

Effects of interleukin (IL)-3 and IL-5 on human eosinophil degranulation induced by complement components C3a and C5a*

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It is suggested that eosinophils (Eos) play an important role in the pathogenesis of bronchial asthma by releasing cytotoxic cationic eosinophil granule proteins and damaging bronchial epithelial cells. However, the exact nature of the actual inducer of eosinophil degranulation *in vivo* is unclear. We examined eosinophil cationic protein (ECP) release from human Eos in response to soluble agonists such as C5a, C3a, platelet-activating factor, and FMLP with or without interleukin (IL)-3 or IL-5 priming. Eosinophil degranulation induced by these soluble agonists required the pretreatment of Eos by cytochalasin B even in IL-3 priming. Among four agonists, C5a was the most effective stimulus of ECP release either with or without IL-5 priming. IL-3 and IL-5 remarkably enhanced ECP release in Eos triggered by C3a and C5a. The enhancement of ECP release by IL-3 and IL-5 occurred at 0.1–0.3 ng/ml and became maximal at 10–30 ng/ml, concentration-dependently. The enhancement of ECP release by cytokines became optimal at an interval of 10 min. Our data support the importance of complement components and cytokines in eosinophil degranulation *in vivo*.

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Eosinophils (Eos) are believed to play an important role in the pathogenesis of bronchial asthma. It has been shown that levels of eosinophil granule proteins in the bronchoalveolar lavage fluids are elevated in the pulmonary late-phase reaction induced by challenge with ragweed extract (1). Tissue eosinophilia and the deposition of eosinophil granule proteins in the affected tissues are observed in bronchial asthma (2). Moreover, in bronchial asthma, major basic protein (MBP) is present in increased concentrations in the sputum (3). It is suggested that Eos contribute to bronchial asthma by releasing cytotoxic cationic eosinophil granule proteins and damaging bronchial epithelial cells (4).

Concerning eosinophil degranulation, platelet-activating factor (PAF) induces eosinophil peroxidase release from human Eos (5). It has also been reported that sIgA- or IgG-coated Sephadex beads trigger eosinophil-derived neurotoxin (EDN) release, which is enhanced by cytokines such as interleukin (IL)-3, granulocyte/macrophage colony-stimulating factor (GM-CSF), and IL-5 (6, 7). Furthermore, serum-opsonized Sephadex particles induce eosinophil degranulation (8). Although studies done to date have mainly focused on degranulation induced by the respective stimuli, little information is available on the comparison of eosinophil degranulation among different stimuli. Therefore, it is still unclear what actually induces eosinophil degranulation *in vivo* and which mechanism is involved in it.

The role of complement components in bronchial asthma has not been thoroughly elucidated. However, it has been reported that some asthmatic

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patients developed reduced hemolytic-component activity or C4 in arterial or venous blood after allergen provocation (9). Complement deposition has been found in the bronchial mucosa of asthmatic patients (10). Some *in vitro* studies have demonstrated that the complement system can be activated by allergens via the classical pathway (11), the alternative pathway (12), or both pathways (13) in normal human serum and in the sera of asthmatic patients. Complement components C5a and C3a cause bronchoconstriction, increase vascular permeability, and induce mast-cell degranulation. It cannot be excluded that there may be local complement activation within the asthmatic airway and that the generated anaphylatoxins C5a and C3a may affect inflammatory cells.

In this study, we selected the complement components C5a and C3a, PAF, and FMLP (the last two are eosinophil chemoattractants like C5a) as the stimuli which activate Eos. We examined eosinophil degranulation induced by these soluble agonists with or without IL-3 or IL-5 priming. Our results show that, among these soluble agonists, C5a most effectively triggers eosinophil cationic protein (ECP) release from human Eos, and cytokine priming further potentiates its release.

Material and methods

Purification of human Eos

Eos were prepared from venous blood of informed healthy volunteers with eosinophil levels of 2–6%. Eosinophil preparations were purified by two-step density centrifugation exactly as described previously (14). Erythrocytes in the preparation were lysed by hypotonic water lysis. Eos were further purified by removal of CD16-positive cells (neutrophils) by immunomagnetic selection, as previously described (15). The purity of Eos was >95%, as determined by microscopy of cytocentrifuge preparations stained with May-Giemsa, according to the method described previously (14). The yield of Eos was $36.5 \pm 6.3\%$. Purified Eos 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 CaCl_2 and 1 mM MgCl_2 at a concentration of 1.5×10^5 Eos/ml.

Reagents

C5a, PAF, and FMLP were obtained from Sigma Chemical Co. (St Louis, MO, USA). C3a was purified from yeast-activated human serum as pre-

viously described (16), the kind gift of Dr Clemens A. Dahinden (Institute of Clinical Immunology, Inselspital, Bern, Switzerland). Recombinant human (rh)IL-3, rhIL-5, and rh tumor necrosis factor- α (TNF- α) were obtained from Genzyme (Cambridge, MA, USA). rh nerve growth factor- β (NGF- β) was obtained from Genentech, Inc. (South San Francisco, CA, USA). Cytochalasin B (cytoB) was obtained from Sigma Chemical Co. All compounds were stored in small aliquots at -70°C and thawed just before use.

ECP release by human Eos

Human Eos suspended in HA buffer with 1 mM CaCl_2 and 1 mM MgCl_2 were warmed up to 37°C for 10 min, and then incubated with or without IL-3 or IL-5 for 60 min. In some experiments, the effects of TNF- α and NGF- β were examined instead of IL-3 and IL-5. Then, C5a, C3a, PAF, or FMLP was added 5 min after the addition of $5 \mu\text{g}/\text{ml}$ cytoB. The reaction was stopped 20 min after stimulus addition by placing the tubes in ice water. In preliminary experiments, this incubation time was found sufficient for the completion of the ECP release reaction. After centrifugation, ECP in supernatants was measured by RIA (Pharmacia, Uppsala, Sweden). For measurement of the total content of ECP, Eos were lysed with 0.5% *N*-cetyl-*N,N,N*-trimethylammonium bromide, as previously described (8).

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. Effects of treatment examined within the same experiments were compared by using Wilcoxon's signed-rank test for pairs.

Results

Effects of IL-3 and IL-5 on ECP release induced by soluble agonists

ECP release in response to C5a from normal human Eos preincubated with or without cytoB was examined with or without IL-3 priming (Fig. 1). Without cytoB, C5a at a concentration of 10^{-7} M did not induce a significant release of ECP compared to buffer control, even if Eos were primed by IL-3. In contrast, when Eos were preincubated with cytoB, ECP release was induced by C5a alone and further enhanced by IL-3 priming. Similar results were observed also when C3a,

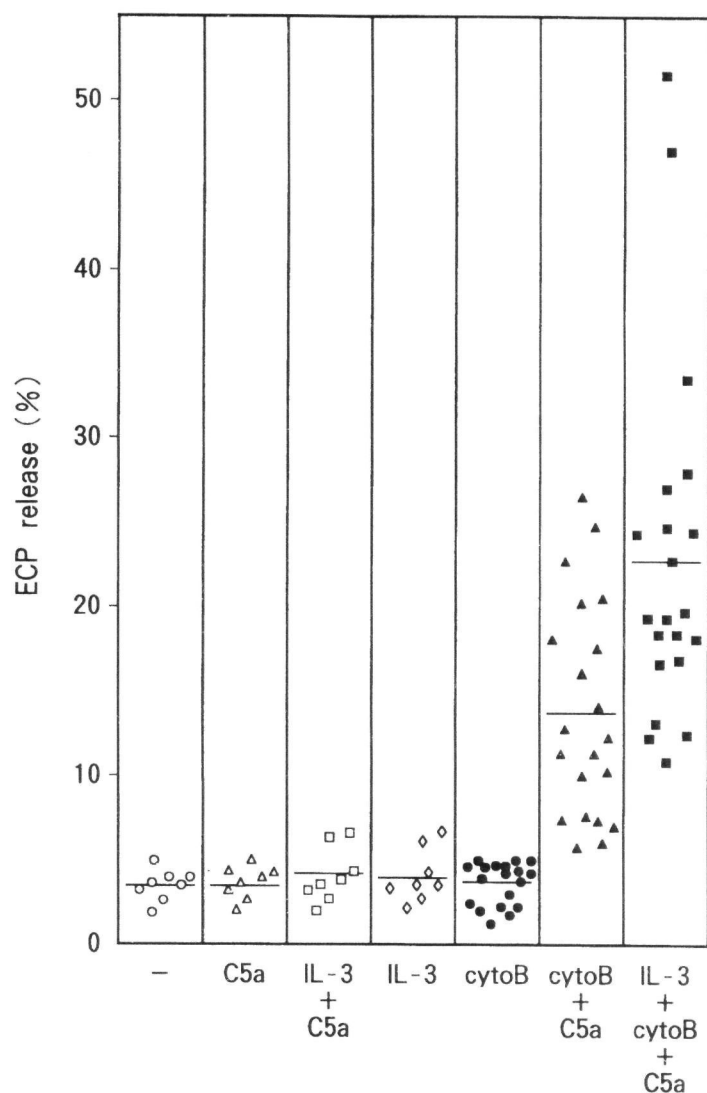


Fig. 1. ECP release by human Eos from various donors. After warming-up period of 10 min (37°C), cells were incubated as follows: 85 min in buffer (-); 65 min in buffer, then C5a at 10^{-7} M for 20 min (C5a); IL-3 at 30 ng/ml for 65 min, then C5a at 10^{-7} M for 20 min (IL-3 + C5a); IL-3 at 30 ng/ml for 85 min (IL-3); 60 min in buffer, then cytoB at 5 μ g/ml for 25 min (cytoB); 60 min in buffer, cytoB at 5 μ g/ml for 5 min, then C5a at 10^{-7} M for 20 min (cytoB + C5a); and IL-3 at 30 ng/ml for 60 min, cytoB at 5 μ g/ml for 5 min, then C5a at 10^{-7} M for 20 min (IL-3 + cytoB + C5a). ECP release in supernatants is shown as percentage of total cellular ECP content. Each symbol represents mean of duplicates from separate experiments performed with cell preparations from different donors. Horizontal bars indicate means of all results.

FMLP, or PAF up to a concentration of 10^{-7} M was used as a stimulus (not shown). IL-3 alone did not induce ECP release in cytoB-pretreated Eos.

Fig. 2 compares ECP release induced by C5a, C3a, FMLP, or PAF at a concentration of 10^{-7} M from Eos preincubated with cytoB, with or without IL-5 priming. Without IL-5 priming, C5a induced more ECP release than C3a ($n=6$, $P<0.05$). ECP release induced by C3a was not significantly different from that induced by FMLP; however, it was greater than that induced by PAF ($n=6$, $P<0.05$). Therefore, without IL-5 priming, the rank order of potency was C5a > C3a = FMLP > PAF. With IL-5 priming, C5a induced more ECP release than C3a ($n=6$, $P<0.05$). ECP release induced by C3a was greater than that induced by FMLP or PAF ($n=6$,

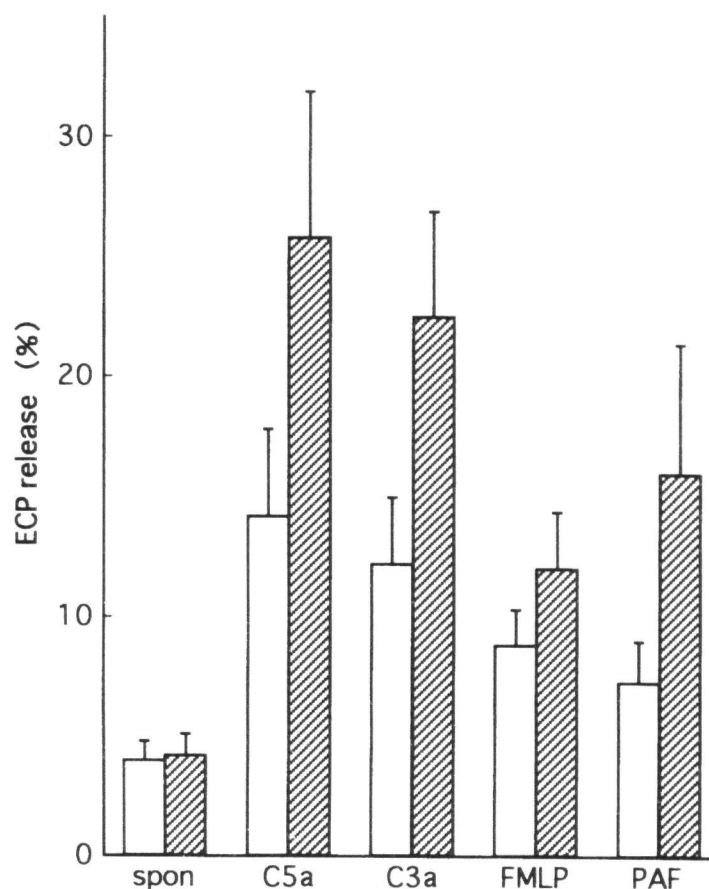


Fig. 2. ECP release by human Eos preincubated with cytoB in response to C5a, C3a, FMLP, and PAF with or without IL-5 priming. Eos were preincubated in buffer alone (open columns) or with 30 ng/ml IL-5 (hatched columns) for 60 min and then stimulated with C5a, C3a, FMLP, or PAF at 10^{-7} M for 20 min after preincubation with 5 μ g/ml cytoB for 5 min. Each column represents mean $\pm$ SEM obtained from six experiments performed in duplicate. spon: spontaneous release.

$P<0.05$). Therefore, with IL-5 priming, the rank order of potency was C5a > C3a > FMLP = PAF. Thus, among four agonists, C5a is the most effective stimulus of ECP release.

Fig. 3 summarizes the effect of IL-3 and IL-5 upon C5a-induced ECP release by cells from different donors, and also shows donor variability. In all cases, IL-3 as well as IL-5 enhanced ECP release within a range of 37–92% ($n=8$, $P<0.05$) and 41–123% ($n=8$, $P<0.05$) for IL-3 and IL-5, respectively. TNF- α and NGF- β exerted no significant effect on ECP release (not shown).

Dose response and kinetics of IL-3 and IL-5 priming

Experiments in which Eos were incubated with various concentrations of IL-5 for 60 min and then stimulated with C5a for 20 min after cytoB pretreatment showed that the enhancement of ECP release by IL-5 preincubation was concentration-dependent. Similar results were observed in IL-3 priming. The enhancement occurred for both cytokines at 0.1–0.3 ng/ml, and became maximal at 10–30 ng/ml (Fig. 4). In a kinetic study, when the time interval between addition of cytokines (IL-3 or IL-

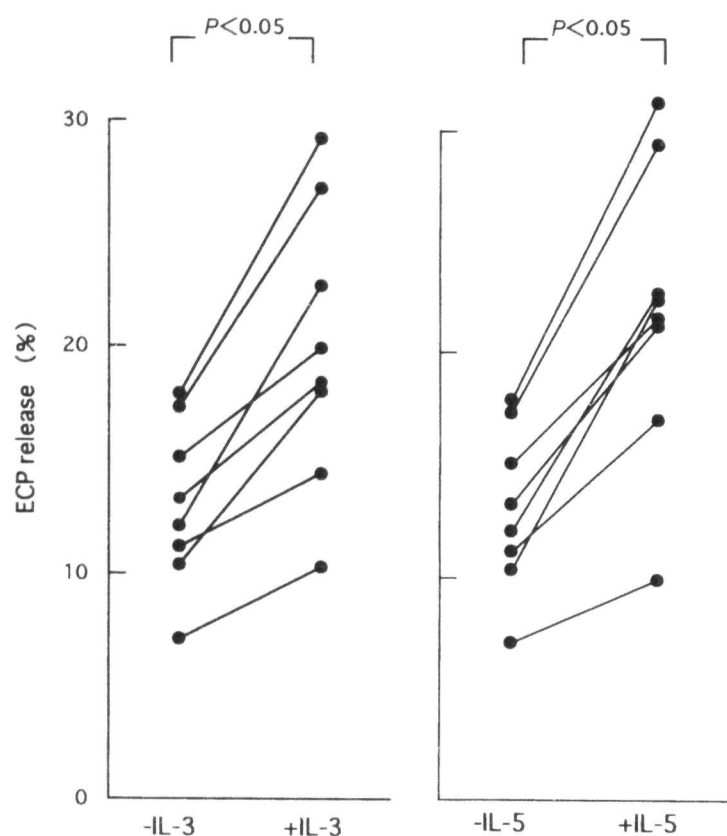


Fig. 3. Variability of cytokine effect upon C5a-induced ECP release by normal Eos. Eos were preincubated for 60 min in buffer alone or in presence of cytokine, and then stimulated with 10^{-7} M C5a for 20 min after preincubation with 5 μ g/ml cytoB for 5 min. Priming effect of IL-3 (30 ng/ml, eight experiments) and IL-5 (30 ng/ml, eight experiments) is shown. All experiments were performed with different donors. Each circle represents mean of two determinations.

5) and agonists was varied, the enhancement of ECP release became optimal at an interval of 10 min and continued afterward (Fig. 5).

Discussion

Our results indicate that soluble agonists such as C5a, C3a, FMLP, and PAF induced ECP release from cytoB-pretreated human Eos. Both with and without IL-5 priming, C5a was the most effective stimulus of eosinophil degranulation among four agonists. IL-3 and IL-5 remarkably enhanced ECP release in Eos triggered by C3a and C5a. Therefore, it is suggested that complement components and cytokines may play an important role in eosinophil degranulation *in vivo*.

So far, sIgA- or IgG-coated beads and serum-opsonized Sephadex particles have been reported to be the stimuli of eosinophil degranulation (6–8). When we compare the released amount of eosinophil granule proteins between cytoB plus soluble stimuli in our experiments and the particles described above, the amount induced by C5a is comparable to that induced by sIgA-coated beads (10–20% of the total) (this study, 7). The amount induced by C3a, FMLP, and PAF is comparable to that induced by serum-opsonized Sephadex parti-

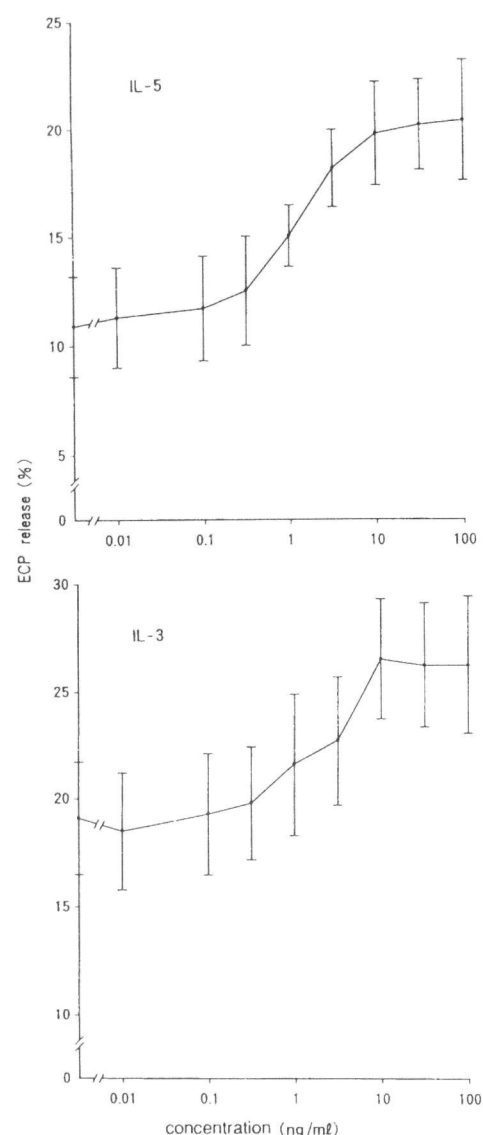


Fig. 4. Effect of IL-5 or IL-3 concentration on C5a-induced ECP release by human Eos. Eos were preincubated with different concentrations of IL-5 or IL-3 for 60 min and then stimulated with 10^{-7} M C5a for 20 min after preincubation with 5 μ g/ml cytoB for 5 min. ECP release is expressed as percentage of total cellular ECP content. Data are presented as mean $\pm$ SEM of three experiments performed in duplicate with cells from three different donors. IL-5 or IL-3 by itself did not induce significant ECP release compared to control within concentration range indicated (not shown).

cles and IgG-coated beads (5–15% of the total) (this study, 7, 8). Therefore, C5a is one of the most effective stimuli of eosinophil degranulation among those reported so far.

Leukotriene C_4 (LTC_4) is the important mediator released from Eos in addition to granule proteins. In this study, among C5a, C3a, FMLP, and PAF, the most effective stimulus of ECP release by Eos was C5a. However, our previous study of LTC_4 production showed that FMLP was the most effective of these four agonists (14). Furthermore, $TNF-\alpha$ and $NGF-\beta$ exerted no significant effect on ECP release (not shown). However, concerning LTC_4 production, enhancing and suppressive effects were observed for $TNF-\alpha$ and $NGF-\beta$, respectively (17). The experiments, in which the kinetics of IL-3 or IL-5 priming was examined, also showed that the optimal preincubation time was 10 min and 90 min

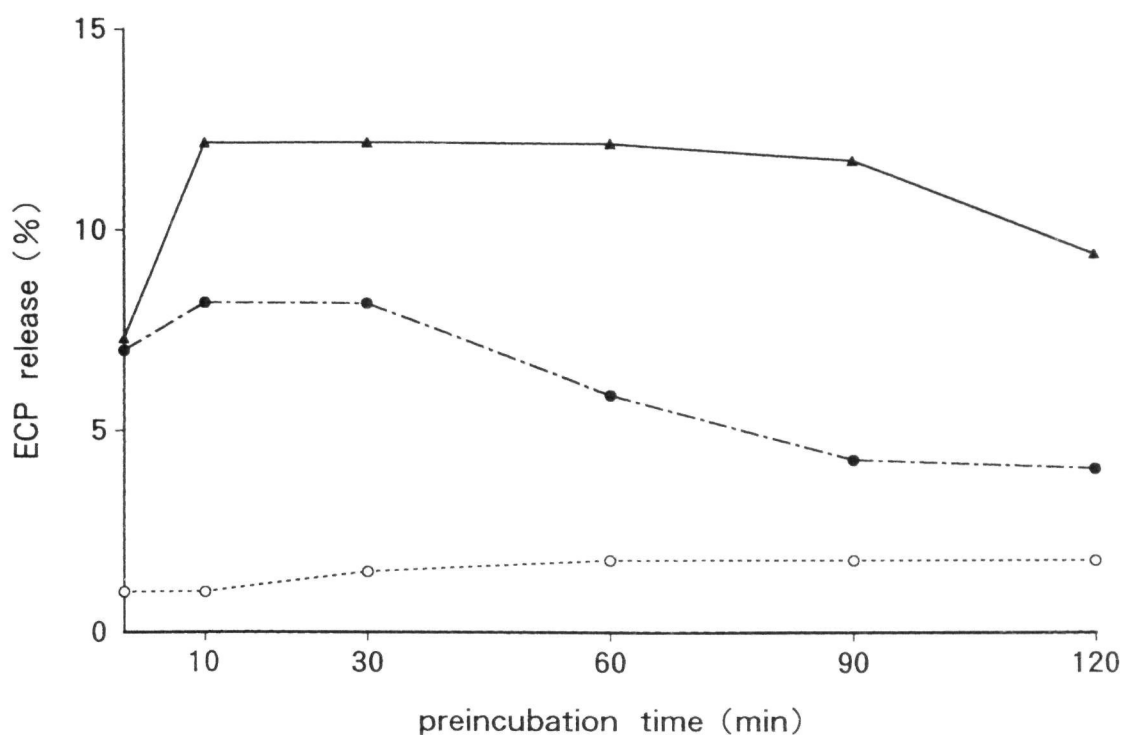


Fig. 5. Variation of time interval between addition of IL-3 and C5a. Eos were preincubated with (closed triangles) or without (closed and open circles) 30 ng/ml IL-3 for time indicated, and then stimulated with 10^{-7} M C5a (closed triangles and circles) or incubated in buffer (open circles) for 20 min after cytoB addition. One representative experiment out of three is shown. Similar results were observed in IL-5 priming.

for ECP release and LTC₄ production, respectively (this study, 14). These results indicate that degranulation and LTC₄ generation are clearly separately regulated.

Even in IL-3 or IL-5 priming, the presence of cytoB was required in ECP release induced by soluble agonists. Similar results were observed in EPO release (18, 19). It has been demonstrated that cytoB disrupts the "barrier" of the actin filament system which limits the access of granules to the cytoplasmic face of the plasma membrane (20, 21). Furthermore, it is suggested that cytoB effects on neutrophils mimic those of cell-surface interaction (22). Recently, it has been shown that CD11b/CD18-dependent cellular adhesion is involved in the degranulation from human Eos incubated in albumin-coated polystyrene plates and stimulated with GM-CSF and PAF (23). CD11/CD18 dependency has also been demonstrated in phorbol ester-induced degranulation from adherent human Eos (24). We have recently found that CD18-dependent adhesion may be involved in the enhancement of human eosinophil degranulation in the presence of human bronchial epithelial cells (25). Thus, cytoB pretreatment may exert effects similar to cellular adhesion through cytoskeletal alteration. Since Eos appear primarily to function on surfaces or to interact with particulate material such as parasites *in vivo*, degranulation from Eos pretreated with cytoB may be of pathophysiologic relevance.

In this study, soluble agonists such as C5a, C3a, FMLP, and PAF induced ECP release from cytoB-

pretreated Eos with or without IL-5 priming. Especially, C5a and C3a were the effective stimuli of eosinophil degranulation. Furthermore, IL-3 and IL-5, which did not alone induce eosinophil degranulation, remarkably enhanced ECP release induced by C5a and C3a. The contribution of these complement components and cytokines to eosinophil degranulation may be important in the pathogenesis of allergic inflammatory diseases.

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