Blockade of CD49d inhibits allergic airway pathologies independent of effects on leukocyte recruitment

MICHAEL T. BORCHERS,1 J. CROSBY,1 S. FARMER,1 J. SYPEK,2 T. ANSAY,1 N. A. LEE,1 AND J. J. LEE1
1Department of Biochemistry and Molecular Biology, Mayo Clinic Scottsdale, Scottsdale, Arizona 85259; and 2Genetics Institute, Incorporated, Cambridge, Massachusetts 02140
Received 29 August 2000; accepted in final form 1 November 2000

Borchers, Michael T., J. Crosby, S. Farmer, J. Sypek, T. Ansay, N. A. Lee, and J. J. Lee. Blockade of CD49d inhibits allergic airway pathologies independent of effects on leukocyte recruitment. Am J Physiol Lung Cell Mol Physiol 280: L813–L821, 2001.—Lymphocyte and/or eosinophil recruitment is dependent on the sequential interactions between adhesion molecules expressed on activated endothelial cells and both leukocyte subtypes. Endothelial P- and E-selectins mediate tethering and rolling of leukocytes through interactions with P-selectin glycoprotein ligand-1 (PSGL-1), and diapedesis subsequently occurs by engagement of endothelial vascular cell adhesion molecule-1 and CD49d (α4-integrins). The anti-inflammatory potential of interfering with these adhesive interactions was assessed with an ovalbumin challenge mouse model of asthma. Administration of a soluble form of PSGL-1 reduced eosinophils (80%) and lymphocytes (50%) in bronchoalveolar lavage fluid without affecting epithelial changes or airway hyperreactivity (AHR). In contrast, although administration of anti-CD49d monoclonal antibodies (PS/2) resulted in similar reductions in eosinophils (75%) and lymphocytes (50%), PS/2 reduced and abolished mucous cell metaplasia and AHR, respectively. Administration of both PSGL-1 and PS/2 had the additive effect of eliminating eosinophils from the airways (96% decrease), with few or no additional reductions (relative to PS/2 administration alone) in lymphocyte recruitment, mucous cell metaplasia, or AHR. These data show that eosinophils and lymphocytes differentially utilize adhesive interactions during recruitment and that the inhibition of AHR is independent of this recruitment.

airway hyperreactivity; α4-integrin; P-selectin glycoprotein ligand-1

ALLERGIC AIRWAY INFLAMMATION is characterized by airway and peribronchial leukocyte accumulation that consists of eosinophils and lymphocytes. It is widely believed that lymphocytes, through the production of Th2 cytokines [i.e., interleukin (IL)-4, -5 and -13], mediate the recruitment and activation of eosinophils (34). In allergic airway diseases such as asthma, eosinophils have been hypothesized to secrete toxic granule proteins and other inflammatory mediators that contribute to the hallmark features of the disease, such as excess mucus production and airway dysfunction (8). Sensitization and aerosol challenge of mice with ovalbumin (Ova) is a common and well-characterized method used to produce phenotypic changes associated with human asthma, including mucous cell metaplasia, airway hyperreactivity (AHR), and accumulation of eosinophils and lymphocytes in the lung (7, 10, 11, 24). The protocols used to generate these phenotypes vary in timing and the number of sensitizations and challenges required to elicit these phenotypes. Although specific studies may be limited because they present data from a single strain of mice under one set of conditions, these studies nonetheless display features of human asthma and thus constitute relevant models of allergic pulmonary inflammation.

The current paradigm of eosinophil-mediated inflammation in response to allergen involves the increased production, maturation, and release of eosinophils from the marrow into the blood and increased adhesion to and migration through the capillary endothelium. The cell-cell interactions that mediate selective eosinophil migration are complex, overlapping processes controlled by families of cytokines, adhesion molecules, and chemoattractants (32). Leukocyte adhesion and migration through the vascular endothelium involve the sequential tethering, rolling, and firm adhesion along the endothelium followed by diapedesis between endothelial cells and directed migration into the tissue (27). The initial binding and subsequent firm adhesion of eosinophils have been shown to be mediated by binding to P-selectin and vascular cell adhesion molecule-1 (VCAM-1) receptors on endothelial cells, respectively (14). Eosinophils bind to these receptors, predominantly through their cell surface ligands P-selectin glycoprotein ligand-1 (PSGL-1) (29) and heterodimeric integrins comprised of the α4-subunit (CD49) (33).

Previous studies with P-selectin-deficient mice have demonstrated an important role for P-selectin in allergic pulmonary inflammation, including tissue eosino-
ligand interactions in a murine model of allergic pulmonary inflammation significantly reduces observed leukocyte recruitment, mucous cell metaplasia, and AHR compared with the disruption of either interaction individually. The data presented demonstrate that intravenous coadministration of a soluble recombinant form of human PSGL-1 (rsPSGL-Ig) (28) and rat anti-mouse CD49d IgG (PS/2) before allergen challenge had the additive effect of eliminating airway and parenchymal eosinophilia, without additional reductions of recruited lymphocytes, compared with either antagonist administered alone. Administration of rsPSGL-Ig had no effect on mucous cell metaplasia or AHR. In contrast, PS/2 significantly reduced mucous cell metaplasia and abolished AHR. Coadministration of these antagonists further inhibited the development of mucous cells along the airways and also abolished AHR. These

Fig. 1. Human recombinant P-selectin glycoprotein ligand (rsPSGL-Ig) and rat anti-mouse CD49d monoclonal antibody (PS/2) equally inhibit ovalbumin (Ova)-induced eosinophil (A) and lymphocyte (B) accumulation in the airway lumen but differentially affect leukocyte trafficking when used in combination. Bronchoalveolar lavage (BAL) was performed 48 h after the last of 3 Ova challenges, and total recovered eosinophils and lymphocytes were enumerated. Values are means ± SE; n = 8–12 mice/group. The administration of PS/2 alone or PS/2 and rsPSGL-Ig together had no effect on leukocyte counts in saline-challenged mice (PS/2 = 0 eosinophils and 1.7 ± 0.28 × 10⁵ lymphocytes; PS/2 plus rsPSGL-Ig = 0 eosinophils and 1.6 ± 0.3 × 10⁵ lymphocytes). *Significantly different from BSA/Ova, P < 0.05. †Significantly different from PS/2/Ova, P < 0.05.

Fig. 2. rsPSGL-Ig and PS/2 inhibition of Ova-induced eosinophil (A) and lymphocyte (B) accumulation in the lung tissue parallels effects observed on BAL fluid accumulations. Collagenase digestion of perfused lungs was performed 48 h after the last challenge, and total recovered eosinophils and lymphocytes were enumerated. Values are means ± SE; n = 8–12 mice/group. The administration of PS/2 alone or PS/2 and rsPSGL-Ig together had no effect on leukocyte counts in saline-challenged mice (PS/2 = 0.20 ± 0.04 × 10⁶ eosinophils and 0.84 ± 0.11 × 10⁷ lymphocytes; PS/2 plus rsPSGL-Ig = 0.20 ± 0.03 × 10⁶ eosinophils and 0.90 ± 0.15 × 10⁷ lymphocytes). *Significantly different from BSA/Ova, P < 0.05. †Significantly different from PS/2/Ova, P < 0.05.
observations suggest that blockade of CD49d reduces allergic pulmonary pathologies through a mechanism(s) independent of its effects on leukocyte recruitment.

MATERIALS AND METHODS

Experimental design. BALB/cJ mice (female, 6–8 wks; Jackson Laboratories, Bar Harbor, ME) were sensitized by an intraperitoneal injection (100 μl) of 20 μg of chicken Ova (Sigma, St. Louis, MO) emulsified in 2 mg of Imject Alum [Al(OH)₃-Mg(OH)₂] (Pierce, Rockford, IL) on days 0 and 14. Mice were subsequently challenged with an aerosol generated from 1% Ova in saline or saline alone for 20 min by ultrasonic nebulization (DeVilbiss, Somerset, PA) on days 24, 25, and 26. Assessments of inflammation and pulmonary pathology, including mucous cell metaplasia and AHR, were made on day 28 (n = 8–12 mice/group). rsPSGL-Ig is a recombinant, chimeric construct containing the 47 NH₂-terminal amino acid sequence of human PSGL-1 fused to a mutated human IgG1 Fc heavy chain fragment (Genetics Institute, Cambridge, MA). The molecule is predominantly dimeric and has been demonstrated to have binding activity to P-selectin in vitro (26). rsPSGL-Ig and/or PS/2 (American Type Culture Collection, Manassas, VA) was administered via the tail vein 3 h before each aerosol challenge. The optimal doses for in vivo neutralization of murine allergic inflammation (rsPSGL-Ig, 100 μg in 0 μl; PS/2, 200 μg in 0 μl) were determined in earlier studies (11, 19, 28). All mice were maintained in microisolator cages housed in a specific pathogen-free animal facility. The sentinel cages within this animal colony were negative for viral antibodies and the presence of known mouse pathogens. Protocols and studies involving animals were conducted in accordance with National Institutes of Health and Mayo Clinic Foundation guidelines.

Assessment of allergic inflammation. Allergic inflammation was assessed on day 28 by enumerating peripheral blood, lung, and bronchoalveolar lavage (BAL) fluid leukocytes. Assessments of blood leukocytes were determined by recovering blood from the tail vein and removing contaminating red blood cells by hypotonic lysis. Total white blood cell counts were quantified with a hemacytometer, and cell

Fig. 3. Localization of tissue eosinophils within compartments of the lung were unaffected by either rsPSGL-Ig or PS/2. Eosinophils were identified in the perivascular and peribronchial regions of the lung by immunohistochemistry with a rabbit polyclonal antiserum against murine eosinophil major basic protein. A: BSA/saline. (agonist pretreatment/experimental provocation). B: BSA/Ova. C: rsPSGL-Ig/saline. D: rsPSGL-Ig/Ova. E: PS/2/Ova. F: rsPSGL-Ig plus PS/2/Ova. Each section is representative of 5 mice/group. Arrows, eosinophils. Bar, 100 μm.
differentials were performed on Wright-stained blood films by counting ≥300 cells. Lungs were lavaged three times with 0.5 ml of Hanks’ balanced salt solution (HBSS; GIBCO BRL, Life Technologies, Gaithersburg, MD) containing 2% FCS. Individual BAL fluid returns were pooled and stored at 4°C until the cells were counted. Total cell counts were determined with a hemacytometer, and cell differentials were performed on Wright-stained cytospin slides (CytoSpin 3, Shandon Scientific, Pittsburgh, PA) by counting ≥300 cells.

Leukocytes within the lung parenchyma were assessed by collagenase digestion of perfused lungs and immunohistochemistry to determine the localization of infiltrating eosinophils. Isolation of lung cells was performed as previously described (10). Briefly, perfused lungs were removed and diced into 100–200-m pieces. Four milliliters of Hanks balanced salt solution (HBSS, Mediatech, Herndon, VA) containing 3% FCS were added per gram of tissue and incubated for 60 min at 37°C in an orbital shaker. The digested lungs were sheared with a 20-gauge needle and filtered through 45- and 20-μm filters. Cells were washed, resuspended in HBSS, and counted with a hemacytometer. Differential cell counts were performed on Wright-stained cytospin slides (≥300 cells). Immunohistochemistry was performed with a rabbit polyclonal antibody against murine major basic protein (15). Murine basic protein antigen-antibody complexes were detected in 4-μm sections of formalin-fixed, paraffin-embedded sections of mouse lungs (n = 5/group) with the Vectastain ABC Elite goat IgG kit (Vector Laboratories, Burlingame, CA). Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 20 min at 25°C. Sections were treated with 3% hydrogen peroxide in methanol for 20 min at 25°C. Sections were treated with 0.05% trypsin in 0.02 M Tris·HCl, pH 7.8) for 30 min at 25°C and washed in PBS (GIBCO BRL) for 15 min. Sections were blocked in 1.0% normal goat serum in PBS with 1% BSA for 30 min at 25°C and subsequently incubated with primary antibody (1:2,000 dilution) for 60 min at 25°C. Antibody-bound slides were washed in PBS-1.0% BSA and incubated (30 min at 25°C) with biotinylated goat anti-rabbit antibody (1:500 dilution). Sections were incubated first with avidin-biotin-peroxidase complex (30 min at 25°C; Vector) and then with diaminobenzidine (3 min at 25°C) for the development of a colored reaction product before being counterstained with methyl green.

Mucous cell metaplasia. Mucous cell development along the airway epithelium was quantified in paraffin-embedded tissue sections (4 μm) stained with periodic acid–Schiff reagent. Parasa golden sections (n = 5 mice/group) were analyzed by bright-field microscopy with an image analysis software program (ImagePro Plus, Media Cybernetics, Silver Spring, MD) to derive an airway mucus index that was reflective of both the amount of mucus per airway and the number of airways affected. The airway mucus index was calculated by summing the ratio of the periodic acid–Schiff-positive epithelial area to the total epithelial area per section and dividing by the number of airways per section.

Measurements of AHR. AHR was determined by inducing airflow obstruction with a methacholine aerosol. Total pulmonary airflow in unrestrained conscious mice was estimated with a whole body plethysmograph (Buxco Electronics, Troy, NY). Pressure differences between a chamber containing the mice and a reference chamber were used to extrapolate minute volume, tidal volume, breathing frequency, and enhanced pause (P<sub>enh</sub>). P<sub>enh</sub> is a dimensionless parameter that is a function of total pulmonary airflow in mice during the respiratory cycle. This parameter closely correlates with airway resistance in BALB/c mice as measured by traditional invasive techniques performed with ventilated mice (10).

Statistical analysis. Data are means ± SE. Statistical analysis was performed on parametric data with t-tests, with differences between means considered significant when P < 0.05.

RESULTS

rsPSGL-Ig and PS/2 administration differentially reduce leukocyte infiltration of the lung. Ova-challenged mice exhibited an increase in both airway eosinophils and lymphocytes recovered in the BAL fluid 48 h after the last challenge (Fig. 1, A and B, respectively). Administration of either rsPSGL-Ig or PS/2 before the Ova challenges resulted in a significant reduction in airway eosinophils (80 and 76%, respectively) recovered in the BAL fluid (Fig. 1A). Moreover, administration of rsPSGL-Ig and PS/2 in combination further reduced eosinophil accumulation (~95% reduction) in the BAL fluid compared with PS/2 alone (Fig. 1B). In contrast, although treatment with either rsPSGL-Ig or PS/2 alone significantly reduced (~50%) the number of lymphocytes in the BAL fluid (Fig. 1B), treatment with both rsPSGL-Ig and PS/2 did not further reduce the number of lymphocytes recovered in the BAL fluid. The administration of either antagonist alone or in combination had no effect on BAL fluid leukocyte counts in saline-challenged mice.

The effects of rsPSGL-Ig and PS/2 on Ova-induced lung inflammation was further examined by analyses of leukocytes recovered from collagenase-digested lungs. These experiments demonstrated that treatment with either rsPSGL-Ig or PS/2 alone significantly inhibited tissue eosinophil accumulation (76 and 70%, respectively), whereas treatment with rsPSGL-Ig in combination with PS/2 further inhibited the number of
eosinophils recruited to the lung (Fig. 2A). Similar to what was observed with BAL fluid lymphocytes, treatment with either rsPSGL-Ig or PS/2 alone significantly reduced the number of lymphocytes in the lung (Fig. 2B). However, treatment with both rsPSGL-Ig and PS/2 did not further attenuate the number of lymphocytes recovered from the lung.

The spatial distribution of eosinophils within the lung was determined by immunohistochemistry of paraffin-embedded sections (Fig. 3). These data demon-
strate that eosinophils were localized predominantly in the peribronchial and perivascular regions of the lung after Ova sensitization and challenge (Fig. 3, A and B). Administration of rsPSGL-Ig and PS/2, alone or in combination, had no effect on mucus production in saline-challenged mice (PS/2 = 0.55 ± 0.20 mucus index and PS/2 plus rsPSGL-Ig = 0.43 ± 0.06 mucus index).*Significantly different from BSA/Ova, P < 0.05.
†Significantly different from PS/2/Ova, P < 0.05.

Peripheral blood eosinophilia is augmented as a consequence of administering rsPSGL-Ig and/or PS/2. Relative to saline control mice, Ova-challenged mice exhibited a significant increase in peripheral blood eosinophils (73 ± 17 vs. 262 ± 25 cells/mm³) 48 h after the last challenge (Fig. 4). Administration of rsPSGL-Ig or PS/2, alone or in combination, inhibited the accumulation of eosinophils in these regions of the lung without any noticeable changes in their relative localization (i.e., eosinophils remained predominantly in peribronchial and perivascular regions).

**DISCUSSION**

The migration of leukocytes from the vasculature into the lung tissue in response to allergen provocation involves tethering, rolling, and firm adhesion to the...
endothelial cell surface. These processes are controlled by cell surface receptor-ligand interactions that include the binding of leukocyte-expressed PSGL-1 with endothelial cell P-selectin and the binding of leukocyte CD49d-containing integrins with VCAM-1 (27). PSGL-1 is a sialomucin consisting of two 120-kDa subunits expressed on the cell surface of most leukocytes, including eosinophils and T cells (18), and is thought to be the principal ligand responsible for eosinophil tethering on endothelial cell-expressed P-selectin (20, 29). The importance of P-selectin in eosinophil and lymphocyte recruitment has been established with P-selectin-deficient mice (5, 7, 9). These studies showed that in Ova-induced models of allergic airway inflammation, the onset and magnitude of leukocyte accumulation in the airways were inhibited in P-selectin-deficient compared with wild-type mice. This has also been demonstrated with P-selectin antibodies in vitro eosinophil adhesion assays (20, 30). CD49d (α4-integrin) is the common subunit of two cell surface heterodimers (α4β1 and α4β7) expressed on leukocytes that mediate cell-cell adhesion to VCAM-1 and cell-matrix adhesion to fibronectin (17). The importance of CD49d expression on eosinophils and lymphocytes and its role in Ova-induced allergic inflammation has previously been established by antibody depletion studies in several species (19, 23, 25). These studies demonstrated that inhibition of CD49d with blocking antibodies inhibited both eosinophil and lymphocyte accumulation in the lung, with a concomitant increase in the circulating levels of these cells.

The incomplete inhibition of pulmonary inflammation commonly observed by blocking either P-selectin binding (tethering) or VCAM-1 binding (adhesion) suggests that there are other mechanisms for tethering and adhesion (i.e., E-selectin, alternate integrin-adhesion molecule interactions) and/or overlapping functions between P-selectin and VCAM-1 (i.e., VCAM-1 can support initial attachment of eosinocytes to the endothelium, and P-selectin is sufficient to mediate some degree of adhesion to the endothelium). Previous evidence demonstrating that initial attachment and subsequent firm adhesion are not independently controlled is extensive. 1) Eosinophil adhesion to nasal polyp endothelium is P-selectin dependent and can occur independent of VCAM-1 (30). Conversely, eosinophils can tether via CD49d to purified VCAM-1 under flow conditions (3), and CD49d was shown to mediate the initial attachment of eosinophils to tumor necrosis factor-α (TNF-α)-activated endothelium under flow conditions (31). 2) Hickey et al. (12) showed that residual eosinophil tethering was completely abolished by CD49d-blocking antibodies in P-selectin-deficient mice. 3) Overlapping and cooperative effects between these two receptors have also been demonstrated in double-knockout P- and E-selectin-deficient mice (13) and in in vitro assays with neutralization antibodies and IL-4-stimulated endothelium (20). Collectively, these experiments do not exclude the contribution of intercellular adhesion molecule (ICAM)/CD18 interactions, also known to mediate adhesion of eosinophils to vascular endothelium (5); however, these studies suggest that interfering with both P-selectin and VCAM-1 effectively eliminates eosinophilia because of the greater specificity toward eosinophils (as opposed to neutrophils) displayed by these two receptors (21). The data presented here (BAL fluid and collagenase digestion of lung tissue) support the idea that P-selectin interactions are required for leukocyte accumulation in allergic airway inflammation and also demonstrate that administration of rsPSGL-Ig is equally effective as a blockade of CD49d. Interestingly, simultaneous inhibition of both interactions effectively eliminated (96% reduction) BAL fluid eosinophilia, likely due to the inability of circulating cells to interact with the endothelium. In contrast, a further inhibition of lymphocytes was not observed after the administration of both antagonists, suggesting that lymphocytes are less dependent on PSGL-1 and CD49d interactions with the vascular endothelium in order to accumulate in the lung during allergic inflammation. In addition to direct effects on relative adhesive interactions, the differential accumulation of eosinophils and lymphocytes may also reflect differences in systemic recirculation (i.e., lymphocytes are able to leave mucosal tissues and return to the circulation, whereas eosinophils cannot recirculate) or in the local expansion of lymphocytes that occurs within the lung parenchyma of allergen-exposed mice (6). Mucous cell metaplasia and AHR are two primary pathological and physiological abnormalities associated with allergic pulmonary inflammation. Our results clearly demonstrate that inhibition of leukocyte recruitment does not correlate with the obviation of these allergic pathophysiologies. Equivalent inhibition of eosinophil and lymphocyte accumulation by blocking distinct receptor-ligand interactions produced different effects on mucous cell development and AHR. Furthermore, only a nominal further decrease in mucous cell development was observed when eosinophilia was abolished with both antagonists in combination. These differential effects on pulmonary pathologies by inhibition of α4-receptor-ligand interactions alone suggest that these interactions mediate a generalized effect on the leukocyte activation state or perhaps mediate specific effects on unique leukocyte subpopulations in the lungs. The role of P-selectin/PSGL-1 appears to be limited to mediating extravasation from the circulation because leukocytes entering the tissue under conditions in which PSGL-1 binding was limited maintained the capacity to become activated, as evidenced by the development of mucous cell metaplasia and AHR. Conversely, the α4-integrin system appears to have dual roles in allergic airway disease. In addition to mediating leukocyte recruitment from the circulation, these data demonstrate that α4-integrin activation is necessary for the development of mucous cell metaplasia and AHR. The mechanism by which and the extent to which CD49d binding mediates activation and/or survival of either eosinophils or lymphocytes remain problematic. However, CD49d binding of extravascular leukocytes to other cells and/or extracellular matrix
components may be required for the functional release of mediators that subsequently lead to AHR. This is supported by data that demonstrate that eosinophils are activated, as assessed by increased survival in vitro, after CD49d binding to fibronectin (4) and by studies (2, 22) suggesting that eosinophil activation as determined by granule protein release rather than the number of cells recruited is associated with the development of bronchial hyperreactivity in sheep and guinea pigs. Furthermore, small-molecule peptide inhibitors of CD49d, which selectively block the fibronectin binding domain without interfering with VCAM binding, effectively inhibited the early- and late-phase airway responses in allergic sheep (1, 16). In addition, Henderson et al. (11) have shown inhibition of Th2 cytokines (IL-4 and IL-5) in the BAL fluid of allergic mice after intranasal administration of CD49d antibody, suggesting that T-cell activation and associated functions depend on CD49d interactions.

The different effects on eosinophil and lymphocyte infiltration observed in our study may also be a consequence of perturbing unique subsets of the heterogeneous lymphocyte populations in the lung. Although the total lymphocyte accumulation is equally reduced after rsPSGL-Ig or PS/2 treatment, one or more subtypes that contribute to the observed pulmonary pathologies may have been preferentially inhibited by PS/2. For example, Nakajima and colleagues (19) demonstrated an equal reduction (~75%) in CD4+ and CD8+ T cells after PS/2 administration in Ova-challenged mice, whereas De Sanctis et al. (7) reported a 40% reduction in CD4+ subsets without a change in CD8+ T cells in Ova-challenged P-selectin-deficient mice. Whether these different responses can be attributed to the selective inhibition of unique T-cell subpopulations remains to be determined.

These data demonstrate that administration of rsPSGL-Ig is as effective as CD49d blockade in reducing eosinophil and lymphocyte accumulation in the lungs of Ova-challenged mice, whereas, in combination, the antagonists completely block eosinophil accumulation without additional effects on the accumulation of lymphocytes. This suggests that leukocyte subtypes capable of utilizing both adhesive interactions nonetheless migrate to the lung via independent mechanisms. Moreover, blockade of these cell adhesion interactions also has different effects on the inhibition of airway mucous cell development and AHR, demonstrating that inhibition of these pathologies occurs independent of leukocyte recruitment. These findings indicate that the extent of activation is critical to the development of mucous cell metaplasia and AHR and may represent alternative therapeutic targets of pulmonary pathologies associated with chronic allergic inflammation.

We acknowledge the technical assistance of the Mayo Clinic Scottsdale Histology Facility (Anita Jennings, Director) and the Mayo Clinic Scottsdale Graphic Arts Department (Mrv Ruona). We also thank Edith Hines and Katie O’Neill for excellent technical assistance and Linda Mardel, research program assistant.

This study was supported by funds from the Mayo Clinic Foundation; National Heart, Lung, and Blood Institute (NHLBI) Grant HL-60793-01S (to N. A. Lee), NHLBI Training Grant HL-07897 (to M. T. Borchers); and an individual National Research Service Award (to J. Crosby).

REFERENCES


