Mechanisms of Selective Leukocyte Recruitment from Whole Blood on Cytokine-Activated Endothelial Cells Under Flow Conditions

Kamala D. Patel

*J Immunol* 1999; 162:6209-6216; http://www.jimmunol.org/content/162/10/6209

**References**

This article cites 50 articles, 29 of which you can access for free at: http://www.jimmunol.org/content/162/10/6209.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Mechanisms of Selective Leukocyte Recruitment from Whole Blood on Cytokine-Activated Endothelial Cells Under Flow Conditions

Kamala D. Patel

Selective recruitment of eosinophils to sites of allergic and parasitic inflammation involves specific adhesion and activation signals expressed on or presented by stimulated endothelial cells. Here we examined leukocyte recruitment on cytokine-activated HUVEC under flow conditions. We perfused whole blood through a flow chamber to examine mechanisms of selective leukocyte recruitment. Although there was substantial recruitment of leukocytes on TNF-α-stimulated HUVEC, we found no selective accumulation of any particular leukocyte subpopulation. In contrast, fewer leukocytes were recruited to IL-4-stimulated HUVEC, but the recruitment was selective for eosinophils. We examined the role of adhesion molecules in these interactions and found that eosinophil recruitment was completely blocked with an α4 integrin mAb at the shear rates examined. A significant number of neutrophils were also recruited to IL-4-stimulated HUVEC, and these interactions required P-selectin and P-selectin glycoprotein ligand-1. Thus, whole blood perfusion over cytokine-activated endothelium revealed that IL-4-stimulated HUVEC support selective recruitment of eosinophils, whereas TNF-α-stimulated HUVEC lack selectivity for any leukocyte subclass. The Journal of Immunology, 1999, 162: 6209–6216.

Leukocyte recruitment occurs in a stepwise fashion beginning with leukocyte tethering to the endothelial cells lining the blood vessels (1). This is frequently followed by leukocyte rolling, activation, firm adhesion, and emigration (1). Although the adhesion proteins participating in leukocyte recruitment have been identified, determining the mechanisms underlying selective recruitment of leukocyte subpopulations to sites of injury or inflammation has continued to be elusive. Many groups have used in vitro flow chambers to examine the interactions of particular leukocyte subpopulations with purified proteins or activated HUVEC (2–7). These studies have attempted to compare the efficiency of tethering and rolling of different leukocyte subclasses to understand how these cells might interact with a given substrate in vivo. Unfortunately, isolation procedures for different leukocyte subclasses vary dramatically, possibly altering leukocyte function. Thus, making comparisons of adhesion efficiency between these laboratories becomes essentially impossible.

In this study, we used a technique recently described by Reinhardt and Kubes (8) to examine leukocyte interactions with TNF-α- or IL-4-stimulated HUVEC. This technique does not rely on isolated populations of leukocytes; instead, whole blood is perfused through the flow chamber. After chasing the whole blood with buffer, interacting leukocytes are examined and quantified using bright-field optics, and the leukocytes recruited to the surface are identified by Wright-Giemsa staining of the plate. This technique has the advantage of observing interactions between leukocytes and endothelial cells in the context of the whole blood. Additionally, this method allows for the immediate identification of the leukocytes that have accumulated on a given substrate (8), unlike current in vivo models.

We found that TNF-α-stimulated HUVEC recruited all classes of leukocytes from whole blood; however, there was no selective accumulation of any particular leukocyte subclass. In contrast, IL-4-stimulated HUVEC recruited fewer leukocytes from the whole blood than TNF-α-stimulated HUVEC; however, there was a 4-fold enrichment in eosinophils. Surprisingly, IL-4-stimulated HUVEC also supported significant accumulation of neutrophils. This manuscript details the variable adhesion molecule combinations used by each leukocyte subclass for optimal recruitment from whole blood.

Materials and Methods

Materials

IL-4 and TNF-α were obtained from R & D Systems (Minneapolis, MN). Anti-P-selectin mAbs G1 and S12 (9, 10), anti-E-selectin mAbs ES1 (7), and anti-P-selectin glycoprotein ligand-1 (PSGL-1)3 mAbs PL1 and PL2 (11) were all kindly provided by Dr. Rodger McEver (University of Oklahoma, Oklahoma City, OK). Anti-VCAM-1 mAb 1.G11B1 and anti-α4 integrin mAb H2/1 were both purchased from Serotec (Oxford, U.K.). Anti-CD16 mAb conjugated to paramagnetic beads was purchased from Miltenyi (Auburn, CA).

Cell culture and isolation

HUVEC were isolated from individual umbilical cords and grown in Media 199 (M199) with 20% human serum as first passage cultures in 35-mm dishes (12). Neutrophils were isolated from normal human donors by dextran sedimentation, hypotonic lysis, and density centrifugation on lymphoprep 1077 (12). Eosinophils were isolated from granulocytes by negative selection with CD16 microbeads using magnetic cell separation (13).

Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

Received for publication November 5, 1998. Accepted for publication March 2, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work is supported by grants from the Medical Research Council of Canada (MT-14180), the Alberta Lung Association, and the Alberta Heritage Foundation for Medical Research (970234).

2 Address correspondence and reprint requests to Dr. Kamala D. Patel, Department of Physiology and Biophysics, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1. E-mail address: kpatel@acs.ucalgary.ca

3 Abbreviations used in this paper: PSGL-1, P-selectin glycoprotein ligand-1; M199, media 199; HSA, human serum albumin; R-factor, recruitment factor.
LEUKOCYTE RECRUITMENT FROM WHOLE BLOOD UNDER FLOW CONDITIONS

Adhesion under flow conditions
HUVECs were treated with 20 ng/ml of IL-4 in M199 with 0.5% human serum albumin (HSA) for 24 h or with 20 ng/ml TNF-α in M199/0.5% HSA for 6 h and then washed once with HBSS. Isolated eosinophils or neutrophils (5 × 10^6/ml) in HBSS/0.5% HSA were perfused through a parallel plate flow chamber at the desired wall shear stresses, and leukocyte accumulation (rolling plus firmly adherent) was determined as described (7, 11). Alternatively, freshly drawn, heparinized whole blood was perfused through the flow chamber at the specified shear rates for 4 min followed by perfusion of HBSS as described (8). Shear rates were used for experiments with whole blood, as determining the precise viscosity of blood to calculate wall shear stress is problematic. The number of interacting leukocytes were counted within 30 s of the HBSS perfusion using a 20× objective and bright-field optics. All experiments were performed at 37°C. In certain experiments, whole blood or HUVECs were preincubated for 10 min with the specified mAb, and accumulation was assessed in the continued presence of the mAb. mAbs were used at concentrations previously shown to be optimal.

Analysis of accumulated leukocytes
Leukocytes accumulated on cytokine-stimulated HUVEC were characterized as described (8). Briefly, the inlet lines were removed from the flow chamber, allowing air to enter the chamber. This did not remove the interacting leukocytes, as we counted the number of leukocytes associated with the monolayer before and after the introduction of air into the system. The plates were removed from the chamber, allowed to air dry for 30 min, Wright-Giemsa stained, and then at least a 200-cell differential was performed in a blinded fashion. Using this technique, we were unable to differentiate monocytes from T cells due to leukocyte transmigration that led to distortion of the nucleus and cytoplasm, thus in all of the figures these cells are referred to as mononuclear cells (PBMCs).

Statistics
Statistical differences between experimental groups were evaluated using the two-tailed Mann-Whitney U test. Values of p < 0.05 were considered significant.

Results
Leukocytes accumulate on TNF-α- or IL-4-stimulated HUVEC
TNF-α- and IL-4-stimulated HUVEC both express adhesion molecules that support accumulation (rolling and adhesion) of isolated leukocyte subpopulations under flow conditions. We and others have independently examined the recruitment of neutrophils (14), eosinophils (15), T cells (16), and monocytes (17) on TNF-α-stimulated HUVEC and found that all of these cell types can adhere under flow conditions. In contrast, IL-4-stimulated HUVEC support the interaction of isolated eosinophils (18), T cells (19), and monocytes (6) but do not support neutrophil adhesion (20) under flow conditions. Fig. 1A compares neutrophil and eosinophil accumulation on TNF-α- or IL-4-stimulated HUVEC under flow conditions. This data confirms previous observations, with eosinophils accumulating on both of these surfaces, but neutrophils only accumulating on TNF-α-stimulated HUVEC (Fig. 1A). Using isolated eosinophils, we compared eosinophil accumulation on TNF-α- and IL-4-stimulated HUVEC side by side. We found that isolated eosinophils bind equally well to both TNF-α- and IL-4-stimulated HUVEC, with nearly identical shear dependence (Fig. 1B). These data together show the potential interactions between leukocytes and endothelial cells following cytokine stimulation. However, these experiments do not address whether these interactions are favored in the context of the whole blood.

We addressed this question by examining leukocyte accumulation on cytokine-stimulated HUVEC using whole blood instead of isolated leukocyte subpopulations. HUVEC stimulated with either TNF-α for 6 h or IL-4 for 24 h were placed in a parallel plate flow chamber. Whole blood was perfused across these surfaces at the specified shear rates and the number of accumulated leukocytes, including both rolling and firmly adherent, was determined as described in Materials and Methods. Leukocytes interacted with both TNF-α- and IL-4-stimulated HUVEC, but did not interact with control HUVEC (Fig. 2). TNF-α-stimulated HUVEC supported more leukocyte accumulation than IL-4-stimulated HUVEC at all of the shear rates examined (Fig. 2 and data not shown). The increased number of leukocytes recruited from whole blood as compared with isolated leukocyte populations likely reflects the increased leukocyte concentration in whole blood (4–10 x 10^6 cells).
cells/ml in whole blood vs 0.5–1 × 10^6 cells/ml in isolated leukocyte experiments) and/or the role of RBC in enhancing leukocyte-endothelial cell interactions (21).

All leukocytes accumulate proportionately on TNF-α-stimulated HUVEC, but eosinophils are selectively recruited on IL-4-stimulated HUVEC.

We next examined the leukocyte subclasses recruited on TNF-α-stimulated HUVEC and compared these numbers to the percentages of each subclass normally found in the peripheral blood. This was done to determine whether there was differential recruitment of particular leukocyte subclasses. We found that neutrophils, PMCs, and eosinophils were all recruited to TNF-α-treated HUVEC (Fig. 3, A–F). The percentage of each leukocyte subclass on TNF-α-stimulated HUVEC was virtually identical to that found in the peripheral blood at all of the shear rates examined. Therefore, dividing the percent of leukocytes accumulated by the percent of leukocytes in the whole blood differential to determine the R-factor. An R-factor of 1 would indicate no selective recruitment. The total number of leukocytes recruited at each shear rate is provided in Fig. 2. All data are mean ± SEM of at least six experiments. C and D, n = 18 for TNF-α and n = 22 for IL-4. *, p < 0.05 as compared with the percentage in the whole blood.

FIGURE 3. Eosinophils are differentially recruited to IL-4-stimulated HUVEC, but not to TNF-α-stimulated HUVEC. HUVEC were stimulated and whole blood was perfused over the surface as described in Fig. 1 at the specified shear rates. Air was allowed to enter the chamber, the flow cell was disassembled, and between a 200- and 500-cell differential was performed on Wright-Giemsa-stained cells accumulated on the plate. A, C, and E, The percentage of each leukocyte subclass bound on the plate was determined. B, D, and F, The percentage of each leukocyte subclass bound on the plate was divided by the total percentage of each leukocyte subclass in the whole blood differential to determine the R-factor. An R-factor of 1 would indicate no selective recruitment. The total number of leukocytes recruited at each shear rate is provided in Fig. 2. All data are mean ± SEM of at least six experiments. C and D, n = 18 for TNF-α and n = 22 for IL-4. *, p < 0.05 as compared with the percentage in the whole blood.
examined (Fig. 2). The most striking observation was the 3- to 5-fold increase in eosinophil recruitment during the 4 min of whole blood perfusion (Fig. 3, B, D, and F). The percentage of eosinophils recruited remained constant at all of the shear rates examined (Fig. 3, A, C, and E). Isolated neutrophils did not interact with IL-4-stimulated HUVEC (Fig. 1A); however, neutrophils were efficiently recruited to IL-4-stimulated HUVEC from whole blood in parallel experiments (Fig. 3). Unlike eosinophil accumulation, this recruitment was shear dependent, as many neutrophils were recruited at lower shear rates and few were recruited at higher shear rates. This decrease in percentage translated to a decrease in the total number of neutrophils on the surface. Based on the total cells recruited (Fig. 2), neutrophils represented 186 cells/mm² at 100 s⁻¹ but only represent 8 cell/mm² at 400 s⁻¹. Consistent with this is the fact that IL-4-stimulated HUVEC preferentially recruited neutrophils at a lower shear rate (R-factor > 1; Fig. 3B) but discriminated against neutrophils at higher shear rates (R-factor < 1; Fig. 3F). Thus IL-4-stimulated HUVEC support selective recruitment of eosinophils at all shear rates but only support neutrophil recruitment at lower shear rates.

The role of adhesion molecules in leukocyte accumulation on TNF-α- or IL-4-stimulated HUVEC under flow conditions

TNF-α-stimulated HUVEC up-regulate VCAM-1, ICAM-1, E-selectin (reviewed in Ref. 1), and in some studies P-selectin (22), whereas IL-4-stimulated HUVEC up-regulate VCAM-1 (23) and P-selectin (20). To examine the role of these adhesion molecules in leukocyte recruitment to either TNF-α- or IL-4-stimulated HUVEC, we used a series of mAb to inhibit leukocyte recruitment from whole blood. For these experiments, we used an intermediate shear rate of 200 s⁻¹. Fig. 4 shows that leukocyte recruitment on TNF-α-stimulated HUVEC was dependent on both E-selectin and VCAM-1/α4 integrin interactions, and together these mAbs almost completely blocked accumulation (Fig. 4A). Anti-P-selectin and anti-PSGL-1 mAbs had no effect on recruitment compared with their isotype-matched nonblocking control mAbs (data not shown), suggesting that P-selectin does not participate in leukocyte recruitment on this surface.

We next examined the role of these adhesion molecules in leukocyte recruitment on IL-4-stimulated HUVEC. Abs directed against VCAM-1, α4 integrins, or VCAM-1 and α4 integrins together attenuated total leukocyte accumulation on IL-4-stimulated HUVEC. Anti-P-selectin or PSGL-1 mAbs also decreased accumulation to varying degrees, and an anti-P-selectin mAb together with an anti-α4 integrin mAb blocked almost all accumulation (Fig. 4B). As expected, an anti-E-selectin mAb had no effect on leukocyte accumulation on IL-4-stimulated HUVEC (data not shown).

Effect of adhesion molecules on leukocyte recruitment profiles on TNF-α- or IL-4-stimulated HUVEC

We next determined the effect of adhesion molecule mAbs on the recruitment of individual leukocyte classes from the whole blood. As in Fig. 3, we performed between a 200- and 500-cell differential for each experiment and determined the percentage of each leukocyte subclass on the cytokine-treated HUVEC following mAb treatment. This data provided two types of information. First, the effect of each mAb on the overall recruitment of each leukocyte subclass could be determined. In addition, this analysis allowed us to examine the effect of each mAb on selective recruitment of particular leukocyte subpopulations.

We first examined leukocyte recruitment on TNF-α-stimulated HUVEC. We found that an anti-E-selectin mAb decreased recruitment of all leukocyte subclasses (Table I). However, we found that an anti-E-selectin mAb did not effect the percentage of each leukocyte subpopulation found on TNF-α-stimulated HUVEC (Table I). This suggests that E-selectin generally participates in the recruitment of all leukocytes. In contrast an anti-VCAM-1 mAb

![Table 1](http://www.jimmunol.org/)

**Table 1. The role of adhesion molecules in leukocyte adhesion and recruitment on TNF-α-stimulated HUVEC**

<table>
<thead>
<tr>
<th>Cells/mm²</th>
<th>E-Selectin mAb</th>
<th>VCAM-1 mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC</td>
<td>178.0 ± 14.8</td>
<td>92.1 ± 8.9*</td>
</tr>
<tr>
<td>PMN</td>
<td>332.0 ± 14.9</td>
<td>200.5 ± 10.4*</td>
</tr>
<tr>
<td>Percent leukocytes bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC</td>
<td>34.0 ± 2.8</td>
<td>31.0 ± 3.1</td>
</tr>
<tr>
<td>PMN</td>
<td>63.5 ± 2.9</td>
<td>67.5 ± 3.5</td>
</tr>
</tbody>
</table>

*H UVEC were stimulated as in Fig. 1, and Abs were used as described in Fig. 4. Cell/mm² for PBMC and PMN was determined by multiplying the percent of leukocytes bound by the total number cells on the plate. The percent of leukocytes bound was determined as described in Materials and Methods. The data represent the mean and SEM of between three and six experiments. 

* * p < 0.05 as compared to control.
decreased recruitment of PBMCs both in total number and as a percentage of the total leukocytes (Table I), but did not affect neutrophil accumulation. So few eosinophils were recruited on TNF-α-activated HUVEC that inhibition data was not statistically significant.

We next examined the effect of these mAbs on leukocyte recruitment to IL-4-stimulated HUVEC. As with TNF-α-treated HUVEC, we looked both at the total numbers of leukocytes on this surface and at the percentage each leukocyte subclass. VCAM-1 mAb decreased accumulation of all leukocyte subclasses, including neutrophils (Fig. 5, A–C). Inhibition of total neutrophils was unexpected; however, Reinhardt and Kubes have recently shown that purified VCAM-1 can recruit neutrophils from whole blood under flow conditions (8) and that all of this recruitment is inhibitable by VCAM-1 mAb with neutrophils from whole blood (26). Thus, inhibition of recruitment of neutrophils may reflect the role of RBC in endothelial cell contacts, thus facilitating neutrophil interactions with the low levels of P-selectin being expressed on these HUVEC. Neither P-selectin nor PSGL-1 mAbs had any effect on neutrophil accumulation (Fig. 5B), consistent with data obtained from whole blood rolling over P-selectin coated surfaces (8). Eosinophil accumulation was attenuated with these mAbs, suggesting that P-selectin and PSGL-1 play a partial role in eosinophil accumulation on IL-4-stimulated HUVEC (Fig. 5A).

Fig. 5 compares the recruitment of each leukocyte subclass on IL-4-stimulated HUVEC following mAb treatment. As with Fig. 3, the data are presented both as the percent of each leukocyte subclass present on IL-4-stimulated HUVEC and as the R-factor based on the percent of each leukocyte in the peripheral blood. These data reflect the role specific adhesion molecules play in selective recruitment of particular leukocyte subclasses. VCAM-1 in combination with an anti-α4 integrin mAb dramatically decreased PBMC recruitment (Fig. 6, B and E). In contrast, neither VCAM-1 nor α4 integrins had any effect on neutrophil recruitment; instead, P-selectin and PSGL-1 were responsible for selective neutrophil recruitment (Fig. 6, C and F). Eosinophils were unique in that α4 integrins alone were responsible for selective recruitment (Fig. 6, A and D). As with accumulation, this may reflect differential utilization of VCAM-1 domain 4 by α4 integrins on eosinophils or may suggest that there is an α4 integrin ligand other than VCAM-1 on IL-4-stimulated HUVEC responsible for selective eosinophil recruitment.

Discussion

Airway inflammation is considered a hallmark of bronchial asthma (27). Eosinophils represent only a few percent of the circulating leukocytes; however, in both human asthma and animal models of asthma, there is a massive increase in eosinophil recruitment to the lungs (27–29). The mechanisms governing this selective infiltration of eosinophils are currently being explored using both in vivo and in vitro models of inflammation.

In vivo models of airway hyperresponsiveness have demonstrated a role for Th2 cytokines in the development of eosinophilia and airway hyperresponsiveness (30, 31). In particular, IL-4 (32), IL-13 (33, 34), and IL-5 (35) have all been shown to participate in the recruitment of eosinophils. Furthermore, roles for adhesion molecules such as VCAM-1, α4 integrins (36) and the endothelial selectins (37, 38) have been suggested in mediating the initial attachment of leukocytes in these and other models of allergic inflammation. These adhesion molecules have been suggested to play either a direct role in eosinophil infiltration or an indirect role.

FIGURE 5. The role of adhesion molecules in total leukocyte recruitment on IL-4-stimulated HUVEC. HUVEC were stimulated, and whole blood was perfused over the surface as described in Fig. 1. mAbs were used to modulate leukocyte recruitment as described in Fig. 4. Air was allowed to enter the chamber, the flow cell was disassembled, and between a 200- and 500-cell differential was performed on Wright-Giemsa-stained cells accumulated on the plate. The effect of the specific adhesion molecule mAbs on total eosinophils (A), PBMCs (B), and neutrophils (C) was determined by multiplying the percent of each leukocyte subclass bound by the total number of cells on the plate (shown in Fig. 4). The data represent the mean and SEM of between three and six experiments. *, p < 0.05; **, p < 0.01 as compared with control. †, Not significant as compared with VCAM-1 or α4 integrin alone. ††, Not significant as compared with P-selectin.
by blocking the recruitment of Th2 cells and, by extension, the infiltration of eosinophils.

The molecular mechanisms that govern the selective recruitment of eosinophils have also been examined using in vitro models of inflammation. In these studies, isolated eosinophils have been perfused over endothelial cells stimulated with cytokines under flow conditions (15, 18, 39, 40). Using this model, two cytokines have come to the forefront for their ability to up-regulate VCAM-1 and bind eosinophils: TNF-α and IL-4.

TNF-α is a multifunctional cytokine that plays a key role in the pathogenesis of both acute and chronic diseases including septic shock, adult respiratory distress syndrome, rheumatoid arthritis, and asthma (reviewed in Ref. 41). TNF-α can be generated by a multitude of cells and exerts its effects by interacting with at least two classes of receptors present on many cells, including endothelial cells (42). TNF-α-stimulation of HUVEC results in increased expression of several adhesion molecules including E-selectin, VCAM-1, ICAM-1, and P-selectin (reviewed in Ref. 1). IL-4 is a cytokine frequently associated with the development of atopy and allergic inflammation (43). IL-4 is produced primarily by Th2 cells, but is also synthesized and released by mast cells and eosinophils (43). IL-4 maintains the Th2 phenotype of CD4+ T cells, leads to class switching in B cells, and stimulates HUVEC (44–47). IL-4-stimulated HUVEC up-regulate adhesion molecules such as VCAM-1 (23) and P-selectin (20). TNF-α and IL-4 are both commonly used in vitro to examine leukocyte-endothelial cell adhesion and activation, and HUVEC treated with either of these cytokines have been shown to effectively recruit isolated eosinophils under flow conditions (15, 18). However, these experiments have not examined the ability of these cytokines to mediate the selective accumulation of eosinophils.

In this study, we found that TNF-α-stimulated HUVEC accumulated a tremendous number of leukocytes from whole blood. This recruitment used both E-selectin and VCAM-1, but not P-selectin. Despite the numbers of leukocytes recruited to TNF-α-stimulated HUVEC, there was no selective increase in any one leukocyte subclass. This suggests that TNF-α alone may act in a general way to recruit leukocytes to the vessel wall. Once leukocytes are rolling along the endothelium, other factors such as chemokines or additional cytokines may modulate subsequent firm adhesion and transmigration of specific leukocyte subpopulations into sites of inflammation.

In this study, we also examined whole blood interactions with IL-4-stimulated HUVEC and found that IL-4-stimulated HUVEC supported selective accumulation of eosinophils from the peripheral blood. A 4.5-fold increase in eosinophil accumulation occurred in the few minutes that whole blood was perfused over this surface. One can envision that over a 24-h period this would lead to a profound increase in eosinophil recruitment. At higher shear stresses, there was also a selective increase in PBMC recruitment. These data are consistent with IL-4 playing a role in selective recruitment of these cells in vivo. At the same time, it is striking that this level of selectivity was seen in a model system in which only the endothelial cells were present. In the context of the whole animal, for example during hypersensitivity, other cell types such as mast cells and resident T cells may further amplify this response by the synthesis of chemokines and other chemoattractants.
The α₄ integrins alone were responsible for the selective recruitment of eosinophils (Fig. 6). This is in contrast to PBMCs, where both VCAM-1 and α₄ integrins affected selective PBMC recruitment (Fig. 6). The α₄ integrins can bind to two domains on VCAM-1, domains 1 and 4 (24, 25), but our VCAM-1 mAb only binds to domain 1. Abe et al. recently demonstrated that T cells use domain 1 for attachment under flow conditions and then use domain 4 to stabilize these interactions (48). Eosinophil utilization of domains 1 and 4 under flow conditions has not been examined, and it may be that eosinophils are interacting with domain 4 on VCAM-1 in these experiments. Alternatively, IL-4-stimulated HUVEC may express another ligand for eosinophil α₄ integrins as has been suggested by Vonderheide et al. (26). Thus, these data show that P-selectin can mediate the attachment of eosinophils under flow conditions in the context of whole blood, but it is the engagement of the α₄ integrins that dictates the selective accumulation of eosinophils in this system.

Unlike isolated neutrophils, neutrophils from whole blood were also recruited on IL-4-stimulated HUVEC at lower shear rates, and these interactions required P-selectin and PSGL-1. Several factors may play a role in these interactions. First, increased encounters between neutrophils and the vessel wall due to the presence of RBC (21) may facilitate interactions between neutrophils and the low levels of P-selectin present on the surface of IL-4-stimulated HUVEC. Interestingly, visual observation of these plates after staining suggests that PBMCs and eosinophils were actively spreading and transmigrating on IL-4-stimulated HUVEC; whereas, neutrophils remained rounded on the surface (data not shown). Because P-selectin mediates only rolling, it is tempting to conclude that neutrophils were only participating in rolling interactions with IL-4-stimulated HUVEC. Indeed, Warnock et al. demonstrated that neutrophils roll in lymph nodes but unlike lymphocytes do not become firmly adherent (49). Additionally, P-selectin expressed on platelets in suspension or platelets accumulated on the plate may act to recruit neutrophils. Recently, Diacovo et al. have demonstrated that P-selectin expressed on platelets can facilitate leukocyte interactions with the vessel wall in vivo (50). Although we did see a few platelets binding to the plate in areas of endothelial cell retraction, we never observed leukocytes interacting with these platelets. Furthermore, blood was maintained in heparin to prevent platelet activation; thus, it is unlikely that activated platelets were responsible for neutrophil accumulation.

Surprisingly, VCAM-1 and α₄ integrins also decreased the total accumulation of neutrophils in this system (Fig. 5). These interactions were not observed using isolated populations of neutrophils (Fig. 1A) in side-by-side experiments. However, unactivated neutrophils have been shown to interact with purified VCAM-1 using whole blood perfusion (8). This may reflect a small sub-population of immature neutrophils that still express α₄ integrins. Consistent with this, we frequently find 1–2% of neutrophils expressing α₄ integrins using flow cytometry (data not shown).

These data show that IL-4-stimulated HUVEC support the selective accumulation of eosinophils from whole blood in the absence of signals from other cell types such as mast cells, resident Th2 cells, or macrophages. In contrast, TNF-α-stimulated HUVEC generally recruit all types of leukocytes from the whole blood and may rely on signals from the tissue to result in selective accumulation of any particular class of leukocytes.

Acknowledgments

We thank Evelyn Lailey for her excellent technical assistance; Dr. Rodger McEver for his generous gifts of reagents; Drs. John Reynolds and Paul Kubes for critical reading of this manuscript; and the Labor and Delivery Unit at the Foothills Hospital in Calgary for their assistance in providing umbilical cords.

References

domain of vascular cell adhesion molecule 1 and an additional α4 integrin counter-receptor on stimulated endothelium. J. Exp. Med. 175:1435.


42. Mackay, F., H. Lootscher, D. Stueber, G. Gehr, and W. Lesslauer. 1993. Tumor necrosis factor α (TNF-α)–induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. J. Exp. Med. 177:1277.


