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Stimulation of Human Endothelium with IL-3 Induces Selective Basophil Accumulation In Vitro

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Basophils have been shown to accumulate in allergic airways and other extravascular sites. Mechanisms responsible for the selective recruitment of basophils from the blood into tissue sites remain poorly characterized. In this study, we characterized human basophil rolling and adhesion on HUVECs under physiological shear flow conditions. Interestingly, treatment of endothelial cells with the basophil-specific cytokine IL-3 (0.01–10 ng/ml) promoted basophil and eosinophil, but not neutrophil, rolling and exclusively promoted basophil adhesion. Preincubation of HUVECs with an IL-3R-blocking Ab (CD123) before the addition of IL-3 inhibited basophil rolling and adhesion, implicating IL-3R activation on endothelial cells. Incubation of basophils with neuraminidase completely abolished both rolling and adhesion, indicating the involvement of sialylated structures in the process. Abs to the β1 integrins, CD49d and CD49e, as well as to P-selectin and P-selectin glycoprotein ligand 1, inhibited basophil rolling and adhesion. Furthermore, blocking chemokine receptors expressed by basophils, such as CCR2, CCR3, and CCR7, demonstrated that CCR7 was involved in the observed recruitment of basophils. These data provide novel insights into how IL-3, acting directly on endothelium, can cause basophils to preferentially interact with blood vessels under physiological flow conditions and be selectively recruited to sites of inflammation. The Journal of Immunology, 2006, 176: 5346–5353.

The infiltration and activation of basophils are characteristic hallmarks of allergic diseases, including asthma. Indeed, basophils have been found to accumulate extensively at sites of allergic inflammation (1). However, the events leading to the selective extravasation of basophils during allergic inflammation are not well understood.

Leukocyte recruitment involves interactions between adhesion molecules on the leukocyte and endothelium and is critically dependent on conditions of shear flow, or pressures created by faster erythrocyte flow on the leukocyte. Sequential steps in leukocyte transmigration include rolling and tethering; activation and triggering by chemokines presented on the endothelial surface; firm adhesion; and transmigration across the endothelium and basement membrane. These steps occur in a cascade-like manner, mediated by specific adhesion molecules present on both the leukocyte and the endothelium (2). The selectins (P- and E-selectins on the endothelium, and L-selectin on the leukocyte) and their sialylated fucosylated carbohydrate ligands such as P-selectin glycoprotein ligand 1 (PSGL-1), regulate the initial leukocyte capture and rolling on the endothelium, whereas firm adhesion and transendothelial migration are mediated through overlapping sets of adhesion molecules such as integrins and their Ig-family ligands (e.g., ICAM-1 and VCAM-1). These events have been studied in detail for neutrophil and eosinophil recruitment using both in vitro flow systems (3, 4) and in vivo models of leukocyte trafficking (5, 6) such as intravital microscopy. However, relatively little is known about the mechanisms of basophil recruitment under physiological flow conditions. Phenotypic analysis and static adhesion assays from our laboratory have shown function and expression of several basophil adhesion molecules, including PSGL-1 (7), sialyl Lewis X, as well as β1, β2, and β3 integrins (including αL) (8, 9). In vitro studies performed with eosinophils under flow conditions have shown an important role for VCAM-1, binding to CD49d/CD29 (α5/β1, VLA-4), which may also be important for basophil trafficking because Abs to CD49d inhibit basophil adhesion to endothelial cells under static conditions (10). In addition, chemokines such as MIP1α (11), RANTES (12), and eotaxin (13) can be released from, or displayed on, activated endothelium. These chemokines have been shown to promote basophil activation, histamine release, and chemotaxis via specific G-protein-coupled receptors such as CCR2 and CCR3 expressed on the basophil cell surface. Basophils have been shown to express CCR1, CCR2, CCR3, and CCR5 and CX3C chemokine receptors CXCR1, CXCR2, and CXCR4 (14).

Selective activation of basophil adherence to endothelial cells has been reported with IL-3 (15) and FcεRI triggering (16), which causes basophil adhesion at levels of activation below those that cause histamine release. IL-3 is a potent priming factor and activator of basophils, also known as multilineage CSF. Both IL-3 and IgE-dependent activators, such as allergen, have been shown to cause selective basophil adherence to resting endothelium in vitro, which could be important for preferential basophil recruitment in vivo. In vivo studies using rIL-3 in primates have shown that IL-3 is a basophil differentiation factor (17). In addition, basophils express the IL-4R (18) and prostaglandin receptors (19), which may play important roles in basophil migration. In support of this, IL-4
(which induces endothelial VCAM-1) has been shown to enhance basophil adhesion (20).

In this study, we compared basophil, neutrophil, and eosinophil rolling and adhesion to cytokine-activated endothelium under physiological flow conditions in a parallel plate flow chamber to search for conditions resulting in preferential effects on basophils. In this regard, we discovered unique properties of IL-3 in these assays. Subsequently, the adhesion molecules and chemokine receptors involved in this unique adhesion of basophils to endothelium were dissected using mAb-blocking studies. We found that the adhesion molecules P-selectin and \( \beta_2 \)-integrins CD49d, CD49e, and CD49f, and the CCR7 chemokine receptor were involved in the recruitment of basophils on IL-3-activated endothelium in vitro.

**Materials and Methods**

**Reagents and Abs**

IL-3, IL-4, and TNF-α were obtained from R&D Systems. The IL-3R CD123 was obtained from BD Pharmingen; CD49e (P1B5) was acquired from Invitrogen Life Technologies: CD29 (4B4), CD18 (7E4), CD49d (HP1/2), CD49e (Sam-1), CD49f (GoH3), CD62L (DREG-56), and PSGL-1 (PL1) were obtained from Immunotech (Beckman Coulter). These Abs were chosen because of their blocking abilities (Refs. 8, 21; our unpublished observations). CD62P (G1 F(ab\(^{-1}\))\(^{2}\)) was provided by Centocor (22), whereas preparations of F(ab\(^{-1}\))\(^{2}\) versions of blocking Abs to CD62E were described previously (21) for expression of chemokine receptors CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CXCR1, CXCR2, CXCR3, CXCR4, and CXCR5.

**Cell culture and isolation**

HUVECs were isolated from individual umbilical cords and grown to confluence in Medium 199 with 20% FCS and endothelial cell growth factor as described previously (21). After the first passage, cells were seeded into 35-mm diameter tissue culture dishes for use in the flow-based assay. No cells were used after the first passage, because P-selectin diminishes with passage.

Basophils were isolated from whole blood (120 ml), using density gradient centrifugation at room temperature and removal of CD16-positive cells which was sufficient to perform up to 10 runs through the in vitro flow assay between 80 and 95%, and the yield was 95%, and viability (by dye exclusion) was 100%.

**Cytokine and Ab treatments**

HUVECs were stimulated with 5 ng/ml TNF-α for 4 h, 20 ng/ml IL-4 for 24 h, or 0.01–10 ng/ml IL-3 at 37°C for 4 or 24 h before the flow assay. mAb blockade of adhesion molecules (L-selectin, PSGL-1, CD29, and CD18; 10 \( \mu \)g/ml) and chemokine receptors (CCR2, CCR3, and CCR7; 10 \( \mu \)g/ml) was performed at 4°C for 30 min, and blockade of endothelial cell adhesion molecules (P-selectin, E-selectin, 10 \( \mu \)g/ml with F(ab\(^{-1}\))\(^{2}\) Abs to reduce leukocyte Fcγ-receptor binding) was performed at 37°C for 15 min, before the flow assay. To remove terminal cell surface sialic acid residues, basophils were incubated with 16 U/ml neuraminidase (Roche Molecular Biochemicals) for 60 min at 37°C. In some experiments, basophils were treated with 250 ng/ml pertussis toxin for 60 min at 37°C.

**Parallel plate flow assay**

The assembled parallel plate flow assay system consisted of a 35-mm diameter transparent Plexiglass flow chamber (Glycotech) with inlet/outlet ports and vacuum lines attached to a vacuum pump. The inlet and outlet lines were filled with buffer (dPBS with calcium and magnesium + 0.1% BSA) before assembly of the flow chamber and air removed from the system. A plate with confluent first passage HUVECs was placed on the top of the chamber and held in place by vacuum. The assembled flow chamber, together with the lines and 35-mm tissue culture plate (Corning) was placed in a microscope stage holder maintained at 37°C, and cells perfused smoothly through the chamber. Interactions between basophils and endothelial cells were visualized in real time using video microscopy (Olympus) and a CCD camera (Hamamatsu). A single field of view (\( \times 10; 0.97 \) mm\(^2\)) was monitored on a black and white high-resolution Sony monitor for the entire observation period and recorded on a videotape recorder for later analysis. A representative field of view was found, and a 4-min run of cellular interaction was monitored and recorded. After the run, recordings of 30-s duration were made in five different fields of view. Leukocyte trafficking parameters were quantified during offline analysis, examining 1) rolling (number of rolling cells in the first 4 min of run); 2) adhesion (number of adherent cells/mm\(^2\)); and 3) leukocyte rolling velocity (μm/s). Firmly adherent cells were considered as those that remained stationary for at least 10 s at the end of the 5-min run.

In some experiments, a detachment assay was performed to monitor the strength of leukocyte adhesion. In these studies, the flow rate was doubled every 30 s to a maximum of 32 dyn/cm\(^2\). The percentage of adherent cells was quantified as those firmly adherent cells remaining in the \( \times 10 \) field of view at the end of each 30-s period relative to the number at the start of the detachment assay.

**Flow cytometry (FACS)**

Indirect immunofluorescence and flow cytometry was performed as described previously (21) for expression of chemokine receptors (Table I) using a BD Biosciences FACSCaliber flow cytometer.

**Statistics**

Data are expressed as mean ± SEM. Statistical differences were calculated on original values using unpaired \( t \) tests or one-way ANOVA followed by a Bonferroni/Dunn post hoc test for intergroup comparisons. A value of \( p < 0.05 \) was accepted as significant.

**Results**

Initial studies were performed to compare rolling and attachment of basophils and neutrophils over 4-h TNF-α-stimulated and 24-h IL-4-stimulated endothelial cells under a range of shear forces. These pilot experiments, designed based on basophil adhesion data from static assays (16), demonstrated that basophils, like neutrophils and eosinophils, can interact with stimulated endothelial cells at a physiologically relevant shear stress of 1 dyn/cm\(^2\) (Fig. 1).

Leukocyte rolling and adhesion on IL-3-stimulated HUVECs

Perfusion of basophils over untreated HUVECs at 1 dyn/cm\(^2\) displayed few rolling and adherent cells (Fig. 2). HUVECs were then stimulated with increasing concentrations of IL-3. Fig. 2A illustrates that treatment of HUVECs for 24 h with increasing concentrations of IL-3 (0.01–10 ng/ml) significantly increased the number of rolling basophils (from 6 ± 1 to 39 ± 8 cells/mm\(^2\) at 10 ng/ml), together with an increase in adhesion (from

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Basophil accumulation on TNF-α and IL-4-treated HUVECs to a similar degree as neutrophils and eosinophils, respectively. Confluent monolayers of HUVECs were stimulated for 4 h with 5 ng/ml TNF-α (A) or 24 h with 20 ng/ml IL-4 (B). Purified leukocytes (1 × 10⁶/ml) were perfused over the HUVECs for 4 min at a shear stress of 1 dyn/cm². The number of accumulated cells was quantified as described in Materials and Methods. Data are mean ± SEM of n = 3–4 experiments.

FIGURE 1. Basophils accumulate on TNF-α and IL-4-treated HUVECs to a similar degree as neutrophils and eosinophils, respectively. Confluent monolayers of HUVECs were stimulated for 4 h with 5 ng/ml TNF-α (A) or 24 h with 20 ng/ml IL-4 (B). Purified leukocytes (1 × 10⁶/ml) were perfused over the HUVECs for 4 min at a shear stress of 1 dyn/cm². The number of accumulated cells was quantified as described in Materials and Methods. Data are mean ± SEM of n = 3–4 experiments.

4 ± 1 to 45 ± 8 cells/mm²). In addition, the basophil rolling velocity decreased consistently in a concentration-dependent manner (from 43.4 ± 8.4 μm/s for 0.01 ng/ml IL-3 to 20.3 ± 3.0 μm/s for 10 ng/ml IL-3). A detachment assay was performed to evaluate the strength of firm adhesion. Shear stress doublings resulted in a significant reduction in the number of adherent cells, with only ~30% of cells remaining adherent at the end of the detachment assay (Fig. 2B), regardless of the IL-3 concentration tested. Therefore, a submaximal concentration of 0.1 ng/ml IL-3 was used for endothelial stimulation in all subsequent experiments.

Basophil accumulation compared with eosinophils and neutrophils

When neutrophils were perfused over IL-3-stimulated endothelium, there was no significant increase in rolling or accumulation. In contrast, purified eosinophils exhibited an enhanced level of rolling (45 ± 14 cells) but not a significant increase in firm adhesion. These findings suggest that IL-3 treatment of HUVECs induces eosinophil and basophil rolling, with only selective accumulation of basophils (Fig. 3). It also suggests that basophils and eosinophils use different molecules for adhesion to IL-3-activated HUVECs.

Involvement of the IL-3R on HUVECs

Because IL-3 can directly activate basophil adhesion (16), experiments were performed to verify that IL-3 effects observed in this study were directed toward the HUVECs. Preincubation of HUVECs with a CD123 (IL-3R)-blocking Ab 30 min before the addition of IL-3 for 18 h inhibited both basophil adhesion (32.6 ± 10.4% inhibition) and rolling (42.2 ± 16.8% inhibition), indicating that the basophil accumulation on HUVECs was evoked in part through the IL-3R on the HUVECs (Fig. 4). The effect of IL-3 on basophil rolling and adhesion was not due to direct stimulation of basophils with the cytokine itself during the flow assay, because 5-min incubation of HUVECs or basophils with 0.1 ng/ml IL-3 did not induce rolling or adhesion (data not shown).

Role of selectins and integrins in basophil accumulation on IL-3-stimulated HUVECs

Next, the molecules mediating basophil rolling and adhesion on IL-3-treated HUVECs were investigated using Ab blockade. To examine the role of selectins, blocking mAbs to basophil (PSGL-1 and L-selectin) and HUVECs (P-, E-selectin) adhesion molecules were used. Fig. 5A illustrates that treatment of basophils with PSGL-1, or HUVECs with anti-P-selectin Abs, significantly inhibited basophil rolling and adhesion, whereas L-selectin or E-selectin blockade did not result in any alterations in basophil rolling or adhesion. In addition, treatment of basophils with neuraminadase (Fig. 5B) completely abolished basophil rolling and adhesion, indicating the importance of sialylated structures in IL-3-induced basophil-endothelium interaction. In contrast, the blockade of β₂ integrins with anti-CD18 mAbs did not inhibit either basophil rolling or adhesion on IL-3-stimulated HUVECs (Fig. 6). However, treatment of basophils with an anti-CD29 (β₁ integrin) mAb significantly reduced basophil rolling by 56.6 ± 12.5% (Fig. 6; p < 0.05), without affecting resulting accumulation. We further investigated the regulation of basophil rolling and accumulation by β₁ integrins, using a panel of Abs specific for CD49c, -d, -e, and
Incubation of basophils with an Ab against CD49c (\(\alpha_3\) integrin, VLA-3) did not inhibit basophil rolling or accumulation on the IL-3-stimulated endothelium (Fig. 7). However, when basophils were treated with CD49d (\(\alpha_4\), VLA-4), CD49e (\(\alpha_5\), VLA-5), or CD49f (\(\alpha_6\), VLA-6) Abs, both rolling and adhesion of basophils were significantly inhibited. The most pronounced inhibition was noted for CD49d (10\(^{-11}\) g/ml rolling cells and 12\(^{-10}\) g/ml adherent cells). A combination of Abs to CD49d and CD49e did not inhibit rolling and adhesion more than when compared with the individual treatments. However, addition of PSGL-1 blockade to these Abs completely abrogated the rolling and adhesion observed with IL-3 stimulation. This result indicates that CD49d, CD49e, CD49f, and PSGL-1 are all involved in the basophil rolling as well as accumulation on IL-3-stimulated endothelial cells.

Involvement of G protein-coupled chemokine receptors

To further decipher the mechanisms leading to basophil accumulation on IL-3-activated endothelium, basophils were treated with pertussis toxin before the flow perfusion. Pertussis toxin treatment completely inhibited basophil rolling and adhesion to the endothelium (Fig. 8A). However, treatment of eosinophils with pertussis toxin did not significantly inhibit IL-3-mediated rolling or adhesion, inducing a 2.7% change in rolling and a 29.1% increase in adhesion. This indicates that G protein-linked receptors may be involved in this interaction for basophils but not for eosinophils. Fig. 9 illustrates the chemokine receptor profile of basophils, which includes CCR1, CCR2, CCR3, CCR7, CXCR1, and CXCR4 but not CCR4, CCR5, CCR6, CXCR2, CXCR3, or CXCR5. We next proceeded to decipher which chemokine receptors were involved in IL-3-stimulated recruitment. Neutralizing Abs to CCR2 did not inhibit basophil rolling and adhesion under these conditions.

FIGURE 3. Basophils and eosinophils, but not neutrophils, interact with IL-3-treated HUVECs. HUVECs were incubated for 24 h with either vehicle (□) or 0.1 ng/ml IL-3 (■). Basophils, eosinophils, or neutrophils were perfused over the HUVECs in the flow system as described in Fig. 1. The numbers of rolling (A) and adherent (B) cells were quantified as described in Materials and Methods. Data are mean ± SEM of n = 3 for neutrophil and eosinophil experiments and n = 10 for basophil experiments.

FIGURE 4. Inhibition of the IL-3R on endothelial cells reduces basophil adhesion and rolling. Confluent monolayers of HUVECs were stimulated for 24 h with 0.1 ng/ml IL-3 (IL-3 (0.1), with or without a 30-min pretreatment with 5 \(\mu\)g/ml anti-CD123. After the incubation the flow chamber was assembled, and purified basophils (5 \(\times\) 10\(^{7}\)/ml) were perfused over the HUVECs. The number of rolling (clear bars) and adherent (striped bars) cells were quantified offline as described in Materials and Methods. Data are mean ± SEM of n = 3 experiments. §, p < 0.05 vs no Ab conditions.

FIGURE 5. Effect of selectin Abs and neuraminidase treatment on basophil rolling on and adhesion to IL-3-treated HUVECs. HUVECs were treated for 24 h with IL-3 as described in Fig. 4. Basophils were preincubated with (A) either 10 \(\mu\)g/ml anti-PSGL-1 (PL-1) or anti-L-selectin (DREG) Abs for 15 min at 4°C or (B) neuraminidase (1.6 U/ml) for 30 min at 37°C before perfusion over the HUVECs. Alternatively, HUVECs were pretreated for 15 min at 37°C with 5 \(\mu\)g/ml F(ab\(^{-}\))2 mAbs directed against E-selectin or P-selectin. Perfusion in the flow system was then performed as described in Fig. 4. The number of rolling (clear bars) and adherent (filled bars) cells were quantified as described in Materials and Methods. Data are mean ± SEM of five experiments. §, p < 0.05 vs 0.1 ng/ml IL-3-treated cells.
rolling and adhesion, whereas CCR3 inhibition induced a modest inhibition in rolling (39.1 ± 13.2%) while it did not affect adhesion. Inhibition of CCR7 also induced a modest inhibition of basophil rolling (39.8 ± 10.7%) but uniquely also significantly inhibited basophil adhesion by 59.8 ± 5.3% (Fig. 8B).

Discussion
In this study, we used a model of basophil rolling and adhesion to study and optimize basophil accumulation on activated endothelial cells. IL-3, a potent cytokine for basophil priming and activation, was among the cytokines used to stimulate human endothelial cells and postulated to play a role in selective basophil accumulation. The adhesion molecules and chemokine receptors involved in these leukocyte-endothelial interactions were elucidated using Ab interference. This is the first study to show indirect activation of selective basophil adhesion by a cytokine, and could be useful in understanding the accumulation of basophils in allergic disease.

To begin to characterize basophil adhesion, TNF-α was used initially as the endothelial stimulus. Basophils accumulated well on 4-h TNF-α-stimulated endothelial cells, to a similar degree as neutrophils. This suggests that during an acute inflammatory response, basophils can roll, accumulate, and migrate as effectively as neutrophils in response to TNF-α. Similarly, incubation of HUVECs with 20 ng/ml IL-4 induced basophil accumulation similar to eosinophils, consistent with the previous report of Kepley et al. (27). In this study, both peripheral blood basophils and cord blood-derived basophils were shown to use CD49d at different affinities for rolling on IL-4-stimulated HUVECs.

As we wished to identify conditions that were selective for basophil accumulation, we focused our efforts on IL-3, a potent basophil activator, as well as a basophil differentiation factor, important in the proliferation and differentiation of basophils from hemopoietic stem cells. Based on our previous studies on IL-3, which was shown to directly induce basophil activation and adhesion onto resting endothelium under static conditions,
(15), we hypothesized that IL-3 could also activate the endothelium directly to induce basophil accumulation. Indeed, this study demonstrates that IL-3 stimulation of endothelium induced resting basophils and eosinophils, but not neutrophils, to roll and also induced selective basophil adhesion. This indicates that IL-3 not only induces basophil activation and adhesion to resting endothelium, but also induces basophil and eosinophil-selective rolling on activated endothelium, while not affecting neutrophils. This could be particularly interesting in the allergic response, where basophils and eosinophils are selectively recruited to sites of inflammation. In particular, this activation of endothelial cells by IL-3 appears to be a direct effect on the endothelium, rather than the basophil. This was confirmed with basophils treated with IL-3 for 5 min and no rolling or adhesion was seen (data not shown). Similarly, basophils stimulated with IL-3 for 5 min did not roll or adhere on untreated HUVECs. In addition, the endothelial cells were washed before the rolling assay, so any residual IL-3 was removed. Furthermore, pretreatment of endothelial cells with a blocking Ab to the IL-3R reduced the degree of rolling and attachment observed with IL-3 treatment, albeit not to basal levels. The reason for achieving only partial inhibition is unknown but could be the result of a suboptimal concentration of the Ab used, reduced Ab binding with shear, or the efficacy of the Ab blockage. Another possibility is that the CD123 mAb was intact, unlike the F(ab')2 mAbs used to block E- and P-selectin. This may allow CD32-dependent adhesion to endothelium, something we have observed with other intact anti-endothelial mAb (data not shown).

To elucidate the adhesion molecules involved in IL-3-induced rolling and adhesion, characterization using blocking Abs were performed. These blocking Abs have previously been shown to be effective in blocking their respective adhesion molecules in leukocytes, namely eosinophils and platelets, inhibiting rolling and adhesion in static assays (21, 22) as well as similar flow-based rolling systems (23, 28). P-selectin, but not E-selectin or L-selectin, were important in basophil rolling on IL-3-stimulated HUVECs. It has been shown that basophils (both cord blood-derived and peripheral blood) can roll and attach to IL-3-stimulated HUVECs and Chinese hamster ovary (CHO) cells expressing P-selectin and E-selectin (27). Previous studies have demonstrated that IL-3-treated endothelial cells do up-regulate and express P-selectin (29). Inhibition of PSGL-1 activity with a blocking Ab also significantly reduced basophil rolling and adhesion, indicating that ligation with P-selectin is essential for the basophil rolling and adhesion observed.

Further investigation into the adhesion molecules involved in the IL-3-induced rolling and adhesion revealed that blocking β2 integrins did not affect basophil accumulation, whereas inhibition of β1 integrins using a CD29-blocking Ab inhibited basophil rolling. β2 integrins have been recently shown to be important in the transendothelial migration of basophils across IL-1β-stimulated endothelium, whereas β1 integrins play a smaller role in this migration (30). However, it is known that β2 integrins are involved in IL-4-stimulated basophil adhesion. Thus, it may be possible to speculate that the involvement of the β1 or β2 integrin ligands depends on the endothelial stimulus and the expression of either β1 or β2 integrin ligands (VCAM-1, or ICAM-1, respectively). Under static conditions, Abs to CD49d, an α subunit of the β1 integrin, inhibits basophil adhesion to endothelial cells (10). Furthermore, Kepley et al. (27) also demonstrated that cord blood-derived basophils, but not peripheral blood basophils, could roll and adhere on VCAM-1-expressing CHO cells. However, when CD49d (VLA-4) was activated by Mn2+, peripheral blood basophils could adhere on VCAM-1-expressing CHO cells. This is similar in our study, where the inhibition of CD29 (the β subunit) resulted in an inhibition in rolling, but not subsequent adhesion. It may be possible that the α subunit (CD49 a-f) may play a larger role in specific attachment of basophils. Thus, more stringent assessment of the involvement of the α subunit demonstrated that CD49d, CD49e, and CD49f played a major role in basophil rolling and adhesion observed with IL-3 treatment of HUVECs. Inhibition of CD49d, CD49f, and PSGL-1 resulted in a synergistic abolishment of the rolling and adhesion observed. Since IL-3 does not induce VCAM-1, we speculate that IL-3 induces endothelial surface fibronectin and laminin expression, because these are ligands for CD49d and CD49e and CD49f, respectively. This effect is much more pronounced than that of Kepley et al. (27), where P-selectin and VCAM-1 inhibited IL-4-induced attachment to only 50%, whereas inhibition of both P-selectin and E-selectin completely

![Image](http://www.jimmunol.org/Downloadedfrom/226x504to550x742.png)
abolished IL-4-induced attachment. This indicates that integrins play an equal, if not larger, role in the adhesion induced by IL-3 than IL-4.

Pretreatment of basophils with pertussis toxin, an inhibitor for G-protein-coupled receptors, completely inhibited basophil rolling and adhesion. These data strongly implicate G protein-linked receptors in mediating basophil accumulation. The finding that eosinophil rolling was not inhibited by pertussis toxin treatment indicates that a specific G protein-coupled receptor, possibly a chemokine receptor selectively expressed by basophils, mediates these responses. CCR3, the chemokine receptor found on both basophils and eosinophils did not play a major role in IL-3-mediated recruitment, with the addition of the blocking Ab only inducing a slight inhibition of rolling without affecting adhesion. A recent study has shown the importance of CCR3 and its ligands in basophil transendothelial migration across IL-1-stimulated endothelium. The expression of CCR3 on eosinophils may be responsible for the eosinophil rolling, but not adhesion on IL-3-stimulated endothelium. We have shown in this study, similar to a previous study (31), that basophils express CCR1, CCR2, CCR3, CXCR1, and CXCR4. Furthermore, a unique CC receptor found on basophils, but not reported to be expressed on eosinophils or neutrophils, is CCR7. Indeed, this chemokine receptor seems to be involved in the observed recruitment induced by IL-3. This is an interesting and novel observation, because CCR7 is the chemokine receptor involved in lymphocyte trafficking to lymph nodes. Although the inhibition studies demonstrate that CCR7 is involved in IL-3-stimulated recruitment of basophils, more transcription-based inhibition studies such as silencing-interfering RNA or antisense oligonucleotides designed specifically for CCR7 should be performed to verify the role of CCR7 in basophil accumulation in allergic disease. It is interesting to note that a recent study showed that IL-3 stimulation of dendritic cells enhances expression of CCR7 and sensitivity to CCL19, a chemokine that binds to CCR7 (32). CCL19 and CCL21 (another CCR7-binding chemokine) are prominently expressed in the high endothelial venules in lymph nodes, guiding naive lymphocyte and dendritic cell trafficking into the lymph nodes (33), as well as in the systemic endothelium in autoimmune diseases (34). It will be interesting to see whether basophils can respond to CCL19 and CCL21, or whether CCR7 can be up-regulated upon stimulation with IL-3 or other inflammatory cytokines. Furthermore, if basophils can be recruited to the lymph node, it is tempting to speculate that basophils may possibly play a role in Ag presentation or possess other functions in the adaptive immune response.

In conclusion, we have shown that treatment of endothelium with IL-3 can, among granulocytes, induce basophil adhesion. This data also suggest that basophil accumulation on IL-3-stimulated HUVECs is mediated in part by P-selectin and its ligand PSGL-1, whereas the β1 integrins CD49d, CD49e, and CD49f also contribute to this interaction. In addition, a chemokine receptor is also involved in this interaction, namely CCR7. This information could potentially be useful in understanding and altering basophil trafficking and recruitment in vivo.

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Disclosures

The authors have no financial conflict of interest.

References


8. Grayson, M. H., M. Van der Vieren, S. A. Sterbinsky, W. M. Gallant, P. D. Thomas, D. A. Klunk, and B. S. Bochner. 1999. IL-3-stimulated recruitment of basophils, more transcription-based inhibition studies such as silencing-interfering RNA or antisense oligonucleotides designed specifically for CCR7 should be performed to verify the role of CCR7 in basophil accumulation in allergic disease. It is interesting to note that a recent study showed that IL-3 stimulation of dendritic cells enhances expression of CCR7 and sensitivity to CCL19, a chemokine that binds to CCR7 (32). CCL19 and CCL21 (another CCR7-binding chemokine) are prominently expressed in the high endothelial venules in lymph nodes, guiding naive lymphocyte and dendritic cell trafficking into the lymph nodes (33), as well as in the systemic endothelium in autoimmune diseases (34). It will be interesting to see whether basophils can respond to CCL19 and CCL21, or whether CCR7 can be up-regulated upon stimulation with IL-3 or other inflammatory cytokines. Furthermore, if basophils can be recruited to the lymph node, it is tempting to speculate that basophils may possibly play a role in Ag presentation or possess other functions in the adaptive immune response.

In conclusion, we have shown that treatment of endothelium with IL-3 can, among granulocytes, induce basophil adhesion. This data also suggest that basophil accumulation on IL-3-stimulated HUVECs is mediated in part by P-selectin and its ligand PSGL-1, whereas the β1 integrins CD49d, CD49e, and CD49f also contribute to this interaction. In addition, a chemokine receptor is also involved in this interaction, namely CCR7. This information could potentially be useful in understanding and altering basophil trafficking and recruitment in vivo.

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Disclosures

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