Loss of PECAM-1 Function Impairs Alveolarization*\[3\]

Horace M. DeLisser\[1,1\], Brian P. Helme\[1,1,2\], Gaoyuan Cao\[‡\], Patricia M. Egan\[‡\], Darren Taichman\[‡\], Melane Fehrenbach\[‡\], Aisha Zaman\[‡\], Zheng Cui\[‡\], Gopi S. Mohan\[‡\], H. Scott Baldwin\[‡\], Peter F. Davies\[‡\], and Rashmin C. Savani\[‡\]

From the Pulmonary, Allergy and Critical Care Division, Department of Medicine, Institute for Medicine and Engineering, the Division of Neonatology, Department of Pediatrics, and the Division of Cardiology, Department of Pediatrics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4318

Received for publication, November 1, 2005, and in revised form, December 22, 2005 Published, JBC Papers in Press, December 23, 2005, DOI 10.1074/jbc.M511798200

The final stage of lung development in humans and rodents occurs principally after birth and involves the partitioning of the large primary saccules into smaller air spaces by the inward protrusion of septae derived from the walls of the saccules. Several observations in animal models implicate angiogenesis as critical to this process of alveolarization, but all anti-angiogenic treatments examined to date have resulted in endothelial cell (EC) death. We therefore targeted the function of platelet endothelial cell adhesion molecule, (PECAM-1), an EC surface molecule that promotes EC migration and has been implicated in in vivo angiogenesis. Administration of an anti-PECAM-1 antibody that inhibits EC migration, but not proliferation or survival in vitro, disrupted normal alveolar septation in neonatal rat pups without reducing EC content. Three-dimensional reconstruction of lungs showed that pups treated with a blocking PECAM-1 antibody had remodeling of more proximal branches resulting in large tubular airways. Subsequent studies in PECAM-1-null mice confirmed that the absence of PECAM-1 impaired murine alveolarization, without affecting EC content, proliferation, or survival. Further, cell migration was reduced in lung endothelial cells isolated from these mice. These data suggest that the loss of PECAM-1 function compromises postnatal lung development and provide evidence that inhibition of EC function, in contrast to a loss of viable EC, inhibits alveolarization.

Lung development represents a carefully coordinated process of airway morphogenesis and concurrent vascular development (1). For humans and rodents, the final or alveolar stage of lung development occurs principally following birth and involves the partitioning of the large primary saccules into smaller air spaces by the inward protrusion of septae derived from the walls of the saccules (2, 3). This secondary septation greatly increases alveolar surface area, enabling efficient gas exchange. The septae begin as invaginations from the saccular wall and consist of a core of connective tissue flanked on either side by capillary vessels. In time, there is stretching and thinning of the septae, accompanied by fusion of the septal capillaries. This remodeling ultimately produces alveolar walls composed of endothelia and epithelia with interstitial tissue interposed between the two cell layers. Identifying the mechanisms that underlie the process of alveolarization may help to increase our understanding of lung diseases such as emphysema or bronchopulmonary dysplasia that are characterized by destruction or failure of development of alveoli (4, 5). The promise of alveolarization as a therapeutic target is seen in the studies demonstrating that retinoic acid reverses emphysema and rescues failed septation in relevant animal models (6, 7).

The completion of alveolarization must necessarily involve the expansion of the vasculature (8, 9) a process that, at this stage of lung development, involves the angiogenic sprouting of new vessels from preexisting ones. The importance of the vascular component to alveolar development is demonstrated by the fact that rat alveolarization is inhibited by treatment with anti-angiogenesis agents (e.g. fumagillin, thalidomide, or SU5416, a vascular endothelial growth factor (VEGF) receptor antagonist) (10). However, all of these treatments result in endothelial cell (EC) apoptosis, making the contribution of ECs to alveolarization difficult to interpret. Nevertheless, these data suggest that molecules that regulate EC activity required for angiogenesis are likely to be important for alveolar development.

One of the endothelial-expressed molecules that appears to play a role in angiogenesis, and thus might be important to the process of alveolarization, is platelet endothelial cell adhesion molecule (PECAM-1) (11–13). Several studies have demonstrated that PECAM-1 promotes EC migration (14–16), and treatment with anti-PECAM-1 antibody inhibits vessel formation in rodent models of cytokine or tumor-induced angiogenesis (14, 17, 18). Further, angiogenesis is reduced in PECAM-1-null mice in a model of chronic inflammation (19). Studies were therefore done in rat and mouse neonatal pups to investigate the involvement of PECAM-1 in alveolarization. Using an antibody blocking strategy, we showed that an anti-PECAM-1 antibody that inhibits EC migration, but not EC proliferation or survival in vitro, disrupts normal alveolar septation in neonatal rat pups without reducing EC content. Subsequent studies in PECAM-1-null mice confirmed these initial findings. Specifically, the absence of PECAM-1 impaired murine alveolarization, without affecting EC content, proliferation, or survival. Finally, cell migration by lung ECs isolated from PECAM-1-deficient mice was decreased in comparison with ECs from the lungs of

8 This work was supported by Department of Defense Grant PR043482 (to H. M. D.), National Institutes of Health Grant HL79090 (to H. M. D.), Grants HL62472, HL62868, and HL075930 (to R. C. S.), and Grant NRSA HL10058 (to B. P. H.), and the Philadelphia Veterans Medical Center (to H. M. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article therefore must be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

9 The online version of this article (available at http://www.jbc.org) contains supplemental text, a supplemental figure, and four supplemental movies.

1 Both authors contributed equally to this work.

2 Present address: Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908.

3 Present address: Vanderbilt University Medical Center, Department of Pediatric Cardiology, Vanderbilt University, Nashville, TN 37232.

4 To whom correspondence should be addressed: MBChB, Rm. 416F, Abramson Research Center, Children’s Hospital of Philadelphia, 3516 Civic Center Blvd., Philadelphia, PA 19104-4318. Tel.: 215-590-5507; Fax: 215-590-4267; E-mail: rsavani@mail.med.upenn.edu.

5 The abbreviations used are: VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; EC, endothelial cell; PECAM-1, platelet endothelial cell adhesion molecule; RLMEC, rat lung microvascular EC; H&E, hematoxylin and eosin; mAb, monoclonal antibody; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling; PCNA, proliferating cell nuclear antigen.
PECAM-1 and Alveolarization

EXPERIMENTAL PROCEDURES

Cells and Antibodies—Rat lung microvascular EC (RLMEC) was cultured in MCDB-31 medium (Invitrogen) supplemented with L-glutamine, hydrocortisone, heparin, endothelial growth factor supplement (Discovery Laboratories, Warrington, PA), and 10% serum. Lung endothelial cells were isolated immunomagnetically from wild type and PECAM-null mice using published protocols (20) and were cultured in EGM-2 MV medium from Cambrex Bio Science Walkersville, Inc (Walkersville, MD). The binding of monoclonal antibody (mAb) 37 and mAb 62 to RLMEC was determined by fluorescence-activated cell sorting analysis using previously described procedures (21).

In Vitro Wounding Assay—Wound-induced migration was performed as described previously (14). Twenty-thousand ECs were added to 24-well tissue culture plates and allowed to grow to confluence. Linear defects were then made in the monolayer. The wounded culture was washed with phosphate-buffered saline and then incubated for 24 h in medium (with 1% serum). Using computer-assisted image analysis and the Image-Pro Plus program (Media Cybernetics, Silver Spring, MD) images were obtained immediately after wounding and then 24 h later, and the distance migrated by cells at the wound edge was determined. For each condition, 3–5 wounds were analyzed.

Cell Proliferation Assay—Cells were cultured for 24 h in 96-well plates, and the number of viable cells was determined using a commercially available non-radioactive colorimetric assay according to the manufacturer’s instructions (CellTiter 96 AQ
compass non-radioactive cell proliferation assay, Promega, Madison WI).

In Vitro Cell Death Detection—For the studies of apoptosis, confluent cells were exposed for 5, 24, or 48 h to serum-free medium or complete medium, with or without antibody. Apoptosis was then assessed using the APOPercentage apoptosis assay (Bicolor Ltd., Belfast, Northern Ireland) with the absorbance measured at 530 nm.

Animals—The Institutional Animal Care and Utilization Committees at both The Children’s Hospital of Philadelphia and The University of Pennsylvania School of Medicine approved all animal care procedures. For studies of postnatal alveolarization, rat pups derived from Sprague-Dawley rats (Charles River Breeding Laboratories, North Wilmington, MA) were untreated or injected intraperitoneally with saline, non-immune IgG, or anti-PECAM-1 antibody every other day beginning on day 1. The animals were sacrificed on day 15. Separate cohorts of rat pups were also treated with 0.1 μg of dexamethasone subcutaneously from days 1–14, a treatment known to inhibit alveolarization. PECAM-1-null mice, on a C57/Bl6 background, were the kind gift of Steven Albelda (University of Pennsylvania, Philadelphia, PA). Wild type mice, also on a C57/Bl6 background, were obtained from Taconic (Germantown, NY). Lungs were harvested after inflation of alveolar cells of the non-pleura) (22). Using paraffin tissue sections, in situ cell proliferation was determined by immunohistochemical staining for PCNA, whereas apoptotic cells were identified by TUNEL assay (R&D Systems, Minneapolis, MN). For the PCNA staining of the endothelial cells of the non-capillary vessels, the percentage of total EC that were PCNA-positive was determined for at least 30 vessels for each mouse. For the quantitation of alveolar cell proliferation, the percentage of total alveolar cells that were PCNA-positive on a ×40 field was determined for at least 60 fields for each mouse.

Real-time Reverse Transcription-PCR—For rat Tie-1 quantitative real-time reverse transcription-PCR, relative mRNA expression was assessed using polymerase-activated fluorescent PCR probes providing continuous message quantification during amplification (TaqMan, Applied Biosystems, Foster City, CA). Differences in gene expression were determined by comparing the number of PCR cycles required to achieve a threshold of fluorescent activity above background during the exponential phase of the reaction. Normalization of sample loading was performed by the simultaneous amplification of GAPDH (with its own fluorescent probe) in each well. All reactions were carried out in at least triplicate. Specific primers/probes were synthesized by Applied Biosystem’s oligo synthesis service as follows: Tie1 forward primer, 5’-GCCTTTTTACGCTTTGTTG-3’; reverse primer, 5’-TTCACCGCAGTCAGTGTGA-3’; and probe, (5’-FAM fluorescent-reporter)-AGCTGCTTATCGGAGAATCCGACCT3’; GAPDH and specific primers/probes were purchased from Applied Biosystems. For mouse Tie-1 and β-actin, reverse transcription-PCR was performed using primer pairs obtained from R&D Systems Inc and the Superscript first-strand synthesis system from Invitrogen.

Computer-generated Three-dimensional Reconstructions—Sixty 5-μm-thick H&E tissue sections, taken every 5 μm, were imaged on an Axiophot Zeiss microscope at low magnification using Image software (Scion, Fredrick, MD). Serial images were then imported into SoftWoRx software (Applied Precision, Issaquah, WA) to form three-dimensional images. Polygons were then created that outlined the limits of specific structures, and then each polygon was assigned as a volume object that could be viewed and analyzed in three-dimensions. More details are provided in the supplemental data online.

Statistical Analyses—Differences among groups were analyzed using one-way analysis of variance. Results are presented as means ± S.E. When statistically significant differences were found (p < 0.05), individual comparisons were made using the Bonferroni/Dunn test.

RESULTS

Effect of Anti-PECAM-1 Antibody, mAb 62, on Rat Lung Microvascular Endothelial Cells—Initial studies were done to determine the effects of monoclonal antibodies against human PECAM-1, mAb 37 and mAb 62, on RLMEC function in vitro. These functionally distinct antibodies cross-react with rat PECAM-1 (23), bind to RLMEC (Fig. 1a), and inhibit tumor-induced human angiogenesis (14). However, migration of RLMEC in a wound assay was inhibited by mAb 62 in a dose-dependent manner (data shown for 100 μg/ml) but was unaffected by mAb 37 (Fig. 1b). Further, neither of these antibodies had an effect on RLMEC proliferation and/or survival (Fig. 1, c and d).

mAb 62 Inhibits Postnatal Rat Alveolarization—In rodents, the first 2 weeks after birth is the critical period for alveolarization (1). Therefore, to investigate the potential involvement of PECAM-1 in rat alveolar development, anti-PECAM-1 antibodies were administered every other day to neonatal rat pups from birth to day 14, and lungs were examined...
on day 15 of life. Untreated and normal IgG-treated animals served as controls. By light microscopy of equivalently inflated lungs, control animals, as well as mAb 37-treated animals, showed thinning of the mesenchyme and increasing septal formation with time over the first 2 weeks of life (Fig. 2a; data shown for IgG). In contrast, treatment with mAb 62 resulted in distorted alveolar architecture, with alveolar sacs demonstrating poorly developed and/or incomplete alveoli. Electron microscopy of control lungs showed appropriate alveolar development and normal secondary septation (Fig. 2b). However, mAb 62-treated animals had large saccular structures with invaginations of secondary crests but few complete alveoli, suggesting that appropriate initiation of secondary crest formation had occurred but the process had failed to be completed. Consistent with these morphological observations was the finding that the mean cross-sectional alveolar area (μm²) determined from H&E sections was 50% greater in mAb 62-treated animals when compared with untreated control rats or rats treated with IgG or mAb 37 (Fig. 2c; 333 ± 30 mm² (control) versus 486 ± 84 μm² (mAb 62); p < 0.001, n = 4–8). Further, radial alveolar counts, another index of progressive alveolarization demonstrated significantly fewer intercepts in mAb 62-treated animals (Table 1).

Endothelial Content Is Unaffected by mAb 62—To understand the possible in vivo mechanism of action of mAb 62, Tie1 mRNA levels, as an index of endothelial cell content (21), were measured on day 15 of life using real time reverse transcription-PCR. The expression of this specific endothelial cell marker increased with age in control animals consistent with the formation of new vessels (data not shown). Significantly, in our studies, no differences were noted in any PECAM-1 antibody-treated group (Fig. 2d), indicating that EC content was not affected by these treatments. As expected, animals treated with dexamethasone, a known inhibitor of alveolarization (3), had significantly decreased Tie1 mRNA content on day 15. As EC content was unaffected by mAb 62 and the antibody inhibited in vitro wound-induced migration (Fig. 1b), but not EC proliferation or survival (Fig. 1, c and d), these data suggest that the disruption of alveolarization resulted from an inhibition of PECAM-1-dependent EC function (migration) and not the loss of viable ECs.

Three-dimensional Reconstructions Reveal Additional Effects of mAb 62 on Airway Architecture—Upon closer examination of serial sections, it became evident that some of the enlarged air spaces were seen on multiple sections and appeared contiguous. We therefore used computer-assisted three-dimensional reconstruction of 300-μm lung blocks from untreated controls and mAb 62- and mAb 37-treated animals to further analyze airway morphology (Fig. 3). Objects identified by this process were differentiated using colors, with alveolar structures designated green, blood vessels designated red, normal airways designated gray, and abnormal contiguous air spaces designated yellow.

FIGURE 1. The effect of anti-PECAM-1 antibodies on RLMEC. a, fluorescence-activated cell sorting analysis was performed on RLMEC using mAb 37 and mAb 62 anti-PECAM-1 monoclonal antibodies. b, the closure of linear wounds made in confluent cell monolayers, reflected by the change in wound area (μm²), was determined as a measure of cell migration. Data represent mean ± S.E. (n = 4; *, p < 0.005 when compared with control or IgG). c, cell proliferation of RLMEC cultured in the presence of antibody. Data represent mean ± S.E. (n = 4–8). d, apoptosis of control untreated RLMEC or cells exposed to serum deprivation or antibody at 5, 24, and 48 h. Data represent mean ± S.D. (n = 25–30; *, p < 0.001 when compared with control). Antibody concentrations were 100 μg/ml for the wounding, proliferation, and apoptosis studies.
sections were confirmed to contribute to the three-dimensional picture by comparison of image stacks to the three-dimensional reconstruction (Fig. 3). Both untreated controls and mAb 37-treated animals had normal-sized alveoli and normal airway structures (Fig. 3, a and c). However, mAb 62-treated animals showed that although some alveoli of appropriate sizes were present, larger tubular structures spanned the whole length of the tissue block examined (Fig. 3, b). Incomplete budding from the large tubules as well as abnormal septal formation were noted. These data indicate that even more proximal areas of saccular and alveolar development were affected by mAb 62, suggesting ongoing EC contribution to remodeling of more proximal airways during development. Movies of these reconstructions that allow for better visualization of the structures can be found in the supplemental data on-line.

**TABLE 1**

Radial alveolar counts

Radial alveolar counts were determined for lungs obtained on day 15. Data represent mean ± S.E. (n = 5–8; *, p < 0.05 when compared with control).

| Antibody | Control | IgG | mAb 37 | mAb 62 |
|----------|---------|-----|--------|--------|
| Radial counts | 8.03 ± 0.03 | 7.90 ± 0.12 | 7.83 ± 0.07 | 7.50 ± 0.1* |

**FIGURE 2.** The effect of anti-PECAM-1 antibody on rat alveolarization. a, H&E sections of day 15 lung from IgG- and mAb 62-treated animals. In the IgG-treated animals alveolar sacs with well developed alveoli are present (black asterisks). In contrast, alveolar sacs of the mAb 62-treated animals (red asterisks) are notable for incomplete, poorly developed alveoli (magnification = ×200). AD = alveolar duct. b, electron micrographs of alveoli from IgG- and mAb 62-treated animals obtained on day 15. The arrows indicate arrested septae. Scale bar, 10 μm. c, mean alveolar area expressed as a percentage of control. Data represent mean ± S.E. (n = 4–8; *, p < 0.001 when compared with control). d, tie-1 mRNA content in the lung expressed as a percentage of control normalized to GAPDH. Data represent mean ± S.E. (n = 2–3, *, p < 0.05 when compared with control).
FIGURE 3. Three-dimensional volume projected images of models computed from histological serial sections. In volume rendered models, green objects indicate alveoli, and semitransparent gray objects indicate airways or terminal bronchioles. Scale bar, 100 μm. a, untreated control lung. In this region, near the pleural surface, alveoli (green) had similar size and shape and were arranged around a connecting terminal bronchiole (gray). An arteriole (red) is visible crossing through part of this tissue volume. b, lung treated with mAb62. Although several alveoli (green) were observed, larger “tubule” structures (yellow) traversed a relatively large thickness of tissue. Incomplete septum formation was observed along the walls of these structures, indicating initiation of alveolar formation. c, lung treated with mAb37. Alveoli (green) were observed surrounding airway structures (gray), as in the control lung.

FIGURE 4. Alveolarization in PECAM-1-null mice. a, H&E sections of lungs from wild type and PECAM-1-null mice obtained from day 1, day 7, and 8-week-old animals. The mutant animals show evidence of arrested/delayed alveolarization (magnification ×100). b, the mean area of alveolar spaces determined by computer-assisted image analysis. Data represent mean ± S.E. (n = 4; *, p < 0.001 when compared with wild type). c, radial alveolar counts. Data represent mean ± S.E. (n = 4; *, p < 0.001 when compared with wild type).
Impaired Alveolarization in Mice Deficient in PECAM-1—PECAM-1-null mice are viable (24) and demonstrate an inhibition of angiogenesis (19). Given the finding that antibody inhibition of PECAM-1 disturbs alveolarization in rats, studies were also done to determine whether secondary septation was affected in PECAM-1-deficient mice. Appropriately inflated lungs from wild type and PECAM-1-null mice were examined on day 1 or day 7 and at 8 weeks after birth (Fig. 4a). When compared with wild type mice on day 1, the lungs of knock-out mice were notable for a very disorganized architecture with larger primary alveolar sacs and thickened alveolar walls. On day 7, whereas some secondary septation was noted in mutant mice, the process appeared to be retarded in comparison with wild type mice. By 8 weeks, the differences in alveolarization between wild type and PECAM-1-null mice were smaller but still present. Using computer-assisted image analysis, the mean areas of alveolar spaces were measured to assess alveolarization. We found that immediately after birth (days 1 and 7) and at 8 weeks after birth (Fig. 4a), the mean alveolar areas were significantly greater in PECAM-1-deficient mice when compared with wild type animals (Fig. 4b). Further, at each time point, the radial counts were significantly lower in the PECAM-1-null mice (Fig. 4c). As normal alveolarization is characterized by a decrease in the cross-sectional area of the alveoli and a progressive increase in radial alveolar counts, these quantitative data provide evidence of an impairment of alveolarization in PECAM-1-null mice.

Endothelial Content Is Preserved in PECAM-1-null Mice during Alveolarization—As noted above, mAb 62 inhibited rat postnatal alveolarization without altering EC content. Given this, Tie-1 expression in the lungs of wild type and PECAM-1 knock-out animals was also assessed to determine whether the loss of PECAM-1 affected EC content. Although the absence of PECAM-1 was associated with impaired alveolarization, Tie-1 mRNA levels in wild type and PECAM-1-null mice were comparable on days 1 and 14 after birth (Fig. 5a). This was confirmed at the protein level by immunoblotting (data not shown). Consistent with this was the finding that the proliferation of ECs in small and medium sized vessels (Fig. 5b), as well as that of cells (including the ECs of the microvessels) within the walls of the alveoli (Fig. 5c), were similar in wild type and PECAM-1-null animals. Further, no differences in alveolar cell apoptosis (detected by TUNEL staining) were noted between wild type and PECAM-1-deficient mice (Fig. 5d). However, wound-induced migration by ECs isolated from the lungs of neonatal PECAM-1-null mice was significantly reduced when compared with that of comparable lung endothelial cells obtained from wild type animals (Fig. 5e). Cell proliferation was similar in both cell types (data not shown). These data therefore suggest that functional impairments in EC motility, rather than a loss of viable ECs, may be a mechanism for the disruption of alveolarization in PECAM-1-deficient mice.

DISCUSSION

Studies were performed with rat and mouse neonatal pups to investigate the involvement of PECAM-1 in alveolarization. We found that an anti-PECAM-1 antibody that inhibits EC migration but not prolifer-
PECAM-1 and Alveolarization

ation or survival in vitro disrupts normal alveolar septation in neonatal rat pups without reducing EC content. Further, in PECAM-1-null mice, the absence of PECAM-1 impaired murine alveolarization, without affecting EC content, proliferation, or survival. Additionally, lung ECs from these PECAM-1 mutant mice demonstrated reduced cell migration. Thus, by two complimentary approaches, we showed that the loss of PECAM-1 function disturbs postnatal lung development and implicated the importance of the pulmonary vasculature, and endothelial function in particular, to alveolar development.

In the embryo, the vasculature of the lung develops initially from two separate but concurrent processes (9). Peripherally, the capillary network arises through a process of vasculogenesis in which endothelial precursors, recruited to the pulmonary mesenchyme, differentiate and then organize into isolated sinusoidal spaces. These “blood lakes” undergo subsequent remodeling to yield the connected capillary vascular tree. Centrally, angiogenesis gives rise to the proximal precapillary vasculature through the budding and branching of new vessels from preexisting ones. Subsequent fusion and coalescence of these peripheral and central systems completes the initial establishment of the pulmonary circulation. Near term, and particularly during the period of alveolar septation, there is a significant increase in the density of intrapulmonary vasculature, the result of angiogenesis in these peripheral vessels (8).

The effects of inhibition of angiogenesis on rat neonatal alveolarization was studied previously by Jakkula et al. (10), who reported that administration of three antagonists of angiogenesis (thalidomide, fumagillin, and SU5416, a VEGF-2 inhibitor) to neonatal animals leads to alveolar simplification and attenuated lung vascular development. These studies, although suggestive of the importance of EC to alveolarization, must be interpreted with caution. First, fumagillin and thalidomide may have effects on lung growth independent of their antiangiogenic activities (25–27). Second, VEGF is a known survival factor for endothelial cells (28–30) and also promotes alveolar type 2 cell growth and differentiation (31), effects that are mediated by VEGFR-2 (29). Thus, the inhibition of alveolarization by SU5416 might be due to the loss of viable ECs and/or impaired alveolar epithelial cell maturation and not the direct result of reduced angiogenesis. Lastly, SU5416 blocks platelet-derived growth receptor signaling in addition to that of VEGFR-2 (32). In contrast to these antagonists with potentially multiple and/or undefined effects, mAb 62, an anti-angiogenic reagent (14) that inhibits EC function during alveolarization might initiate signals that regulate lung epithelial differentiation and airway morphogenesis. This possibility is suggested by our finding in rat pups that anti-PECAM-1 antibody treatment had effects on the architecture of the larger, more proximal conducting airways (Fig. 3).

Alveolar abnormalities with vascular pathologies are the hallmark of emphysema in adults (42) and bronchopulmonary dysplasia in preterm infants (43). That the targeting of the vasculature may well be part of the pathogenesis of these diseases is suggested by animal studies. In rats, emphysema occurs with induction of alveolar cell apoptosis, after treatment with a VEGF receptor antagonist (4), and decreased expression of VEGF and its Flt-1 receptor are associated with abnormal development of alveolar capillaries in a baboon model of bronchopulmonary dysplasia (5). Thus, endothelial cell death and/or the loss of functionally intact EC (as suggested by this report) may interfere with the host response to environmental insults or lung injury and in turn contribute to the development of certain forms of parenchymal lung disease.

Acknowledgment—We are grateful to Donald Massaro for the review of the manuscript and helpful comments.

REFERENCES

1. Burri, P. H. (1997) in Lung Growth and Development (McDonald, J. A., ed) pp. 1035, Marcel Dekker, Inc. New York.
2. Massaro, D., and Massaro, G. D. (1986) Am. J. Physiol. 251, R218–R224
3. Massaro, D., and Massaro, G. D. (1986) Am. Rev. Physiol. 58, 73–92
4. Kasahara, Y., Tudor, R. M., Taraseviciene-Stewart, L., 2000) J. Clin. Invest. 106, 751
5. Healy, D. M., and Ewart, T. H. (1981) Science 213, 751
6. H. M. DeLisser, unpublished data.
