Human eosinophils induce histamine release from antigen-activated rat peritoneal mast cells: A possible role for mast cells in late-phase allergic reactions

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Background: Mast cells and eosinophils are believed to interact during the late and the chronic stages of allergic inflammation.
Objective: In this study we investigated whether eosinophils can cause activation and consequent histamine release of already challenged mast cells, a situation likely to take place during the allergic late-phase reaction.
Methods: Rat peritoneal mast cells presensitized with IgE anti-dinitrophenol-human serum albumin and challenged by dinitrophenol-human serum albumin or compound 48/80 were incubated with either eosinophil sonicate or major basic protein (MBP). Eosinophils were purified from the peripheral (>98%) blood of mildly allergic patients. Heparin and pertussis toxin and different extracellular Ca^{2+} concentrations were used to modulate mast cell reactivation by MBP. Histamine release was assessed as a marker of mast cell activation.
Results: IgE-challenged mast cells were sensitive to reactivation induced by eosinophil sonicate and MBP. Reactivation was not cytotoxic for the mast cells. Mast cells previously challenged with compound 48/80 did not respond to subsequent MBP activation. Furthermore, heparin and pertussis toxin both inhibited mast cell reactivation induced by MBP. The ability of eosinophil sonicate and MBP to activate mast cells was not significantly affected at the different Ca^{2+} concentrations.
Conclusions: In summary, we have shown a direct activating activity of eosinophils, partially due to MBP, toward IgE-challenged and immunologically desensitized mast cells. This suggests that in vivo mast cells can be reactivated during a late-phase reaction to release histamine by a non–IgE-dependent mechanism. (J Allergy Clin Immunol 2001;107:993-1000.)

Key words: *Rat peritoneal mast cells, eosinophils, major basic protein, IgE-dependent activation, IgE-independent activation, histamine, heparin, pertussis toxin*

Abbreviations used
DMEM: Dulbecco modified Eagle medium
DNP-HSA: Dinitrophenol-human serum albumin
ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
MBP: Major basic protein
PAF: Platelet-activating factor
Ptx: Pertussis toxin
Tg: Tyrode's buffer containing 0.1% gelatin

Mast cells play a main role in the early stages of type I hypersensitivity reactions, in which their activation is triggered by the cross-linking of IgE antibodies by the antigen. Mast cell degranulation also induces the onset of a second late-phase reaction a few hours later,[1] characterized by tissue infiltration and activation of various inflammatory cells such as neutrophils, macrophages, lymphocytes, and eosinophils. Although the role of these inflammatory cells during the late and chronic phases of the allergic process has been studied extensively, the possibility that activated mast cells still play an important role in these phases by its interaction with the infiltrating cells has not yet been considered. We as well as other groups have recently become interested in the role that mast cells might have in these later stages of allergic inflammation.[2-4] Some evidence would suggest that a cross-talk occurs between mast cells and eosinophils that infiltrate the tissue in increased numbers during the late-phase reaction. For example, eosinophils are activated by cytokines produced by lung mast cells,[4] and their in vitro survival is enhanced by mast cell–derived TNF-α.[5] Eosinophils during the late-phase reaction are likely to encounter mostly allergen-activated mast cells that might be desensitized to allergen. In fact, we have shown that rat peritoneal mast cells, activated by an IgE-dependent mechanism, are partially desensitized to a similar reactivation for a few hours. However, they still release residual histamine when subsequently exposed to a non–IgE-dependent activator such as compound 48/80 or substance P.[6] In addition, it is known that incubation of rat peritoneal mast cells with native major basic protein (MBP) and eosinophil cationic protein (ECP), but not eosinophil-derived neurotoxin (EDN), results in a concentration-dependent histamine release that requires both Ca^{2+} and metabolic energy.[7] Altogether this suggests that antigen-

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desensitized mast cells can react to nonimmunologic secretagogues, and consequently they might respond to basic proteins released by activated eosinophils.

Therefore the aims of this study were to investigate whether human peripheral blood eosinophils can induce resting or IgE-activated rat peritoneal mast cells to release histamine and also to partially characterize the signal transduction pathway of this event.

METHODS

Rat peritoneal mast cell/3T3 cocultures

Rat peritoneal mast cells were cocultured with 3T3 fibroblasts at 37°C in a humidified atmosphere of 5% CO_2 to ensure optimal conditions of survival and functional activity of the mast cells.[8] The rats were cared for according to the Guidelines of the Animal Committee of the Hebrew University of Jerusalem, Israel. Highly purified rat peritoneal mast cells (metrizamide gradient, 22.5%, >96%) were seeded on confluent monolayers of the Swiss albino mouse embryonic 3T3 fibroblast cell line (American Type Culture Collection, Rockville, Md) in 12 wells at a density of 6×10^4/0.5 mL in Dulbecco modified Eagle medium (DMEM) with heat-inactivated FCS (10% vol/vol) (56°C, 30 minutes), penicillin (100 U/mL), streptomycin (100 μg/mL), and L-glutamine (2 mmol/L) (DMEM+; Biological Industries, Beith Haemek, Israel).

Isolation of human peripheral blood eosinophils

Eosinophils were purified as previously described[9] from the peripheral blood of human volunteers, normal or mildly allergic, who were not taking any oral treatment for their condition and whose blood eosinophilia ranged from 4% to 10%. Written informed consent was obtained from all volunteers according to the guidelines established by the Hadassah–Hebrew University Human Experimentation Helsinki Committee, Jerusalem, Israel. Eosinophils were collected at a purity of 97% to 100%, as assessed by Kimura staining, and at a viability of 99%, as assessed by trypan blue staining.

Eosinophil sonicate was obtained by continuous sonication of 1×10^6 eosinophils/100 μL DMEM for 3 minutes in ice (W-380 Sonicator, 50% duty cycle, output 5; Heat System Ultrasonics, Misonix Inc, Farmingdale, NY). The sonicate was microcentrifuged (12,000*g,* 7 minutes, 4°C). Debris-free supernatants were collected and stored in aliquots at –70°C until being used.

To activate eosinophils, we incubated the cells (2×10^6 eosinophils/500 μL) with platelet-activating factor (PAF) (1×10^{-6} mol/L) for 1 hour in enriched medium (RPMI 1640 containing 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine) at 37°C in a humidified atmosphere of 5% CO_2. After centrifugation (150*g,* 5 minutes, 4°C) supernatants were stored in aliquots at –70°C until being used.

Purification of human eosinophil granule proteins

MBP, ECP, and EDN were purified from eosinophils obtained from patients with marked eosinophilia as described.[10,11] The purified proteins were stored at –70°C, and samples were thawed immediately before use. Each of the proteins was judged pure by its banding pattern on SDS-PAGE after staining with Coomassie brilliant blue R. Protein concentrations were determined with the appropriate E277 values.[12-14]

Rat peritoneal mast cell activation

Rat peritoneal mast cell cocultures were passively sensitized by incubation with mouse monoclonal anti-dinitrophenol-human serum albumin (DNP-HSA) IgE antibodies (10 μg/mL) (kindly donated by Dr T. Ishizaka, Japan) in DMEM+, for 18 hours at 37°C to saturate IgE receptors. Sensitized cultures were washed twice with 1 mL DMEM+. Thereafter rat peritoneal mast cells were incubated for 30 minutes with one of the following: DNP-HSA (10 ng/mL), compound 48/80 (3 μg/mL; Sigma Chemical Co, Jerusalem, Israel), eosinophil sonicate (mast cell/eosinophil ratios = 1:5, 1:10, 1:20, and 1:40), MBP (3×10^{-6} mol/L), EDN (1×10^{-6} mol/L), ECP (5×10^{-7} mol/L) in Tyrode's buffer containing 0.1% gelatin (Tg), $CaCl_2$ (1.8 mmol/L), and $MgCl_2$ (0.9 mmol/L) (Tg^{++}) at 37°C in humidified atmosphere containing 5% CO_2. In a few experiments, rat peritoneal mast cells were incubated with 3T3 fibroblast sonicate (mast cell/fibroblast ratio = 1:5) as control or with supernatant of nonactivated and PAF-activated eosinophils (2×10^6/0.5 mL), or PAF (1×10^{-6} mol/L). To assess the effect of increasing concentrations of extracellular Ca^{2+} on mast cell activation by eosinophil sonicate (mast cell/eosinophil ratio = 1:5), MBP (3×10^{-6} mol/L), and compound 48/80 (3 μg/mL), mast cell stimulation was carried out in Ca^{2+}-free buffer and buffer containing Ca^{2+} (0.18, 1.8, and 18 mmol/L).

Reactivation of rat peritoneal mast cells

Rat peritoneal mast cell cultures were passively sensitized as described above and, after 2 washings with 1 mL DMEM+, were incubated with either DNP-HSA (10 ng/mL) or compound 48/80 (3 μg/mL) in 0.5 mL Tg^{++} for 30 minutes at 37°C (first activation). After 30 minutes, the medium was removed by aspiration, and 0.5 mL Tg^{++} was added to the cocultures. Three hours later, the cultures were reactivated with DNP-HSA (10 ng/mL), compound 48/80 (3 μg/mL), eosinophil sonicate (mast cell/eosinophil ratio = 1:5; this ratio was found optimal in the reactivation experiments), or MBP (3×10^{-6} mol/L), or they were reactivated with heparin (100 μU/mL, Sigma) and eosinophil sonicate or MBP in 0.5 mL Tg^{++} for 30 minutes at 37°C (second activation). In some experiments to assess the effects of pertussis toxin (Ptx) in mast cell activation, cultures were incubated after the first activation with Tg^{++} containing Ptx (1 μg/mL, Sigma) and then reactivated as described.

Confocal microscopy experiments for intracellular Ca^{2+} detection

Freshly isolated rat peritoneal mast cells (1×10^5 cells) were adhered to the surface of 35-mm petri dishes that were previously coated with human fibronectin (5 μg/mL) (Biological Industries). Rat peritoneal mast cell adhesion was completed after 3 hours of incubation at 37°C in DMEM+. A hole (1.5 cm diameter) in the plastic plate was closed at one side by a glass coverslip, and this allowed the confocal imaging of living rat peritoneal mast cells on an inverted microscope at physiologic temperature. After 2 washings with DMEM+, rat peritoneal mast cells were labeled with Fluo-3AM (4 μmol/L) (Molecular Probes, Eugene, Ore). Before imaging, the cells were preincubated for 15 minutes at 37°C in Tg containing EDTA (4 mmol/L) or Tg^{++}. Changes in intracellular Ca^{2+} were observed with a Zeiss LSM 410 confocal laser scanning system attached to the Zeiss Axiovert 135M inverted microscope with 63x/1.2. C-Apochromat water immersion lens was utilized. The system was equipped with a 25-mW air-cooled argon laser (488-nm excitation line with 515-nm long-pass barrier filter) for the excitation of green fluorescence. In some experiments, differential interference contrast images according to Nomarski were collected with a transmitted light detector. Confocal images were taken at 2-second intervals after addition of compound 48/80 (3 μg/mL), eosinophil sonicate (mast cell/eosinophil ratio = 1:5), or MBP (3×10^{-6} mol/L) in Tg containing EDTA (4 mmol/L) or Tg^{++}. Confocal images were converted to TIF format and transferred to a Zeiss imaging workstation for pseudocolor representation. Brightness and contrast level were adjusted with the Zeiss and Adobe Photoshop 3.0 programs.

Histamine assay

Histamine was measured in the culture supernatants and in the mast cells after the cells were scraped from the plates, resuspended in 0.5 mL Tg^{++}, and sonicated for 1 minute. Histamine was determined by a radioenzymatic assay with ^{14}C methyl-S-adenosyl-L-methionine and crude preparation of rat kidney histamine methyl transferase.[15] Histamine was calculated as a percentage as follows:

$$\% \text{ Release} = S/(S + P) \times 100,$$

where *S* is the content of histamine in the supernatant and *P* is the content of histamine in the cells. This percentage was always assessed on the samples at the indicated times and calculated consequently.

Detection of rat peritoneal mast cell viability

Rat peritoneal mast cell viability was assessed by the Trypan blue exclusion test. Rat peritoneal mast cell cocultures were examined blindly 10 minutes, 60 minutes, and 24 hours after incubation with the different activators immediately after addition of 0.1 mL trypan blue (0.4%) (Sigma). The percentage of viable cells was calculated as follows:

$$\% \text{ Viable rat peritoneal mast cells} = (\text{No. of trypan blue–negative cells/No. of total cells}) \times 100.$$

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed by the Student paired *t* test. A *P* value of $< .05$ was considered statistically significant.

RESULTS

Eosinophils and MBP activate antigen-challenged mast cells

We evaluated whether mast cells were susceptible to activation by eosinophils after an IgE-dependent challenge. Rat peritoneal mast cells cocultured with 3T3 fibroblasts were activated by DNP-HSA after presensitization with IgE anti-DNP. Three hours later, to mimic a time point at which eosinophils accumulate after mast cell activation,[12] the cultures were incubated with eosinophil sonicate (mast cell/eosinophil ratio = 1:5). Mast cells activated at time 0 by the antigen released 65.5% ± 7.3% histamine (n = 5) (Fig 1, *A*). Three hours later, the same cell population still released a significant percentage of histamine when incubated with eosinophil sonicate 57.4% ± 12.8% ($P < .02$). In contrast, these mast cells were significantly desensitized to a second activation by the antigen and released only 29.8% ± 4.9% histamine ($P < .01$). Cultures incubated first with buffer and 3 hours later with eosinophil sonicate released 37.5% ± 4.6% histamine.

Eosinophil sonicate also activated unchallenged mast cells, causing a maximal stimulating activity at the mast cell/eosinophil ratio of 1:20 (16.5% ± 3.2% for mast cells incubated with eosinophil sonicate vs 3.6% ± 1.6% for mast cells incubated in buffer; $P < .05$) (n = 4). A further increase of eosinophils (1:40), did not result in a significant increase in histamine release. Similarly, eosinophil supernatant activated by an optimal concentration of PAF (1×10^{-6} mol/L) for 60 minutes induced 24.5% ± 8.9% (n = 5) histamine release from unchallenged mast cells (9.5% ± 3.4% histamine release from mast cells incubated with 1×10^{-6} mol/L PAF).

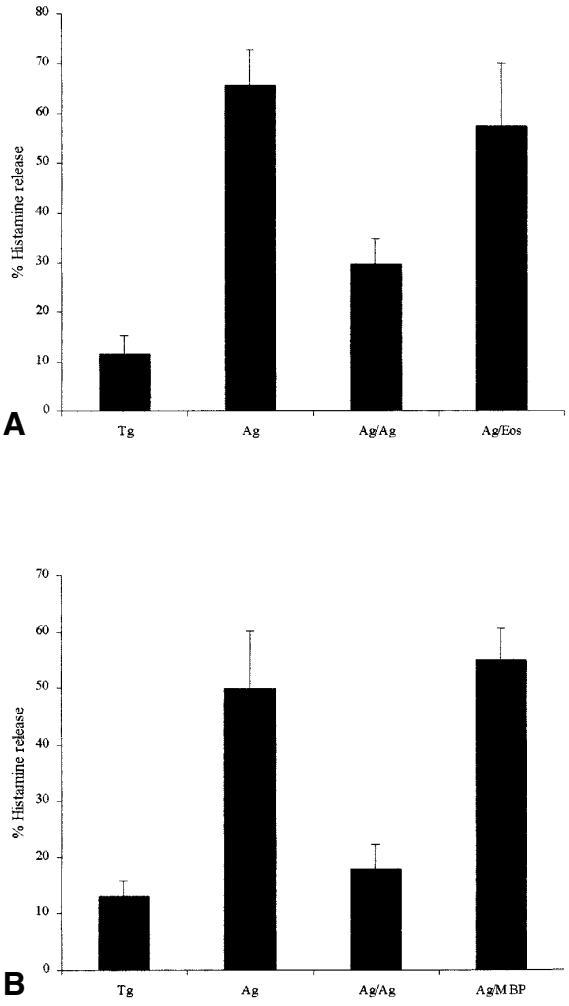

FIG 1. Eosinophil sonicate and MBP activate rat peritoneal mast cells previously challenged with antigen. Rat peritoneal mast cells, sensitized with IgE anti-DNP-HSA antibodies, were incubated (30 minutes) with either Tg^{++} alone *(Tg)* or DNP-HSA *(Ag)*. Three hours later, the cultures previously incubated with antigen were incubated with either DNP-HSA *(Ag/Ag)* or eosinophil sonicate *(Ag/Eos)* **(A)** or MBP *(Ag/MBP)* **(B).** Data are the mean ± SEM of 5 **(A)** and 6 **(B)** experiments performed in quadruplicate.

It has been previously demonstrated that MBP and ECP, but not EDN, stimulate histamine release from naive rat peritoneal mast cells.[7] We reconfirmed these results, obtaining 39.5% ± 5.8% and 33.6% ± 2.4% (n = 3) histamine release from mast cells incubated with optimal concentrations of MBP (3×10^{-6} mol/L) and ECP (1×10^{-6} mol/L), respectively. EDN did not induce a significant histamine release. Therefore in subsequent experiments MBP was used to stimulate previously immunologically challenged mast cells. As shown in Fig 1, *B,* mast cells, stimulated at time 0 by the antigen (49.9% ± 10.3% histamine release, n = 6), were reactivated by MBP 3 hours later to release 54.9% ± 5.6% his-

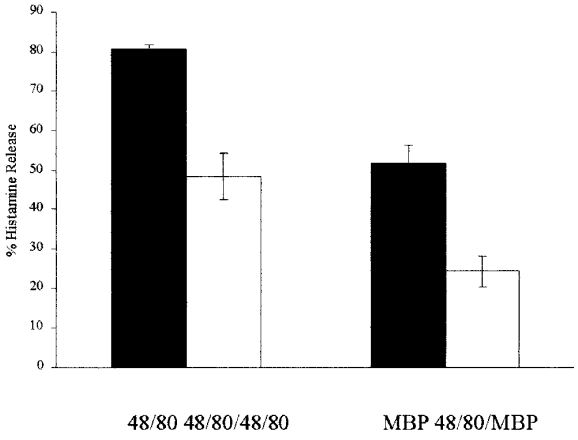

FIG 2. Rat peritoneal mast cells stimulated by compound 48/80 are desensitized to reactivation by MBP. Rat peritoneal mast cells were incubated (30 minutes) with either Tg^{++} alone or compound 48/80 *(48/80)*. Three hours later, the cultures previously incubated with compound 48/80 were incubated with either compound 48/80 *(48/80/48/80)* or MBP *(48/80/MBP)*. Data are the mean ± SEM of 3 experiments performed in quadruplicate.

tamine ($P < .0005$), and these mast cells were partially desensitized to a second activation by antigen (17.9% ± 4.4% histamine release). Cultures incubated first with buffer and 3 hours later with MBP released 47.5% ± 5.5% histamine (n = 6).

Effect of mast cell reactivation by eosinophil sonicate and by MBP on mast cell viability

Because high concentrations of MBP and ECP have been shown to be cytotoxic for some transformed but also for some normal mammalian cells,[16-18] the viability of mast cells incubated with eosinophil sonicate or MPB was evaluated. Viability of naive and rechallenged mast cells 30 minutes, 60 minutes, and 24 hours after their incubation with eosinophil sonicate or MBP was always greater than 85% and similar to the viability of cultures incubated in buffer alone (n = 3).

Mast cell reactivation by MBP has characteristics similar to those of compound 48/80 reactivation

To investigate whether MBP effects on rat peritoneal mast cell histamine release are similar to those induced by compound 48/80, a well-characterized mast cell activator,[19] we performed cross-desensitization experiments. Mast cells previously exposed to compound 48/80 were incubated 3 hours later with MBP. As shown in Fig 2, mast cells stimulated at time 0 by compound 48/80 released 80.5% ± 1.9% histamine ($P < .005$, n = 3). Three hours later this mast cell population was significantly desensitized to a second activation by MBP (24.3% ± 3.8% histamine release, $P < .005$). Similarly, mast cells were desensitized to a second activation by compound 48/80 (48.4% ± 4.5% histamine release, $P < .01$) (n = 4).

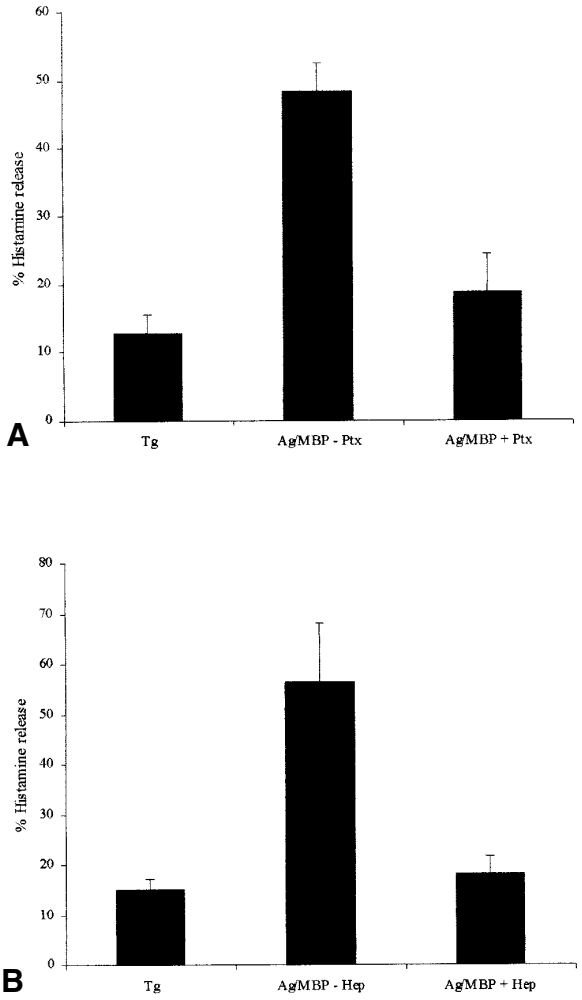

FIG 3. Ptx and heparin inhibit rat peritoneal mast cell activation and reactivation induced by MBP. Rat peritoneal mast cells sensitized with IgE anti-DNP-HSA antibodies were incubated (30 minutes) with either Tg^{++} or DNP-HSA. After this first activation, supernatants were removed, and Ptx *(+ Ptx)* or Tg^{++} *(– Ptx)* was added to the cultures **(A)**. Three hours later, the cultures previously incubated with antigen were incubated with MBP *(Ag/MBP)*. In another set of experiments, the cultures previously incubated with antigen were incubated with either MBP *(Ag/MBP – Hep)* or MBP and heparin *(Ag/MBP + Hep)* **(B)**. Data are the mean ± SEM of 3 experiments performed in quadruplicate.

Ptx and heparin inhibit mast cell reactivation induced by MBP

Because compound 48/80 causes histamine release from mast cells through interaction with Ptx-sensitive G_i proteins,[20] we examined whether preincubation with this toxin would inhibit mast cell reactivation induced by MBP. Preincubation with Ptx inhibited histamine release induced by MBP after activation with the antigen (Fig 3, *A*). Similarly, mast cell reactivation with compound 48/80 after immunologic challenge was also partially inhibited by Ptx (88.0% of inhibition histamine release, $P < .05$) (n = 2) (data not shown).

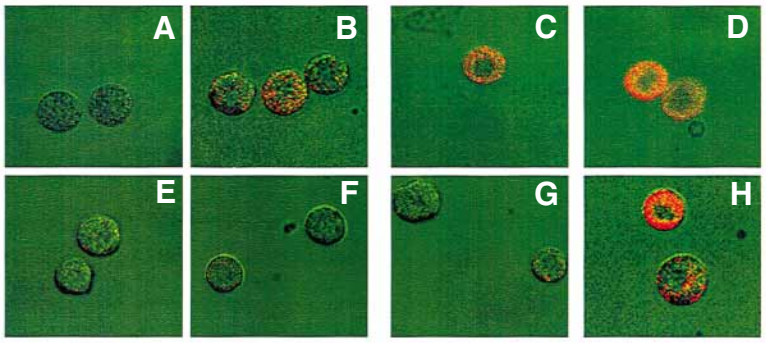

FIG 4. Confocal images of rat peritoneal mast cells stimulated by compound 48/80, eosinophil sonicate, and MBP in Ca^{2+}-free buffer and in buffer containing Ca^{2+}. Rat peritoneal mast cells were incubated with buffer compound 48/80, eosinophil sonicate, or MBP in Ca^{2+}-free buffer (**A, B, C,** and **D,** respectively) and with the same activators in buffer containing 1.8 mmol/L Ca^{2+} (**E, F, G,** and **H,** respectively). The mast cells were observed 15 minutes after stimulation at 10-second intervals. Intracellular Ca^{2+} release is observed as an increase in orange fluorescence after mast cell activation in comparison with nonactivated cells *(green).*

It has been also shown that heparin is able to inhibit histamine release from rat peritoneal mast cells induced by compound 48/80 and by the polyethyleneimine PEI6.[21-24] Therefore we evaluated whether heparin could inhibit rat peritoneal mast cell reactivation induced by MBP. For this purpose MBP was preincubated with heparin and then added to IgE-challenged mast cells. The reactivation of immunologically activated mast cells by MBP was significantly inhibited by the presence of heparin (93.0% inhibition, $P < .05$) (Fig 3, *B*). Heparin added to eosinophil sonicate inhibited its histamine-releasing activity but to a lesser degree than that observed with MBP (37.4% inhibition, n = 3). Addition of heparin alone to mast cells did not affect their basal histamine release (data not shown).

Rat peritoneal mast cell activation by eosinophil sonicate and by MBP is independent on extracellular Ca^{2+} concentration

In the next set of experiments, the effect of extracellular Ca^{2+} on the activation of mast cells by eosinophil sonicate or by MBP was evaluated. In Ca^{2+}-free buffer, eosinophil sonicate (mast cell:eosinophil ratio = 1:5) and MBP (3×10^{-6} mol/L) were slightly more activated, releasing 27.5% ± 7.2% and 40.8% ± 6.9% histamine, respectively, than in buffer containing higher amounts of Ca^{2+}. In fact, at 1.8 mmol/L Ca^{2+} their histamine-releasing capacities actually decreased slightly (although not significantly) to 24.3% and to 38.2%, respectively. Histamine release from mast cells incubated with compound 48/80 similarly diminished from 80.1% ± 1.6% in Ca^{2+}-free buffer to 34.5% ± 3.5% in buffer containing 18 mmol/L Ca^{2+}.

By confocal microscopy, mast cells loaded with Fluo-3AM showed an increase in cytosolic Ca^{2+} after addition of compound 48/80, eosinophil sonicate, or MBP in Ca^{2+}-free buffer (Fig 4, *B, C,* and *D,* respectively). Almost no fluorescence was obtained when mast cells were incubated in buffer containing 1.8 mmol/L Ca^{2+} with compound 48/80 or eosinophil sonicate (Fig 4, *F* and *G*, respectively). Incubation of the mast cells with MBP in Ca^{2+}-free buffer or in buffer containing Ca^{2+} revealed similar increases in intracellular Ca^{2+} (Fig 4, *D* and *H*).

DISCUSSION

In this study we have shown that human peripheral blood eosinophil sonicates and MBP activate immunologically challenged and IgE-desensitized rat peritoneal mast cells to release histamine. Mast cell reactivation is induced through G-Ptx–sensitive proteins in an extracellular Ca^{2+}-independent fashion.

In allergic responses, mast cell activation by antigen cross-linking of IgE bound to FcεRI results in the release of preformed and newly synthesized mediators and the clinical symptoms of the early phase of allergy.[25] A few hours later, a late-phase reaction takes place. In asthma, this results in bronchial hyperresponsiveness and in an increase in its severity.[26] The late-phase reaction and chronic allergic inflammation are characterized by infiltration of eosinophils and $CD4^+$ T cells.[27] Nevertheless, the presence of eosinophils has been recognized as the critical element inducing the inflammatory process and causing tissue damage.[28] During these stages, mast cell–eosinophil interactions are likely to take place, and they would amplify the local allergic inflammation. Mast cells, for example, by synthesizing and releasing the eosinophilotrophic cytokines IL-5, GM-CSF, and TNF-α, promote eosinophil recruitment, activation, and survival.[4,29] In addition to their contribution to the onset of the late phase, mast cells can also affect late and chronic stages by releasing histamine and/or other preformed and newly produced mediators.[30] Indeed, mast cells have been found degranulated in the airways of patients with chronic asthma, as shown directly by electron microscopy and by immunocytochemistry.[31] In addition, increased levels of tryptase, histamine, and prostaglandin D_2 have been found in this condition in the bronchoalveolar lavage fluid.[32]

In this study, to assess whether mast cells can be activated by eosinophils, we used rat peritoneal mast cells as a well-characterized source of highly purifiable mast cells. Rat peritoneal mast cells were cocultured with 3T3 fibroblasts to ensure optimal conditions of survival and functional activity of the mast cells.[8] Because in the late phase and chronic stage of allergic inflammation, eosinophils encounter a majority of previously IgE-activated mast cells, we wondered whether these mast cells are able to be reactivated by eosinophils to release the residual histamine. In fact, we had previously shown that rat peritoneal mast cells activated by IgE-dependent mechanisms are partially desensitized to similar activation for 3 hours.[6] Therefore eosinophil sonicate was added to rat peritoneal mast cells previously activated by the antigen, with a resting time of 3 hours between both activations to mimic the lapse of time between the early and the late phases of an allergic response. Eosinophil sonicate was able to induce histamine release from previously activated mast cells in a significant fashion. To the best of our knowledge, this is the first in vitro report of direct activating activity of eosinophils on immunologically challenged mast cells. Our data would suggest therefore that histamine detected during a late-phase allergic reaction can also be derived from mast cells and not only from basophils, as proposed by several authors.[33,34] Fibroblasts are known to produce a number of cytokines that can modify the response of mast cells (ie, stem cell factor and nerve growth factor). However, in our system fibroblasts were incubated with eosinophils for 30 minutes. This lapse of time is probably not enough to induce fibroblasts to synthesize and secrete cytokines that can either directly stimulate mast cells or prime them to secrete histamine. Furthermore, mast cells incubated with fibroblast sonicate did not release significant amounts of histamine in comparison with mast cells incubated with medium alone (Piliponsky AM and Levi-Schaffer F, 1998). Therefore it can be inferred that the activation of mast cells by eosinophils is specific and not mediated or modulated by mediators from stimulated fibroblasts.

Eosinophil sonicate and supernatants from PAF-activated eosinophils were also able to activate the rat mast cells, demonstrating that the responsible mediator or mediators are indeed preformed and quickly released. Because rat peritoneal mast cells were desensitized to the IgE-dependent reactivation but not to the one mediated by eosinophils, we hypothesized that the eosinophil mediator or mediators involved have a signaling pathway different from that of IgE-dependent stimulation. We considered as a relevant eosinophil mediator one of the ECPs that was present in the eosinophil granules. In previous studies the effect of the 4 eosinophil basic proteins—MBP, ECP, eosinophil peroxidase, and EDN—on histamine release from unchallenged rat and human mast cells has been analyzed.[7,35,36] MBP and ECP were found to cause histamine release from rat peritoneal mast cells and more recently demonstrated to activate human heart mast cells as well.[7,35] We reconfirmed the activating properties of MBP and ECP on rat peritoneal mast cells and found that MBP also reactivated IgE-desensitized mast cells to release histamine similarly to eosinophil sonicate, by a noncytotoxic event.

Interestingly, we found that IgE-challenged mast cells, incubated with either eosinophil sonicate or MBP, released higher percentages of histamine in comparison with naive mast cells. A possible explanation for what we found is that IgE-mediated activation of mast cells triggers the upregulation of signal transduction mechanisms involved in mast cell activation by nonimmunologic secretagogues. Indeed, our observation seems to be in agreement, for example, with the enhanced releasability of mast cells to substance P in patients with atopy and asthma in comparison with subjects without atopy or asthma.[37]

Some of the characteristics of mast cell activation mediated by eosinophils and that mediated by MBP can be similar to those produced by other polycationic compounds such as compound 48/80, substance P, Mastoparan, and bradykinin.[20] We decided to further analyze this point, especially considering that rat peritoneal mast cells become unresponsive to a second activation by compound 48/80.[38] Therefore we evaluated whether rat peritoneal mast cells already activated by compound 48/80 were desensitized to reactivation by MBP. Indeed, compound 48/80–activated mast cells were desensitized to a second activation by MBP. This result suggests that MBP shares with compound 48/80 the signal-transduction mechanism involved in mast cell activation. Pretreatment of rat peritoneal mast cells with Ptx is known to inhibit histamine release induced by compound 48/80,[39] an effect that is due to the interaction of compound 48/80 with Ptx-sensitive G_i proteins, particularly G_{i3}.[40,41] We found that Ptx significantly inhibited MBP-mediated rat peritoneal mast cell activation and reactivation, demonstrating that the Ptx-sensitive G proteins are involved in this activation as well.

Previous studies were undertaken to determine the role of the cationic charge in the effects of polycationic compounds on mast cells. For example, heparin, a negatively charged molecule, dose dependently inhibited histamine release from rat peritoneal mast cells induced by compound 48/80 and by PEI6.[20-23] It was also found that heparin inhibits production of IL-11 by fibroblasts stimulated by MBP and IL-1.[42] However, heparin can also decrease histamine release in IgE-activated mast cells by inhibiting binding of 1,4,5-inositoltriphosphate to the endoplasmic reticulum, thus preventing the subsequent internal release of calcium necessary for degranulation.[43] In our experiments we observed that heparin added to either eosinophil sonicate or MBP-inhibited histamine release from naive and already activated rat peritoneal mast cells. However, the inhibition obtained by addition of heparin to eosinophil sonicate was lower than that obtained by its addition to MBP. This suggests the presence of other nonbasic mediators in the eosinophil sonicate involved in rat peritoneal mast cell activation.

The ion requirements for rat peritoneal mast cell activation by eosinophils were also assessed and compared with

those for compound 48/80. Rat peritoneal mast cells are known to be less sensitive to activation by polycationic compounds in the presence of millimolar concentrations of Ca^{2+}.[20] Eosinophil sonicate and MBP induced histamine release from rat peritoneal mast cells independently of external Ca^{2+}. Histamine release from mast cells incubated with MBP was less influenced by increasing Ca^{2+} concentrations than was release from mast cells incubated with compound 48/80. By confocal microscopy, an increase in the cytosolic Ca^{2+} concentration was detected in mast cells incubated with eosinophil sonicate and MBP in Ca^{2+}-free medium, further indicating that these activators are not dependent on extracellular Ca^{2+} and that they mobilize this ion from intracellular stores.

In summary, we have reported evidence that eosinophils activate antigen-activated mast cells to release histamine. This activation is mediated in part by MBP and shares with polycationic compounds some of the characteristics of non–IgE-dependent mast cell activation.

The fact that eosinophils can cause histamine release from previously antigen-stimulated mast cells provides novel evidence of a mast cell–eosinophil cross-talk that likely takes place in the late-phase and chronic stages of allergic reactions.

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