

Original article

Effects of human lung fibroblasts on eosinophil degranulation*

Background: Although eosinophils and fibroblasts are thought to contribute to the mechanisms of chronic asthmatic inflammation, the interaction between eosinophils and fibroblasts has not been thoroughly clarified. We examined eosinophil cationic protein (ECP) release from human eosinophils cultured in the presence of human lung fibroblast HFL-1.

Methods: Eosinophils from healthy donors were cultured with or without C5a for 16 h in the presence of human fetal lung fibroblasts which had previously been incubated with or without TNF for 4 h. ECP in supernatants was measured by RIA.

Results: ECP release was potentiated only when both eosinophils and fibroblasts were activated by C5a and TNF, respectively, while it was not significantly potentiated when either eosinophils or fibroblasts were activated.

Paraformaldehyde fixation of fibroblasts had some suppressive effect on ECP enhancement, and mAb against GM-CSF partly inhibited ECP enhancement. Coculture of eosinophils and fibroblasts with stimulus treatment resulted in the enhancement of both eosinophil adhesion and ECP release. The potentiation of ECP release was partially inhibited by anti-ICAM-1 mAb, anti-CD18 mAb, and anti-CD29 mAb, which caused partial and comparable inhibition of the enhancement of eosinophil adhesion.

Conclusions: This study suggests that the activation of fibroblasts may have some role in the potentiation of ECP release from cocultured eosinophils, and that adhesion of eosinophils to fibroblasts may partly be involved in the mechanism of ECP potentiation.

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It has been suggested that eosinophils contribute to bronchial asthma by releasing cytotoxic cationic eosinophil granule proteins and damaging tissue (1, 2). In recent years, adhesion has been supposed to play an important role in eosinophil degranulation. CD11/CD18-dependent adhesion is involved in human eosinophil degranulation induced by immobilized IgG (3) or by recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) and PAF in albumin-coated polystyrene plates (4). Phorbol ester-induced degranulation in adherent eosinophils is also CD11/CD18-dependent (5). Our previous study shows that CD18-dependent adhesion of eosinophils to epithelial cells is involved in the enhancement of eosinophil degranulation in coculture of these cells (6).

It has been suggested that fibroblasts are involved in the chronicity of asthmatic inflammation and bronchial hyperreactivity. Activation of fibroblasts is associated with an increased deposition of collagen and fibronectin, leading to the thickening of the basal membrane and the appearance of a diffuse subepithelial fibrosis (7). In addition, fibroblasts produce various cytokines that can serve to amplify and/or perpetuate tissue inflammation (8).

Some studies were performed to investigate the interaction of eosinophils and fibroblasts. Shock et al. have shown that eosinophils are capable of both adhering to and releasing mitogens for fibroblasts *in vitro* (9). Furthermore, it has been reported that eosinophil major basic protein interacts synergistically with IL-1 α or TGF- β to augment fibroblast IL-6-type cytokine production (10). On the other hand, it has been shown that fibroblasts may be involved in eosinophil activation (accelerated hypodensity, augmented leukotriene C₄

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production, and enhanced antibody-dependent cytotoxicity) and may increase eosinophil viability; this involves 3T3 fibroblasts plus GM-CSF or IL-5 (11, 12). Moreover, Spoelstra et al. have reported that lung fibroblasts modulate both CD11b and L-selectin expression of eosinophils by both adhesive interactions and soluble factors, especially GM-CSF (13). However, the influence of fibroblasts on eosinophil degranulation has not yet been elucidated.

In this study, we examined eosinophil cationic protein (ECP) release from human eosinophils cultured in the presence of human lung fibroblasts. We investigated the effects of cytokines and adhesion on eosinophil degranulation. As a result, we have found that the activation of fibroblasts may have some role in the potentiation of ECP release from cocultured eosinophils, and that adhesion may partly be involved in the potentiation of eosinophil degranulation.

Material and methods

Purification of human eosinophils

Eosinophils were prepared from venous blood of informed healthy volunteers with eosinophil percentages in the range of 2–6%. Eosinophil preparations were purified by a two-step density centrifugation as described previously (6). Erythrocytes in the preparation were lysed by 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) (14). The purity of eosinophils was over 95%, as determined by microscopy of cytocentrifuge preparations stained with May-Giemsa. Purified eosinophils were suspended in RPMI 1640 (Life Technologies, Inc., Grand Island, NY, USA) supplemented with 1% penicillin/streptomycin, 0.1% human serum albumin (Miles, Inc., Kankakee, IL, USA), 1 mM CaCl_2 , and 1 mM MgCl_2 .

Reagents

rhTNF- α , rhIL-1 β , and rhIL-6 were obtained from Genzyme (Cambridge, MA, USA) and TGF- β from Life Technologies, Inc. (Grand Island, NY, USA). rhIFN- γ , rhC5a, PAF, and FMLP were obtained from Sigma Chemical Co. (St Louis, MO, USA). C3a was purified from yeast-activated human serum, as previously described (15), the kind gift of Dr Clemens A. Dahinden (Institute of Clinical Immunology, Inselspital, Bern, Switzerland). Anti-hGM-CSF, anti-hSCF, and anti-hIL-6 were purchased from Genzyme, and anti-hIL-11 was obtained from Sigma Chemical Co. These antibodies were selected because of their ability to neutralize the bioactivity of their cytokines, i.e., GM-CSF, SCF, IL-6, and IL-11, respectively. These compounds were stored in small aliquots at -70°C and thawed just

before use. Anti-ICAM-1 mAb (British Biotechnology, Oxford, UK), anti-CD18 mAb (Immunotech SA, Marseille, France), and anti-CD29 mAb (Seikagaku Corp., Tokyo, Japan) were selected because of their ability to block adhesion. Anti-HLA class I Ag mAb, the isotype-matched control Ab for anti-ICAM-1, anti-CD18, and anti-CD29 (IgG1), was purchased from PharMingen (San Diego, CA, USA). These mAbs were stored at 4°C .

Culture of HFL-1

A human lung fibroblast HFL-1 (American Type Culture Collection, Rockville, MD, USA) was cultured in DME medium containing 10% fetal calf serum and 1% penicillin/streptomycin. Cells in their 15th to 20th passages, which have the same characteristics as other soft-tissue fibroblasts, i.e., production of collagens type I and type III (16), were plated on 24-well tissue culture plates at 1.5 to 2×10^5 per well for ECP and eosinophil adhesion assays, and cultured in a humidified atmosphere at 37°C with 5% CO_2 . The monolayer achieved confluency at days 2–3.

Eosinophil degranulation

When confluency was achieved, fibroblast monolayers were washed with PRMI 1640 containing 0.1% human serum albumin. After incubation with or without TNF (30 ng/ml) for 4 h, the monolayers were washed. Then, to each well was added 500 μl of eosinophils (5×10^4). The wells were gently agitated, and eosinophils were cocultured with or without C5a (10^{-7} M) for 16 h at 37°C and 5% CO_2 . Coculture for 16 h was sufficient to cause the effect of stimulus on eosinophil degranulation, and that for longer periods made spontaneous release too high, resulting in less effect of stimulus on eosinophil degranulation. In some experiments, mAbs (10 $\mu\text{g/ml}$) were added to the well at the start of the culture. At the end of the culture period, supernatants from wells were collected and stored at -70°C until ECP measurements were performed. Eosinophil viability after the culture for 16 h was over 95%, as assessed by the exclusion of trypan blue dye. Moreover, fibroblast monolayers were still almost confluent after culture for 16 h. ECP was measured by RIA (Pharmacia, Uppsala, Sweden). For the measurement of the total content of ECP, eosinophils were lysed with 0.5% *N*-cetyl-*N,N,N*-trimethylammonium bromide, as previously described (17).

Fixation of fibroblasts

In some experiments, fibroblast monolayers were fixed by addition of 1% paraformaldehyde (Sigma Chemical Co.) in PBS for 15 min at room temperature (18). After three washings with PBS, the monolayers were incubated with 1% glycine and 1% BSA in PBS for 30 min to quench unreacted paraformaldehyde.

Adhesion assay

To evaluate adhesion and degranulation simultaneously from the same samples, eosinophil adhesion was examined by measuring the ECP content of adherent cells by RIA. At the end of the culture period, supernatants were collected and stored for ECP assay. The plates were gently rinsed with RPMI 1640 to remove nonadherent cells. Then, adherent cells were lysed with 0.5% *N*-cetyl-*N,N,N*-trimethylammonium bromide. The ECP content was measured by RIA. The percentage of adhesion was calculated by the method of Horie & Kita (4).

Flow cytometry analysis

HFL-1 cells that had been cultured with various cytokines or medium control were harvested by treatment with trypsin and EDTA and gentle scraping from the culture dish. From 0.5 to 1.0×10^6 cells were incubated with FITC-conjugated anti-ICAM-1 mAb (Immunotech SA) or R-PE-conjugated anti-VCAM-1 mAb (PharMingen International, San Diego, CA, USA) for 30 min. After being washed, the cells were resuspended in PBS, and 10 000 cells were analyzed for fluorescent intensity by a flow cytometer (EPICS-XL; Coulter Corp., Hialeah, FL, USA).

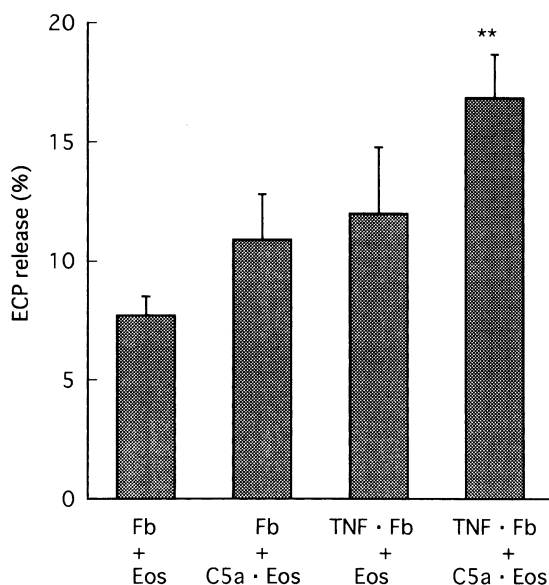


Figure 1. Effect of stimulus treatment on ECP release in coculture of eosinophils and fibroblasts. Confluent fibroblast monolayers were preincubated with culture medium alone (control) or medium containing 30 ng/ml TNF for 4 h and washed before addition of eosinophils. Eosinophils (5×10^4 cells) were cultured with or without 10^{-7} M C5a in the presence of control fibroblasts or TNF-treated fibroblasts for 16 h. Each column represents mean $\pm$ SEM obtained from five experiments performed in duplicates with cells from five different donors. Double asterisk signifies $P < 0.01$ significance from values without stimulus treatment. Fb: fibroblasts; Eos: eosinophils.

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 the paired Student's *t*-test.

Results

ECP release in coculture of C5a-activated eosinophils and TNF-treated fibroblasts

We examined the effect of stimulus treatment on ECP release in coculture of eosinophils and fibroblasts. In coculture of eosinophils and fibroblasts without stimulus, $7.7 \pm 0.8\%$ of the total (percentage of ECP release, mean $\pm$ SEM, $n = 5$) were released spontaneously. When eosinophils alone were activated by C5a or when fibroblasts alone were activated by TNF, no significant enhancement of ECP release was observed. However, when both eosinophils and fibroblasts were activated by C5a and TNF, respectively, ECP release was significantly potentiated (Fig. 1). Unstimulated fibroblasts or TNF-stimulated fibroblasts alone released no detectable amounts of ECP.

Effects of stimuli for eosinophils on ECP release in coculture of eosinophils and TNF-activated fibroblasts

Fig. 2 shows the effect of various soluble agonists added to eosinophils on ECP release in coculture of TNF-activated fibroblasts and eosinophils. C5a, C3a, PAF, and FMLP induced significant potentiation of ECP release from eosinophils cocultured with TNF-activated fibroblasts, compared to control ($n = 4$, $P < 0.05$). Our previous study indicated that, of four agonists, C5a was the most effective stimulus of ECP release from cytochalasin B-pretreated eosinophils (19). In coculture of TNF-activated fibroblasts and eosinophils, C5a was also the most effective stimulus of ECP release of four agonists ($n = 4$, $P < 0.05$). There was no significant difference in ECP release among C3a, PAF, and FMLP.

Effects of cytokines for fibroblasts on ECP release in coculture of C5a-activated eosinophils and fibroblasts

TNF, IFN- γ , IL-1 β , TGF- β , and IL-6 were added to fibroblasts and tested for their effects on ECP release in coculture of C5a-activated eosinophils and fibroblasts (Fig. 3). When IL-1 β , as well as TNF, was added to fibroblasts, ECP release was clearly and significantly enhanced. The other three cytokines showed no effects on ECP release.

Dose response of C5a and TNF

Fig. 4 shows the dose dependence of the effect of C5a on

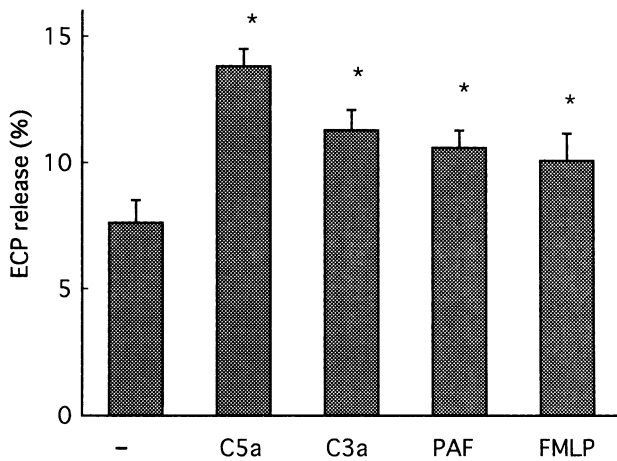


Figure 2. Effects of soluble agonists for eosinophils on ECP release in coculture of eosinophils and TNF-activated fibroblasts. Confluent fibroblast monolayers were preincubated with medium containing 30 ng/ml TNF and washed before addition of eosinophils. Eosinophils were cultured without (control) or with C5a, C3a, PAF, or FMLP at 10^{-7} M in presence of TNF-treated fibroblasts for 16 h. Each column represents mean $\pm$ SEM obtained from four experiments performed in duplicates. Asterisk signifies $P < 0.05$ difference between control and stimulus treatment.

ECP release in coculture of eosinophils and TNF-treated fibroblasts. The enhancement of ECP release by C5a was concentration dependent, occurring from 10^{-10} to 10^{-9} M, and becoming maximal at 10^{-7} M. The enhancement of ECP release by TNF added to fibroblasts was also concentration dependent, occurring from 0.1 to 1 ng/ml and becoming maximal at 30 ng/ml (Fig. 5).

GM-CSF from fibroblasts partly accounts for ECP enhancement

When fibroblasts were fixed by addition of paraformaldehyde after TNF treatment, eosinophils added to fixed fibroblasts released significantly less ECP than eosinophils added to control fibroblasts after the culture with C5a (fixed fibroblasts, 11.7 ± 3.2 ; control fibroblasts, 15.6 ± 2.8 [percentage of ECP release, mean $\pm$ SEM, $n = 3$, $P < 0.05$]). This result suggests that soluble factors from fibroblasts stimulated with TNF may have some effect on ECP potentiation. Therefore, we investigated whether mAbs against some cytokines released from fibroblasts inhibit ECP potentiation or not. Among mAbs against GM-CSF, SCF, IL-6 and IL-11, mAb against GM-CSF partly inhibited ECP enhancement (anti-GM-CSF, 11.0 ± 1.4 ; control, 13.7 ± 0.8 [percentage of ECP release, mean $\pm$ SEM, $n = 4$, $P < 0.05$]). Thus, GM-CSF from fibroblasts may partly be involved in the potentiation of eosinophil degranulation.

Relationship between eosinophil adhesion and ECP release

To investigate the relationship between eosinophil adhesion and degranulation, we measured both eosinophil adhesion and ECP release from the same samples.

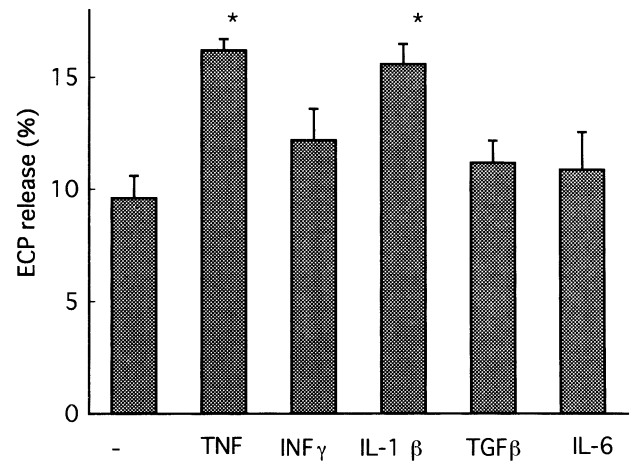


Figure 3. Effects of cytokines for fibroblasts on ECP release in coculture of C5a-activated eosinophils and fibroblasts. Confluent fibroblast monolayers were preincubated with culture medium alone (control) or medium containing 30 ng/ml TNF, 10^3 U/ml IFN- γ , 10 ng/ml IL-1 β , 10^{-9} M TGF- β , or 10^3 U/ml IL-6, and washed before addition of eosinophils. Eosinophils were cultured with 10^{-7} M C5a in presence of control fibroblasts or cytokine-treated fibroblasts for 16 h. Each column represents mean $\pm$ SEM obtained from four experiments performed in duplicate. Asterisk signifies $P < 0.05$ difference between control fibroblasts and cytokine-treated fibroblasts.

When eosinophils alone were activated by C5a or when fibroblasts alone were activated by TNF, significant enhancement of eosinophil adhesion was observed. Furthermore, stimulus treatment of both eosinophils and fibroblasts resulted in the enhancement of both adhesion of eosinophils to fibroblasts and ECP release (Table 1).

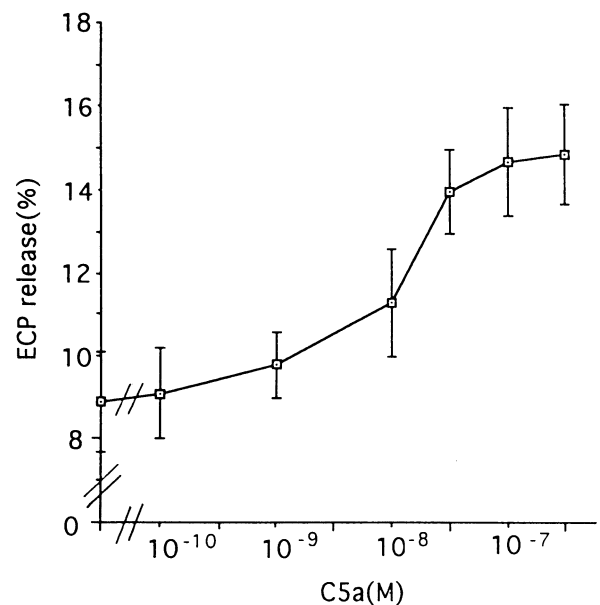


Figure 4. Dose dependence of C5a on ECP release in coculture of eosinophils and TNF-treated fibroblasts. Eosinophils were cultured with C5a at concentrations indicated for 16 h in presence of TNF (30 ng/ml)-treated fibroblasts. Data are means $\pm$ SEM of three separate experiments performed in duplicate.

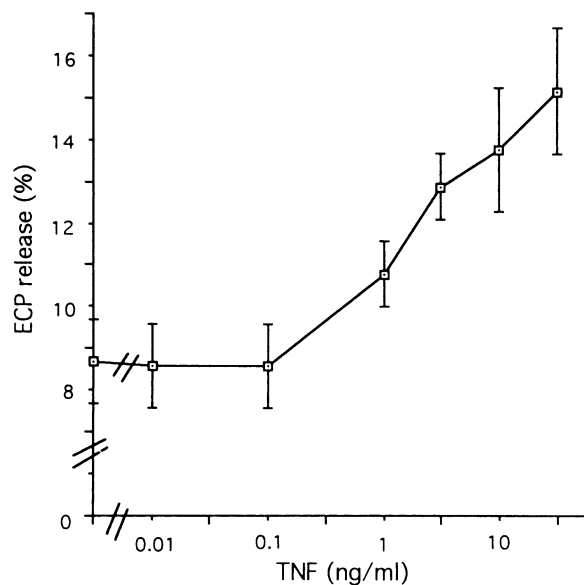


Figure 5. Dose dependence of TNF on ECP release in coculture of C5a-activated eosinophils and fibroblasts. Fibroblasts were preincubated with TNF at concentrations indicated for 4 h. Eosinophils were then added and cultured with C5a (10^{-7} M) for 16 h in presence of TNF-treated fibroblasts. Data are means $\pm$ SEM of three separate experiments performed in duplicate.

Effects of cytokines on ICAM-1 expression on fibroblasts

To examine further the effects of cytokines on fibroblasts, we investigated ICAM-1 expression on fibroblasts cultured with some cytokines. Flow cytometry analysis showed that ICAM-1 was constitutively expressed on HFL-1. ICAM-1 expression was clearly enhanced by TNF and IL-1 β (Fig. 6). The expression of VCAM-1 constitutively expressed on HFL-1 was also enhanced by TNF and IL-1 β (not shown). Similar results were observed with cytokine effects on eosinophil degranulation and those on adhesion molecule expression.

Effects of mAbs against adhesion molecules on ECP potentiation

Since the adhesion of eosinophils to fibroblasts appears to be related to the potentiation of ECP release, we examined the role of adhesion molecules in the response (Fig. 7). Anti-ICAM-1 mAb, anti-CD18 mAb, and anti-CD29 mAb significantly inhibited ECP enhancement by 56%, 36%, and 51%, respectively, whereas control mAb anti-HLA was ineffective.

Anti-ICAM-1 mAb, anti-CD18 mAb, and anti-CD29 mAb also caused comparable inhibition of the enhancement of eosinophil adhesion. These results suggest that the enhancement of ECP release in coculture of eosinophils and fibroblasts with stimulus treatment is partly dependent on the adhesion of eosinophils to fibroblasts mediated by the ICAM-1/CD18-dependent and CD29-dependent mechanism.

Table 1. Effects of stimuli on eosinophil adhesion and ECP release^a

	Eosinophil adhesion (%)	ECP release (%)
Fb + Eos	34.3 $\pm$ 4.4	7.7 $\pm$ 0.8
Fb + C5a $\cdot$ Eos	38.6 $\pm$ 4.8*	10.9 $\pm$ 1.9
TNF $\cdot$ Fb + Eos	47.7 $\pm$ 4.6*	12.0 $\pm$ 2.8
TNF $\cdot$ Fb + C5a $\cdot$ Eos	61.9 $\pm$ 3.9**	16.9 $\pm$ 1.8**

^a Eosinophils were cultured with or without C5a (10^{-7} M) in presence of TNF-treated (30 ng/ml for 4 h) fibroblasts or control fibroblasts for 16 h. Adhesion assay and ECP assay were performed simultaneously from same sample, as described in Material and methods. Data are mean $\pm$ SEM of five separate experiments performed in duplicates.

* and ** signify $P < 0.05$ and $P < 0.01$ significance, respectively, from values without stimulus treatment.

Eos: eosinophils; Fb: fibroblasts.

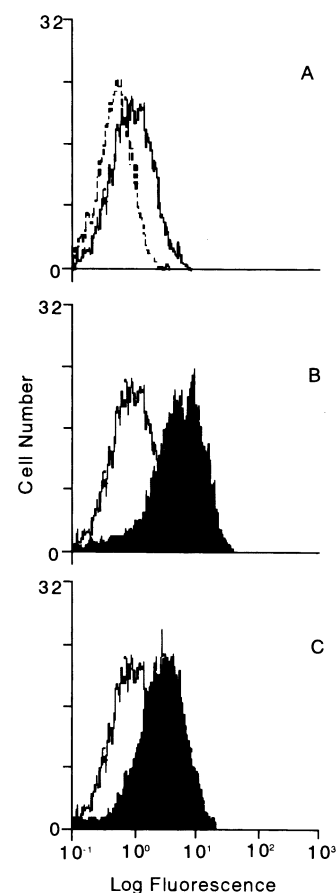


Figure 6. Regulation of ICAM-1 expression on HFL-1 by cytokines. HFL-1 were stained with 84H10 (anti-ICAM-1), as described in Material and methods. Stained cells (solid line) were compared to controls prepared by incubating cells with irrelevant isotype-matched mAb (broken line) (A). Next, cells were incubated for 4 h in presence of 30 ng/ml TNF (B) or 10 ng/ml IL-1 β (C). Histograms representing ICAM-1 staining of cells treated for 4 h with cytokines (shaded portion) were compared to histograms representing ICAM-1 labeling of cells incubated for 4 h in culture medium alone (open portion). Results are shown as cell number (y axis) vs log fluorescence intensity (x axis), and are representative of at least three different experiments.

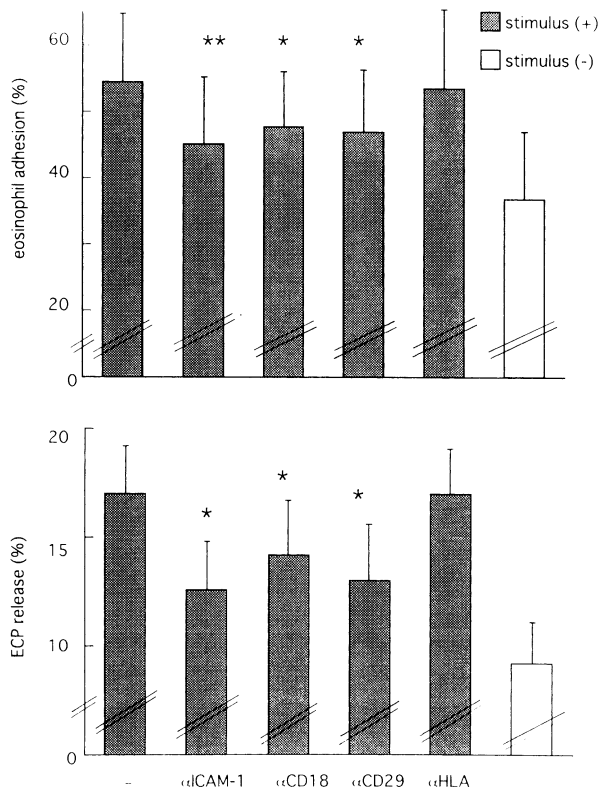


Figure 7. Effects of mAbs against adhesion molecules on eosinophil adhesion and ECP release. At start of coculture of eosinophils and fibroblasts with stimulus treatment, mAbs against ICAM-1, CD18, or CD29 were added to wells, and their effects on eosinophil adhesion (upper panel) and ECP release (lower panel) were examined. Anti-HLA mAb was used as control mAb. Each column represents mean $\pm$ SEM of four separate experiments using eosinophils obtained from different donors. One asterisk and two asterisks signify $P < 0.05$ and $P < 0.01$ significance, respectively, from values without mAb.

Discussion

In this study, we examined eosinophil degranulation in the presence of lung fibroblasts. ECP release was enhanced only when both eosinophils and fibroblasts were activated by C5a and TNF, respectively, while it was not enhanced when either eosinophils or fibroblasts were activated. ECP release from eosinophils activated by C5a was also potentiated when fibroblasts were stimulated by IL-1 β . The enhancement of ECP release was partly dependent on GM-CSF from fibroblasts and on the adhesion of eosinophils to fibroblasts mediated by the ICAM-1/CD18-dependent and CD29-dependent mechanism.

Eosinophil degranulation in the presence of lung fibroblasts with stimulus treatment in this study was only partial in contrast to eosinophils found in airway biopsies that showed strong and even total degranulation. The discrepancy between *in vitro* and *in vivo* findings may be due to the fact that not only fibroblasts and soluble factor used in this study but also other cells, cytokines, chemical mediators, and the extracellular

matrix may be involved in eosinophil degranulation *in vivo*. Investigation of the effects of cells other than fibroblasts and other soluble factors on eosinophil degranulation is needed.

In recent years, it has been suggested that tissue cells such as epithelial cells and fibroblasts may act as effector cells in asthmatic inflammation. It is reported that cultured human epithelial cells can support eosinophil survival via the elaboration of GM-CSF (20). Our previous study showed that the activation of epithelial cells affects eosinophil degranulation through the adhesion of eosinophils to epithelial cells (6). On the other hand, fibroblasts produce various cytokines that can contribute to tissue inflammation (8) and also express ICAM-1 (21). This study suggests that the activation of fibroblasts may be involved in the enhancement of eosinophil degranulation through a soluble factor from fibroblasts and/or adhesion between eosinophils and fibroblasts.

The role of complement components in the atopic asthmatic response has not been thoroughly elucidated. However, it has been reported that some asthmatic patients developed reduced hemolytic component activity or C4 in arterial or venous blood after allergen provocation (22). Complement deposition has been found in the bronchial mucosa of asthmatic patients (23). Some *in vitro* studies have demonstrated that the complement system can be activated by allergens via the classical pathway (24), the alternative pathway (25), or both pathways (26) 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 including eosinophils.

In our previous study, C5a was the most effective stimulus of ECP release of the four agonists C5a, C3a, PAF, and FMLP in cytochalasin B-pretreated eosinophils (19). It is suggested that cytochalasin B disrupts the barrier of the actin filament system, which limits the access of granules to the cytoplasmic face of the plasma membrane (27, 28), and that the effects of cytochalasin B on neutrophils mimic those of cell-surface interaction (29). In fact, in this study, C5a was the most effective stimulus of four agonists also in coculture of TNF-activated fibroblasts and eosinophils as it was in the above experiment with cytochalasin B.

Our previous study using bronchial epithelial cells has shown that, in coculture of epithelial cells and IL-5-activated eosinophils, ECP release was enhanced when epithelial cells were stimulated by IFN- γ as well as TNF (6). On the other hand, in this study, when IL-1 β , and not IFN- γ , as well as TNF, was added to

fibroblasts, ECP release was enhanced in coculture of fibroblasts and C5a-activated eosinophils. These results indicate the difference of the activator between epithelial cells and fibroblasts in ECP release from cocultured eosinophils.

It is well known that hematopoietic growth factors and other cytokines not only promote the proliferation and differentiation of progenitor cells but also modulate the response of mature inflammatory effector leukocytes. With regard to eosinophils, it has been demonstrated that GM-CSF, IL-3, and IL-5 all enhance cytotoxicity, superoxide production, phagocytosis (30–32), and degranulation in response to particulate stimuli (33). In this study, paraformaldehyde fixation of fibroblasts had some suppressive effect on ECP enhancement, and mAb against GM-CSF partly inhibited ECP potentiation in coculture of eosinophils and fibroblasts with stimuli. Therefore, GM-CSF from fibroblasts may have some influence on eosinophil degranulation.

There is increasing evidence that adhesion to extracellular matrix components or tissue cells modulates eosinophil function. It has been demonstrated that adhesion to fibronectin prolonged eosinophil survival by triggering autocrine generation of cytokines by eosinophils (34). Adhesion to extracellular matrix proteins and endothelial cells also modulates production of O_2^- in response to physiologic soluble stimuli (35). It has been reported that CD11b/CD18 (Mac-1)-dependent cellular adhesion plays an important role in the degranulation of human eosinophils induced by GM-CSF, PAF, or IgG (3, 4). In our previous study, we reported that CD18-dependent adhesion of eosinophils to epithelial cells is involved in the potentiation of eosinophil degranulation in coculture of these cells (6). It has been shown that lung fibroblasts and dermal fibroblasts express adhesion molecules such as ICAM-1 and VCAM-1 (36, 37). Our present study indicates that adhesion of eosinophils to fibroblasts may have some influence on eosinophil degranulation.

In this study, the enhancement of ECP release in coculture of eosinophils and fibroblasts with stimuli was accompanied by that of adhesion of eosinophils to fibroblasts, and was partially inhibited by anti-ICAM-1 mAb, anti-CD18 mAb, and anti-CD29 mAb. We found ICAM-1 expression on fibroblasts by flow cytometry analysis, and this expression was enhanced by addition of TNF and IL-1 β to fibroblasts, as was eosinophil degranulation in coculture of eosinophils and fibroblasts. VCAM-1 expression on fibroblasts was also enhanced by TNF and IL-1 β . Therefore, we suggest that ICAM-1 is a ligand for CD18 in our system. On the other hand, it is likely

that VCAM-1 or fibronectin on the surface produced by fibroblasts is a ligand for CD29. Thus, the potentiation of ECP release in coculture of eosinophils and fibroblasts with stimulus treatment may be partly dependent on the adhesion of eosinophils to fibroblasts mediated by the ICAM-1/CD18-dependent and VCAM-1 or fibronectin/CD29-dependent mechanism.

The mechanism of adhesion-dependent enhancement of ECP release is unclear. First, it is possible that cytoskeletal alterations in adherent eosinophils may lead to granule translocation and fusion of granules with plasma membrane (19). Second, the Ca^{2+} signal may be involved in adherent-dependent activation, because CD11b/CD18 integrins can trigger Ca^{2+} level oscillations and elevate cytosolic free Ca^{2+} levels after cell adhesion in neutrophils (38, 39). Third, cellular adhesion may affect phospholipase C activation because anti-CD18 mAb inhibits the production of inositol phosphates from eosinophils stimulated by immobilized IgG (3). Further investigation of the mechanism of adhesion-dependent activation is needed.

This study indicates that fibroblasts may influence eosinophil degranulation through a soluble factor from fibroblasts and/or adhesion of eosinophils to fibroblasts. On the other hand, it has been reported that eosinophils can release mitogens for fibroblasts *in vitro* (9) and that eosinophil major basic protein interacts in a synergistic fashion with IL-1 α or TGF- β to augment fibroblast IL-6-type cytokine production (10). It is possible that activated fibroblasts lead to the enhancement of eosinophil granule protein release, which results in the further activation of fibroblasts, leading to chronic asthmatic inflammation.

In summary, the present study suggests that the activation of airway fibroblasts that come into contact with activated eosinophils may play some role in the potentiation of eosinophil degranulation in the pathogenesis of bronchial asthma. Furthermore, the data show that the enhancement of eosinophil degranulation may be partly dependent on GM-CSF from fibroblasts and on the adhesion of eosinophils to fibroblasts mediated by the ICAM-1/CD18-dependent and CD29-dependent mechanism.

Acknowledgments

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