

Release of granule proteins from human eosinophils stimulated with mast-cell mediators

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Background It has been suggested that mast cells and eosinophils are major effector cells in the pathogenesis of allergic diseases. However, the interaction of these cells has not been thoroughly elucidated. We examined eosinophil cationic protein (ECP) release and cytosolic free calcium concentration ($[Ca^{2+}]_i$) in human eosinophils induced by the major mast-cell mediators including cytokines.

Methods Eosinophils from healthy donors were stimulated with the major mast-cell mediators for 20 min after preincubation with cytochalasin B for 10 min. ECP in supernatants was measured by radioimmunoassay. Moreover, to examine changes of $[Ca^{2+}]_i$ in eosinophils, Fura-2-loaded eosinophils were monitored for fluorescence changes after stimulus addition.

Results Of the tested mediators (prostaglandin $[PG]D_2$, leukotriene $(LT)B_4$, platelet-activating factor (PAF), histamine, LTC_4 , and eosinophil chemotactic factor of anaphylaxis [ECF-A]), LTB_4 and PAF induced ECP release from eosinophils. Any cytokines produced by human mast cells, i.e., interleukin (IL)-4, IL-5, IL-8, tumor necrosis factor (TNF), or granulocyte-macrophage colony-stimulating factor (GM-CSF), did not induce ECP release in our system. ECP release triggered with LTB_4 and PAF occurred at concentrations of 10^{-8} – 10^{-6} M concentration-dependently. LTB_4 and PAF also elicited a rise in $[Ca^{2+}]_i$ in eosinophils. Neither PGD_2 , histamine, nor LTC_4 induced ECP release, although they increased cytosolic calcium in eosinophils.

Conclusions Of mast-cell mediators, LTB_4 and PAF induced eosinophil degranulation. The contribution of LTB_4 and PAF from mast cells to eosinophil degranulation may be important in the pathogenesis of allergic inflammatory diseases.

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The correlation of eosinophils with mast cells has been proposed in the pathogenesis of allergic diseases. The number of eosinophils and the level of mast-cell tryptase in bronchoalveolar lavage fluid were correlated with bronchial hyperresponsiveness in patients with chronic asthma (1). Activated (EG2⁺) eosinophil counts correlated with tryptase-positive mast-cell counts in the nasal mucosa of patients with allergic rhinitis (2). Furthermore, in mast-cell-deficient mice, eosinophil peroxidase levels in lavage fluid were reduced compared with normal mice after antigen challenge in sensitized mice (3).

Mast cells release various mediators. They include prostaglandin $(PG)D_2$, leukotriene $(LT)B_4$, platelet-activating factor (PAF), histamine, LTC_4 ,

and eosinophil chemotactic factor of anaphylaxis (ECF-A). The effects of each mast-cell mediator on eosinophils have been reported. It has been shown that LTB_4 has chemotactic activity and produces H_2O_2 in eosinophils (4, 5). PAF also induces eosinophil chemotaxis and H_2O_2 generation in eosinophils (6, 7). Furthermore, PAF induces eosinophil granule protein release in eosinophils pretreated with cytochalasin B (cyto B) and LTC_4 production in eosinophils primed by IL-3 or IL-5 (8, 9). A recent *in vitro* study has indicated that LTD_4 is a chemoattractant for eosinophils (10). Histamine and ECF-A also have chemotactic effects on eosinophils *in vitro* (11, 12). Recently, it has been reported that mast cells produce some

cytokines, i.e., IL-4, IL-5, IL-8, tumor necrosis factor (TNF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (13, 14). However, the effects of mast-cell mediators described above, including those of cytokines, on eosinophil degranulation have not been thoroughly examined.

In this study, we examined the effects of the major mast-cell mediators on eosinophil cationic protein (ECP) release from normal human eosinophils. As a result, we have found that, in addition to PAF, LTB₄ induces eosinophil degranulation. PGD₂, histamine, and LTC₄ did not induce eosinophil degranulation in spite of eliciting a rise in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in eosinophils.

Material and methods

Purification of human eosinophils

Eosinophils were prepared from venous blood of informed healthy volunteers with eosinophil percentages of 2–6%. Eosinophil preparations were purified by two-step density centrifugation, as described previously (9). Erythrocytes in the preparation underwent hypotonic water lysis. Eosinophils were further purified by removal of CD16-positive cells (neutrophils) with immunomagnetic selection by a magnetic activated cell sorter system (Miltenyi Biotec, Bergisch-Gladbach, Germany) (15). The purity of eosinophils was >95%, as determined by microscopy of cytocentrifuge preparations stained with May-Giemsa. Purified eosinophils were suspended in HA buffer (20 mM HEPES [Calbiochem-Behring Corp., La Jolla, CA, USA], 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, and 0.025% bovine serum albumin, fatty acid-free [Boehringer Mannheim, Mannheim, Germany]) supplemented with 1 mM CaCl₂ and 1 mM MgCl₂ at a concentration of 1.5×10^5 eosinophils/ml.

Reagents

PGD₂, LTB₄, PAF, LTC₄, val-ECF-A, ala-ECF-A, rhIL-8, and cyto B were purchased from Sigma Chemical Co. (St Louis, MO, USA). rhIL-5, rhTNF, and rhGM-CSF were from Genzyme (Cambridge, MA, USA). Histamine dihydrochloride, PGE₂, and rhIL-4 were from Wako Pure Chemical (Osaka, Japan), Funakoshi (Tokyo, Japan), and Becton Dickinson Labware (Bedford, MA, USA), respectively. PGD₂, PAF, and PGE₂ were dissolved in ethanol at concentrations of 10^{-3} , 10^{-2} , and 10^{-2} M, respectively. val-ECF-A, ala-ECF-A, IL-4, and IL-8 were dissolved in PBS containing 1 mg/ml BSA at concentrations of 10^{-2} M, 10^{-2} M, 5 µg/ml, and 10^{-5} M, respectively. Histamine and cyto B were

dissolved in 0.01 N HCl and dimethylsulfoxide at concentrations of 10^{-2} M and 5 mg/ml, respectively. Histamine solution was stored at 4°C. Other compounds than histamine were stored in small aliquots at -70°C and thawed just before use. Fura-2/AM (Dojindo, Kumamoto, Japan) was stored in small aliquots at -20°C and thawed just before use.

ECP release by human eosinophils

Human eosinophils suspended in HA buffer with 1 mM CaCl₂ and 1 mM MgCl₂ were warmed to 37°C for 10 min. Then, the various agents described above were added 10 min after the addition of 5 µg/ml cyto B. The reaction was stopped 20 min after stimulus addition by placing the tubes in ice water. After centrifugation, ECP in supernatants was measured by RIA (Pharmacia, Uppsala, Sweden). For measurement of the total content of ECP, eosinophils were lysed with 0.5% *N*-cetyl-*N,N,N*-trimethylammonium bromide, as previously described (16).

$[Ca^{2+}]_i$ changes

Eosinophils were suspended at 10^6 cells/ml in HA buffer and loaded with 3 µM Fura-2/AM for 1 h at 37°C. After centrifugation, the loaded cells were resuspended at 10^6 cells/ml in HA buffer. Fura-2 fluorescence changes of the cell suspension in response to cell agonists were continuously monitored and analyzed with a fluorometer (CAF-100, Japan Spectroscopic, Tokyo). Changes in $[Ca^{2+}]_i$ were calculated as described previously (17) with 0.2% Triton X-100 (Wako Pure Chemical) and 10 mM EGTA (Sigma Chemical Co.) for calibration.

Presentation of data

All experiments were performed in duplicate and repeated at least three times with cell preparations from different donors. ECP data were presented as percentage of the total cellular content. The effects of treatment examined within the same experiments were compared by Wilcoxon's signed-rank test for pairs.

Results

ECP release in response to mast-cell mediators

We examined the effects of the major mast-cell mediators (PGD₂, LTB₄, PAF, histamine, LTC₄, val-ECF-A, and ala-ECF-A) on ECP release from normal human eosinophils preincubated with cyto B (Fig. 1). LTB₄ or PAF at a concentration of 10^{-6} M induced remarkable ECP release. None of

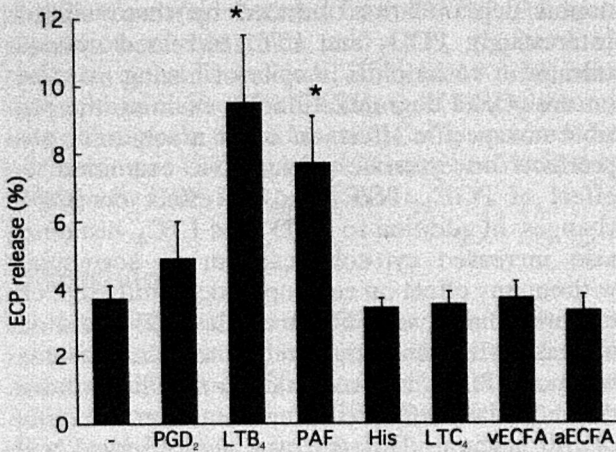


Fig. 1. ECP release by human eosinophils preincubated with cyto B in response to PGD₂, LTB₄, PAF, histamine, LTC₄, val-ECF-A, and ala-ECF-A. Eosinophils were stimulated with PGD₂, LTB₄, PAF, histamine, LTC₄, val-ECF-A, or ala-ECF-A at 10⁻⁶ M for 20 min after preincubation with 5 µg/ml cyto B for 10 min. Each column represents mean ± SEM obtained from six experiments performed in duplicate. **P* < 0.05 significance from buffer control (-).

the other mediators tested induced a significant release of ECP compared to buffer control. Without cyto B, ECP release was not observed in eosinophils triggered with LTB₄ or PAF, as described before in eosinophils triggered with C5a (8). Ethanol, which was used to dissolve PGD₂ and

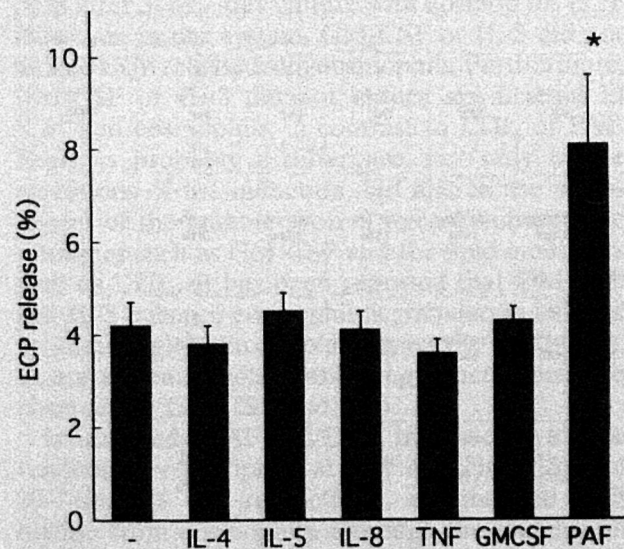


Fig. 2. ECP release by human eosinophils preincubated with cyto B in response to IL-4, IL-5, IL-8, TNF, GM-CSF, and PAF. Eosinophils were stimulated with IL-4 (50 ng/ml), IL-5 (10 ng/ml), IL-8 (10⁻⁷ M), TNF (30 ng/ml), GM-CSF (10 ng/ml), or PAF (10⁻⁶ M) for 20 min after preincubation with 5 µg/ml cyto B for 10 min. Each column represents mean ± SEM obtained from three experiments performed in duplicate. **P* < 0.05 significance from buffer control (-).

PAF, had no effect on ECP release (not shown). Next, we examined the effects of some cytokines which have so far been reported to be produced by human mast cells (Fig. 2). IL-4, IL-5, IL-8, TNF, or GM-CSF by itself did not induce a significant release of ECP compared to buffer control, although PAF induced ECP release. However, IL-5 enhanced PAF-induced ECP release, as described before in C5a-induced ECP release (8) (Fig. 3). GM-CSF also showed a similar effect (not shown).

Dose response and kinetics of LTB₄ or PAF-induced ECP release

Experiments in which eosinophils were stimulated with various concentrations of LTB₄ or PAF for 20 min after cyto B pretreatment showed that LTB₄ or PAF-induced ECP release was concentration dependent. ECP release occurred at LTB₄ or PAF concentrations of 10⁻⁸–10⁻⁶ M (Fig. 4). When the time course of LTB₄ or PAF-induced ECP release was examined, it was found that ECP release occurred within 30 s very rapidly after LTB₄ or PAF addition (not shown).

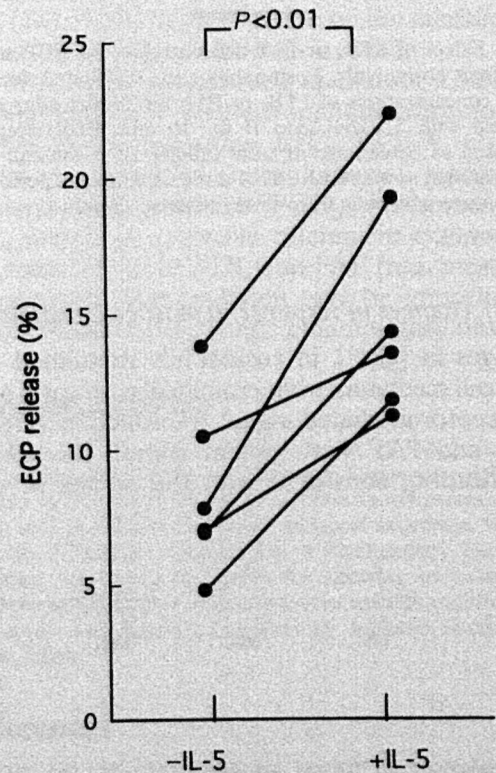


Fig. 3. IL-5 effect upon PAF-induced ECP release by human eosinophils. Eosinophils were preincubated for 60 min in buffer alone or in presence of IL-5 (30 ng/ml), and then stimulated with 10⁻⁶ M PAF for 20 min after preincubation with 5 µg/ml cyto B for 10 min. All experiments were performed with different donors. Each circle represents mean of two determinations.

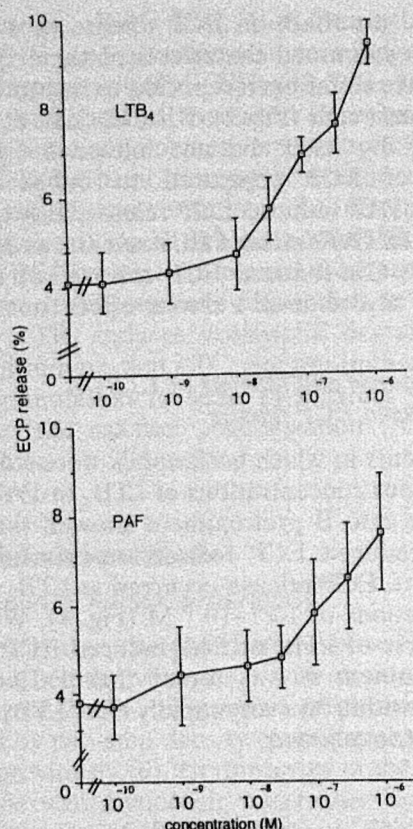


Fig. 4. Effect of LTB₄ or PAF concentration on ECP release by human eosinophils. Eosinophils were stimulated with different concentrations of LTB₄ or PAF for 20 min after preincubation with 5 μ g/ml cyto B for 10 min. ECP release is expressed as percentage of total cellular ECP content. Data are presented as mean $\pm$ SEM of three experiments performed in duplicate with cells from three different donors.

[Ca²⁺]_i changes in response to mast-cell mediators

Changes in [Ca²⁺]_i in eosinophils stimulated with mast-cell mediators were examined as an early event of receptor-mediated signal transduction (Fig. 5). LTB₄ and PAF were potent stimuli for calcium mobilization, consistent with the results of eosi-

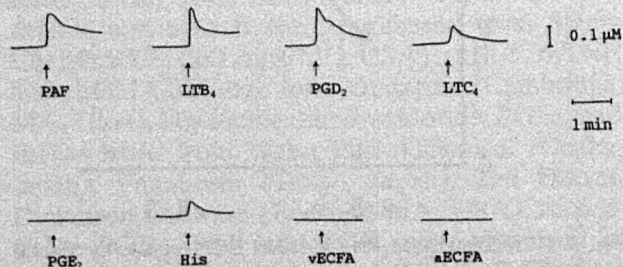


Fig. 5. Changes of [Ca²⁺]_i in human eosinophils induced by PAF, LTB₄, PGD₂, LTC₄, PGE₂, histamine, val-ECF-A, and ala-ECF-A. Fura-2-loaded cells were suspended at 2 $\times$ 10⁶/ml and monitored for fluorescence changes. Various agonists (10⁻⁶ M) were added as indicated (arrows). Initial [Ca²⁺]_i level was 103 nM.

nophil degranulation induced by these stimuli. Interestingly, PGD₂ and LTC₄ increased cytosolic calcium in eosinophils in spite of having no effect on eosinophil degranulation. To examine the possible nonspecific effects of other arachidonic acid products on cytosolic calcium, we examined the effect of PGE₂. PGE₂ had no effect on [Ca²⁺]_i changes. In addition to PGD₂ and LTC₄, histamine also increased cytosolic calcium in eosinophils without any effect on eosinophil degranulation. On the other hand, val-ECF-A or ala-ECF-A did not increase cytosolic calcium in eosinophils. A characteristic of PGD₂-induced calcium mobilization was the secondary rise in calcium seen after the initial rise in calcium. This rise was not observed with other stimuli. It may have been due to the PGD₂ metabolite, 9 α , 11 β -PGF₂ (18). When we examined the effects of the cytokines produced by human mast cells (IL-4, IL-5, IL-8, TNF, and GM-CSF), we found that no cytokine increased cytosolic calcium in eosinophils (not shown).

Dose-response of mast-cell mediators on the concentration of cytosolic calcium

Fig. 6 shows the dose dependence of the effects of PAF, LTB₄, PGD₂, histamine, and LTC₄ on [Ca²⁺]_i. The changes in [Ca²⁺]_i occurred from 10⁻⁹, 10⁻⁸, and 10⁻⁹ M for PAF, LTB₄, and PGD₂, respectively, and became maximal at 10⁻⁷ M for these three agonists. The secondary rise in PGD₂-induced calcium

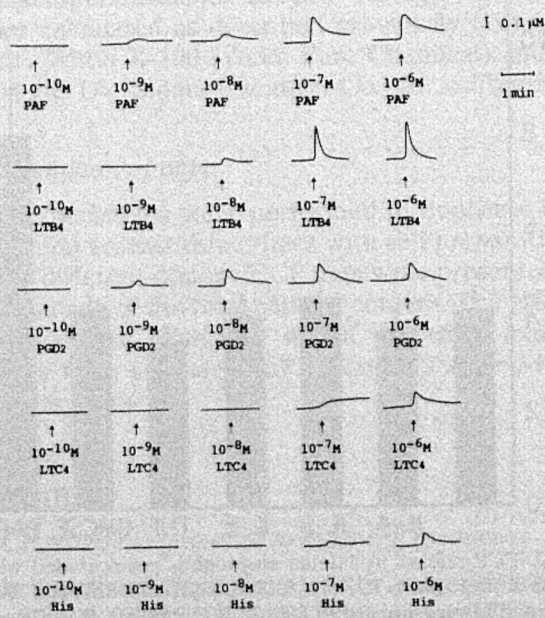


Fig. 6. Effect of PAF, LTB₄, PGD₂, LTC₄, or histamine concentration on changes of [Ca²⁺]_i in human eosinophils. Fura-2-loaded cells were suspended at 2 $\times$ 10⁶/ml and monitored for fluorescence changes. Different concentrations of agonists were added as indicated (arrows). Initial [Ca²⁺]_i level was 153 nM.

mobilization was observed from 10^{-7} M. The concentration of histamine and LTC₄ required to increase cytosolic calcium (10^{-7} to 10^{-6} M) was higher than that of PAF, LTB₄, and PGD₂. Moreover, the peak elevation of $[Ca^{2+}]_i$ induced by histamine and LTC₄ was approximately 40% of that induced by PAF, LTB₄, and PGD₂.

Discussion

In this study, we examined eosinophil degranulation in response to the major mast-cell mediators, including cytokines. Concerning chemotaxis, some mast-cell mediators, i.e., LTB₄, PAF, LTD₄, histamine, and ECF-A, have been reported so far to have this activity for eosinophils (4, 6, 10–12). Concerning eosinophil degranulation, of the major mast-cell mediators, only PAF was examined, and it has been reported to induce the release of eosinophil granule protein (8, 19), although the effects of other mast-cell mediators are unclear. Our study has shown that, in addition to PAF, LTB₄ also can induce ECP release from eosinophils and that LTC₄, histamine, or ECF-A does not induce eosinophil degranulation although they are chemotactic for eosinophils (10–12).

It has been shown that mast cells produce some cytokines, i.e., IL-4, IL-5, IL-8, TNF, and GM-CSF (13, 14). Of these cytokines, GM-CSF induces eosinophil degranulation after 4-h incubation with eosinophils in albumin-coated plates (20). IL-5 induces the release of granule proteins from eosinophils after 3- or 7-day culture with eosinophils (21). However, in our system, GM-CSF or IL-5 did not induce ECP release from eosinophils. Furthermore, GM-CSF or IL-5 did not induce an increase in $[Ca^{2+}]_i$ in eosinophils, in contrast to LTB₄ or PAF. There is probably a difference, not only in the conditions of the induction, but also in the mechanisms of the granule protein release between the cytokines such as GM-CSF and the lipid mediators such as LTB₄. It has been reported that GM-CSF and IL-5 enhance eosinophil degranulation induced by immunoglobulin-coupled Sepharose beads (22). In our system, similar enhancing effects were also observed in GM-CSF and IL-5.

In our study, PAF and LTB₄ increased cytosolic calcium in eosinophils at low concentrations of 10^{-9} and 10^{-8} M, respectively, and induced ECP release from eosinophils, although the increase in $[Ca^{2+}]_i$ in response to LTC₄ and histamine required more than 10^{-7} M of these agonists and did not result in eosinophil degranulation. The PAF concentration required for ECP release was similar to that for LTC₄ production from eosinophils (10^{-8} – 10^{-6} M) (23). When we compared ECP release in response to LTB₄ and PAF with that in response

to complement component C5a, LTB₄ and PAF were weaker than C5a on both a molar basis and the amount of released ECP. C5a induced ECP release at 10^{-9} – 10^{-7} M, and the amount of ECP in response to C5a was $16.0 \pm 3.6\%$, while those in response to LTB₄ and PAF were $9.5 \pm 2.0\%$ and $8.1 \pm 1.4\%$, respectively (percent ECP release, mean $\pm$ SEM, four donors) (this study, 8). However, since mast cells are initially activated in response to the antigen and the mediators, such as LTB₄ and PAF, are released in allergic inflammation, it cannot be excluded that LTB₄ and PAF are initial triggers for eosinophil degranulation.

Previous studies have suggested that eosinophil activation induced by LTB₄ and PAF is due to the receptor-mediated mechanism that involves Ca^{2+} and protein kinase C (24–27). In our study also, LTB₄ and PAF induced an increase in $[Ca^{2+}]_i$ in eosinophils at low concentrations. PGD₂ increased cytosolic calcium similarly, but it did not induce eosinophil degranulation. We examined whether PGD₂ affects eosinophil degranulation induced by the other stimulus or not. PGD₂ had no effect on C5a-induced ECP release (not shown), although it has been reported to have a priming effect on LTC₄ release in response to calcium ionophore in eosinophils (18). Further investigation on the mechanisms of eosinophil degranulation is needed.

In this study, of mast-cell mediators, LTB₄ and PAF induced ECP release from human eosinophils. Neither PGD₂, histamine, LTC₄, ECF-A, nor any cytokines examined here induced eosinophil degranulation, although PGD₂, histamine, and LTC₄ increased cytosolic calcium in eosinophils. The contribution of LTB₄ and PAF from mast cells to eosinophil degranulation may be important in the pathogenesis of allergic inflammatory diseases.

Acknowledgments

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References

1. Ferguson AC, Whitelaw M, Brown H. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J Allergy Clin Immunol* 1992;90:609–13.
2. Bentley AM, Jacobson MR, Cumberworth V, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increase in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992;89:877–83.

3. Kung TT, Stelts D, Zurcher JA, et al. Mast cells modulate allergic pulmonary eosinophilia in mice. *Am J Respir Cell Mol Biol* 1995;12:404-9.
4. Erger RA, Casale TB. Comparative studies indicate that platelet-activating factor is a relatively weak eosinophilotactic mediator. *Am J Respir Cell Mol Biol* 1995;12:65-70.
5. Rabe KF, Giembycz MA, Dent G, Barnes PJ. Activation of guinea pig eosinophil respiratory burst by leukotriene B₄: role of protein kinase C. *Fundam Clin Pharmacol* 1992; 6:353-8.
6. Casale TB, Erger RA, Little MM. Platelet-activating factor-induced human eosinophil transendothelial migration: evidence for a dynamic role of the endothelium. *Am J Respir Cell Mol Biol* 1993;8:77-82.
7. Kroegel C, Giembycz MA, Matthys H, Westwick J, Barnes PJ. Modulatory role of protein kinase C on the signal transduction pathway utilized by platelet-activating factor in eosinophil activation. *Am J Respir Cell Mol Biol* 1994; 11:593-9.
8. Takafuji S, Tadokoro K, Ito K. Effects of IL-3 and IL-5 on human eosinophil degranulation induced by complement components C3a and C5a. *Allergy* 1996;51:563-8.
9. Takafuji S, Bischoff SC, de Weck AL, Dahinden CA. IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C₄ in response to soluble agonists. *J Immunol* 1991;147:3855-61.
10. Spada CS, Nieves AL, Krauss AH, Woodward DF. Comparison of leukotriene B₄ and D₄ effects on human eosinophil and neutrophil motility *in vitro*. *J Leukoc Biol* 1994; 55:183-91.
11. Clark RAF, Gallin JI, Kaplan AP. The selective eosinophil chemotactic activity of histamine. *J Exp Med* 1975;142: 1462-76.
12. Goetzl EJ, Austen KF. Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: identification as eosinophil chemotactic factor of anaphylaxis. *Proc Natl Acad Sci U S A* 1975;72:4123-7.
13. Okayama Y, Semper A, Holgate ST, Church MK. Multiple cytokine mRNA expression in human mast cells stimulated via Fc epsilon RI. *Int Arch Allergy Immunol* 1995;107:158-9.
14. Bradding P, Roberts JA, Britten KM, et al. Interleukin-4, -5 and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10:471-80.
15. Hansel TT, de Vries IJ, Iff T, et al. An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Methods* 1991;145: 105-10.
16. Carlson M, Hakansson L, Peterson C, Stålenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* 1991; 87:27-33.
17. Mitsui M, Abe A, Tajimi M, Karaki H. Leakage of the fluorescent Ca²⁺ indicator Fura-2 in smooth muscle. *Jpn J Pharmacol* 1993;61:165-70.
18. Raible DG, Schulman ES, Dimuzio J, Cardillo R, Post TJ. Mast cell mediators prostaglandin-D₂ and histamine activate human eosinophils. *J Immunol* 1992;148:3536-42.
19. Kroegel C, Yukawa T, Dent G, Venge P, Chung KF, Barnes PJ. Stimulation of degranulation from human eosinophils by platelet-activating factor. *J Immunol* 1989; 142:3518-26.
20. Horie S, Kita H. CD11b/CD18 (Mac-1) is required for degranulation of human eosinophils induced by human recombinant granulocyte-macrophage colony-stimulating factor and platelet-activating factor. *J Immunol* 1994;152: 5457-67.
21. Kita H, Weiler DA, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Release of granule proteins from eosinophils cultured with IL-5. *J Immunol* 1992;149:629-35.
22. Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990;144:642-6.
23. Tamura N, Agrawal DK, Townley RG. Leukotriene C₄ production from human eosinophils *in vitro* - role of eosinophil chemotactic factors on eosinophil activation. *J Immunol* 1988;141:4291-7.
24. Perkins RS, Lindsay MA, Barnes PJ, Giembycz MA. Early signalling events implicated in leukotriene B₄-induced activation of the NADPH oxidase in eosinophils: role of Ca²⁺, protein kinase C and phospholipase C and D. *Biochem J* 1995;310:795-806.
25. Bach MK, Brashler JR, Petzold EN, Sanders ME. Super-oxide production by human eosinophils can be inhibited in an agonist-selective manner. *Agents Actions* 1992;35:1-11.
26. Zeck-Kapp G, Kroegel C, Riede UN, Kapp A. Mechanisms of human eosinophil activation by complement protein C5a and platelet-activating factor: similar functional responses are accompanied by different morphologic alterations. *Allergy* 1995;50:34-47.
27. Kroegel C, Pleass R, Yukawa T, Chung KF, Westwick J, Barnes PJ. Characterization of platelet-activating factor-induced elevation of cytosolic free calcium concentration in eosinophils. *FEBS Lett* 1989;243:41-6.

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