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Eosinophil Tissue Recruitment to Sites of Allergic Inflammation in the Lung Is Platelet Endothelial Cell Adhesion Molecule Independent

Marina Miller,* K.-L. Paul Sung,† William A. Muller,‡ Jae Youn Cho,* Mark Roman,* Diego Castaneda,* Jyothi Nayar,* Thomas Condon,§ John Kim,‡ P. Sriramarao,§ and David H. Broide*‡§

Platelet endothelial cell adhesion molecule (PECAM or CD31) is a cell adhesion molecule expressed on circulating leukocytes and endothelial cells that plays an important role in mediating neutrophil and monocyte transendothelial migration in vivo. In this study, we investigated whether eosinophils, like neutrophils and monocytes, utilize PECAM for tissue recruitment to sites of allergic inflammation in vivo. Eosinophils express similar levels of PECAM as neutrophils as assessed by FACS analysis. RT-PCR studies demonstrate that eosinophils like neutrophils express the six extracellular domains of PECAM. Eosinophils exhibit homophilic binding to recombinant PECAM as assessed in a single-cell micropipette adhesion assay able to measure the biophysical strength of adhesion of eosinophils to recombinant PECAM. The strength of eosinophil adhesion to recombinant PECAM is the same as that of neutrophil binding to recombinant PECAM and can be inhibited with an anti-PECAM Ab. Although eosinophils express functional PECAM, anti-PECAM Abs did not inhibit bronchoalveolar lavage eosinophilia, lung eosinophilia, and airway hyperreactivity to methacholine in a mouse model of OVA-induced asthma in vivo. Thus, in contrast to studies that have demonstrated that neutrophil and monocyte tissue recruitment is PECAM dependent, these studies demonstrate that eosinophil tissue recruitment in vivo in this model is PECAM independent. *The Journal of Immunology, 2001, 167: 2292–2297.

Eosinophils are bone marrow-derived leukocytes that circulate through the blood stream and egress from the vasculature at sites of allergic inflammation (1). Eosinophil adhesion and transmigration across endothelium is dependent upon several eosinophil-expressed adhesion receptors which mediate distinct steps in eosinophil adhesion to endothelium. For example, initial eosinophil rolling along endothelium is mediated by eosinophil cell surface receptors including L-selectin (2), P-selectin glycoprotein ligand 1 (3, 4), α4β1 integrins and α4β2 integrins (2, 5), whereas subsequent eosinophil-firm adhesion to endothelium is mediated by eosinophil β1 and β2 integrins (6–9). On the endothelial cell surface P-selectin (3, 4, 6) and VCAM (5) mediate eosinophil rolling, while ICAM-1 (10) and VCAM-1 (5) mediate eosinophil-firm adhesion. In contrast to the prominent recruitment of eosinophils to sites of allergic inflammation, neutrophils are recruited in particular to sites of bacterial infection, suggesting differential regulation of the recruitment of these two circulating leukocyte populations. The selective recruitment of either circulating eosinophils or neutrophils in response to a variety of stimuli suggests a preference for neutrophil as opposed to eosinophil rolling on endothelium (13) and tissue recruitment (14). Differential recruitment of eosinophils compared with neutrophils may also be accounted for by the differential response of eosinophils and neutrophils to chemokine signals released at sites of inflammation (i.e., eotaxin stimulates eosinophil but not neutrophil chemotaxis) (15). The adhesion molecule platelet endothelial cell adhesion molecule (PECAM1 or CD31) (16, 17) is not to play an important role in neutrophil, monocyte, and NK cell transendothelial migration (18–23), whereas its role in eosinophil transendothelial migration in vivo has not been reported. PECAM is a cell adhesion molecule that belongs to the Ig superfamily and is expressed on endothelial cells as well as circulating leukocytes including neutrophils (18–21), monocytes (22), NK cells (23), and eosinophils (24). PECAM is a large gene encoded by 16 exons with 6 extracellular domains (16, 17). The predicted size of the 711 aa PECAM is ~80 kDa, with 9 asparagine-linked glycosylation sites distributed over the molecule (17). The fully processed PECAM molecule is 130 kDa on an immunoblot with carbohydrate residues accounting for ~40% of the molecular mass of PECAM (17). In mice, a homologous molecule exists that shares the physiologic features of human PECAM (25). The homophilic PECAM interaction of neutrophil or monocyte PECAM with endothelial

Departments of *Medicine and ‡Orthopedics and Bioengineering, University of California at San Diego, La Jolla, CA 92093; †Department of Pathology, Weill Medical College of Cornell University, New York, NY 10021; §Division of Vascular Biology, La Jolla Institute for Molecular Medicine, La Jolla, CA 92121; and ¶ISIS Pharmaceuticals, Carlsbad, CA 92008

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2 Address correspondence and reprint requests to Dr. David H. Broide, Division of Allergy and Immunology, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0635. E-mail address: dbroide@ucsd.edu

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PECAM is very important to neutrophil and monocyte transendothelial migration as demonstrated in studies in several different laboratories (18–22). Neutralizing Abs to PECAM inhibit neutrophil and monocyte transendothelial migration by 80% in vitro and in vivo (18–22). The anti-PECAM Abs inhibit tissue recruitment of neutrophils and monocytes in vivo by inhibiting the transmigration of neutrophils and monocytes between endothelial cells. Studies using intravital videomicroscopy have demonstrated that anti-PECAM Abs do not inhibit other steps in neutrophil or monocyte recruitment such as rolling on endothelium or activation-dependent adhesion to endothelium (19). The interaction between neutrophil or monocyte PECAM and endothelial PECAM is a homophilic interaction which may be mediated by interdigitating PECAM molecules from neutrophils or monocytes and endothelial cells forming a zipper which promotes their adhesion (26, 27).

In this study, we have investigated the role of PECAM in eosinophil tissue recruitment following allergen challenge to determine whether the PECAM pathway is also utilized by eosinophils as previously described for neutrophil and monocyte tissue recruitment, or whether the PECAM pathway is selective for neutrophils and monocytes. Using a mouse model of allergen-induced recruitment of eosinophils to the lung, we demonstrate that eosinophil tissue recruitment is PECAM independent, suggesting an important additional difference in the mechanism of leukocyte tissue recruitment utilized by eosinophils as compared with neutrophils and monocytes.

Materials and Methods

Eosinophils and neutrophils

Human. Eosinophils were purified from the peripheral blood of subjects with mild allergic rhinitis as previously described in this laboratory (2, 5) in a protocol approved by the University of California at San Diego human subjects committee. None of the subjects were asthmatic or on any medications at the time of the study. Eosinophils with >95% purity and >95% viability were recovered by negative selection using a magnetic assembly cell separator (MACS, Miltenyi Biotec, Burlingame, CA) and magnetized anti-CD16 Abs (2, 5).

Mouse. Mouse eosinophils were purified from IL-5-transgenic mice kindly provided by Dr. C. Sanderson (Perth, Australia) using a Percoll gradient as previously described in this laboratory (10). FACs analysis of the purified mouse eosinophils (>90% purity, >95% viable) demonstrate that they express L-selectin, express very late Ag 4, and can up-regulate β2 integrin expression in response to PMA stimulation (10).

FACS analysis of PECAM expression

Purified populations of either eosinophils or neutrophils were incubated for 30 min with 10 μg/ml of an anti-PECAM primary Ab. A hamster anti-mouse PECAM IgG1 mAb 2H8 (28) was used for mouse cells, whereas a mouse anti-human PECAM IgG1 (BD PharMingen, San Diego, CA) was used for human studies (29). Species- and isotype-matched Abs were used as controls. The cells were then washed and incubated with a FITC-labeled secondary Ab. PECAM expression was assessed using a FACSCalibur instrument (BD Biosciences, Mountain View, CA) with CellQuest software (San Jose, CA). A minimum of 5000 eosinophils or neutrophils was analyzed for each determination.

RT-PCR analysis of eosinophil, neutrophil, and mouse lung PECAM expression

Total cellular RNA was isolated from mouse eosinophils (purity >95%), mouse neutrophils (purity >95%), and mouse lung (naive and OVA-challenged) using TRIzol reagent (Life Technologies, Gaithersburg, MD) following the manufacturer’s instructions. In brief, the cell pellet was suspended in 500 μl of TRIzol reagent and allowed to stand at room temperature for 5 min for lysis. Chloroform (100 μl) was added to each tube for 15 s and samples were centrifuged at 12,000 rpm for 15 min at 4°C. The clear aqueous supernatant was transferred to a fresh tube and 250 μl of isopropanol was added overnight at 4°C. Samples were centrifuged at 12,000 rpm for 15 min at 4°C. The RNA was washed with 500 μl of 75% ethanol, air dried, and dissolved in diethyl pyrocarbonate-treated water.

For the synthesis of the first-strand cDNA, 1–5 μg of total RNA was mixed with 500 ng of oligonucleotide, 12 μl of sterile distilled water, heated to 70°C for 10 min, and then quick chilled on ice. Four microliters of 5× first-strand buffer, 2 μl of 0.1 M DTT, 0.1 mM dNTP mix were added and incubated at 42°C for 2 min. Two hundred units of SuperScript II (Life Technologies) was added and the mixture was incubated at 42°C for 50 min. The reverse transcriptase reaction was terminated by the incubation of the reaction tubes samples at 70°C for 15 min and the first-strand cDNA was cooled to 4°C. RT-PCR was conducted using mouse PECAM-1-specific primers (PECAM-1561–S, 5'-CGGTGGATGAAAGTTGGTATTG; PECAM-2181–AS, 5'-ACCCGTCTCTTGCTGCCTCTG) (30). These primers span the six extracellular domains of mouse PECAM (30) and were used to determine whether the exons encoding the PECAM codoemts were the same in eosinophils and neutrophils. PCR amplification was conducted with a 50-μl reaction volume consisting of a PCR buffer containing 1.5 mM MgCl2, 10 mM of each dNTP, 50 μM of each primer, and 2 U of Taq DNA polymerase (all reagents were obtained from Life Technologies). The reaction mixture was denatured at 95°C for 30 s, annealed at 50°C for 30 s, and extended at 72°C for 40 s. The cDNA was amplified for 30 cycles, followed by an extension step of 8 min at 72°C to extend the partially amplified products. The PCR products (620 bp) were analyzed by electrophoresis on 1.5% agarose gels and the products were visualized by staining with ethidium bromide. PCR products for eosinophil, neutrophil, or lung genes were normalized to housekeeping genes (L32 for mouse) before PECAM mRNAs were quantified by densitometry.

Single-cell adhesion assay of eosinophils or neutrophils to PECAM

Recombinant PECAM (10 μg/ml; R&D Systems, Minneapolis, MN) was adhered to a coverslip before use of the PECAM-coated coverslip in the single-cell adhesion assay. We have previously studied eosinophil adhesion to several different individual adhesion proteins (i.e., VCAM, CS-1) using this assay (31, 32). In each experiment, we incubated eosinophils with an anti-PECAM Ab (10 μg/ml for 30 min) before incubation of the leukocytes with the PECAM-coated coverslip to demonstrate the specificity of the observed PECAM eosinophil adhesion. The single-cell adhesion assay was performed as previously described (31, 32). In brief, a micropipette with an internal radius (Rp) of 1.6–3.3 μm was manipulated using a hydraulic micromanipulator mounted on the stage of an inverted microscope. The tip of the pipette was positioned in the cell chamber and the wide end of the pipette was connected to a pressure regulation system. Eosinophils (or neutrophils) incubated on the PECAM-coated coverslip were randomly chosen and held at the tip of the pipette by the application of an initial aspiration pressure. The aspiration pressure in the pipette was increased stepwise with increments of 100–500 mdyne/cm2. At each pressure level, the pipette was pulled away gradually by micromanipulation. If the cell was adherent to PECAM, such pulling caused the cell to slip out of the pipette while remaining adherent to PECAM. At a certain pressure level, the cell becomes completely separated from PECAM. The minimum aspiration pressure that leads to the total separation of the eosinophil from PECAM is referred to as the critical separation force. The critical separation force (Fc) is calculated as in Fc = (Pc)(πR2p), where Pc is the critical separation pressure and Rp is the radius of the pipette (31, 32). The adhesion force between an eosinophil (or neutrophil) and PECAM is measured at 1-min intervals by micromanipulation and recorded on video tape.

Mouse model of eosinophilic pulmonary inflammation

Female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were used when they reached 8–10 wk of age. All animal experimental protocols were approved by the University of California at San Diego animal institutional committee. Pulmonary eosinophilia in mice was induced as previously described in this laboratory (33). In brief, BALB/c mice (n = 4 mice per group) were sensitized by s.c. injection of 25 μg of OVA/1 mg of alhydrogel (Aldrich, Milwaukee, WI) in 0.9% sterile saline on days 0, 7, 14, and 25. Nonsensitized mice received 1 mg of alhydrogel in 0.9% saline subcutaneously (control mice received saline only). Twenty-four hours after the final OVA-inhalation challenges, mice were killed by CO2 asphyxiation.
viability and total number of BAL white blood cells were counted with a hemocytometer. Differential leukocyte counts were performed after brief acetone fixation and staining of the BAL cells with May-Grünwald-Giemsa stains. The percentage of eosinophils, neutrophils, and mononuclear cells present on each slide were assessed by counting a minimum of 100 cells in random high-power fields using a light microscope (×40 magnification).

Lung tissue eosinophils
Lung tissues embedded in OCT in 10 × 50 × 50-mm tissue wells were cryosectioned at 10 μm and acetone fixed onto poly-L-lysine-coated slides (33). Total eosinophil numbers were enumerated by detection of eosinophil peroxidase using diaminobenzidine staining and microscopic examination, as described in this laboratory (33). Slides were incubated at room temperature for 1 min in the presence of cyanide buffer (10 mM potassium cyanide, pH 6), rinsed in PBS, and incubated for 10 min with the peroxidase substrate diaminobenzidine (Vector Laboratories, Burlingame, CA). Slides were subsequently washed in PBS, counterstained with hematoxylin, air dried, and examined by light microscopy (×40 magnification). Five random fields were selected and eosinophils were counted (cells staining brown) to determine total eosinophil number per microscope field.

Peripheral blood eosinophils
Blood was collected from the carotid artery. RBC were lysed using a 1:10 solution of 100 mM potassium carbonate:1.5 M ammonium chloride (33). The remaining cells were cyto spun (3 min at 500 rpm) onto microscope slides and air dried. Eosinophil counts were performed as described above.

Determination of airway responsiveness to methacholine (MCh)
Airway responsiveness was assessed on day 31 twenty-four hours after completion of the OVA-inhalation challenges, immediately before the mice were sacrificed using a single chamber whole-body plethysmograph obtained from Buxco (Troy, NY), as previously described (33). In this system, an unrestrained, spontaneously breathing mouse is placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber are recorded. In the plethysmograph, mice were exposed for 3 min to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (Sigma, St. Louis, MO) in PBS using an aerosonic ultrasonic nebulizer (DeVilbiss, Sommerset, PA). After each nebulization, recordings were taken for 3 min. The enhanced pause (Penh) was used to monitor airway responsiveness. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated mice (34). Penh values measured during each 3-min sequence are expressed for each MCh concentration (3–24 mg/ml) (33). The PC200 concentration of MCh is the concentration of MCh that causes a 200% increase in Penh from baseline Penh measurements.

Pretreatment of mice with an anti-PECAM Ab
Abs to PECAM (hamster anti-mouse PECAM Ab 2H8, or species- and isotype-matched IgG Ab) (21) were administered i.p. (100 μg) on days 25 and 29 twenty-four hours before each OVA inhalation. The anti-PECAM Ab administration was timed to occur after BALB/c mice were sensitized to OVA and before the OVA-inhalation Ag challenge. The dose and route of administration of the anti-PECAM Ab were similar to those used in studies to inhibit neutrophil migration in vivo (35).

Statistical analysis
Statistical analysis was performed using Instat software (San Diego, CA). Results are expressed as mean ± SEM unless otherwise indicated. Values of p < 0.05 was considered to be statistically significant.
Results

FACS analysis of eosinophil and neutrophil PECAM expression

FACS analysis revealed that both eosinophils and neutrophils expressed PECAM at approximately equivalent levels (Fig. 1 and 2). This was true both for mouse (Fig. 1. n = 3, p = NS) and human eosinophils (Fig. 2, n = 3, p = NS). The mean fluorescent intensity of PECAM expression by eosinophils (67.3 ± 7.9) and neutrophils (73.4 ± 1.2) was similar, but significantly less than that expressed by mononuclear cells (342.6 ± 122.7, n = 3, p < 0.05).

RT-PCR demonstration of eosinophil PECAM extracellular domain expression

To determine whether eosinophils like neutrophils expressed the standard six extracellular domains of PECAM, we isolated mRNA from mouse eosinophils and neutrophils and converted it to cDNA. PCR studies demonstrate that mouse eosinophils like neutrophils express equivalent levels of all six extracellular domains of PECAM (Fig. 3A, n = 3).

PECAM was expressed constitutively by endothelial cells and resident leukocytes in mouse lung, and the levels of mouse lung PECAM expression did not change significantly following OVA sensitization and OVA-inhalation challenge as assessed by RT-PCR (Fig. 3B). Levels of PECAM expressed in naive mouse lung were significantly greater than levels of PECAM expressed by equivalent concentrations of RNA derived from mouse eosinophils or mouse neutrophils (Fig. 3B). This may explain why levels of PECAM in mouse lung did not change significantly following OVA challenge when eosinophils expressing low levels of PECAM compared with lung were recruited to the lung.

Single-cell adhesion assay of eosinophil or neutrophil binding to recombinant PECAM

We investigated the strength of adhesion of eosinophils or neutrophils to recombinant endothelial PECAM coated on a coverslip using our single-cell adhesion assay. These studies demonstrate that eosinophils bind with similar affinity as neutrophils to endothelial PECAM (eosinophil mean adhesion strength, 0.21 ± 0.06 mdyne/cm²; neutrophil mean adhesion strength, 0.23 ± 0.04 mdyne/cm² (p = NS); Fig. 4). The specificity of the eosinophil-PECAM interaction was demonstrated by studies in which eosinophils incubated with an anti-PECAM Ab exhibited >80% inhibition of binding to recombinant endothelial PECAM (eosinophil mean adhesion strength with anti-PECAM Ab, 0.04 ± 0.01 mdyne vs eosinophil mean adhesion strength, 0.21 ± 0.06 mdyne with control Ab; Fig. 4).

Effect of anti-PECAM Ab on eosinophil lung recruitment

Sensitization and OVA allergen challenge of wild-type mice (n = 3 experiments) induced a significant BAL eosinophilia (31.4 ± 5.7% BAL eosinophils) compared with mice that were not sensitized or challenged with OVA (0.2 ± 0.1% BAL eosinophils, p = 0.001), or compared with mice immunized with OVA and challenged with PBS diluent (0.8 ± 0.2% BAL eosinophils, p = 0.001). Neutrophils comprised <1% of BAL cells preallergen, postallergen, or postdiluent challenge. Mononuclear cells comprised the remainder of the BAL cells. There was no significant difference in pulmonary BAL eosinophilia in OVA-challenged mice that had received anti-PECAM compared with species- and

![Image 3](https://www.jimmunol.org/)

![Image 4](https://www.jimmunol.org/)

![Image 5](https://www.jimmunol.org/)
isotype-matched control Abs before OVA allergen challenge (anti-PECAM Ab 35.4 ± 6.9% BAL eosinophils vs control Ab 31.4 ± 5.7% BAL eosinophils, n = 3, p = NS; Fig. 5).

Similarly, the anti-PECAM Ab did not inhibit the accumulation of lung eosinophils (anti-PECAM Ab, 29.6 ± 3.7 lung eosinophils/high-power field vs control Ab, 21.6 ± 0.5 lung eosinophils/high-power field, n = 3, p = NS).

Effect of anti-PECAM Ab on blood and bone marrow eosinophils

If the anti-PECAM Ab inhibited eosinophil transendothelial migration in vivo, one might note this reflected in an increase in peripheral blood eosinophils following OVA challenge in anti-PECAM Ab-treated mice. The number of peripheral blood eosinophils following OVA challenge were not significantly different in anti-PECAM Ab- vs control Ab-treated mice (9.1 ± 2.1% blood eosinophils vs 9.5 ± 2.6% blood eosinophils, n = 3, p = NS). Similarly, the number of bone marrow eosinophils were not significantly different following OVA challenge in anti-PECAM Ab- vs control Ab-treated mice (12.2 ± 2.0% bone marrow eosinophils vs 11.0 ± 1.4% bone marrow eosinophils, n = 3, p = NS).

Effect of anti-PECAM Ab on airway hyperreactivity to MCh

Airway responsiveness to MCh was significantly increased in mice following OVA sensitization and OVA challenge (Fig. 6). Mice sensitized to OVA without inhalation challenge or mice OVA challenged without sensitization showed minimal change in Penh in response to MCh (data not shown). The anti-PECAM Ab did not inhibit airway hyperresponsiveness to increasing concentrations of MCh (Fig. 6). There was no significant difference in the PC200 in anti-PECAM Ab-treated mice (6.5 ± 1.0 mg/ml MCh) compared with control Ab-treated mice (8.1 ± 3.5 mg/ml MCh, n = 3, p = NS).

Discussion

We have used the same anti-PECAM domain 1 Ab previously shown to inhibit neutrophil and monocyte tissue recruitment in vivo (35) to determine whether these anti-PECAM Abs also inhibit eosinophil tissue recruitment in vivo in a mouse model of asthma. Despite eosinophils expressing similar levels of cell surface PECAM as compared with neutrophils, the anti-PECAM Ab did not inhibit eosinophil tissue recruitment. These studies suggest that eosinophils differ from neutrophils and monocytes in their requirement for PECAM for tissue recruitment to sites of inflammation in vivo. Although eosinophils, neutrophils, and monocytes share several adhesion receptor pathways in mediating their tissue recruitment, the different requirement for the PECAM pathway defines an additional difference to utilization of very late Ag 4/VCAM (eosinophils and monocytes, but not neutrophils) (8, 9), E-selectin (neutrophils, monocytes) (13), and chemotaxotactants (eotaxin attracts eosinophils, but not neutrophils and monocytes) (15) in leukocyte tissue recruitment.

The presence of a PECAM-independent pathway for leukocyte recruitment has been suggested from studies of PECAM-deficient mice (36). PECAM-deficient mice are viable and undergo normal vascular development. Although electron microscopic examination of mesenteric postcapillary venules in PECAM-deficient mice revealed a transient accumulation of neutrophils at the perivascular basement membrane, similar numbers of leukocytes migrate into the peritoneal cavity in both wild-type and PECAM-deficient mice after challenge with IL-1β or thioglycolate (36). In addition, PECAM-deficient mice showed similar levels of neutrophil migration compared with wild-type mice in several different models of inflammation in other vascular beds (34). PECAM-deficient neutrophils exhibit no defect in migration in vitro (34). Leukocyte recruitment in PECAM-deficient mice may be mediated by compensatory induction of normally redundant PECAM-independent pathways in these mutant mice. Similar and/or different PECAM-independent pathways may be mediating eosinophil tissue recruitment in vivo.

Our FACS experiments demonstrated that eosinophils expressed similar levels of PECAM as did neutrophils and that the anti-PECAM Ab used in the mouse experiments in vivo recognized mouse eosinophil PECAM. To determine whether eosinophil PECAM can engage in homophilic binding to PECAM, we used a single-cell adhesion assay able to measure the strength of eosinophil homophilic binding to recombinant PECAM. These studies demonstrated that eosinophils bound with a similar affinity as neutrophils to recombinant PECAM and that the anti-PECAM Ab inhibited eosinophil binding to PECAM in vitro. These PECAM functional studies are consistent with the RT-PCR studies demonstrating that eosinophils and neutrophils both express the six extracellular domains of PECAM. Taken together, these studies suggest that neither the structure of PECAM, level of expression of PECAM, and more importantly function of PECAM differ significantly between eosinophils and neutrophils in vitro. In light of these in vitro studies, the in vivo demonstration that anti-PECAM Abs do not inhibit eosinophil tissue recruitment would not be predicted. To reconcile the in vitro and in vivo observations, one would need to postulate that in vivo eosinophils either utilize a PECAM-independent adhesion pathway (as yet uncharacterized but alluded to in studies of PECAM-deficient mice) and/or that mediators released at sites of allergic inflammation inhibit the eosinophil PECAM interaction with endothelial PECAM requiring eosinophils to utilize the non-PECAM pathway.

In summary, our studies demonstrate that eosinophil tissue recruitment in vivo is PECAM independent and in this regard differs significantly from neutrophil and monocyte tissue recruitment which is PECAM dependent. Neither the structure nor the function of eosinophil PECAM explain the differences in PECAM-dependent tissue recruitment in vivo. The improved characterization of this yet unknown PECAM-independent pathway (?) receptor or nonreceptor mediated) will improve our understanding of eosinophil tissue recruitment.

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References


FIGURE 6. Effect of anti-PECAM Ab on airway hyperreactivity to MCh. There was no significant difference in airway responsiveness to MCh (n = 3 experiments, p = NS) assessed in OVA-challenged mice before sacrifice on day 30 that had received the anti-PECAM compared with control Ab.


