

Original Article

Role of adhesion molecules in eosinophil activation: A comparative study on the effect of adhesion molecules on eosinophil survival

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ABSTRACT

Background: Adhesion molecules participate in an important part of inflammatory process in relation to the accumulation of inflammatory cells, such as eosinophils. The expression of adhesion molecules differs depending upon the cells and tissue. In the present study, to elucidate these differences, a comparative study was performed on the prolongation of eosinophil survival via adhesion molecules.

Methods: Blood eosinophils were purified using Percoll and anti-CD16 antibody coated magnetic beads. Eosinophils were incubated with or without the various concentrations of adhesion molecules for 18 or 36 h. Eosinophil survival was analyzed by a flow cytometer with staining by annexin V (AV) and propidium iodide (PI).

Results: Intercellular adhesion molecule (ICAM)-1, fibronectin (FN) and cellular fibronectin (cFN), but not vascular cell adhesion molecule (VCAM)-1, significantly prolonged eosinophil survival compared with control. The present comparative study for eosinophil survival showed the following tendency: cFN = FN > ICAM-1 > VCAM-1. Moreover, enhancement of prolonged eosinophil survival by connecting segment-1 was greater than that by FN and cFN.

Conclusions: The regulation of adhesion molecules, by not only preventing eosinophil adhesion but also

eosinophil activation, may be a potential target in the treatment of allergic inflammatory disorders.

Key words: adhesion molecule β integrin, eosinophil, fibronectin, survival.

INTRODUCTION

Inflammatory responses are now believed to play a primary role in immunologic and allergic responses. Eosinophils are considered to be a major type of inflammatory cell, because they cause tissue damage with their granular proteins and also modulate inflammation by producing cytokines.¹

Adhesion molecules participate in an important part of inflammatory process, including eosinophil migration and accumulation into inflamed sites.² Among the β 1 integrins, very late antigen (VLA)-4 binds vascular cell adhesion molecule (VCAM)-1 and fibronectin (FN), and the β 2 integrins Mac-1 and LFA-1 bind to intercellular adhesion molecule (ICAM)-1, expressed on eosinophils. These integrins have been considered to play a role in cell activation as well as cell adhesion. Signals via adhesion molecules augment the release of granular proteins, the production of reactive oxygen species (ROS)^{3–5} and eosinophil survival.^{6,7}

The expression of adhesion molecules differs depending upon the cells and tissue. For example, VCAM-1 is mainly expressed on the surface of vascular endothelial cells, ICAM-1 is expressed on respiratory epithelium and vascular endothelial cells and FN is expressed in the interstitium.⁸ However, differences among the effects of various adhesion molecules on eosinophil survival have

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not been studied sufficiently. In the present study, to elucidate these differences, the survival of eosinophils cultured with adhesion molecules was examined by flow cytometry analysis. Moreover, a comparative study on the prolongation of eosinophil survival via adhesion molecules was performed.

METHODS

Eosinophil preparation

Peripheral venous blood was obtained from subjects with mild to moderate eosinophilia. Eosinophils were isolated by sedimentation with 6% dextran followed by

centrifugation on 1.088 Percoll (Pharmacia, Uppsala, Sweden) density gradients,⁹ as modified from the method of Hansel *et al.*¹⁰ Cells were further purified by negative selection using anti-CD16 immunomagnetic beads and a MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany). Eosinophils (>99% purity) were then suspended in Hank's balanced salt solution (HBSS) with 1% fetal calf serum (FCS) in tubes coated with 3% human serum albumin.

Eosinophil culture

A 48-well flat-bottom tissue culture-treated plate was coated with recombinant soluble (rs) ICAM-1 (R&D

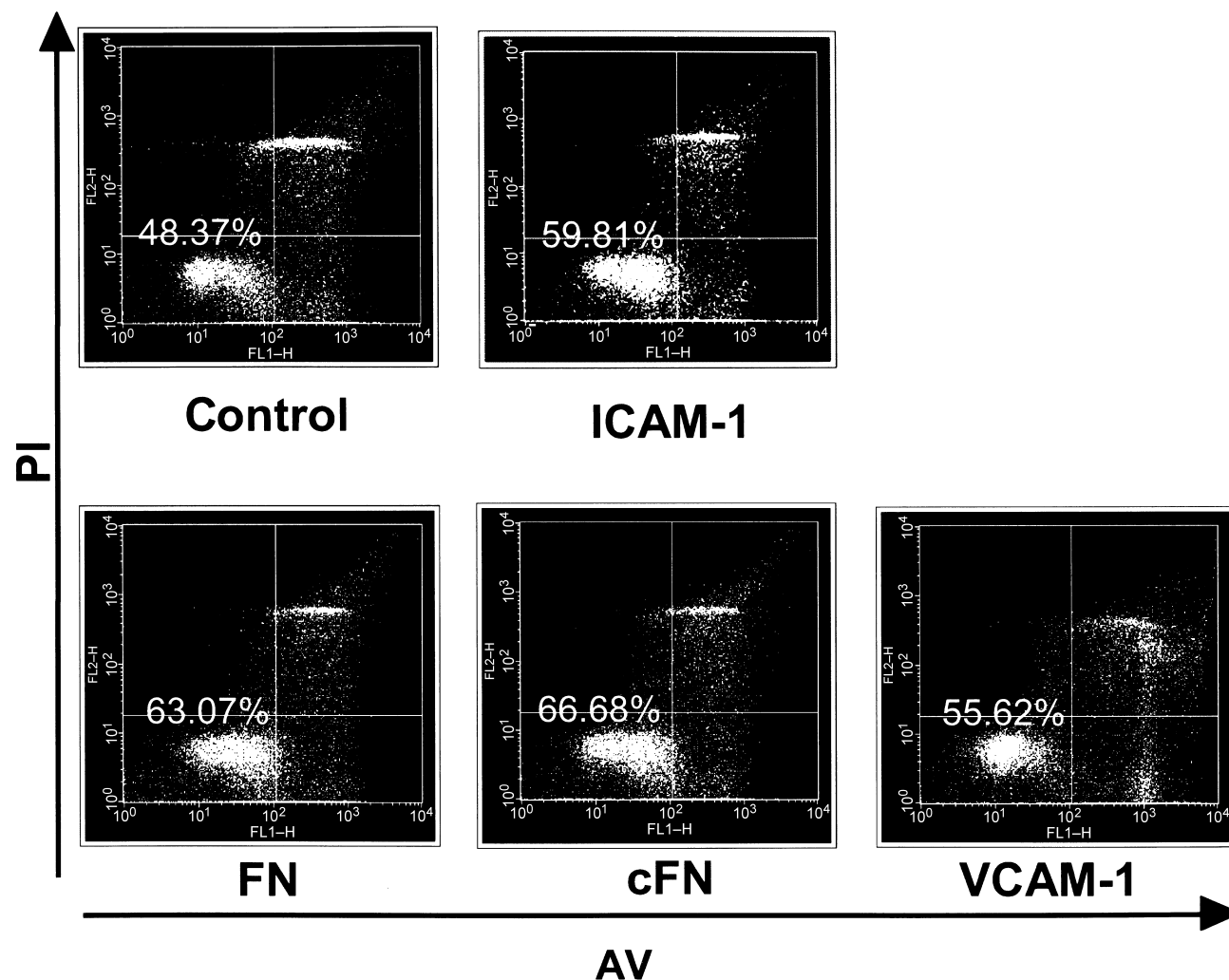


Fig. 1 Dot plots of FACSscan analysis on the prolongation of eosinophil survival. This figure shows dot plots after cells had been incubated with or without adhesion molecules for 18 h. Values in the figure denote the percentage of living cells (annexin V (AV)-negative and propidium iodide (PI)-negative cells). VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; FN, fibronectin; cFN, cellular fibronectin.

Systems, Minneapolis, MN, USA), rs VCAM-1 (R&D Systems), FN (Sigma-Aldrich, St Louis, MO, USA), cellular fibronectin (cFN; Sigma-Aldrich) and connecting segment-1 (CS-1; Sigma-Aldrich) at 37°C for 1 h. Blocking was then performed with 1% bovine serum albumin (BSA). Eosinophils were suspended in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 3% FCS at a concentration of 1×10^6 /mL for culture in a 5% CO₂ atmosphere at 37°C. The plate was coated with 1% BSA as a control.

Assay for eosinophil survival

Eosinophils were washed twice in cold phosphate-buffered saline (PBS) after incubation with or without various concentrations of adhesion molecules (0.1, 1, 10 and 50 µg/mL) for 18 or 36 h. Then, double staining with propidium iodide (PI) and annexin V (AV) (Apoptosis Detection Kit; MBL, Nagoya, Japan) and FACSscan assay (Becton-Dickinson, San Jose, CA, USA) were performed.¹¹ The percentage of viable cells was given by the percentage of double-negative cells depicted in the lower left quadrant.

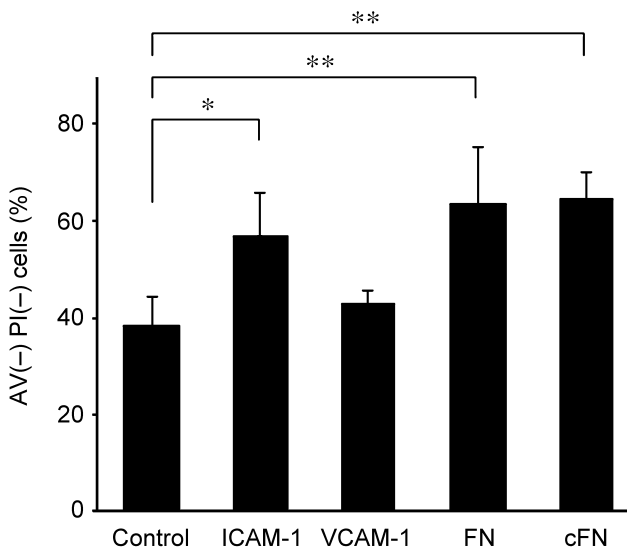


Fig. 2 Effects of adhesion molecules on the prolongation of eosinophil survival. Eosinophils were cultured with or without 10 µg/mL adhesion molecules for 18 h and their effects were compared with control. Data are expressed as the mean ± SEM ($n = 3-5$). * $P < 0.05$, ** $P < 0.01$ compared with control (Fisher's multiple comparison test). AV(-)PI(-) cells, annexin V-negative and propidium iodide-negative cells; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; FN, fibronectin; cFN, cellular fibronectin.

Statistical analysis

Results are expressed as the mean ± SEM. An ANOVA was used for group analysis. For Fisher's multiple comparison test, $P < 0.05$ was considered significant.

RESULTS

Effects of adhesion molecules on eosinophil survival

Eosinophils were cultured with or without 10 µg/mL ICAM-1, VCAM-1, FN or cFN for 18 h. The percentage of viable cells was then determined using a flow cytometer, after eosinophils were double stained with AV and PI (Fig. 1). Intercellular adhesion molecule-1 ($56.9 \pm 8.8\%$; $P < 0.05$), FN ($63.8 \pm 11.2\%$; $P < 0.01$) and cFN ($64.6 \pm 5.3\%$; $P < 0.01$), but not VCAM-1 ($42.9 \pm 2.6\%$; $P > 0.05$), significantly prolonged eosinophil survival compared with control ($38.6 \pm 6.0\%$; Fig. 2). With regard to the number of apoptotic cells, indicated by the percentage of AV-positive and PI-negative cells, there were no significant differences among groups cultured with various adhesion molecules (data not shown). The percentage of viable cells after 36 h was also examined, but no significant differences were observed (Table 1).

Effect of various concentrations of adhesion molecules on eosinophil survival

To investigate the effect of various concentrations of adhesion molecules on eosinophil survival, eosinophils were cultured with adhesion molecules at concentrations of 0.1, 1, 10 and 50 µg/mL for 18 h on the basis of

Table 1 Effects of adhesion molecules on prolongation of eosinophil survival

	Survival rate (%)	<i>P</i>
Control	47.70 ± 5.74	
VCAM-1	46.41 ± 5.55	0.443
Fibronectin	48.90 ± 6.42	0.562
Cellular fibronectin	52.88 ± 5.27	0.592
ICAM-1	51.62 ± 2.43	0.433

Data are the mean ± SEM ($n = 3-5$). Fisher's multiple comparison test was used for P values.

Eosinophils were cultured with or without 10 µg/mL adhesion molecules for 36 h and their effects were compared with control.

VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

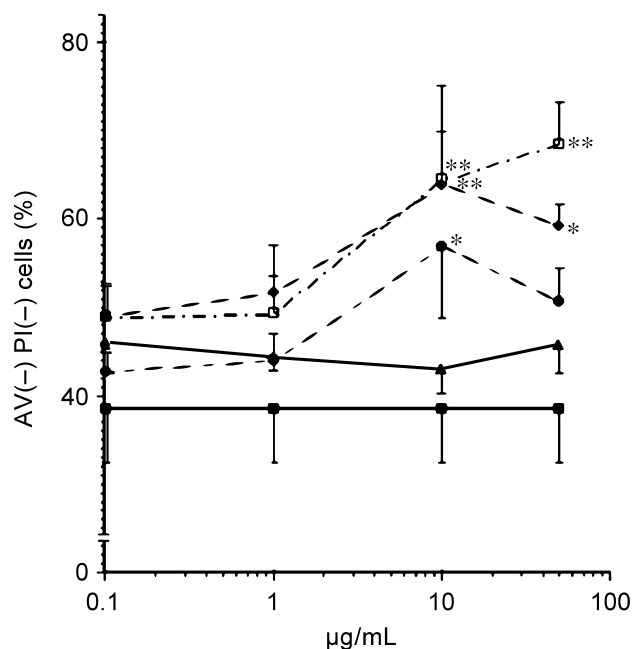


Fig. 3 Effect of various concentrations of adhesion molecules on eosinophil survival. Eosinophils were incubated with or without 0.1, 1, 10 and 50 $\mu\text{g/mL}$ adhesion molecules for 18 h. The percentages of viable cells were compared with control. Data are expressed as the mean $\pm$ SEM ($n = 4$). * $P < 0.05$, ** $P < 0.01$ compared with control (Fisher's multiple comparison test). (■), control; (●), intercellular adhesion molecule-1; (▲), vascular cell adhesion molecule-1; (◆), fibronectin; (□), cellular fibronectin.

results described above (Fig. 3). Fibronectin and cFN prolonged eosinophil survival, with the peak effect at concentrations of 10 $\mu\text{g/mL}$ FN ($63.8 \pm 11.3\%$; $P < 0.01$) and 50 $\mu\text{g/mL}$ cFN ($68.4 \pm 4.9\%$; $P < 0.01$). Intercellular adhesion molecule-1 showed the highest prolongation of eosinophil survival at 10 $\mu\text{g/mL}$ ($56.9 \pm 8.8\%$; $P < 0.05$). Regarding the effect of VCAM-1 on prolonged eosinophil survival, no significant differences were observed with any concentration used.

Effect of the CS-1 region on eosinophil survival

To elucidate the effect of the CS-1 region on eosinophil survival, a comparative study between CS-1, FN and cFN was performed using concentrations of 1 $\mu\text{g/mL}$ (Fig. 4). At 1 $\mu\text{g/mL}$, CS-1 significantly prolonged eosinophil survival ($71.3 \pm 5.2\%$; $P < 0.01$) compared with control.

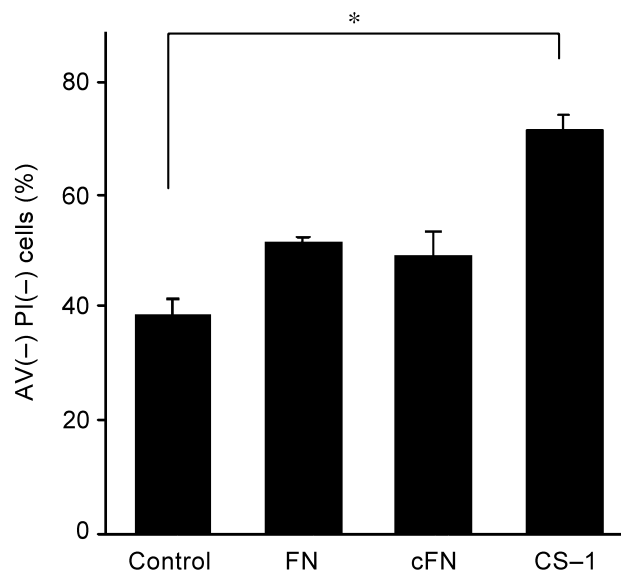


Fig. 4 Effect of the connecting segment-1 (CS-1) region on eosinophil survival. Eosinophils cultured with or without 1 $\mu\text{g/mL}$ of cellular fibronectin (cFN), fibronectin (FN) and CS-1 for 18 h were compared with control. The percentages of viable cells were compared with control. Data are expressed as the mean $\pm$ SEM ($n = 4$). * $P < 0.05$ compared with control (Fisher's multiple comparison test).

DISCUSSION

In the present study, it was demonstrated that ligands of both $\beta 1$ and $\beta 2$ integrins prolonged eosinophil survival. We have reported previously that prolonged survival of eosinophils induced by ICAM-1 was completely blocked by anti-granulocyte-macrophage colony stimulating factor (GM-CSF)⁶ and FN also prolonged eosinophil survival, which was related to the production of GM-CSF and interleukin (IL)-3.¹² Thus, these reports suggested that GM-CSF and other cytokines may be produced by eosinophils themselves in response to signals by adhesion molecules. Further investigations should clarify whether cytokines may be produced to prolong eosinophil survival.

Furthermore, the present comparative study on eosinophil survival showed the following tendency: cFN = FN > ICAM-1 > VCAM-1. Intercellular adhesion molecule-1 and VCAM-1 are mainly expressed in vascular endothelium, whereas FN and cFN are expressed in the interstitium.⁸ In this regard, this tendency may reflect a pathophysiologic process during activation of eosinophils at the site of inflammation. Moreover, Wu *et al.* have reported that VCAM-1 robustly adheres to

VLA-4 more than FN.¹³ In addition to FN, Meerschaert *et al.* have reported that eosinophil survival is prolonged by VCAM-1.¹⁴ In the present study, FN prolonged eosinophil survival more than VCAM-1. In addition, to confirm this finding, the effect of the CS-1 region, which is the binding site for FN, was investigated. The CS-1 region, as well as FN, prolonged eosinophil survival more than VCAM-1. Taken together, these findings suggest that the mechanism of cell activation, such as the prolongation of eosinophil survival via adhesion molecules, may be different from that of cell adherence.

We reported previously that eosinophils release eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) in response to signaling through the adherence to ICAM-1.⁴ Reactive oxygen species, as well as eosinophil granule proteins, from eosinophils are considered inflammatory mediators that cause tissue injury at the inflamed site. In this context, we also reported previously the possible involvement of adhesion molecules in ROS production from eosinophils.¹⁵ The present findings suggest that adhesion molecules may promote tissue damage by eosinophil adherence to the inflamed site and prolongation of eosinophil survival. Symptomatic improvement by the preventing migration and/or activation of eosinophils at the site of airway inflammation was achieved in an animal model of asthma after treatment with an anti-ICAM-1 monoclonal antibody (mAb) or anti-VLA-4 mAb.^{16–18} Therefore, the regulation of adhesion molecules, by not only preventing eosinophil adherence but also activation, such as prolongation of survival, may be a potential target for the treatment of allergic inflammatory disorders.

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