

Signaling through the $\beta 2$ integrin prolongs eosinophil survival

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Background: Recently, adhesion molecules have been suggested to play an important role in allergic inflammatory diseases such as bronchial asthma. It is unclear whether eosinophil activation and paracrine or autocrine synthesis of eosinophilopoietic growth cytokines is mediated through signaling by intercellular adhesion molecule-1 (ICAM-1) and the $\beta 2$ integrin family.

Objective: We examined whether signaling by ICAM-1 and its ligands ($\beta 2$ integrins) could prolong eosinophil survival.

Methods: Eosinophils were isolated from patients with hypereosinophilia by modified CD16 negative selection. After culture with or without recombinant soluble ICAM-1, eosinophil viability was measured by trypan blue dye exclusion.

Results: Eosinophil survival was prolonged in cultures with recombinant soluble ICAM-1 compared with cultures without it ($P < .01$ on days 2, 4, and 6); this effect was dose-dependent. Eosinophil survival in cultures with recombinant soluble ICAM-1 was significantly inhibited by antibodies against ICAM-1 ($P < .01$), complement receptor 3 ($P < .01$), and lymphocyte function-associated antigen-1 β ($P < .01$). Anti-IL-3 showed no effect on eosinophil survival, whereas anti-IL-5 caused partial inhibition of survival. Interestingly, anti-granulocyte/macrophage colony-stimulating factor caused the complete inhibition of eosinophil survival in cultures with recombinant soluble ICAM-1.

Conclusion: These results suggested the importance of the $\beta 2$ integrins in eosinophil-mediated allergic inflammation. (*J Allergy Clin Immunol* 2000;106:S99-103.)

Key words: Adhesion molecules, integrins, eosinophil survival, cytokines

Attention has recently been focused on allergic inflammatory reaction in asthma, especially in the late asthmatic response and intractable asthma. Many types of cells, including eosinophils, macrophages, lymphocytes, and platelets, participate in inflammatory reactions that occur in the respiratory tract. Eosinophils are considered to be the major type of inflammatory cell involved in bronchial asthma¹ because eosinophil-specific granule proteins can damage the bronchial mucosa and

Abbreviations used

ICAM-1: Intercellular adhesion molecule-1

LFA-1: Lymphocyte function-associated antigen-1

RPMI: Roswell Park Memorial Institute

cause bronchial hyperresponsiveness.² The effector functions of eosinophils have been extensively studied in parasitic diseases and have also been suggested to have a role in asthma.³⁻⁵ The detection of a wide variety of membrane receptors and the identification of several cytotoxic molecules (mainly the granule basic proteins) over recent years have led eosinophils to be viewed as effector cells that release active mediators after interaction with specific antibodies and antigens.⁶ In the light of the well-known cytotoxicity of granule proteins for epithelial or lung cells in vitro, their detection in the tissues or bronchoalveolar lavage fluid of patients with asthma strongly suggests a pathologic role of eosinophils in asthma.² Both an increase of eosinophil numbers and eosinophil activation can be detected in the blood and tissues of patients with asthma.⁷ Hypodense eosinophils have been suggested to show increased effector activity, not only against parasites but also in various pathologic situations.⁷⁻¹⁴ Hypodense eosinophils exhibit upregulation of the expression of various membrane receptors^{9,15-17} and increased exocytosis of cationic proteins,¹⁸ elevated in vitro leukotriene production,¹⁹ and variability of protein expression.²⁰

Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) have been considered to play an important role in allergic inflammatory diseases such as bronchial asthma, because symptomatic improvement was achieved in an animal model of asthma after treatment with an anti-ICAM-1 mAb.²¹ Moreover, adhesion molecules may play an important role in allergic inflammation, not only through modulating the adherence of inflammatory cells (particularly eosinophils) at the inflamed focus but also through the activation of these cells. Therefore, it is of interest to evaluate eosinophil activation by signaling through ICAM-1 and the $\beta 2$ integrin family (ie, complement receptor 3, macrophage antigen-1, and lymphocyte function-associated antigen-1 [LFA-1]), which are ligands for ICAM-1. For example, it is still unclear whether eosinophil activation mediated through ICAM-1 and the $\beta 2$ integrin family results in an increased release of eosinophil-mediated inflammatory

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0091-6749/2000 \$12.00 + 0 1/0/106884

doi:10.1067/mai.2000.106884

Viability (%)

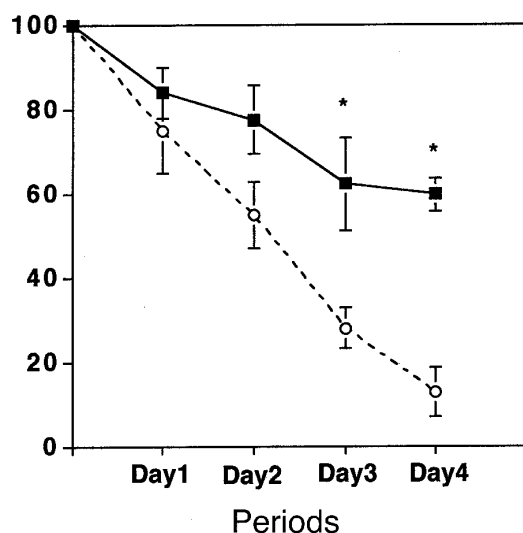


FIG 1. Eosinophil survival in cultures with (■) and without (○) ICAM-1. Viability was examined by trypan blue dye exclusion on days 1 through 4 of culture. * $P < .01$.

proteins. In this context, we have previously reported that an increase of eosinophil oxidative metabolism²² and a release of granule proteins can be mediated by signaling through ICAM-1 and its ligands.²³ It has also been demonstrated that adhesion to fibronectin can prolong eosinophil survival,²⁴ so it was of interest for us to investigate whether signaling through ICAM-1 and its ligands could influence eosinophil viability.

METHODS

Isolation of eosinophils

Eosinophils were isolated from heparinized venous blood samples obtained from patients with hypereosinophilia by a modification of the CD16 negative selection method,²⁵ as described previously.²⁶ In brief, after addition of one-fourth volume of 6% high molecular weight dextran (Wako Chemicals, Osaka, Japan) to the heparinized peripheral venous blood, the mixture was allowed to stand for 40 minutes at room temperature, and the buffy coat was obtained. Cells in the buffy coat were washed twice with Hanks' balanced salt solution (Difco Laboratories, Detroit, Mich), and then were reacted with an anti-CD16 mAb (MG38; Nichirei, Tokyo, Japan), an anti-CD3 mAb (Nu-pan T; Nichirei), an anti-CD20 mAb (Nu-B2; Nichirei), and an anti-CD14 mAb (CLB-Mon/I; Nichirei) for 45 minutes at 4°C. After this, the cells were again washed twice with Hanks' balanced salt solution and were reacted with anti-mouse IgG magnetic beads (Dynabeads; Dynal, Norway; cell:bead ratio, 1:20) at 4°C for 60 minutes. Cells positive for CD16, CD3, CD20, or CD14 were removed with the use of a magnetic particle concentrator (Dynal), and the cells that were negative for these molecules were harvested as eosinophils. This procedure yielded eosinophils at more than 96% purity.²⁶

Recombinant soluble ICAM-1

A soluble form of ICAM-1 that lacked the transmembrane and cytoplasmic domains was expressed by transfection of Chinese hamster ovary cells and was purified from culture supernatants.²²

Eosinophil culture

Freshly isolated eosinophils were suspended at 2×10^6 cells/mL in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco Laboratories, Grand Island, NY). Aliquots of this suspension were seeded into 24-well tissue culture plates (Costar, Cambridge, Mass) with or without coating by recombinant soluble ICAM-1. To coat the plates, 200 μ L of recombinant soluble ICAM-1 (500, 50, 5, or 0.5 mg/mL) was added to each well, and incubation was allowed for 1 hour at 37°C. As a control, the supernatant of nontransfected Chinese hamster ovary cells that were cultured under protein-free conditions was used to coat well in the same manner. Before the addition of the cells, each well was washed twice with RPMI 1640 to remove free soluble ICAM-1. Culturing was done for 1 to 4 days at 37°C in a 5% carbon dioxide atmosphere. To block nonspecific protein-binding, 200 μ L of 1% heat-denatured bovine serum albumin in phosphate-buffered saline solution was added to each well, and the plates were incubated for 2 hours at room temperature. Before the cells were added, the plates were washed twice with RPMI 1640 medium containing 1% bovine serum albumin. The cells were cultured in RPMI 1640 with penicillin and streptomycin, but without fetal calf serum, to avoid the possible influence of matrix proteins contained in serum. In some experiments, mAbs were added to the wells at the start of culture. To determine eosinophil viability, cells were removed from each well by gentle pipetting, stained with trypan blue, and counted by an examiner who was blinded to the specifics of the study.

Statistical analysis

Data were expressed as mean $\pm$ SE. The Student *t* test was used for comparison of means. A probability value of .05 or less was considered significant.

RESULTS

Eosinophil survival was prolonged in cultures with recombinant soluble ICAM-1 when compared with cultures without ICAM-1 ($P < .01$ on days 2-4: 77.6% $\pm$ 8.2% vs 54.9% $\pm$ 7.9% [day 2], 62.3% $\pm$ 11.0% vs 28.0% $\pm$ 4.9% [day 3], 59.8% $\pm$ 4.0% [day 4] vs 12.9% $\pm$ 5.9% [control]; $n = 4$; Fig 1), showing concentration-dependent fashion (Fig 2). Whereas survival on day 3 of culture was not inhibited by a mAb for LFA-1 β (CD11a; Dako Japan, Japan; control, 32.5% $\pm$ 3.6%; ICAM-1, 67.9% $\pm$ 6.9%, vs anti-CD11a, 61.9% $\pm$ 8.5%), eosinophil viability in cultures with recombinant soluble ICAM-1 was significantly reduced by mAbs for ICAM-1 (Immunotech, Marseille, France; 39.7% $\pm$ 7.4%; $P < .01$), complement receptor 3 (CD11b; Ortho Diagnostic Systems, Mass; 34.0% $\pm$ 5.7%; $P < .01$), and LFA-1 β (CD18; Dako Japan; 36.5% $\pm$ 5.4%; $P < .01$; $n = 7$; Fig 3). On day 3 of culture, anti-IL-3 (Cosmobio, Japan) showed no effect on eosinophil survival (control, 39.8% $\pm$ 6.0%; ICAM-1, 70.0% $\pm$ 9.9% vs 70.4% $\pm$ 13.0%; $P > .1$), but anti-IL-5 (NC17; Olympus, Japan) showed inhibition (59.9% $\pm$ 15.6%) in 2 of 6 experiments and had no effect on eosinophil viability in the other 4 experiments. Interestingly, anti-GM-CSF (Cosmobio) completely inhibited eosinophil survival in cultures with recombinant soluble ICAM-1 (37.8% $\pm$ 7.0%; $n = 6$; Fig 4).

ICAM-1 concentration

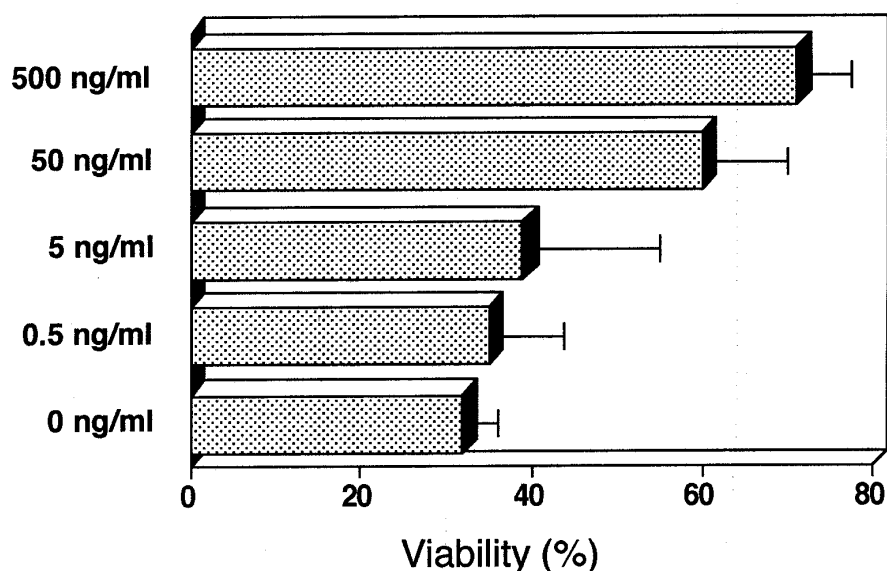


FIG 2. Eosinophil viability after culture with various concentrations of ICAM-1. Eosinophil survival was prolonged in cultures with recombinant soluble (RS) ICAM-1 in a concentration-dependent fashion. Eosinophils cultured with 500 ng/mL and 50 ng/mL of RS ICAM-1 showed high viability compared with eosinophil cultures without RS ICAM-1 ($P < .05$).

Culture Conditions

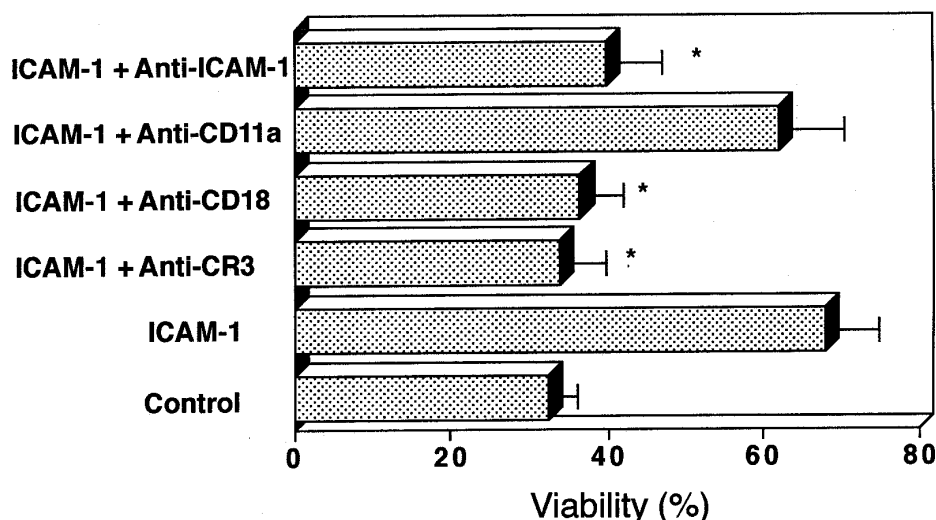


FIG 3. The effect of mAbs directed against various adhesion molecules on eosinophil viability. * $P < .01$.

DISCUSSION

Eosinophil survival was prolonged in recombinant soluble ICAM-1 coated wells. To investigate the mechanism by which recombinant soluble ICAM-1 prolonged eosinophil survival, we examined the inhibitory effect of antibodies directed against various adhesion molecules. Our results suggested the CD11b and CD18 may play an important role in the prolongation of eosinophil survival through the $\beta 2$ integrins.

Eosinophils have recently been shown to produce various cytokines, including IL-5, GM-CSF, and IL-3.⁵ The recent demonstration that eosinophils can synthesize GM-CSF,²⁷ IL-3,^{27,28} IL-5²⁹ and RANTES (regulated on activation, normal T expressed and secreted)^{30,31} indicates that these cytokines may not only have a paracrine but also an autocrine role in the regulation of eosinophil production, differentiation, and activation. We demonstrated the complete inhibition by anti-GM-CSF and par-

Culture Conditions

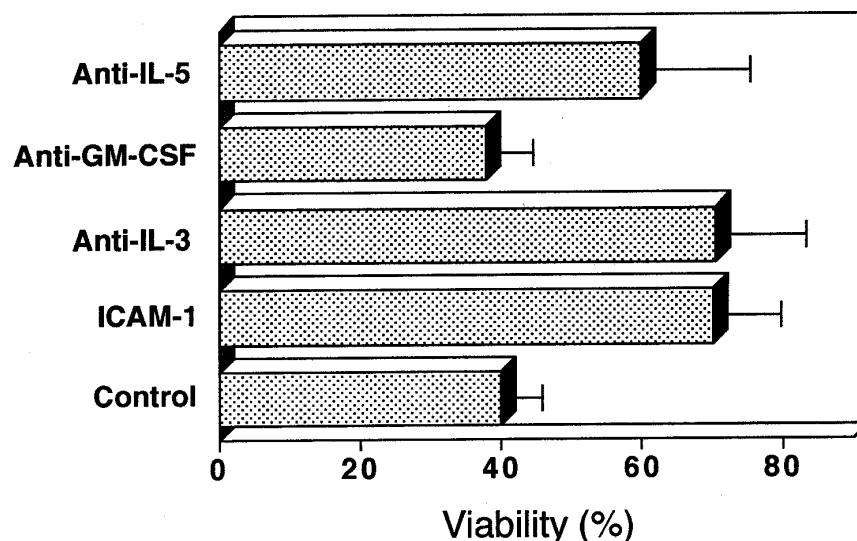


FIG 4. The effect of neutralizing antibodies for eosinophilopoietic cytokines on eosinophil viability. Anti-GM-CSF completely inhibited eosinophil survival in cultures with recombinant soluble ICAM-1 ($P < .01$), and anti-IL-5 showed inhibition in 2 of 6 experiments.

tial inhibition by anti-IL-5 and no inhibition by anti-IL-3 on prolonged survival through the signal of the $\beta 2$ integrin.

These findings demonstrated that the interaction of eosinophils and ICAM-1 resulted in CD11b- and CD18-mediated autocrine generation of GM-CSF, which led to the prolongation of eosinophil survival. Thus, in addition to the reported prolongation of eosinophil survival by adhesion to fibronectin,²⁴ we demonstrated the prolongation of survival by signaling through the $\beta 2$ integrin family. The possible important role of the $\beta 1$ integrin family in the selective infiltration of eosinophils into inflamed foci across the endothelial cell barrier is well known and well emphasized, because of the very late antigen-4 (CD49d), which is a member of the $\beta 1$ -integrin family and is expressed on eosinophils and basophils but not on neutrophils. We have also shown that there is release of eosinophil-specific proteins during culture with recombinant soluble ICAM-1, which leads to the selective induction of vascular cell adhesion molecule-1 by endothelial cells.²³ Taken together, our findings suggest that eosinophil adherence to endothelial cells, even if mediated by ICAM-1 and the $\beta 2$ integrin, may be involved in selective eosinophil recruitment and accumulation at sites of allergic inflammation by the prolongation of eosinophil survival.

In conclusion, these results suggested an important role of the $\beta 2$ integrins in eosinophil-mediated allergic inflammation.

The authors thank Ms Yoko Ohyama for her secretarial assistance.

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