L-Selectin and Very Late Antigen-4 Integrin Promote Eosinophil Rolling at Physiological Shear Rates In Vivo

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Adherence of eosinophils to vascular endothelium and their accumulation at sites of allergen challenge are hallmarks of allergic inflammation. However, the molecular mechanisms mediating eosinophil adhesion under conditions of blood flow are not well understood. The present studies were performed to identify the receptors on human eosinophils involved in initiating adhesion to activated endothelium at physiologic shear rates in vivo. We have compared the relative contribution of L-selectin, VLA-4 (CD49d), and CD18 integrins in mediating eosinophil adhesion to microvascular endothelial cells in the rabbit mesentery by using intravital video microscopy. Eosinophils were found to roll in venules, but not arterioles, and this rolling could be stimulated by activation of endothelium with IL-1. In contrast to neutrophil rolling, which is predominantly L-selectin-dependent, eosinophil rolling was mediated by L-selectin, and also VLA-4. mAbs to L-selectin and VLA-4α, but not CD18, significantly inhibited eosinophil rolling in vivo. The inhibition of VLA-4-mediated eosinophil rolling was not caused by modulation of eosinophil L-selectin or CD18 expression. This inhibition also was not caused by nonspecific inhibitory effect of the Abs studied, because the anti-VLA-4 mAbs inhibited eosinophil (VLA-4+) but not neutrophil (VLA-4−) rolling in the mesenteric venules. These results demonstrate that early events of eosinophil adhesion, i.e., rolling, are mediated by multiple adhesion receptors, including L-selectin and VLA-4, at physiologic shear rates in vivo.

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allergic reactions (9, 15) and nasal biopsies of patients with perennial rhinitis (16). In addition, mAbs to VLA-4 have been shown to be effective in blocking eosinophil accumulation at sites of allergic inflammation in vivo (17). However, the in vivo role of eosinophil adhesion receptors in the pathogenesis of asthma and allergic inflammation, and molecular mechanisms mediating eosinophil adherence to activated EC under conditions of blood flow have not been previously demonstrated. Furthermore, because of lack of appropriate in vivo animal models to study distinct events of eosinophil adhesion, it has not been possible to demarcate clearly the role of specific adhesion receptors in mediating initial events of eosinophil adhesion at physiologic shear rates.

Recent studies in vivo have revealed that adhesive interactions between neutrophils and venular EC involve multiple sequential steps (18–21). At sites of tissue inflammation, circulating neutrophils initially interact with inducible cell adhesion molecules expressed on the surface of venular EC. Under conditions of shear force, this adhesion is weak and transient and results in rolling of neutrophils along venular walls. Neutrophil rolling is predominantly mediated by L-selectin, which is expressed constitutively on most leukocytes and appears to bind carbohydrate ligands through its functional lectin domain (22–26). Furthermore, L-selectin also has been proposed to promote neutrophil rolling by presenting sialyl-Lewis^x^ to vascular E- and P-selectins (19, 20). Rolling is a prerequisite for subsequent firm adhesion of activated neutrophils (19, 27). Activation by chemotactic stimuli results in functional activation and up-regulation of CD11/CD18 integrins, and shedding of surface L-selectin. The rolling neutrophils may firmly adhere or stick to EC, and can subsequently transmigrate across the endothelium into the extravascular space at sites of inflammation.

Although substantial evidence for the role of eosinophils in the pathogenesis of allergic inflammation has been obtained (4–6), the molecular mechanisms of eosinophil adhesion receptors, L-selectin, VLA-4, and CD18, in promoting eosinophil-EC interactions, and their role in mediating selective recruitment of eosinophils during chronic inflammation under conditions of blood flow have not been determined. By using intravital microscopy, we have studied the role of these adhesion receptors in mediating the early events of eosinophil adherence to IL-1-activated venular EC in the rabbit mesentery. In this study, we report that eosinophils “roll” along cytokine-stimulated EC in vivo and, unlike neutrophils, engage at least two adhesion receptors, L-selectin and VLA-4, to participate in rolling along the walls of mesenteric venules.

Materials and Methods

**Study patients**

Blood samples were drawn from atopic subjects who had mild allergic rhinitis and/or asthma (peripheral blood eosinophil count 3 to 20%), and positive immediate hypersensitivity skin tests to environmental allergens.

The patients did not receive any medication other than a β-agonist Metered Dose Inhaler on an as needed basis for 4 wk before blood donation.

**Cell isolation and labeling**

Eosinophils were purified in the cold from peripheral blood, as described by Hansel et al. (28). Heparinized blood was obtained from patients with asthma and/or allergic rhinitis in a protocol approved by the University of California, San Diego, Human Subjects Committee. Mononuclear cells were depleted by layering blood onto a Percoll gradient density of 1.089. The cell pellet, containing neutrophils (CD16^+^) and eosinophils (CD16^-^), was depleted of red blood cells by ammonium-chloride lysis, and then incubated with magnetised anti-CD16 microbeads for 30 min. The cells were then applied to a MACS (magnetic cell separator, Miltenyi Biotech, Sunnyvale, CA), and eosinophils of >95% purity were recovered by a negative selection. Eosinophils had >25% viability, as assessed by trypan blue staining.

Neutrophils were isolated from whole blood by using dextran sedimentation, followed by discontinuous density-gradient centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ) and Percoll (Pharmacia), as described previously (19, 29). Viability and purity of all cell preparations were >95%. Isolated eosinophils and neutrophils were labeled fluorescently with 20 μg/ml carboxyfluorescein diacetate (CFDA; Molecular Probes, Eugene, OR) for 30 min. The cells were washed, resuspended at a concentration of 1 × 10^7^ cells/ml in PBS + 0.01% glucose, and kept at room temperature in the dark until use. Aliquots of eosinophils or neutrophils that were purified identically, but not labeled with CFDA, were used for the quantification of receptor expression by flow cytometry, as described below.

**Flow cytometry**

Expression of L-selectin, VLA-4 (α4 subunit), and CD18 integrins on isolated eosinophils and neutrophils was determined on a FACStar flow cytometer (Becton Dickinson, San Jose, CA). Anti-L-selectin mAb DREG-200 (30), anti-VLA-4 mAb P4G9 (31), anti-CD18 mAb IB4 (32), and an irrelevant, nonbinding, isotype-matched (IgG2b) anti-tenascin control mAb 81C6 (33) were conjugated with FITC and used for flow-cytometric determination of cell surface expression, as described previously (29). A saturating concentration of fluorescent mAb DREG-200, P4G9, or IB4 (5 μg/ml) was added to 50 μl eosinophils (5 × 10^6^ cells) and incubated for 30 min on ice. In some experiments, eosinophils were treated with 10^{-7} M PMA and with 50 μg/ml anti-VLA-4 mAbs for 30 min at room temperature before incubation with FITC-labeled mAbs DREG-200, IB4, or 81C6 on ice. Surface expression was determined after gating for eosinophils or neutrophils by characteristic forward and side light scatter. Results are expressed as percentage of median fluorescence channel numbers.

**Antibody treatment**

Fluorescently labeled eosinophils and neutrophils (0.2 to 1 × 10^7^/ml) were incubated for 20 min with 50 μg/ml DREG-56, which is known to block L-selectin function (34), before injecting in vivo. In addition, some cells also were treated with anti-murine VLA-4α mAb PS2, IgG2b, (with affinity for human VLA-4α) (35), or anti-VLA-4 α mAb P4G9 (IgG2b) and iso-type-matched anti-CD18 mAb IB4 (32).

**Animal preparation**

New Zealand White rabbits (1.2–1.5 kg body weight) were sedated by i.m. injection of 0.1 ml 0.1% droperidol and 0.002% fentanyl (Innovar-Vet, Pitman-Moore, Mundelein, IL). Three thousand units of human rIL-1β (gift from Dan Witt, Repligen, Cambridge, MA) in 5 ml PBS were administered i.p. for local stimulation of mesenteric EC. Two hours later, surgical anesthesia was induced (5–10 ml of 20% urethane i.v.), and animals were prepared for in vivo observation of the mesenteric microvasculature adjacent to the terminal ileum, as described previously in this laboratory (19). Briefly, after placement of a tracheal tube and catheters in the right carotid artery and left jugular vein, a midline abdominal incision was made and the terminal ileum was exteriorized and spread on a heated glass window (37.5°C) of the stage of a Leitz intravital microscope. The rabbit mesentery was superfused continuously with warm endotoxin-free isotonic saline solution (pH 7.4). Parts of the rabbit intestine that were not observed microscopically were covered with cotton pads soaked with isotonic saline. Subsequently, a side branch of the...
termed superior mesentery artery was cannulated with a polyethylene catheter for injection of fluorescently labeled eosinophils or neutrophils.

**Intravital microscopy and image analysis**

Fluorescently labeled eosinophils or neutrophils (0.2 to 1 × 10⁷) were injected consecutively through the catheter into the terminal mesentery artery bloodstream 4 to 6 h after i.p. application of IL-1β. Cells passing through venules of a mesenteric segment in the downstream circulation were made visible by stroboscopic epi-illumination use of a video-triggered Xenon lamp and a Leitz Ploemopak epi-illuminator utilizing an I2 filter block. All images were recorded through a silicon-intensified tube camera (SIT68, Dage MTI, Michigan City, IN) using a ×10/0.13 water immersion objective (Nikon, Nikon, McVille, NY) and an SVHS video recorder (JVC HC-6000).

Video recordings of different venules were analyzed for assessment of rolling of eosinophils, as previously described for neutrophils in this laboratory (19, 25, 36). Briefly, fluorescently labeled cells were injected through a cannulated artery, and their passage through the activated endothelium was observed and recorded. Rolling was analyzed by counting manually the total number of fluorescent eosinophils or neutrophils passing through a venular segment (total flux, Ft). The tapes were rewound, and only those cells were counted that were rolling visibly along the venular wall (rolling flux, Fr). The rolling fraction (Rf) was determined as the percentage of Fr in the Ft of fluorescent eosinophils or neutrophils passing a venule during one injection.

Velocity profiles of rolling and free flowing eosinophils before and after Ab treatment were determined in five venules in two rabbits by frame by frame analysis. The centerline velocity (Vc) in the bloodstream of each venule was determined from the fastest cell, as described earlier (36). Velocities of individual eosinophils (Vc) were normalized to VcL, which was given a value of 1. The normalized velocities (Vc/VcL) of approximately 80 consecutive eosinophils were determined and distributed to successive classes from 0 to <0.1, 0.1 to <0.2, etc., to 0.9 to 1.0. The critical velocity (Vcrit), i.e., the lowest velocity that a free flowing cell can assume in a parabolic flow, in each venule with diameter of approximately 80 consecutive eosinophils were determined and discussed in each venule (34 mm diameter) in two animals. As described previously (18, 19, 36), eosinophils flowing with relative velocities above the relative critical velocity (Vc/VcL = 0.27) were considered to be noninteracting (free flowing), whereas those with relative velocities below Vc/VcL were assumed to be rolling. The rolling velocity of interacting cells was markedly lower than those observed for free flowing eosinophils (Fig. 3). Compared with the mean velocity of rolling eosinophils (0.22 ± 0.18 mm/s), the free flowing eosinophils had mean velocities of 1.11 ± 0.34 mm/s. The normalized velocity (Vc/VcL) of rolling and free flowing eosinophils (in which Vc is velocity of individual cells and VcL is centerline velocity of the fastest cell) was determined in each of the representative venular segments (Fig. 3). The data obtained from these studies demonstrate that eosinophil-EC interactions are mediated by rolling cells, and that mean velocities of rolling eosinophils were comparable with those reported earlier for neutrophils (25).

**Eosinophil rolling in vivo is mediated by cell surface adhesion receptors L-selectin and VLA-4**

The expression of various adhesion receptors on the surface of eosinophils was analyzed by flow cytometry. Eosinophils expressed L-selectin, VLA-4, and CD18, whereas neutrophils from the same donors expressed L-selectin and CD18 integrin, but not VLA-4. We also have determined that 50 μg/ml of each of the mAbs tested were saturating for the expression of different adhesion receptors on human eosinophils (data not shown). To examine the effect of these adhesion receptors on rolling in inflamed venules, eosinophils were preincubated with mAb DREG-56 (anti-L-selectin), mAbs PS/2 or P4G9 (anti-VLA-4α), and mAb IB4 (anti-CD18) before injection into the rabbit mesentery (n = 14/4) (Fig. 4). Compared with untreated eosinophils, preincubation of eosinophils with DREG-56 at a concentration of 50 μg/ml resulted in 41.8 ± 25.7% (p < 0.001 vs control) inhibition in rolling, demonstrating that L-selectin promotes rolling of human eosinophils under conditions of blood flow in IL-1-stimulated mesenteric venules. Pretreatment of eosinophils with anti-VLA-4α mAbs P4G9 or PS/2 at a similar concentration (50 μg/ml) resulted in 48.7 ± 15.8% (p < 0.001 vs control) and 46.1 ± 22.4% (p < 0.001) inhibition in rolling, respectively. In comparison with blocking by anti-L-selectin or VLA-4 mAbs alone, ex vivo treatment of eosinophils with a combination of mAbs DREG-56 and P4G9 before injection into the mesenteric circulation resulted in 58.8 ± 23.5% (p > 0.05 vs P4G9 or DREG-56; p < 0.001 vs
control) inhibition of eosinophil rolling, mAb IB4, in contrast, had no effect on eosinophil rolling (Rf = 25.84 ± 10.9% vs 24.5 ± 8.6% in absence of mAb), suggesting that initial steps of eosinophil adhesion to inflamed venules under conditions of blood flow were mediated by L-selectin and VLA-4, and were CD18-independent.

To establish if the inhibition in eosinophil rolling by mAbs PS/2 and P4G9 was specific for VLA-4, we similarly treated neutrophils with mAbs PS/2 or P4G9, as well as DREG-56 or IB4, and then injected the neutrophils into the terminal mesentery artery bloodstream of IL-1-stimulated rabbits (Fig. 4). In agreement with previous observations (19, 25), mAb DREG-56 blocked L-selectin dependent rolling of human neutrophils in vivo by 64.6 ± 10.5% (n = 6/2), whereas mAbs against VLA-4 (PS/2 and P4G9) and CD18 integrins (mAb IB4) had no effect on neutrophil rolling in mesenteric venules.

Inhibition of eosinophil rolling by anti-VLA-4 mAbs is not caused by modulation of L-selectin or CD18 receptor expression

We next examined whether inhibition of eosinophil rolling by anti-VLA-4 mAbs was caused by L-selectin shedding or CD18 up-regulation. Treatment of eosinophils with anti-VLA-4 mAbs P4G9 or PS/2 (50 μg/ml) for 30 min at
IL-1 activated rabbit mesenteric venules. Fluorescently labeled eosinophils were injected into the terminal mesentery artery of control (PBS) or IL-1-treated rabbits. The flowing cells were made visible by transillumination and stroboscopic fluorescent epi-illumination, and recorded simultaneously. The recorded tapes were then analyzed to determine the percentage of Fr from the Ff (rolling fraction; Rf) during one injection. A total of 17 venules in five IL-1-treated rabbits, and six venules in two control rabbits were analyzed. The bars represent mean ± SD.

Room temperature had no effect on the expression of L-selectin or CD18, as assessed by flow cytometry (Fig. 5). In contrast, ex vivo treatment of eosinophils with PMA (10⁻⁷ M) under the same experimental conditions resulted in a significant decrease in L-selectin and increase of CD18 surface expression (Fig. 5). The median fluorescence intensity of L-selectin after PMA stimulation was only slightly higher than that observed with an irrelevant nonbinding mAb 81C6 (Fig. 5). Furthermore, incubation of eosinophils with mAbs P4G9 or PS/2 did not result in cell aggregation, as determined by flow cytometry and microscopic examination. These results demonstrate that the in vivo inhibition of eosinophil rolling by anti-VLA-4 mAbs is not caused by receptor activation or cell aggregation, but by blocking of VLA-4 receptor function.

**Hematocrit, white blood cell counts, and blood pressure**

Differences in rolling observed in our experiments were not caused by changes in rabbit hematocrit, blood pressure, or differential blood counts. As previously reported (19), i.p. injection of IL-1, as well as injection of mAbs, did not have any effect on these parameters. However, the surgical procedure itself increased the systemic leukocyte count by 1.4- to 1.5-fold (12.75 ± 5.8 × 10⁶ leukocytes/ml), but these levels remained elevated throughout the length of the experiment and did not change during injections in IL-1-stimulated or unstimulated animals.

**Effect of anti-L-selectin and anti-VLA-4 mAbs on the velocity distribution profiles of rolling eosinophils**

In addition to effects of anti-L-selectin and anti-VLA-4 mAbs on eosinophil rolling, we have examined the effect of these mAbs on the velocity distribution profiles of rolling eosinophils in five venular segments in vivo. The mean velocities (mm/s) of rolling eosinophils before and after ex vivo mAb treatment were determined in each of the representative venules (Fig. 6). Fluorescently labeled eosinophils were treated with 50 μg/ml mAbs DREG-56, P4G9, PS/2, or IB4 before injection into the cannulated mesentery artery and the mean velocity of each passing eosinophil in a representative venule was determined by frame by frame analysis. Compared with a mean rolling velocity of 0.22 ± 0.18 mm/s of control eosinophils, mAb
FIGURE 4. Eosinophil rolling in IL-1-treated mesenteric venules is mediated by VLA-4 and L-selectin in vivo. CFDA-labeled eosinophils (a) and neutrophils (b) were incubated with mAbs PS/2 (anti-α1) or P4G9 (anti-α4); DREG-56 (anti-L-selectin) or a combination of DREG-56 and PS/2; or IB4 (anti-CD18) before injecting into mesenteric artery and rolling in venules determined. The results are expressed as percent inhibition in FR, compared with untreated eosinophils or neutrophils. The values represent mean ± SD obtained from 14 venules in four rabbits.

FIGURE 5. Flow-cytometric analyses of surface expression of L-selectin and CD18 after stimulation of human eosinophils with anti-VLA-4 mAbs. Eosinophils were stimulated with mAbs P4G9 and PS/2 (50 μg/ml for 30 min at room temperature), and expression of L-selectin and CD18 was determined on a FACStar flow cytometer (Becton Dickinson) by using FITC-labeled mAbs DREG-200 and IB4. The FITC-mAb binding to eosinophils was determined after gating by characteristic forward and side light scatter. Eosinophils also were stimulated with PMA (10^{-7} M) as a control. PMA stimulation resulted in significant shedding of L-selectin and up-regulation of CD18. The dotted line represents median fluorescence intensity obtained with an irrelevant, nonbinding control mAb, 81C6.

DREG-56 treatment resulted in 40% increase in the mean velocity (0.31 ± 0.19 mm/s) of the rolling eosinophils. Likewise, eosinophils treated with mAbs P4G9 or PS/2 rolled at significantly higher velocities, compared with untreated eosinophils (0.32 ± 0.16 mm/s, p < 0.05 P4G9 vs control; and 0.36 ± 0.17 mm/s, p < 0.05, PS/2 vs control). Treatment with mAbs DREG-56 and P4G9 in combination resulted in a 1.8-fold increase in mean velocity of rolling eosinophils (0.4 ± 0.15 mm/s; p < 0.05 vs control), whereas isotype-matched anti-CD18 mAbIB4 had no significant effect on the mean velocities of rolling eosinophils (0.26 ± 0.14 mm/s).

These studies demonstrate that mAbs to L-selectin and VLA-4 not only inhibit the number of rolling eosinophils, but also increase the mean velocity of interacting (rolling) eosinophils in IL-1-stimulated mesenteric venules. Taken together, these results provide evidence that the earliest event of eosinophil interaction with EC under conditions of blood flow is characterized by rolling, and that these eosinophils engage both L-selectin and VLA-4 to roll in IL-1-stimulated mesenteric venules.

Discussion

Several lines of investigation have revealed the importance of surface receptors in mediating the adhesion and recruitment of eosinophils to extravascular sites during asthma and allergen-induced late phase responses. However, the molecular mechanisms mediating the selective recruitment of eosinophils to sites of tissue inflammation during allergic inflammation and asthma are not clearly understood. Furthermore, because of the lack of appropriate tools to study eosinophil adhesion at physiologic shear rates, it has not been possible to identify and demarcate the functional role of surface receptors in mediating eosinophil adhesion and extravasation in vivo. We have therefore examined the function of eosinophil surface receptors in mediating eosinophil adhesion to IL-1-stimulated EC under conditions of blood flow by using intravital microscopy. The xenogeneic mesentery model has previously provided important insights to the understanding of in vivo interactions of human neutrophils with rabbit mesenteric EC (19, 25, 37). Recent studies have demonstrated interspecies homology and conservation of function between several human, rabbit, and murine adhesion molecules, including human and murine L-selectin; rabbit E-selectin, murine E- and P-selectins, and canine P-selectin (reviewed
mesenteric venules, but not arterioles. In addition, no firm physiologic role of L-selectin, VLA-4, and CD18 in up-regulation of E-selectin and the earliest events of leukocyte interaction with EC are characterized in mesenteric venules has previously been demonstrated to sites of tissue inflammation, we have examined the restricted to venular but not arterial EC, whereas E-selectin expression was observed on both venous and arterial EC (44). Increased expression of these adhesion molecules also has been observed in tissue sites of late phase allergic reactions and in nasal biopsies of patients with perennial rhinitis (9, 15, 16). These studies suggest that inducible adhesion molecules, E-selectin and VCAM-1, may both be involved in mediating eosinophil rolling along venular EC under conditions of shear force in vivo. Previous studies have demonstrated that L-selectin expression on eosinophils is reduced significantly after transendothelial migration (14, 45). Likewise, allergen-challenged bronchoalveolar lavage eosinophils shed L-selectin, compared with peripheral blood eosinophils (46). Although these results support a role for L-selectin in eosinophil-EC interactions, the in vivo role of L-selectin as a rolling receptor under conditions of blood flow has not been demonstrated, and can be appreciated only by direct observation with use of intravital microscopy. Our studies demonstrate a physiologic role for eosinophil L-selectin as a rolling receptor involved in early adhesion of eosinophils to EC in vivo. In contrast to studies with neutrophils (19), eosinophil rolling was not mediated totally by L-selectin (as determined by mAb DREG-56 studies), and is suggestive of the participation of alternate eosinophil epitopes in rolling. In support of this hypothesis, recent studies in vitro have demonstrated differences in the function and expression of sialic acid containing counterligands for E-selectin between neutrophils and eosinophils (47). In addition to these differences, our studies demonstrate that eosinophil rolling is mediated by at least two adhesion receptors (L-selectin and VLA-4), and this rolling could be reduced significantly by treatment with mAbs to L-selectin or VLA-4α, but not by anti-CD18 mAb.

The inhibition of eosinophil rolling brought about by two different anti-VLA-4 Abs was not caused by nonspecific effects of the mAbs P4G9 or PS/2, because in vivo rolling of human neutrophils (which do not express VLA-4) was not affected when treated with these mAbs. The anti-VLA-4 Abs did not inhibit eosinophil rolling by altering the level of receptor expression, as eosinophils treated with anti-VLA-4 Abs expressed the same levels of L-selectin and CD18 as untreated eosinophils. Thus, VLA-4,

**FIGURE 6.** Effect of anti-L-selectin, anti-VLA-4, and anti-CD18 mAbs on the velocity distribution profiles of rolling eosinophils. Fluorescently labeled eosinophils were treated ex vivo with 50 μg/ml mAb DREG-56, P4G9, PS/2, or IB4 for 30 min at room temperature before administration into the mesenteric artery. The passage of the flowing eosinophils was recorded, and velocities of consecutive eosinophils before and after mAb treatment were determined by frame by frame analysis in each of the five representative venules in two rabbits. The dotted line represents the mean rolling velocity of untreated (no mAb) eosinophils.

Human eosinophils were found to roll along the walls of mesenteric venules, but not arterioles. In addition, no firm adhesion or spreading of the rolling eosinophils was observed in the inflamed venules. IL-1 stimulation of mesenteric venules resulted in up to a fourfold increase in eosinophil rolling, and suggests the involvement of inducible EC adhesion molecules in promoting early events of eosinophil adhesion. Several investigators have demonstrated that eosinophil adhesion to activated EC is mediated by E-selectin, VCAM-1, and ICAM-1 in vitro (7–11, 13). In support of our observation of inducible rolling along venular EC are recent studies demonstrating that the up-regulation of E-selectin and VCAM-1 expression is mediated within 4 h of cytokine stimulation (43). Furthermore, the expression of VCAM-1 was found to be restricted to venular but not arterial EC, whereas E-selectin expression was observed on both venous and arterial EC (44). Increased expression of these adhesion molecules also has been observed in tissue sites of late phase allergic reactions and in nasal biopsies of patients with perennial rhinitis (9, 15, 16). These studies suggest that inducible adhesion molecules, E-selectin and VCAM-1, may both be
conceivably through its interactions with VCAM-1, participates along with L-selectin in promoting early events of eosinophil adhesion in vivo. Further indication for the involvement of VLA-4/VCAM-1 interactions during early stages of eosinophil-EC interactions is evident from studies demonstrating that mAbs to VLA-4 inhibit cell adhesion, but not transmigration, across cytokine-activated EC under static conditions (48, 49). In support of these data, electron microscopy studies have revealed that VCAM-1 expression is limited to luminal surface, but not at inter junctional space, of IL-1-stimulated EC (50). Our study demonstrates that VLA-4 can participate as a rolling receptor in conjunction with L-selectin to promote initial events of eosinophil adhesion to cytokine-stimulated EC under conditions of blood flow. It is possible that VLA-4 and L-selectin mediate eosinophil rolling in a sequential manner. Initiation of eosinophil rolling may be dominated by L-selectin in conjunction with vascular selectins, whereas establishment of continued rolling may require engagement of VLA-4 with endothelial ligands. Subsequent activation of eosinophils could then not only result in shedding of L-selectin (51), but also in altering of the activation state of VLA-4 (52), such that VLA-4 could then participate in firm adhesion of eosinophils to cytokine-stimulated EC in vivo. Alternatively, it is possible that VLA-4 can participate as a rolling receptor, along with L-selectin, in parallel rather than in a sequential manner. Recent studies with mouse lymphocytes have demonstrated that a single endothelial ligand MAdCAM (which shares structural homology with VCAM-1 and E-selectin) can bind to two structurally dissimilar lymphocyte receptors (L-selectin and α4β7) (53, 54). Likewise, it is possible that L-selectin and VLA-4 participate in parallel to mediate eosinophil rolling by interacting with a similar/same EC counter receptor. In vivo rolling studies with L-selectin”VLA-4” or VLA-4”L-selectin” transfected cell lines will assist in evaluating this hypothesis.

Although in vitro studies of eosinophils and EC under static conditions in the absence of blood flow have provided important insights into eosinophil adhesion, it has thus far not been possible to elucidate clearly the role of various adhesion receptors in mediating initial and later events of eosinophil adhesion at physiologic shear rates in vivo. In this investigation, we have examined the initial steps of what appears to be a complex series of adhesive events mediating eosinophil-EC interactions in vivo. We demonstrate that eosinophils utilize different cell surface receptors, i.e., L-selectin and VLA-4, as adhesion receptors for rolling along IL-1-stimulated EC. In addition, we demonstrate that the rabbit mesentery preparation is a useful model system to understand eosinophil-EC interactions in asthma or allergic inflammation under conditions of blood flow in vivo.

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References


