a Philips XL 30 scanning electron microscope (Eindhoven, The Netherlands) at 20 kV.

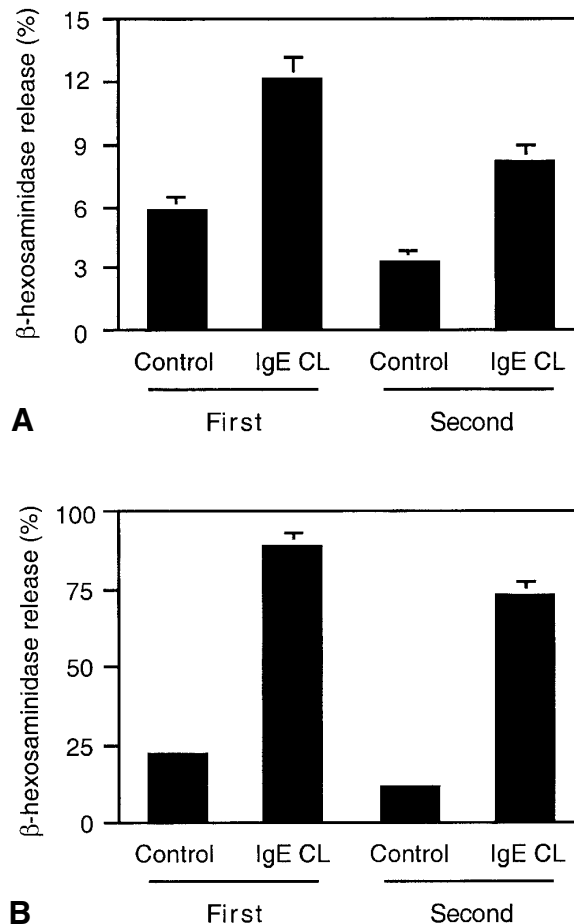


FIG 1. Mast cell activation measured by β -hexosaminidase release. MCP5/L cells (**A**) and BMCMCs (**B**) were activated by IgE-receptor cross-linkage (IgE CL), and β -hexosaminidase release for both resting control cells and activated cells was measured as a percent of the total. Forty-eight hours (MCP5/L) or 24 hours (BMCMCs) after the initial activation, cells were activated again and β -hexosaminidase release was measured.

Time-lapse photography

The video recording was performed basically as described previously.¹⁰ Cultured mast cells were transferred to an inert anodized aluminum chamber containing 0.45 mL of medium and covered with a glass plate. The chamber was transferred to a Labovet inverted microscope (Leitz, Wetzlar, Germany) with an interference lens giving a 10×25 -fold magnification at the ocular. The picture was further enlarged 3 times at the camera lens. The temperature inside the chamber was maintained at 37°C by circulating water. The image from a Sony 3CCD camera (Tokyo, Japan) with a resolution of 710 lines was captured by a PC with a 300-MHz Pentium II processor and a Perception video recorder card (DPS, Markham, Ontario, Canada). The time-lapse procedure was controlled by a computer-controlled device (Jorotech, Uppsala, Sweden), 1 frame being taken every second for 15 minutes. The same computer controlled an X-Y table so that cells in motion could be followed. The same cultures and preparations used in the video recording were fixed for electron microscopy.

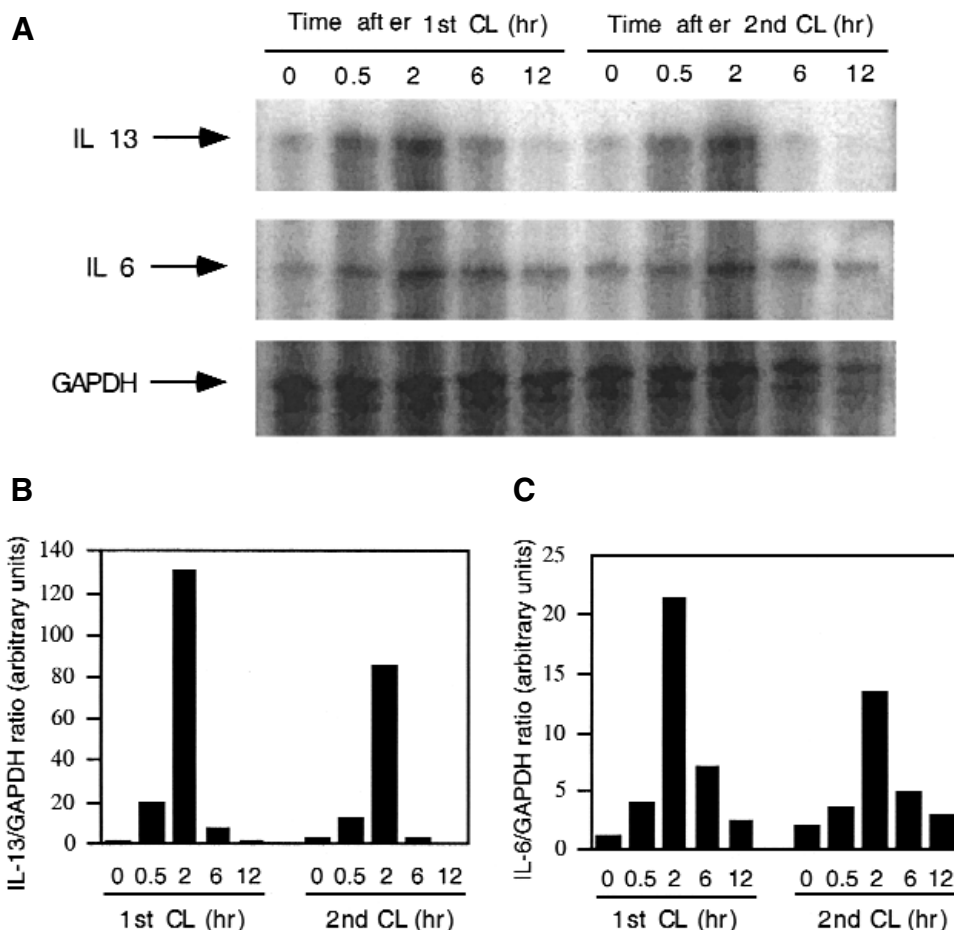


FIG 2. Effects of IgE-receptor cross-linkage (CL) on IL-13 and IL-6 transcript expression levels. **A**, Cells were activated through IgE-receptor cross-linkage at various time points, as indicated. Time 0 stands for control cells without cross-linkage. mRNA transcript levels were measured by ribonuclease protection assay. **B** and **C**, Phosphoimaging signals of IL-13 and IL-6 presented in Fig 2, **A** are normalized to the housekeeping glyceraldehyde-3-phosphate dehydrogenase gene by densitometric analysis.

RESULTS

Re-activation as evidenced by β -hexosaminidase release and cytokine transcript expression

Mast cells contain many preformed mediators that are released on IgE-mediated activation. Here we used β -hexosaminidase as an indicator of proper mast cell activation.⁷ For MCP5/L cells sensitized with specific IgE, addition of the antigen caused an increase of β -hexosaminidase release over time in comparison with the control. At 30 minutes after addition of the stimuli, release of β -hexosaminidase increased to 12.2%; this was in contrast to the spontaneous release of 5.9% (Fig 1, **A**). The cells that had been activated were washed and maintained under normal culture conditions. Forty-eight hours after the initial stimulation, these cells were activated again through cross-linkage of the high-affinity IgE-receptor (Fc ϵ RI). Like the initial stimulation, the second activation triggered the cells to increase release of β -hexosaminidase to 8.2% in comparison with 3.4% for the control (Fig 1, **A**).

MCP5/L is a mast cell line that actively proliferates in WEHI-conditioned medium, and a 2.8-fold increase in the number of cells was noted during the 48-hour interval between the 2 rounds of activation. To avoid the possibility that our data from the MCP5/L cells did not reflect recovery of the same cells as a result of proliferation, we used BMCMCs that maintained a constant cell number over the experimental period. As shown in Fig 1, **B**, a pronounced increase in β -hexosaminidase—from 22.3% to 89.3%—could be observed on activation. Re-activation after 24 hours stimulated the cells again, as evidenced by an increase in β -hexosaminidase release from 11.4% to 72.9%.

To further demonstrate that the cells could be repeatedly activated in the same manner, we performed an antigen dose response at 1, 10, 100, and 1000 ng/mL. The dose-response pattern for β -hexosaminidase release from BMCMCs was similar for the initial and second activations. An antigen concentration of 1 ng/mL gave a measurable release of β -hexosaminidase both at the initial activation and the second activation (33% and 27%,

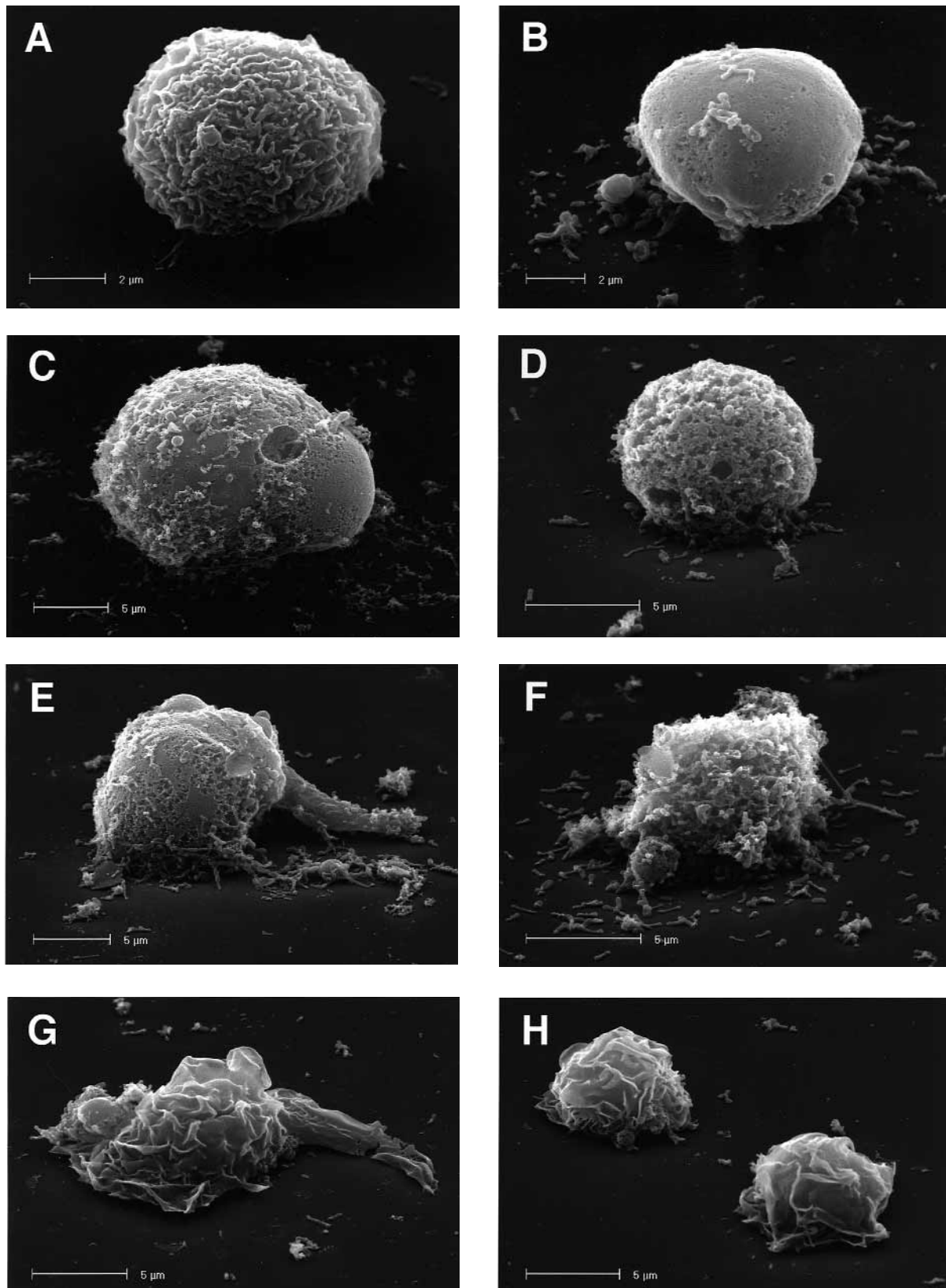


FIG 3. Scanning electron micrographs of control and IgE-receptor activated mouse MCP5/L mast cells. **A**, A resting cell. **B through H**, 30 minutes after FcεRI cross-linkage, cells with various morphologic appearances can be identified.

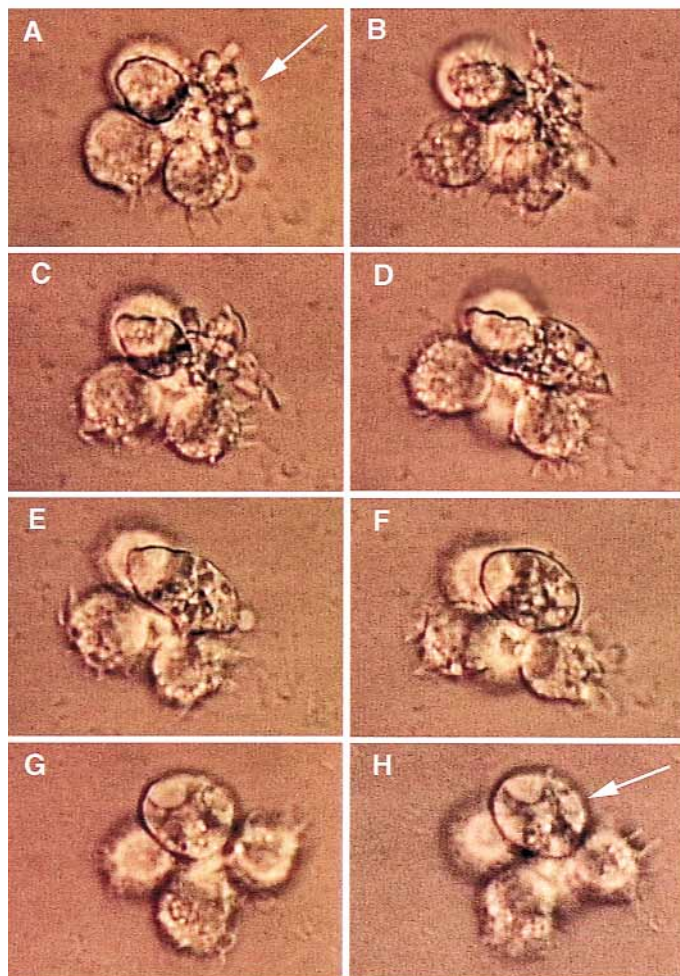


FIG 4. Mast cell degranulation and recovery visualized by means of time-lapse photography. Photos were taken in chronologic order (*A through H*) during 15 minutes. The activated cell is the cell on top of another cell with extensive membranous activity. *D*, The cell has formed an intact cell membrane. (To view a complete videographic file of this figure, go to the JACI website (www.mosby.com/jaci), access this article, and click on the video icon within the article.)

respectively). An optimal effect was achieved at 10 ng/mL with releases of 94% and 72%, respectively.

To assess the cytokine induction at gene transcript levels after mast cell recovery, we undertook to analyze IL-13 and IL-6 mRNA transcript expression during both the initial and the second IgE-mediated activations. IL-13 and IL-6 are well characterized cytokines that are upregulated at both the gene transcription level and the protein release level in mast cells after IgE-mediated activation.^{11,12} As previously described, we could detect IgE-dependent upregulation of steady-state levels of IL-13 and IL-6 transcripts in MCP5/L cells after FcεRI cross-linkage. The peak induction was observed at 2 hours after activation for both cytokines; the induction then returned to basal levels after 12 hours (Fig 2). Re-activation 48 hours after the initial activation led to upregulation of IL-13 and IL-6 transcripts (Fig 2), with exactly the same kinetics shown for the initial activation.

Scanning electron microscopy of activated mast cells

A resting mast cell exhibited a heterogeneous display of membranous ridges (Fig 3, *A*). When the cells are sensitized with IgE and exposed to specific antigen, they are not activated in a synchronized manner—ie, the amounts of time that it takes for the individual cells to undergo exocytosis and recovery differ. Therefore, 30 minutes after the addition of cross-linking stimuli we could identify cells at different degranulation and recovery stages (Fig 3, *B-H*). In Fig 3, *B*, a mast cell that had lost almost all of its surface protrusions is shown. These protrusions could contain its intracellular granules, inasmuch as it has been suggested that granule-plasma membrane fusions can be observed during exocytosis.¹³ In Fig 3, *H*, 2 cells that are probably close to the completion of recovery are shown; membranous ridges are present. Other photographs show intermediate stages of mast cell degranulation (Fig 3, *C-F*).

Time-lapse analysis of mast cell recovery

To confirm that a single cell can recover from degranulation, we used the technique of time-lapse photography to follow 1 cell after its activation by IgE cross-linkage. As shown in Fig 4, a degranulated cell with many membranous activities (the cell at 2 o'clock in Fig 4, *A* and *B*) becomes rounded and recovered. The process presented in Fig 4 took 15 minutes.

DISCUSSION

In this report, we have demonstrated that mast cells can repeatedly be activated to release β -hexosaminidase and express cytokines. Furthermore, for the first time we demonstrated, using time-lapse photography, mast cell degranulation and recovery processes monitored consecutively in the same cell. Through use of this technique we have provided a direct proof that a mast cell can recover after the dynamic process of degranulation; such a proof is not possible with the technique of ordinary electron microscopy.

Mast cells are generally regarded as important effector cells in inflammatory and allergic reactions, because inflammation is characterized mainly by an influx of leukocytes, edema, and higher permeability of the microvasculature; this reflects at least partially the release of chemical mediators derived from injured tissues in which mast cells usually accumulate as a result of active migration.^{14,15} To regulate such inflammatory processes, mast cells need to repeatedly release their cellular contents, and this cannot be achieved without recovery and regranulation. This process is thus one of the most important features that mast cells possess pertaining to the regulation of the allergic and inflammatory processes.

In this report, we examined β -hexosaminidase release as well as mRNA expression kinetics of IL-13 and IL-6 during the initial and second activations to indicate that mast cells really can upregulate the production of the aforementioned mediators during their degranulation, which can be triggered repeatedly after regranulation. Previously, it was reported that BMCMCs upregulate IL-6 transcript levels as well as increase IL-6 and IL-13 release after Fc ϵ RI cross-linkage.^{11,12} Here, we have shown that both IL-13 and IL-6 gene expression levels were upregulated in MCP5/L cells on repeated bouts of IgE-mediated activation. When cells are activated by IgE cross-linkage for a second time, both β -hexosaminidase release and the kinetics of IL-13 and IL-6 upregulation showed the same pattern as in the initial activation (Figs 1 and 2), suggesting that cells have undergone recovery and regranulation.

The mast cell line MCP5/L does not respond and release as readily as BMCMCs (Fig 1). One could argue that if only 12% of the β -hexosaminidase release is achieved at the initial activation of MCP5/L cells, it could be a different cell population that is activated in the second round. However, our data for BMCMCs strongly suggest that the same cell can be activated and release β -hexosaminidase repeatedly. Because BMCMCs are not undergoing mitosis and release approximately 75% to 80% on both rounds, it is most likely that the same cells are involved.

Mast cell degranulation is a tough and very dynamic process that causes extensive morphologic changes in the cell.^{3,4} The present study was intended to clarify whether an individual mast cell, after cross-linkage of the high affinity IgE-receptor, degranulates and then recovers. Previously published studies using light and electron microscopy have not been able to confirm this. By using time-lapse photography we were able to study an individual mast cell that had been activated and to document that this cell recovered afterwards.

In conclusion, we have provided evidence that a mast cell can be morphologically recovered, which is supported by protein release as well as kinetics of cytokine transcript expression. The capacity of mast cells to undergo several cycles of degranulation and regranulation is an important feature of these cells in the induction and perpetuation of an allergic reaction.

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REFERENCES

1. Kobayashi T, Asboe-Hansen G. Degranulation and regranulation of human mast cells. *Acta Derm Venerol* 1969;49:369-81.
2. Kurosawa M, Inamura H, Kanbe N, Igarashi Y, Tomita T, Takeda J, et al. Phase-contrast microscopic studies using cinematographic techniques and scanning electron microscopy on IgE-mediated degranulation of cultured human mast cells. *Clin Exp Allergy* 1998;28:1007-12.
3. Dvorak AM, Schleimer RP, Lichtenstein LM. Morphologic mast cell cycles. *Cell Immunol* 1987;105:199-204.
4. Dvorak AM, Schleimer RP, Schulman ES, Lichtenstein LM. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. In vitro studies with mast cells purified from human lungs. *Lab Invest* 1986;54:663-78.
5. Burwen SJ. Recycling of mast cells following degranulation in vitro: an ultrastructural study. *Tissue Cell* 1982;14:125-34.
6. Arora N, Min KU, Costa JJ, Rhim JS, Metcalfe DD. Immortalization of mouse bone marrow-derived mast cells with Ad12-SV40 virus. *Int Arch Allergy Immunol* 1993;100:319-27.
7. Schwartz LB, Austen KF, Wasserman SI. Immunologic release of beta-hexosaminidase and beta-glucuronidase from purified rat serosal mast cells. *J Immunol* 1979;123:1445-50.
8. Xiang Z, Nilsson G. IgE receptor-mediated release of nerve growth factor by mast cells. *Clin Exp Allergy* 2000;30:1379-86.
9. Juremalm M, Hjertson M, Olsson N, Harvima I, Nilsson K, Nilsson G. The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1 α acts as a mast cell chemotaxin. *Eur J Immunol* 2000;30:3614-22.
10. Lofman C, Rigo R, Block M, Hulten K, Enroth H, Engstrand L. Bacterium-host interactions monitored by time-lapse photography. *Nat Med* 1997;3:930-1.
11. Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995;181:1373-80.
12. Gagari E, Tsai M, Lantz CS, Fox LG, Galli SJ. Differential release of mast cell interleukin-6 via c-kit. *Blood* 1997;89:2654-63.
13. Guo Z, Turner C, Castle D. Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells. *Cell* 1998;94:537-48.
14. Nilsson G, Costa JJ, Metcalfe DD. Mast cells and basophils. In: Gallin JJ, Snyderman R, editors. *Inflammation: basic principles and clinical correlates*. Philadelphia: Lippincott-Raven; 1999. p. 97-117.
15. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000;105:847-59.