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Differential Regulation of Eosinophil Adhesion and Transmigration by Pulmonary Microvascular Endothelial Cells

Hideaki Yamamoto, Julie B. Sedgwick, and William W. Busse

In bronchial asthma, eosinophils (EOS) adhere to, and migrate across, the lung microvasculature to exert their effector functions in the airways. This study was conducted to determine the effect of cytokines on adhesion molecule expression on human pulmonary microvascular endothelial cells (HPMEC) and the influence of these molecules on EOS adhesion and transmigration in vitro. Unlike ICAM-1 expression (>80% positive cytokine-treated HPMEC by flow cytometry), VCAM-1 expression varied with the cytokine(s) pretreatment; the order of potency was: TNF-α + IL-4 (82.2 ± 4.2% positive cells) > TNF-α (41.8 ± 5.1%) > IL-1β (20.8 ± 4.7%). IL-4 alone had no effect on either ICAM-1 or VCAM-1 expression. EOS adhesion to cytokine-treated HPMEC followed the same order as that observed for VCAM-1 expression. Interestingly, EOS migration across cytokine-treated HPMEC varied inversely with VCAM-1 expression on, and EOS adhesion to, HPMEC; IL-1β (21.2 ± 1.4% migration) > TNF-α (12.6 ± 2.6%) > TNF-α + IL-4 (9.1 ± 2.0%). EOS adhesion was greatest with TNF-α + IL-4-treated HPMEC, was dependent on VCAM-1, and inhibited with anti-α4 integrin mAb (67.7 ± 7.5% inhibition, \( p < 0.0005 \)). In contrast, the highest EOS migration occurred across IL-1β-treated HPMEC and was inhibited by anti-β2 integrin mAb (40.4 ± 2.5% inhibition, \( p < 0.005 \)). Viable HPMEC were required for EOS migration but not adhesion. Our results suggest that EOS adhesion and transmigration are differentially regulated by VCAM-1 and ICAM-1 expression and the interaction of these adhesion proteins with their respective counterligands, i.e., α4 and β2 integrins on EOS. The Journal of Immunology, 1998, 161: 971–977.

Eosinophils (EOS) are pivotal effector cells in the development of airway inflammation in bronchial asthma (1). To cause airway inflammation, it is necessary that EOS migrate from the circulation to the lung (2). For this to occur, EOS must first adhere to, and then migrate across, pulmonary microvascular endothelium; this process is regulated by many factors, including cytokines and adhesion proteins. When endothelial cells are exposed to inflammatory cytokines, such as IL-1, TNF-α, and IL-4, the expression of ICAM-1 and VCAM-1 is increased (3–7). It is proposed that EOS then bind to these endothelial adhesion proteins by their counterligands: β2 integrins, α4β1 (LFA-1) and α4β7 (Mac-1), for ICAM-1, and α4 integrins, α4β1 (very late Ag (VLA)-4) and α4β7, for VCAM-1 (8, 9). Since all leukocytes utilize the β2 integrin/ICAM-1 pathway for adhesion and transmigration (3–5, 10, 11), selective recruitment of EOS to the airway occurs by other pathways, such as α4 integrin/VCAM-1 interaction, since the α4 integrin is constitutively expressed on EOS but not on neutrophils (12, 13). Anti-α4 integrin or anti-VCAM-1 mAb have been shown to inhibit EOS, but not neutrophil, adhesion to cytokine-treated endothelial cells (11, 14). The contribution of α4 integrin/VCAM-1 pathway in EOS transendothelial migration, however, remains to be fully defined (5, 7, 15, 16).

The importance of the α4 integrin/VCAM-1 interaction and the contribution of this complex to EOS migration have been largely derived from HUVEC, a macrovascular, nonpulmonary cell (6, 7, 11–15). Since EOS participation in allergic inflammation involves small vessels, we propose that human pulmonary microvascular endothelial cell (HPMEC) monolayers are likely to be more relevant than HUVEC as an in vitro model and to reflect leukocyte trafficking to the lung; this conclusion is based on information that macro- and microvascular endothelial cells have considerable heterogeneity in their biologic properties (17). For example, VCAM-1 is selectively induced by IL-4 in HUVEC but not microvascular endothelial cells from skin or intestine (18, 19). Furthermore, although cytokine-regulated adhesion molecule expression is well documented in HUVEC (20), there is limited information on adhesion molecule expression by HPMEC (21). This report determines the cytokine regulation of ICAM-1 and VCAM-1 expression on HPMEC, the influence of these molecules on EOS adhesion and transmigration, and the involvement of α4 and β2 integrins in these two processes.

Materials and Methods

Reagents and cytokines

Percoll was purchased from Pharmacia (Uppsala, Sweden). HBSS, RPMI 1640 medium, PBS, newborn calf serum (NCS), FCS, trypsin-EDTA, l-glutamine, and penicillin-streptomycin were obtained from Life Technologies (Grand Island, NY). Plasma fibronectin was obtained from Armour Pharmaceutical (Tuckahoe, NY). Recombinant human IL-1β, TNF-α, and IL-4 were purchased from R&D Systems (Minneapolis, MN). Other reagents were purchased from Sigma (St. Louis, MO) unless otherwise stated.
Cell culture

HPMEC cryopreserved as tertiary or quaternary cultures were purchased from Clonetics (San Diego, CA). These cells were isolated from the vascular compartment surrounding the alveolar sacs and were characterized as endothelial cells by Clonetics for acetylated low density lipoprotein uptake, factor VIII-related Ag expression, and positive staining for platelet endothelial cell adhesion molecule (PECAM)-1 (CD31) and Matrigel. Endothelial cell basal culture medium supplemented with 10 ng/ml human recombinant epidermal growth factor, 1 μg/ml hydrocortisone, 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, 12 μg/ml bovine brain extract, and 5% FBS was obtained from Clonetics. To promote HPMEC attachment and growth, all culture surfaces were precoated with 10 μg/ml fibronectin for 1 h at 37°C. Cells were passaged before they reached confluence. The profile of adherent molecules on HPMEC has been determined to be equivalent between passages 3 and 12 (21). Cells derived from different donors were used at passages 5 through 9 in this study and found to give equivalent results.

Expression of ICAM-1 and VCAM-1 on HPMEC

The expression of ICAM-1 and VCAM-1 on cytokine-treated HPMEC was examined by flow cytometric analysis. HPMEC were seeded into fibronectin-coated six-well tissue culture plates (Corning, Corning, NY), and preconfluent monolayers were incubated with medium, IL-1β, TNF-α, IL-4, or TNF-α + IL-4 for the indicated times. Cells were washed twice with HBSS, and cells were detached with 25 mM HEPES-buffered PBS containing 0.1% bovine serum albumin (BSA) for 40 min at 37°C. The cells were washed, resuspended in PBS with 0.2% sodium azide and 2% BSA, and stained with phycoerythrin (PE)-conjugated mouse anti-human ICAM-1 mAb (clone: LB-2, IgG2b, Becton Dickinson, San Jose, CA) and/or FITC-conjugated mouse anti-human VCAM-1 mAb (clone: 1G11B1, IgG1, Southern Biotechnology Associates, Birmingham, AL) for 30 min on ice. Mouse IgG2-PE and IgG1-FITC (Becton Dickinson) were used as isotype controls. Labeled cells were washed and analyzed on a Becton Dickinson FACScan flow cytometer after propidium iodide staining. Initial gating was done using forward scatter and propidium iodide fluorescence to eliminate dead cells and debris. Quadrant lines were determined from the IgG2-PE and IgG1-FITC fluorescence dot plot. At least 5000 events were collected for each sample, and the percentage of cells positive for ICAM-1 and/or VCAM-1 was determined.

IL-4 bioassay

Bioactivity of IL-4 was confirmed by CD23 induction on B lymphocytes (CD19) (22). Briefly, PBMC were separated from healthy subjects using histopaque-1077 (Sigma) and incubated for 48 h at 37°C, 5% CO2, with different concentrations of IL-4 in enriched medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin). Cells were washed and two-color stained with FITC-conjugated mouse anti-human CD23 mAb (clone: B-G6, IgG1, Bio-source International, Camarillo, CA) and PE-conjugated mouse anti-human CD19 mAb (clone: 4G7, IgG1, Becton Dickinson). Mouse IgG1-FITC and IgG1-PE (Becton Dickinson) were used as isotype controls. The percentage of CD19-positive cells also expressing CD23 was determined by flow cytometry.

Human subjects

EOS were isolated from the peripheral blood of subjects with allergic airway disease such as allergic rhinitis and mild asthma. Subjects ranged in age from 22 to 60 years, and gender distribution was equal. Immediate participation in the study, and the study was approved by the University of Wisconsin Human Subjects Committee.

EOS separation

EOS were isolated using negative immunomagnetic bead selection, as previously described (23). Briefly, heparinized blood was diluted with HBSS without Ca2+ and centrifuged for 20 min at 700 × g over 1,090 g/ml Percoll. Plasma, mononuclear cell band, and Percoll were removed, and the RBCs in the pellet were lysed by hypotonic shock. The resulting granulocytes were washed with 4°C HBSS supplemented with 2% NCS (HBSS/NCS). Concurrently, purified anti-CD16 from the mouse myeloma clone 3G8 (a generous gift from Dr. David M. Segal, National Cancer Institute, Bethesda, MD) was incubated with goat anti-mouse IgG-coated magnetic beads (PerSeptive Biosystems, Framingham, MA) for 1 h. The anti-CD16-bound beads were washed and incubated with granulocytes for 40 min at 4°C. Steel wool (size 0) columns prepared in 10-ml syringes were soaked 2 to 4 h in ethanol, washed with HBSS, and filled with 4°C HBSS/NCS. The cells and magnetic beads mixture was filtered through the column in a column of a magnetic field (MACS system, Miltenyi Biotec, Auburn, CA) to remove neutrophils bound to magnetic beads. CD16-negative EOS (>98% purity and >99% viability) were collected, washed, and resuspended in enriched medium.

EOS adhesion assay

EOS adhesion to confluent HPMEC monolayers was determined by measuring the residual EOS peroxidase (EPO) activity of adherent cells, as previously described (24). Briefly, EOS (1 × 107/ml in enriched medium) were placed onto cytokine-treated HPMEC monolayers and incubated for 60 min at 37°C. Plates were washed three times with 37°C HBSS. One hundred μl of enriched medium was added to the reaction wells, and 100 μl of the original EOS suspension was added to empty wells to determine a standard EPO curve. EPO substrate (1 mM H2O2, 1 mM o-phenylenediamine dihydrochloride, and 0.1% Triton X-100 in 55 mM Tris buffer, pH 8.0) was then added to all wells. After a 30-min incubation at room temperature, 50 μl of 4 M H2SO4 was added to stop the reaction. Absorbance was measured at 490 nm in a microplate reader (Bio-Tek Instruments, Winooski, VT). Percent EOS adhesion was calculated from the log-dose response curve.

Transendothelial migration of EOS

HPMEC (2.5 × 105 cells/ml) were cultured on fibronectin-coated transwell inserts (6.5- or 12-mm diameter polycarbonate membrane with 3-μm pores, Costar, Cambridge, MA). Medium was added into the upper compartment only to inhibit the formation of a HPMEC bilayer. HPMEC monolayers formed within 2 days and were confirmed for confluence and cobblestone appearance by Diff-Quik staining (Baxter Scientific Products, McGaw Park, IL). Confluent monolayers were incubated in the presence or absence of cytokines for the indicated times. After cytokine treatment, both upper and lower compartments of the transwell were washed three times with 37°C HBSS. EOS (3–5 × 105/ml) were then added to the upper compartment, and enriched medium to the lower compartment. After a 3-h incubation with 5% CO2 at 37°C, 10 mM EDTA was added in the lower compartment, and plates were gently vibrated to dislodge any migrated EOS that were adherent to the bottom of the filters before the transwell inserts were removed. The number of migrated EOS were counted in triplicate by hemocytometer, and percent migration was determined as: (migrated EOS)/(total EOS added into upper compartment) × 100 (%).

Effect of anti-integrin Ab on EOS adhesion and migration

To define the involvement of α4 and β2 integrins in EOS adhesion to, and migration across, cytokine-treated HPMEC, EOS were preincubated with 1 μg/ml of mouse anti-human α4 integrin mAb (clone: HP1/2, IgG1, a generous gift from Dr. Roy R. Lobb, Biogen, Cambridge, MA) and/or 3 μg/ml of mouse anti-human β2 integrin mAb (clone: L130, IgG1, Becton Dickinson) for 30 min at room temperature before adhesion or migration assays were performed. Optimal mAb concentration was determined by dose-response study. Concentration-matched mouse IgG1 (Becton Dickinson) was used as an isotype control.

Paraformaldehyde fixation of HPMEC

To assess the contribution of endothelial cell viability to EOS adhesion and migration, cytokine-treated HPMEC monolayers were fixed using 1% paraformaldehyde in PBS at ambient temperature for 15 min. After washing the monolayers three times with 37°C HBSS, 1% glycine in enriched medium was added (ambient temperature for 1 h) to quench the residual paraformaldehyde. Plates or transwells were then washed three times before use. As a positive control for EOS migration, RANTES (10 nM, Biosource International) was added to 30°C HBSS for 1 h. As a positive control for EOS migration, RANTES (10 nM, Biosource International) was added to the lower compartment.

Statistics

Data are presented as mean ± SEM, and the groups were analyzed by ANOVA with repeated measures and Scheffe constants, or Student’s t test, for paired comparisons. A p value less than 0.05 was considered significant.
Results

Regulation of HPMEC expression of ICAM-1 and VCAM-1 by IL-1β, TNF-α, and IL-4

HPMEC expression of ICAM-1 was significantly increased (>80% positive cells) by either 100 pM IL-1β or TNF-α (± IL-4) after a 6-h incubation; this level of expression was maintained when evaluated at 24 and 72 h (p < 0.0001 vs baseline at each time point, Figure 1). The kinetics and magnitude of VCAM-1 expression, however, were different when the response to IL-1 was evaluated at 24 and 72 h (Fig. 1a). TNF-α, in contrast, caused a significant increase in HPMEC expression of VCAM-1 following 6 h of incubation (26.5 ± 4.3%, p < 0.01 vs baseline) and reached peak values at 24 h (41.8 ± 5.1%, p < 0.001 vs baseline, Fig. 1b). Based upon these experiments, we selected 6 h for IL-1β and 24 h for TNF-α (± IL-4) for incubation times with HPMEC monolayers.

Although IL-4 alone had no effect on ICAM-1 or VCAM-1 expression (24 h, 10.1 ± 2.7% and 3.1 ± 2.8%, respectively, Fig. 1c), it acted synergistically with TNF-α to increase VCAM-1 expression to 82.2 ± 4.2% by 24 h (p < 0.0001 vs baseline; p < 0.005 vs TNF-α alone, Fig. 1d). When we compared the cytokine effect on HPMEC adhesion molecule expression, the order of potency for VCAM-1 was TNF-α + IL-4 > TNF-α > IL-1β > IL-4; the relative potency for cytokine effects on ICAM-1 expression was IL-1β = TNF-α = TNF-α + IL-4 >> IL-4. Under the conditions of our experiments, VCAM-1 was always expressed on HPMEC in conjunction with ICAM-1; that is, no endothelial cell cultures expressed only VCAM-1 (Fig. 2).

IL-4 is known to stimulate VCAM-1 expression on HUVEC (6). To determine whether the lack of response to IL-4 by HPMEC we observed was related to an inactive cytokine preparation, IL-4 was assessed by a bioassay that utilized its effect on CD23 induction on B lymphocytes (CD19). IL-4 (100 pM) increased the percentage of CD23-positive CD19 cells (Fig. 3).

EOS adhesion to, and transmigration across, cytokine-treated HPMEC

Maximal EOS adhesion to HPMEC occurred after 60 min of incubation; 3 h was optimal for EOS transendothelial migration (data not shown). Pretreatment of HPMEC monolayers with IL-1β or TNF-α (± IL-4) significantly enhanced EOS adhesion (medium: 13.5 ± 1.2% adhesion; IL-4: 14.6 ± 1.1%; IL-1β: 27.9 ± 2.2%; TNF-α: 34.3 ± 2.5%; TNF-α + IL-4: 40.0 ± 3.0%; p < 0.0001 for IL-1β, TNF-α, TNF-α + IL-4 vs medium and IL-4, Fig. 4a). The effect of TNF-α + IL-4 on EOS adhesion was significantly greater than that of either TNF-α alone (p < 0.05) or IL-1β (p < 0.0001). The order of cytokine potency on EOS adhesion was: TNF-α + IL-4 > TNF-α > IL-1β; this was the same order of cytokine potency as VCAM-1 expression on HPMEC.

An inverse relationship was found between EOS transendothelial migration across HPMEC monolayers on transwell filters and EOS adhesion. Pretreatment of HPMEC with IL-1β or TNF-α (± IL-4) significantly enhanced EOS migration (medium: 1.6 ± 0.4% migration; IL-4: 2.8 ± 0.5%; IL-1β: 21.2 ± 1.4%; TNF-α: 12.6 ± 2.6%; TNF-α + IL-4: 9.1 ± 2.0%; p < 0.0001 for IL-1β vs medium and IL-4; p < 0.001 for TNF-α vs medium and IL-4; p < 0.05 for TNF-α + IL-4 vs medium, Fig. 4b). The effect of IL-1β on EOS migration was significantly greater than that of either TNF-α alone (p < 0.02) or TNF-α + IL-4 (p < 0.001). Given these observations, the order of cytokine potency on EOS migration was: IL-1β > TNF-α > TNF-α + IL-4, the inverse order of potency that is observed for VCAM-1 expression on, and EOS adhesion to, HPMEC.
Effect of anti-α₄ and anti-β₂ integrin mAb on EOS adhesion and transmigration

To determine the identity and involvement of EOS adhesion integrins in these processes, EOS were pretreated with anti-α₄ and/or anti-β₂ integrin mAb before adhesion to, or migration across, IL-1β- or TNF-α1IL-4-treated HPMEC. Treatment of EOS with a concentration-matched IgG1-isotype control did not affect EOS adhesion or migration (data not shown). EOS adhesion to IL-1β-treated HPMEC was significantly, but only partially, inhibited by anti-α₄ or anti-β₂ integrin mAb (p = 0.05 and p = 0.005, respectively, Fig. 5a); total inhibition of EOS adhesion to HPMEC required the presence of both mAb. When TNF-α1 + IL-4-treated HPMEC were evaluated, EOS adhesion was significantly inhibited by anti-α₄ integrin (67.7 ± 7.5% inhibition, p < 0.0005). Anti-β₂ integrin alone had no inhibitory effect on EOS adhesion; when the anti-β₂ mAb was combined with anti-α₄ integrin, significantly...
greater inhibition of EOS adhesion was noted when compared with anti-α₄ integrin alone (p < 0.05).

A different and distinct pattern of integrin involvement was found when migration was evaluated; only anti-β₂ integrin significantly inhibited EOS migration across cytokine-treated HPMEC (IL-1β: 40.4 ± 2.5% inhibition, p < 0.005; TNF-α + IL-4: 63.1 ± 9.8% inhibition, p < 0.05, Fig. 5b). Moreover, the presence of anti-α₄ integrin significantly increased EOS transmigration across IL-1β-treated HPMEC (p < 0.05). Furthermore, the addition of anti-α₄ integrin to anti-β₂ integrin mAb had no additional inhibitory effect on EOS transmigration than anti-β₂ integrin alone.

Effect of paraformaldehyde fixation of HPMEC on EOS adhesion and migration

To assess the contribution of endothelial cell viability to EOS adhesion and migration, IL-1β-treated HPMEC were fixed in 1% paraformaldehyde (15 min). Although HPMEC fixation had no effect on EOS adhesion (unfixed: 23.8 ± 3.9% adhesion; fixed: 24.0 ± 6.1%, Fig. 6), EOS transmigration was significantly inhibited in the presence of fixed HPMEC monolayers (unfixed: 17.2 ± 3.8% migration; fixed: 13.1 ± 0.8%, p < 0.05). RANTES (10 nM) stimulated significant EOS transendothelial migration irrespective of HPMEC fixation, suggesting that the fixed monolayer retained the capacity of EOS diapedesis.

Discussion

The mechanisms, and involvement of cytokines and adhesion molecules, that regulate the preferential accumulation of EOS in the airways of patients with bronchial asthma are not completely understood. To begin to understand how cytokine treatment of vascular endothelial cells affects the expression of selected adhesion proteins and subsequent EOS migration, we modeled the process with microvascular cells in a transwell system. To our knowledge, these are the first observations to show that cytokine-treated HPMEC regulated EOS adhesion and transmigration in an inverse manner. More than 80% of HPMEC expressed ICAM-1 when they were treated with either IL-1β or TNF-α (+ IL-4). However, these cytokines had a marked difference in their potency to stimulate VCAM-1 expression on, and EOS adhesion to, HPMEC monolayers: TNF-α > IL-1β > medium. In contrast, when EOS transendothelial migration was evaluated, the order of cytokine effectiveness on cell movement was reversed from that observed for VCAM-1 expression and adhesion. These observations indicate that, under conditions of strong ICAM-1 expression, EOS adhesion to, and migration across, cytokine-treated HPMEC were differentially regulated by the expression status of VCAM-1.

To identify the EOS integrins that interact with HPMEC-expressed ICAM-1 and VCAM-1, and how these interactions determine cell adhesion and migration, we performed experiments to
test the hypothesis that these two HPMEC adhesion proteins modulate different EOS functions. It is generally accepted that firm adhesion of leukocytes to cytokine-stimulated endothelial cells is mediated by α4(β1 and α5β2) and β2(α5β2 and αMβ2) integrins (8). The involvement of these EOS integrins in our in vitro model was determined using anti-α4 and anti-β2 integrin mAbs. Anti-ICAM-1, was equally, but only partially, inhibited by either anti-α4 or anti-β2 integrin mAb. When TNF-α + IL-4 were added to HPMEC, leading to both ICAM-1 and VCAM-1 expression, anti-α4 integrin significantly inhibited EOS adhesion, while anti-β2 mAb alone had no effect. These results confirm the functional importance of the α4 integrin/VCAM-1 pathway for EOS adhesion to HPMEC. When either IL-1β or TNF-α + IL-4 were used to treat HPMEC, a combination of anti-α4 and anti-β2 integrin mAbs was necessary to reduce EOS adhesion to baseline values (EOS adhesion to nonstimulated HPMEC), suggesting that these mAbs are sufficient to completely block β2 integrin/ICAM-1 and α4 integrin/VCAM-1 interactions.

In agreement with studies using HUVEC (5, 16), only anti-β2 integrin mAb significantly inhibited EOS migration across cytokine-treated HPMEC. This finding suggests that the β2 integrin/ICAM-1 interaction, but not α4 integrin/VCAM-1, is required for EOS transendothelial migration. Wegner et al. (25) also reported that ICAM-1 was crucial for EOS infiltration to the lung in a primary model of asthma. Interestingly, in our study, anti-α4 integrin mAb did not inhibit EOS transmigration but actually increased cell migration across IL-1β-treated HPMEC monolayers. Moser et al. (5) and Ebisawa et al. (16) also demonstrated that anti-α4 integrin or anti-VCAM-1 mAb failed to inhibit EOS migration across IL-1β- or TNF-α-treated HUVEC. Moreover, Kuipers et al. (26) reported that “freezing” β1 integrin in a state of high avidity by activating mAb completely inhibited EOS migration across fibronectin or endothelial cell-coated filters. Therefore, it is likely that, although the α4 integrin/VCAM-1 pathway is important for selective EOS adhesion to HPMEC, strong adhesion via this pathway actually inhibits subsequent β2 integrin/ICAM-1-dependent transendothelial migration.

We have reported that rhVCAM-1 alone can activate EOS adhesion and its respiratory burst (27) while EOS interaction with ICAM-1 requires an additional costimulus for activation (28). These findings agree with other reports that Mn²⁺, platelet activating factor, FMLP, or granulocyte-macrophage (GM)-CSF can activate both adhesion and functions dependent on granulocyte β2 integrin interaction with endothelial ICAM-1 (29–31). We now propose that adhesion of EOS to HPMEC via very late Ag (VLA)-4 and VCAM-1 fulfills the role of a second stimulus for ICAM-1-dependent EOS migration. In support of this hypothesis, Weber et al. (32) reported the differential regulation of β1 and β2 integrin avidity of EOS by chemotactractants such as RANTES and MCP-3 (monocyte chemotactrant protein-3). Although these chemokines rapidly increased and then subsequently reduced EOS adherence via α4 integrin and VCAM-1, which was dependent on the actin cytoskeleton, they increased and prolonged EOS conformational changes of β2 integrin, promoting its interaction with ICAM-1. Thus, these particular chemokines may facilitate migration of EOS by first adhering these cells to VCAM-1, then quickly detaching them from VCAM-1 and/or increasing the availability of the β2 integrin/ICAM-1 interaction. Although our model does not involve the addition of chemokines, we observed a similar interrelationship between VCAM-1-dependent EOS adhesion and subsequent migration across cytokine-activated HPMEC monolayers via ICAM-1.

Fibronectin, which coated the surface of the HPMEC culture flask, has also been reported to be an activator of EOS functions (33, 34). To define the possible role of fibronectin as an agonist in our system, each experiment included a control of HPMEC without cytokine treatment (medium controls in Figs. 2, 4, and 5). These data provided the baseline determinants of EOS adhesion and migration and would include any fibronectin activation. In addition, one well of HPMEC was stained and observed microscopically to confirm monolayer confluence in each experiment.

Our cytokine effects on adhesion protein expression by HPMEC were distinct from responses observed with HUVEC; with HUVEC, VCAM-1 expression associated with IL-1β was greater than that caused by TNF-α and was maintained for more than 24 h (19, 20). Carley et al. (17) also demonstrated that HPMEC differ from HUVEC with respect to prostaglandin and plasminogen activator production, the ability of tube-like structure formation, and the distribution of factor VIII-related Ag. Although IL-4 selectively induces VCAM-1 expression by HUVEC or nasal polydederived microvascular endothelium (6, 35), it has no effect on VCAM-1 expression by microvascular endothelial cells from skin or intestine (18, 19). Likewise, IL-4 alone had no effect on VCAM-1 expression on HPMEC in our study. The highest expression of VCAM-1 occurred (82.2 ± 4.2%) when IL-4 was combined with TNF-α. This synergistic effect was also demonstrated in HUVEC culture (36, 37) and confirms the importance of IL-4 for increased and sustained VCAM-1 expression.

Finally, we evaluated the requirement of endothelial cell viability for EOS adhesion and transmigration. Our data indicate that EOS adhesion to cytokine-treated HPMEC occurred regardless of HPMEC viability. However, subsequent transmigration was almost completely inhibited when cytokine-treated HPMEC monolayers were first fixed with paraformaldehyde. This finding suggests that viable HPMEC are essential for adherent EOS to migrate in this model. Similarly, Asako et al. (38) and Huang et al. (39) demonstrated that an alteration of the cytoskeleton, or an increase in cytosolic free calcium in endothelial cells, was required for neutrophil migration, but not adhesion. To determine whether fixation “closed” the endothelial cell tight junctions, thus inhibiting EOS diapedesis, RANTES was added to the bottom wells as a chemoattractant. RANTES induced EOS migration across fixed HPMEC, indicating that a fixed monolayer still retained its capacity for EOS diapedesis, and that RANTES-induced EOS migration is independent of HPMEC viability.

In conclusion, EOS adhesion to, and migration across, cytokine-treated HPMEC appear to be differentially regulated by the expression status of ICAM-1 and VCAM-1 and by the expression and activation of α4 and β2 integrins on EOS. The biologic relevance of these observations is not established but raises a number of possibilities. Our observations suggest that eosinophil interaction with VCAM-1 and ICAM-1 are different and inversely related. The interaction of eosinophil with VCAM-1 is associated with a high level of adhesion but not transendothelial migration. The interaction with ICAM-1 appears important for cell migration but not initial adhesion. However, to direct eosinophils to sites of inflammation, it is essential that both cell responses are achieved. Specifically how these two responses are interrelated, if indeed they are, is the next step to investigate. In that regard, we propose that studies with HPMEC promise to provide a new insight into how EOS, and other inflammatory cells, adhere to and migrate across lung microvasculature, accumulate in the airways, and contribute to allergic inflammation.
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