

Adhesion of Human Basophils, Eosinophils, and Neutrophils to Interleukin 1-activated Human Vascular Endothelial Cells: Contributions of Endothelial Cell Adhesion Molecules

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Summary

Cytokines such as interleukin 1 (IL-1) promote adhesiveness in human umbilical vein endothelial cells for leukocytes including basophils, eosinophils, and neutrophils, and induce expression of adherence molecules including ICAM-1 (intercellular adhesion molecule-1), ELAM-1 (endothelial-leukocyte adhesion molecule-1), and VCAM-1 (vascular cell adhesion molecule-1). In the present study, blocking monoclonal antibodies (mAb) recognizing ICAM-1, ELAM-1, and VCAM-1 have been used to compare their roles in IL-1-induced adhesion of human basophils, eosinophils, and neutrophils. IL-1 treatment of endothelial cell monolayers for 4 hours induced a four- to eight-fold increase in adhesion for each cell type. Treatment of endothelial cells with either anti-ICAM-1 or anti-ELAM-1 mAb inhibited IL-1-induced adherence of each cell type. In contrast, treatment with anti-VCAM-1 mAb inhibited basophil and eosinophil (but not neutrophil) adhesion, and was especially effective in blocking eosinophil adhesion. The effects of these mAb were at least additive. Indirect immunofluorescence and flow cytometry demonstrated expression of VLA-4 α (very late activation antigen-4 α , a counter-receptor for VCAM-1) on eosinophils and basophils but not on neutrophils. These data document distinct roles for ICAM-1, ELAM-1, and VCAM-1 during basophil, eosinophil, and neutrophil adhesion *in vitro*, and suggest a novel mechanism for the recruitment of eosinophils and basophils to sites of inflammation *in vivo*.

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Acute allergic reactions in the skin and airways are often followed by a more sustained inflammatory reaction (the so-called late phase response) characterized by the appearance of eosinophils and basophils, as well as neutrophils and mononuclear cells (1). The fact that increased numbers of eosinophils and basophils appear during these and other inflammatory responses suggests that mechanisms exist which facilitate their activation and recruitment to extravascular sites. Results of studies examining *in vitro* binding of purified leukocyte subtypes to cultured human vascular endothelial cells have suggested that endothelium can play an active role in the recruitment of leukocytes. The cytokines IL-1 and TNF can stimulate cultured endothelial cells to acquire adhesive properties for various peripheral blood leukocytes *in vitro* (2-5). During these treatments, changes in adhesiveness are associated with the induction on endothelial cells of at least three distinct adhesion molecules: ICAM-1, ELAM-1, and

VCAM-1, identical to inducible cell adhesion molecule-110 (INCAM-110) and the 1E7/2G7 protein (6, 7). Both ELAM-1 and ICAM-1 are important ligands in neutrophil adhesion to activated endothelium (7-10), while VCAM-1 does not appear to participate (11, 12). Since the endothelial cell adherence receptors for eosinophils and basophils are unknown, we used blocking mAb to ICAM-1, ELAM-1, and VCAM-1 to compare their effects on the adhesion of basophils, eosinophils, and neutrophils to cytokine-activated endothelium *in vitro*.

Materials and Methods

Antibodies. Murine mAb recognizing ELAM-1 (H18/7, IgG2a [9]), ICAM-1 (Hu5/3, IgG1 [10]), and VCAM-1 (2G7, IgG1 [7]) were generated as previously described. In addition, monoclonal HP2/1, an IgG1 antibody recognizing the α subunit of the leukocyte integrin VLA-4 (CD49d) was purchased (AMAC Inc., West-

brook, ME). Antibody W6/32 (IgG2a, anti-HLA class I antigen) was used as a control antibody (9). F(ab')₂ preparations of the above antibodies (except HP2/1) were generated using pepsin digestion as described (7, 10), and used in all blocking experiments.

Endothelial Cell Cultures. Endothelial cells were isolated following collagenase digestion from human umbilical cord veins, and grown to confluence as previously described (3). No cell lines were used after more than three serial passages.

Isolation of Human Leukocytes. Human neutrophils and basophils were purified from EDTA-anticoagulated venous blood of volunteers using density gradient centrifugation methods (3, 5). Human eosinophils were purified from normal donors or patients with allergic rhinitis or asthma using a discontinuous Percoll gradient method or an immunomagnetic bead technique (4, 13). Purity and viability for neutrophils and eosinophils always exceeded 94%, while basophil purity ranged between 2–10%.

Leukocyte-Endothelial Cell Monolayer Adhesion Assays. Neutrophil and eosinophil adherence to endothelial cells was measured using the ⁵¹Cr leukocyte collection assay (3, 4). Basophil adherence assays were performed as described (5), using the histamine content of perchloric acid lysates of adherent cells to quantitate basophil binding.

Adhesion assays were performed as follows: endothelial cells were preincubated for 4 h with or without 5 ng/ml (500 U/ml) of recombinant human IL-1 (generously provided by Dr. Steven Gillis, Immunex Corp., Seattle, WA). Endothelial cells were washed, then incubated with or without saturating concentrations of appropriate F(ab')₂ preparations of mAb in a final volume of 300 μl (45 min, 37°C). Leukocytes were then added (100 μl, containing 2–4 × 10⁴ basophils, or 2.5 × 10⁵ neutrophils or eosinophils), and allowed to adhere to the monolayers for 10 min at 37°C before removal of nonadherent cells by rinsing. No difference was seen if the antibodies were removed or allowed to remain during the adherence assay (data not shown).

Percent of inhibition of IL-1-stimulated adhesion was calculated according to the following formula: $(1 - [(\% \text{ adherence to IL-1 treated endothelium in the presence of mAb} - \% \text{ adherence to untreated endothelium in the presence of mAb}) / (\% \text{ adherence to IL-1 treated endothelium in the absence of mAb} - \% \text{ adherence to untreated endothelium in the absence of mAb})]) \times 100$. In the absence of IL-1, each of the mAb had no significant effect on leukocyte adhesion to endothelial cells (data not shown). All statistical analyses to determine differences between groups were performed using the Student's paired *t* test.

Indirect Immunofluorescence and Flow Cytometry. Basophils, eosinophils, and neutrophils were evaluated for expression of VCAM-1 and VLA-4α (CD49d) antigen using indirect immunofluorescence and flow cytometry (14). Basophils were specifically labeled by simultaneous incubation with FITC-conjugated polyclonal goat anti-human IgE (Kirkegaard and Perry Labs, Inc., Gaithersburg, MD) as described (14). Leukocytes were washed and resuspended in R-PE-conjugated F(ab')₂ goat anti-mouse IgG (Tago Inc., Burlingame, CA); at least 10,000 leukocytes were evaluated using a Coulter EPICS Profile flow cytometer (Coulter Electronics Inc., Hialeah, FL). Appropriate gating was used to eliminate debris; for basophil studies, further gating was done to restrict analysis to FITC-positive (IgE-bearing) cells (14).

Results and Discussion

Previous studies have established that treatment of human umbilical vein endothelial cells for 4 h with 5 ng/ml (500 U/ml) of IL-1 is optimal for inducing adhesiveness for neu-

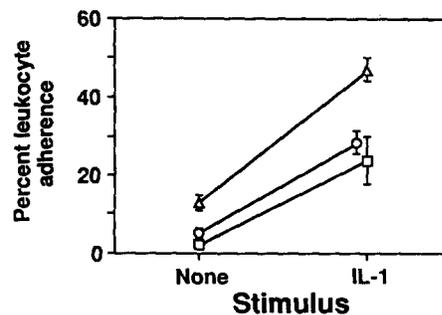


Figure 1. Activation of human endothelial cells by IL-1 (4 h, 5 ng/ml) increases adhesion of human basophils (Δ; n = 17), eosinophils (□; n = 6), and neutrophils (O; n = 8). All values (mean ± SEM) for IL-1-induced adhesion were significantly greater than for unstimulated adherence (*p* < 0.05).

trophils, eosinophils, and basophils (3–5). As shown in Fig. 1, treatment of endothelial cells with IL-1 induced a statistically significant (four- to eight-fold) increase in the adherence of human neutrophils, eosinophils, and basophils.

Using specific mAb, the role of ICAM-1, ELAM-1, and VCAM-1 during granulocyte adhesion to cytokine-activated endothelium was determined. As shown in the upper panel of Fig. 2, F(ab')₂ preparations of mAb to ICAM-1 (Hu5/3) and ELAM-1 (H18/7) significantly inhibited adhesion to IL-1-activated endothelium for all three cell types. In contrast, anti-VCAM-1 (2G7) significantly inhibited adhesion of eosinophils and basophils, but not neutrophils, to cytokine-activated endothelium. Interestingly, 2G7 was more effective than any of the other mAb at inhibiting eosinophil adherence, and was less effective at inhibiting basophil adherence. Inhibition by 2G7 was not due to its ability to directly bind to the eosinophil or basophil, since (a) removal of the antibody before performing the adherence assay did not affect the results and (b) indirect immunofluorescence and flow cytometric anal-

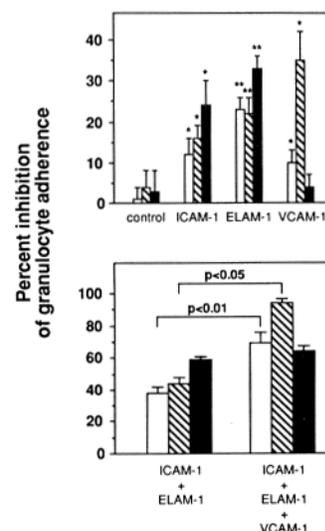


Figure 2. Effect of F(ab')₂ preparations of anti-ICAM-1 (Hu5/3, 10 μg/ml), anti-ELAM-1 (H18/7, 50 μg/ml), anti-VCAM-1 (2G7, 10 μg/ml) or control mAb (W6/32, anti-HLA class I, 50 μg/ml) on adherence of basophils (□; n = 3–17), eosinophils (▨; n = 3–6), and neutrophils (■; n = 4–8) to IL-1-treated (4 h, 5 ng/ml) umbilical vein endothelial cells. After cytokine treatment, endothelial cells were incubated with the indicated antibodies, and inhibition of adherence for each cell type (mean ± SEM) was determined. For individual antibodies (upper panel), values for statistical significance were determined by comparison to the W6/32 control. In the lower panel, values for statistical significance were determined by comparing percent inhibition for the combination of two vs. three blocking antibodies. **p* < 0.05; ***p* < 0.01.

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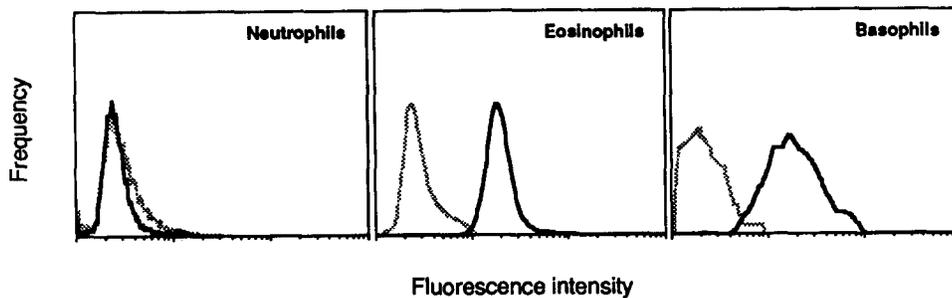


Figure 3. Expression of the VLA-4 α subunit (CD49d) on human neutrophils, eosinophils, and basophils. Leukocytes were purified, then labeled with mAb and analyzed by flow cytometry as described in Materials and Methods. Stippled lines represent labeling with an irrelevant, isotype-matched control antibody. Results shown are from a single experiment representative of three separate experiments.

ysis consistently failed to detect VCAM-1 expression on any of the granulocyte subtypes (data not shown). The effects of combining both anti-ELAM-1 and anti-ICAM-1 antibodies were approximately additive for each of these leukocyte types. However, the addition of anti-VCAM-1 to this antibody combination had a more than additive effect in inhibiting adhesion of eosinophils and basophils (Fig. 2, lower panel).

Since VLA-4 (CD49d/CD29) has recently been shown to be a counter-receptor for VCAM-1 (11), we also measured VLA-4 α expression on granulocytes. Indirect immunofluorescence and flow cytometric analyses (Fig. 3) revealed that basophils and eosinophils expressed VLA-4 α (CD49d); lack of expression by neutrophils confirmed previous reports (11).

These studies demonstrate that IL-1-induced enhancement of endothelial cell adhesiveness for eosinophils and basophils, like neutrophils, occurs in part through ICAM-1 and ELAM-1 mediated mechanisms. Although previous studies have shown that cytokine treatment of vascular endothelial cells facilitates transendothelial migration for neutrophils, an effect also mediated via ICAM-1 and ELAM-1 (10), it remains to be determined whether engagement of these endothelial cell adhesion molecules (and perhaps VCAM-1) promotes transendothelial migration for eosinophils or basophils. These observations also suggest that the coordinate expression of ICAM-1 and ELAM-1 on endothelial cells *in vivo* may result in recruitment of all types of granulocytes, with the pattern of tissue infiltration influenced by the relative number of each leukocyte available in the circulation.

In addition, these experiments clearly demonstrate an important role of VCAM-1 surface proteins for eosinophil and basophil (but not neutrophil) adhesion to IL-1-activated endothelium. Indeed, the potent ability of 2G7 to block eosinophil adhesion suggests that VCAM-1 is a major ligand for eosinophil adhesion to activated endothelium. These findings contrast with a report in which a different mAb recognizing VCAM-1 (1.4C3) failed to inhibit eosinophil adhesion to cytokine-activated endothelium (15). Since mAb 1.4C3 is ineffective at blocking T lymphocyte adhesion to VCAM-1 (15), it is likely that this antibody did not inhibit eosinophil adherence to endothelial cells because it binds to an epitope on VCAM-1 not involved in adhesion that is distinct from the epitope recognized by mAb 2G7. Since pretreatment with a combination of antibodies recognizing ICAM-1, ELAM-1, and VCAM-1 caused >90% inhibition of eosinophil adhe-

sion to cytokine activated endothelium (Fig. 2), these three molecules appear to be the major receptors involved in eosinophil adhesion under these conditions. In contrast, pretreatment with the same combination of antibodies resulted in only 60–70% inhibition of basophil and neutrophil adhesion, suggesting the possible existence of other epitopes and/or endothelial cell ligands for these cell types. Since the eosinophil and basophil, unlike the neutrophil, express VLA-4 (Fig. 3), these data also suggest that eosinophils and basophils, like lymphocytes and monocytes (11), are capable of binding VCAM-1 via this receptor. Thus, interaction of eosinophils and basophils with VCAM-1 expressed locally on endothelial cells may represent a novel mechanism by which the endothelium may directly promote the recruitment of these and other leukocytes without influencing neutrophil emigration. This is of particular interest in light of recent studies suggesting that stimuli such as IL-4 induce expression of VCAM-1 in the absence of induction of ELAM-1 and ICAM-1 (reference 16 and our unpublished observations).

With regard to allergic inflammation, it is interesting to note that at sites of late phase cutaneous reactions induced by allergen in atopic subjects, neutrophils represent the predominant cell type recruited during the initial hours (17). This is in marked contrast to the prominent influx of eosinophils, basophils, and mononuclear cells which occurs 6–12 h after antigen challenge (17). Based on these data, we hypothesize that the sequential local expression of endothelial cell adhesion molecules during these inflammatory events might play an important role in determining the composition of the cellular infiltrate. That endothelial cell activation occurs during allergic reactions is suggested by the observation that IL-1 is released at sites of cutaneous antigen challenge in atopic but not in nonatopic subjects or at control sites (17). In addition, studies using intracutaneous allergen challenge have shown that expression of ELAM-1, ICAM-1, and VCAM-1 on endothelial cells occurs within 2 to 8 h (15, 18), and infusion of anti-ICAM-1 antibodies in asthmatic monkeys reduces airways eosinophilia and airways hyperreactivity (19). Taken together, these results strongly suggest that the local expression of ELAM-1, ICAM-1, and VCAM-1 may contribute to the recruitment of basophils, eosinophils, and neutrophils during experimental allergic reactions and in chronic allergic diseases.

We would like to thank Dr. Steven Gillis for supplying recombinant IL-1, Dr. Donald MacGlashan, Jr. for providing the software to generate the histograms in Figure 3, Dr. Myron Cybulsky for providing several monoclonal reagents used in these studies, and Ms. Bonnie Hebden for assistance in the preparation of this manuscript.

This work was supported by grants AI-27429, AR-31891, AI-20136, and HL-36028 from the National Institutes of Health. Dr. Bochner was also supported in part by a New Investigator Award from the American Lung Association. Publication No. 048 from the Johns Hopkins Asthma and Allergy Center.

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Received for publication 21 December 1990 and in revised form 26 March 1991.

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