Angiopoietin-1 and VEGF in vascular development and angiogenesis in hypoplastic lungs

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Chinoy, Mala R., Megan M. Graybill, Shane A. Miller, C. Max Lang, and Gordon L. Kauffman. Angiopoietin-1 and VEGF in vascular development and angiogenesis in hypoplastic lungs. Am J Physiol Lung Cell Mol Physiol 283: L60–L66, 2002; 10.1152/ajplung.00317.2001.—We hypothesized that exposure of murine fetuses to environmental toxins, such as nitrofen, during early embryogenesis alters vasculogenesis. To address our hypothesis, we assessed protein levels of endothelial cell-selective angiogenic factors: angiopoietin (ANG)-1, vascular endothelial growth factor (VEGF), and mediator of VEGF signaling, VEGF receptor-2 [fetal liver kinase (Flk)-1], a transmembrane receptor tyrosine kinase. VEGF and Flk-1 proteins were lower in hypoplastic lungs from pseudoglandular to alveolar stages than in normal lungs at equivalent developmental time points significant for induction of pulmonary vasculogenesis and angiogenesis. ANG-1 protein was higher in hypoplastic lungs than in normal lungs at all the developmental stages considered in this study, i.e., pseudoglandular, canalicular, saccular, and alveolar stages. We assessed exogenous VEGF-mediated endothelial cell response on extracellular signal-regulated kinase (ERK) 1/2, also referred to as p44/42 mitogen-activated protein kinase. Hypoplastic lungs had more elevated ERK 1/2 protein than normal developing lungs. Exposure to exogenous VEGF activated ERK 1/2 in normal developing lungs but not in hypoplastic lungs. Our results suggest that in hypoplastic lungs: 1) low VEGF signifies negative effects on vasculogenesis/angiogenesis and indicates altered endothelial-mesenchymal interactions; 2) increased ANG-1 protein may be required to maintain vessel integrity and quiescence; and 3) regulation of ERK 1/2 protein is affected in hypoplastic lungs. We speculate that extensive remodeling of blood vessels in hypoplastic lungs may occur to compensate for structurally and functionally defective vasculature.

SEVERAL ANGIOGENIC FACTORS, such as acidic and basic fibroblast growth factor, transforming growth factor-α (TGF-α), TGF-β, vascular endothelial growth factor (VEGF), vascular permeability factor, angiopoietin (ANG)-1, tumor necrosis factor, and platelet-derived growth factor (PDGF) have been identified (13, 14, 18, 21, 25). Members of the VEGF family and the angiopoietins (ANG-1 and ANG-2) specifically act on the endothelial cells. VEGF is a mitogen and angiogenic factor, which was first isolated as an endothelial mitogen factor from pituitary follicular cells (8, 14). During normal embryogenesis, VEGF promotes differentiation and proliferation of endothelial cells and the formation of immature vessels. ANG-1 binds to endothelial cell-specific Tie-2 receptor and stimulates a signal transduction cascade responsible for induction of blood vessel stabilization and maturation (24) and recruits/interacts with periendothelial support cells.

VEGF has two receptors, receptor-1 [fins-like tyrosine kinase (Flt)-1] and receptor-2 [fetal liver kinase (Flk)-1, the kinase domain-containing receptor]. These receptors are regulated by autocrine and paracrine mechanisms. With the use of receptor knockout models, the VEGF receptors have been found to play important roles in regulation of endothelial cell proliferation and vessel formation. The Flt-1 inhibits the excessive proliferation of vascular endothelial cells, whereas Flk-1 allows the proliferation of vascular endothelial cells during mouse embryogenesis (9, 22). Flt-1 knockout models show endothelial cell proliferation and migration, but the cells do not assemble into tubes as functional vessels, whereas Flk-1 knockout models lack endothelial cells and a developing hematopoietic system. VEGF is required to signal to the hematopoietic progenitors to become endothelial cells. The functional significance of the VEGF (ligand), Flt-1 (receptor-1), Flk-1 (receptor-2), ANG-1 (ligand agonist), ANG-2 (ligand antagonist), and Tie-2 (receptor) in vasculogenesis and angiogenesis is summarized in a figure by Hanahan (11).

It is known that the pulmonary vasculature is defective in hypoplastic lungs in human and in animal models of congenital diaphragmatic hernia (CDH). Because endothelial cells line the inner surface of vasculature, which is immediately surrounded by mesenchyme, endothelial-mesenchymal interactions are likely...

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altered, leading to abnormal vascular morphogenesis in hypoplastic lungs. We used our murine model of the human condition of pulmonary hypoplasia and CDH to understand the possible alterations in some of the angiogenic factors. Because of the significance of VEGF and Flk-1 in vasculogenesis and angiogenesis and that of ANG-1 in maintenance of vessel integrity and quiescence, we chose to assess these proteins in developing hypoplastic lungs and compare them with normal lungs of equivalent ages. Mitogen-activated protein kinases (MAPKs), including extracellular signal-related kinase (ERK) 1/2, are activated by a wide variety of extracellular signals such as growth and neurotropic factors, hormones, cytokines, and neurotransmitters. Our data provide some clues to the possible pathways involved in the abnormal formation of pulmonary vasculature in hypoplastic lungs.

CDH is a birth defect observed in human newborns in which the herniation of abdominal organs is observed in the chest cavity through a hole (incomplete formation) of the diaphragm. This condition is accompanied by cardiopulmonary malfunction, diaphragmatic defect, and depending on the severity of the condition, may also involve gastrointestinal anomalies. In the present study, we used a nitrofen-induced murine model of the human newborn condition (7). Nitrofen is an herbicide (2,4,4′-trichloro-2′-chlorophenyl-p-nitrophenyl ether) and an environmental toxicant. In the past 6 yr, research from our laboratory and by others has established that the murine model has several similarities to the human newborn condition, including left-sided pulmonary hypoplasia and CDH, reduced airway branching, cardiac and vascular abnormalities, excessive muscularization of pulmonary vessels, surfactant deficiency, respiratory failures at birth, and several other biochemical similarities (as reviewed in Ref. 7).

We also believe that the murine model is an appropriate model for studying the human condition, because both are results of embryonic insult. It may be of interest to mention that embryological development of lung or the respiratory system has five different stages of development: in mouse, embryonic lung primordium, days 8–9; pseudoglandular lung, days 10–16; canalicular lung, days 16–18; saccular lung, days 18–20; and alveolar stage begins at birth and extends almost 4 wk into the postnatal period. Saccular phase is extended up to 5 days after birth. So, there is a little overlap between sacculare and alveolar stages. The embryonic insult by nitrofen just before the formation of lung primordium results in abnormal lung morphogenesis with structural and vascular defects, suggesting that nitrofen affects cardiopulmonary development.

METHODS

Animals. CD1 time-dated pregnant mice (Charles River Laboratories) were used. Some mice were gavaged with 25 mg of nitrofen in 0.5 ml of olive oil in the latter half of gestational day 8 using previously established methods in our laboratory (7); other mice were left untreated to serve as controls. Previously, we have shown that the mice gavaged with 0.5 ml of olive oil (vehicle alone) were not any different from untreated controls (7). Therefore, in the current experiment we used untreated controls. Both the control and nitrofen-treated dams (mice gavaged with nitrofen early in gestation) were euthanized on gestational days 14, 16, and 19 by halothane overdose while others were allowed to complete gestation. Lungs were harvested from the fetal and neonatal mice and processed for total protein, immunoblotting, and immunohistochemistry. Some normal lungs and some hypoplastic lungs from each stage were processed for assessing VEGF activity or VEGF modulation of ERK 1/2 by detecting p44/42 MAPK protein.

In vitro assay of VEGF-mediated activation of ERK 1/2. Normal and hypoplastic lungs at gestational days 14, 16, and 19, and neonatal stages were individually placed in organ culture dishes in 1 ml of BGJb media with or without recombinant human VEGF (R&D Systems, Minneapolis, MN). VEGF was prepared in 0.5% bovine serum albumin in sterile phosphate-buffered saline, added to each organ culture dish at a concentration of 50 ng/ml, and incubated at 37°C for 15 min. Each lung was then transferred to an appropriately labeled microfuge tube and quick-frozen in nitrofen. Protein assay was performed on these tissues, and immunoblotting was carried out to assess ERK 1/2 activation in the presence of VEGF.

Protein assay and immunoblotting. Total protein content from normal and nitrofen-exposed lungs was determined by Bio-Rad protein microassay (3, 4, 6). Thirty-microgram aliquots of total protein for VEGF and Flk-1 detection and 40-μg aliquots of total protein for ANG-1 from normal and hypoplastic lungs at different developmental stages were separated by SDS-PAGE (12, 6, and 10% gels, respectively) and electroblotted to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), employing the methods published earlier (6). Briefly, the membranes were blocked with 5% nonfat, powdered milk in Tris-buffered saline with TWEEN 20 (TBS-T) and then incubated with ANG-1 (1:200), VEGF (1:300), or Flk-1 (1:200) primary antibody for 3 h. They were washed in TBS-T and further incubated with respective anti-goat or anti-rabbit secondary antibody for 1 h at dilutions of 1:5,000, 1:2,000, or 1:3,000, respectively. Immunoblotting was carried out at room temperature. All primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA), and all secondary antibodies were from Amersham Pharmacia Biotech (Piscataway, NJ). All antibody dilutions were prepared in TBS-T with 2% nonfat, powdered milk.

For detection of antigen-antibody complexes, we used an enhanced chemiluminescence kit (Amersham Pharmacia Biotech), and the membranes were then exposed to X-ray film (Kodak X-OMAT). The signals were normalized using β-actin as an internal control, and a semiquantitative analysis of the signals was done on a 100 Molecular Dynamics densitometer using Protein Data Basis Information software.

Evaluation of ERK 1/2 activation in normal and hypoplastic lungs. Immunoblotting for ERK 1/2 was performed on 30 μg of total protein from each tissue lysate separated on 12% SDS-PAGE. The proteins were electrophoretically transferred as described above. ERK 1/2 primary antibody (Cell Signaling Technology, Beverly, MA) was used at a concentration of 1:1,000 for overnight incubation with immunoblotted membranes at 4°C, and the secondary donkey anti-rabbit antibody (Amersham Pharmacia Biotech) was used at a concentration of 1:4,000 for 1 h at room temperature, where the secondary antibody was prepared in TBS-T with 5% nonfat, powdered milk.

For detection of antigen-antibody complexes, LumiGLO reagent and peroxide were used according to the recommen-
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RESULTS

**Western blot analyses.** ANG-1 protein was the highest at the early developmental stage in hypoplastic lungs, which was persistent up to gestational day 16, and it reduced with increased development but was not significantly different except in the neonatal lungs (Fig. 1). ANG-1 protein essentially showed a plateau in developing normal lungs. Overall, levels of ANG-1 protein were lower in normal lungs throughout development than hypoplastic lungs of equivalent developmental stages. The difference was statistically significant at gestational day 16 (canalicular stage) and gestational day 19 (saccular stage), respectively, the stages that fall into the categories of vascular proliferation and maturation ($P < 0.05$; Fig. 1).

VEGF protein in normal and hypoplastic lungs showed a developmental decrease with a similar trend (Fig. 2). Although VEGF protein levels were lower in the hypoplastic lungs than in the normal lungs during most developmental stages, the differences were statistically significant early in development, i.e., only at pseudoglandular stage (gestational day 14; $P < 0.05$).

Flk-1 protein showed a more significant increase at gestational day 16 (canalicular stage) than the lungs at gestational day 14 (pseudoglandular stage; $P < 0.05$; Fig. 3). Flk-1 at gestational day 19 (saccular stage) was not significantly different than at gestational day 16; however, Flk-1 protein reduced significantly in neonatal lungs (alveolar stage). In hypoplastic lungs, Flk-1 protein showed a similar trend to that of normal lungs of equivalent stages; however, Flk-1 protein was significantly lower in hypoplastic lungs at all four developmental stages studied ($P < 0.05$; Fig. 3).

**VEGF-mediated ERK 1/2 activation assay.** In normal lungs, ERK 1/2 levels were highest at early developmental stages and decreased with increase in age (Fig. 4). In nitrofen-exposed hypoplastic lungs, ERK 1/2 protein was highest at gestational day 14, and a decrease was seen at gestational days 16 and 19; however, neonatal hypoplastic lungs had elevated protein levels comparable to those seen in early stages of development. The difference between normal and hypoplastic lungs was statistically significant at all stages of development of lung (Fig. 4) where the hypoplastic lungs had significantly higher ERK 1/2 protein ($P < 0.05$).

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![Graph showing ANG-1 protein levels](image1)

**Fig. 1.** Angiopoietin (ANG)-1 protein remained at the same level throughout the developmental stages in normal (N) lungs and essentially showed a plateau. In nitrofen-induced hypoplastic lungs (NT), ANG-1 was higher at the early developmental stages up to the canalicular stage and declined with increased age. At gestational days (Gd) 16 and 19, the levels of ANG-1 protein were significantly higher in hypoplastic lungs compared with normal lungs of equivalent age ($P < 0.05$). Neo, neonatal.

![Graph showing VEGF protein levels](image2)

**Fig. 2.** Vascular endothelial growth factor (VEGF) protein was highest at gestational day 16 (canalicular stage) than the lungs at gestational day 14 (pseudoglandular stage). The difference was statistically significant at P < 0.05; Fig. 1).
Normal lungs exposed to exogenous VEGF (VEGF activity assay) showed VEGF-mediated elevation in ERK 1/2 protein at initial stages of development ($P < 0.05$), whereas closer to birth and at birth (neonatal lungs), no alteration was seen in ERK 1/2 protein in the presence of exogenous VEGF. On the other hand, hypoplastic lungs had significantly higher levels of ERK 1/2 protein than normal lungs of equivalent ages ($P < 0.05$). When exposed to exogenous VEGF, the hypoplastic lungs showed no activation of ERK 1/2 protein at any of the developmental stages studied (Fig. 4) but showed a marked decrease in ERK 1/2 protein in neonatal lungs. High levels of ERK 1/2 protein but failure of exogenous VEGF to increase ERK 1/2 suggests disruption of regulation of ERK 1/2 signal transduction pathways and possible effect on downstream pathways in hypoplastic lungs.

**Immunohistochemistry.** ANG-1 was localized in the endothelial cells of both normal and hypoplastic lungs (Fig. 5). However, persistence of ANG-1 protein in the hypoplastic lungs during development was observed compared with normal lungs.

Immunohistochemically, no differences were observed in the localization pattern of VEGF in normal and hypoplastic lungs at any of the developmental stages (data not presented).

**India ink-perfused lungs.** Sections of India ink-perfused lungs at gestational day 16 (canalicular stage) showed predominant differences in vascular development in the peripheral lungs of normal and nitrofen-exposed fetuses (Fig. 6). Overall, the hypoplastic lungs had fewer capillaries, but the most pronounced differences were in the distal lung, with significantly less peripheral capillaries in the hypoplastic lungs (India ink is shown as black staining).

**DISCUSSION**

Development of pulmonary vasculogenesis is not well understood, despite the fact that the endothelial cells are the most abundant cell type in the differentiated lungs. Mechanisms of vessel formation in the developing lung require the coordination of several processes common to all areas of embryonic cardiovascular development (1). Differentiation of endothelial cell precursors, followed by migration and tube formation, creates a complex, highly organized network of vessels. This complicated scheme of vessel formation has been described as occurring by two mechanisms: angiogenesis, the budding and branching of new vessels from preexisting vessels, and vasculogenesis, the de novo organization of blood vessels by differentiation of endothelial cells from mesoderm (16, 17, 19).

VEGF expression pattern in developing lungs indicates its role in lung morphogenesis coinciding with vasculogenesis during early development. Normal lungs show high expression of VEGF at gestational day 14, as endothelial cells proliferate and begin to form vessels. VEGF protein is developmentally downregulated in normal lungs, which may correlate with the shift in developmental activity toward saccularization and surfactant production in preparation of birth.

High expression of VEGF is known to result in high permeability of the vascular endothelial cells (23). In murine hypoplastic lungs, the levels of VEGF protein are downregulated. This suggests that there may be reduced vascular permeability in hypoplastic lungs. We have previously discussed that nitric oxide (NO)/endothelial NO synthase are also reduced in the hypoplastic lungs (5). NO is important for regulation of smooth muscle cell (SMC) proliferation. Together, these two observations suggest not only that NO production is reduced but its diffusion through the endothelial cells of the vasculature may be reduced due to the reduced permeability of the endothelial cells in the hypoplastic lungs. Thus less production and reduced diffusion of NO may also be responsible for excessive proliferation of vascular SMC of the pulmonary vasculature.
lature. Both reduced NO and excessive vascular SMC proliferation are observed in our murine model as well as in the human condition, which may be contributory to the pulmonary hypertension observed in the newborns.

VEGF binding to Flk-1 (VEGF receptor-2) during early embryogenesis sends classic proliferative signals, resulting in generation of endothelial cells. Shalaby et al. (22) demonstrated that mice lacking Flk-1 fail to develop endothelial cells, perhaps owing to lack of differentiation of a common progenitor of the endothelial and hematopoietic lineages, the hemangioblasts. Therefore, the lower levels of VEGF and Flk-1 protein in hypoplastic lungs compared with normal lungs at gestational day 14 (pseudoglandular stage) may suggest reduced endothelial cell production and/or immaturity endothelial cells in hypoplastic lungs during this critical time. The increase in Flk-1 at gestational day 16 in normal and in hypoplastic lungs followed by gradual decline by birth may indicate that the developmental program follows vascular development in hypoplastic lungs. Furthermore, less VEGF protein in developing hypoplastic lungs suggests possible influence on cell-matrix interactions in the absence of growth or survival signals and possible endothelial cell death.

It has been demonstrated that mice lacking Flt-1 and Flk-1 fail to develop endothelial cells or develop endothelial cells that migrate but fail to form tubes and functional vessels, respectively, indicating a role for VEGF in both of these important processes of vasculogenesis (9, 11, 22). Reduced ligand and receptor expression, as seen in hypoplastic lungs, may cause not only the reduced number of endothelial cells but lack of functional vessels during both vasculogenesis and angiogenesis.

VEGF is also described as a permeability factor, and it has ~40% similarity with PDGF. VEGF can be induced by other growth factors via ras-raf-dependent pathway (10). Milanini et al. (15) demonstrated the effect of ERK 1/2 protein, also known as p44/42 MAPK, and constitutively active ras in regulation of VEGF expression. MAPK p44 and 42 (ERK 1/2) function in a protein kinase cascade that plays a critical role in the

Fig. 5. Persistence of ANG-1 protein in the nitrofen-induced hypoplastic lungs during development compared with normal lungs suggested overall developmental delay of hypoplastic lungs. ANG-1 is shown as black staining. Norm, normal lung; Hypo, hypoplastic lung.

Fig. 6. Representative paraffin sections of normal and nitrofen-induced hypoplastic lungs perfused with India ink showed fewer peripheral capillaries in the hypoplastic lung at the canalicular stage (darker staining shows capillaries with India ink).
regulation of cell growth and differentiation. Therefore, the inability of exogenous VEGF to induce an increase in ERK 1/2 protein in hypoplastic lungs at any of the developmental stages suggests that the cell growth and differentiation-related pathways are affected. However, high levels of ERK 1/2 protein in hypoplastic lungs compared with normal lungs at all stages of development in the absence of exogenous VEGF and low levels of endogenous VEGF as well as its receptor Flk-1 in the hypoplastic lung during development suggest that p44/42 is regulated via mechanisms other than VEGF in these lungs. Thus the present study provides evidence that VEGF to ERK 1/2 signaling and, therefore, also the downstream signal transduction pathways, are disrupted in hypoplastic lungs. Because VEGF is a potent mitogen for endothelial cells and since the endothelial cells line the inside of the vasculature, our data support the possible abnormalities in vasculature. VEGF is an inducer of angiogenesis, and lack of ERK 1/2 protein activation to VEGF exposure indicates that the angiogenic pathways are affected in hypoplastic lungs. However, the high levels of ERK 1/2 protein in hypoplastic lungs in the absence of exogenous VEGF also indicate that alternative mechanisms are active in the body to overcome the inhibitory effects on angiogenic activities in hypoplastic lungs. The overall observations of vascular development indicate that these alternative mechanisms are not as effective, and the hypoplastic lungs have poorly developed peripheral capillaries, as seen in Fig. 6. Furthermore, Becker et al. (2) have demonstrated regulation of actin cytoskeletal remodeling by VEGF and MEK. This observation further indicates the implications of disruption of this pathway on endothelial cell permeability, chemotaxis, and cell proliferation.

Clinically, pulmonary hypoplasia is frequently associated with abnormalities of the pulmonary vasculature/circulation. The commonly known conditions are CDH and pulmonary hypoplasia, bronchopulmonary dysplasia, and Down's syndrome (reviewed in Ref. 12). In an elegant study, Jakulla et al. (12) demonstrated that antiangiogenic therapy of postnatal rats during critical lung growth period with thalidomide and fumagillin results in hypoplastic lung formation and affected pulmonary arterial density. They also used a specific inhibitor of Flk-1 to disrupt angiogenesis and demonstrated that it attenuated normal lung development and reduced alveolarization. These studies demonstrate postnatal effects of antiangiogenic pharmacological agents on lung development. Our study demonstrates "embryonic hit" by an environmental toxicant, which results in hypoplastic lung-associated defects because of abnormal vascular development.

In a recent study, Schachtner et al. (20) used the lacZ gene under Flk promoter control to mark endothelial cell differentiation and vessel formation in the lung during murine embryonic development. Their results suggested that vasculogenesis is the mechanism of proximal vessel formation. They quantified the endothelial cell differentiation in the developing mouse lung and showed that vessel development is a continuous process that begins at the earliest stages of development, rather than occurring as a burst at a later developmental stage. Their data suggested that formation of the pulmonary vasculature is vulnerable to perturbation throughout in utero as well as perinatal development.

ANG-1 protein is associated with active recruiting and maintenance of association of periendothelial support cells (pericytes, SMC, myocardocytes) in an effort to solidify and stabilize the newly formed vessels (i.e., to maintain vessel integrity and quiescence) during this active stage of vascular development. Hanahan (11) suggested that ANG-1 action is mediated via Tie-2 receptors, which activate the pathway of maturation of endothelial tubes into elaborate vessel structures involving the above-mentioned multiple cell types.

In hypoplastic lungs, higher levels in ANG-1 were seen at early stages of development, i.e., gestational days 14 and 16; however, in normal lungs levels of ANG-1 protein were much lower and expressed almost the same level throughout the development. ANG-1 protein was higher in hypoplastic lungs during development than lungs of equivalent age; however, at birth there was no difference noted. ANG-1 is also associated with remodeling and sprouting of blood vessels, and it may remain upregulated throughout development of hypoplastic lungs to compensate for the defective vasculogenesis and angiogenesis. These data, along with reduced levels of mediator of vascular endothelial cell proliferation, Flk-1, suggest overall delayed development (delayed proliferation and maturation).

In summary, our results show significantly lower levels of VEGF and its receptor Flk-1 in hypoplastic lungs than in normal lungs of equivalent stages and higher ANG-1 protein in hypoplastic lungs throughout development compared with normal lungs. These observations indicate that ANG-1 may cause leaky vessels, and inhibition of VEGF and its receptor Flk-1 may reduce pulmonary vascular morphogenesis, thus contributing to hypoplastic lung formation. Higher levels of ERK 1/2 protein in hypoplastic lungs compared with normal lungs and inability of exogenous VEGF in our experiment to induce an increase in ERK 1/2 protein in these lungs indicate that ERK 1/2 and downstream pathways of cell growth and differentiation are affected in hypoplastic lungs. Because differentiation involves actin-cytoskeletal remodeling, its attenuation in the absence of ERK 1/2 activation may be speculated in hypoplastic lungs. Thus the present study signifies the abnormalities in VEGF/ANG-1 and related pathways in the developing hypoplastic lungs and provides evidence for structurally and functionally abnormal/defective vasculature in hypoplastic lungs. Furthermore, evaluation of the expression patterns of the downstream genes and proteins in the ERK 1/2 pathway will yield significant information on vascular morphogenesis, which is crucial to the understanding of pulmonary pathogenesis.
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REFERENCES