Vascular endothelial growth factor in the lung

Norbert F. Voelkel, R. William Vandivier, and Rubin M. Tuder

Vascular endothelial growth factor (VEGF) acts as a prototypic growth factor for endothelial cells, but it also has a broad impact on endothelial cell functions. Discovered ~15 years ago, VEGF remains a central cytokine/growth factor for endothelial cells and the focus of intense research during the last 5 years, leading to a brisk pace of discovery of its roles in biology and pathobiology. In addition to VEGF-A, the first member of this family to be discovered (54, 163) due to its vascular permeability and endothelial cell growth functions, the immediate family members include VEGF-B, -C, and -D and placental growth factor (PIGF). A large number of physiological effects of VEGF pertain particularly to the lung, which is one of the organs with the highest expression of VEGF in animal systems. The lung actions of VEGF are overarching since they affect lung development (70, 134) and structural maintenance of the adult lung (192). This review highlights the critical physiological and pathophysiological actions of VEGF-A (hereafter designated as VEGF) in the lung and attempts to reconcile the pathogenetic roles for VEGF in disparate lung diseases as emphysema, pulmonary hypertension, and interstitial lung disease. We propose that effects of VEGF on pulmonary endothelial cells are of particular importance in the pathobiology of lung diseases. To frame the role of VEGF in lung diseases, we review the rapidly expanding literature and highlight its role in lung development, and we postulate that the critical role of VEGF as a pivotal lung structure maintenance factor underlies its role in emphysema, whereas abnormal VEGF and VEGF receptor (VEGFR)-2 signaling is critically involved in severe pulmonary hypertension.

BIOLICAL ACTIVITIES OF VEGF

In the lung, VEGF functions as a mitogen, survival, and differentiation factor for endothelial cells (55). The growth properties of VEGF have been demonstrated in in vitro and in vivo systems. However, the relative contribution of VEGF to endothelial cell growth is organ and context specific, i.e., some endothelial cells respond more robustly to VEGF when cultured under semiconfluent conditions or when they engage in angiogenic activity in tumors or corpus luteum development. Several critical gene products are activated downstream of VEGF (68, 201), which play a contributory role in VEGF-induced angiogenesis and endothelial cell growth. Under specific conditions, particularly those related to tumor vessels, VEGF is a potent permeability factor (163).

Our knowledge of the scope of VEGF’s actions on endothelial cells expanded significantly with the identification of the prosurvival functions of this growth factor in vitro (5, 54, 55, 60) and in seminal studies that explored retinopathy of prematurity and cancer angiogenesis (5, 12).

VEGF profoundly affects several functional properties of endothelial cells, highly relevant to lung function and pulmonary vascular properties, such as nitric oxide (NO) and prostacyclin synthesis. The production of NO and PGI2 leads to vasodilation, as demonstrated in the pulmonary circulation and coronary arteries (117), and systemic hypotension (56). VEGF activates endothelial cell NO synthase (81, 187), which in turn mediates the proangiogenic effects of VEGF (43). NO also mediates the permeability effects of VEGF (62), possibly involving src or Yes kinases (49). The survival properties of VEGF rely on activation of Bcl-2 (69), survivin, inhibitors of apoptosis (50), and vessel morphogenesis, on the activity of the integrin-linked kinase (105), and on serum-response factor (23).

In addition and importantly, several nonendothelial cells also express VEGFRs and bind VEGF (Table 1), which then triggers cell growth and survival. For example, type II pneumocytes undergo growth and differentiation in the presence of VEGF (15, 32). VEGF affects neuronal cells, pancreatic cells, mobilization, and survival of bone marrow progenitor cells (71, 79), and as discussed below, activation of immune cells (25). Interestingly, there is an age-dependent progressive loss of prosurvival effects of VEGF on endothelial and bone marrow progenitor cells (158) that may also impact on aging of the lung.
VEGF or vascular permeability factor or vasculotropin exists in the form of four major splice variants of a single 14-kb gene with eight exons and seven introns on chromosome 6, which encodes the 121-, 165-, 189-, or 206-amino acid forms. The active form of VEGF is a homodimer. The most frequent isoform is the 165-amino acid, which is the most mitogenic of all VEGF isoforms (151). The higher-molecular-weight isoforms 165, 189, and 206 bind to heparin by means of basic arginine residues and are thus retained in the basement membrane (151). The 121 isoform is acidic and easily diffusible.

VEGF is a prototypic member of hypoxia-inducible genes (168). The transcriptional control resides in a key region of the promoter at approximately −930 from the transcriptional start site in a 50-bp region responsive to hypoxia, oncoproteins like c-Myc (140), and growth factor activators (101, 114). This region binds to a heterodimer of hypoxia-inducible factor-1α (HIF-1α) (162) and aryl hydrocarbon nuclear translocator (ARNT or HIF-1β). HIF-1α stability, the rate-limiting step in the activation of the heterodimer transcription factor, relies on reduced proline hydroxylation by decreased proline hydroxylase activity due to low oxygen (8, 162). The lack of proline hydroxylation renders HIF-1α resistant to binding of the von Hippel-Lindau protein and proteosome-mediated degradation (72, 96).

A wide range of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-α and -β, insulin growth factor-I, fibroblast growth factor, and keratinocyte growth factor stimulate VEGF synthesis (2, 53, 102, 156). The fact that platelet-activating factor (PAF) production is stimulated by VEGF (2) illustrates the tight link between inflammation and angiogenesis (98). Estrogens increase VEGF transcription (95, 130) via binding of the estrogen receptor to cognate response elements (142, 209). IL-1β and PDGF increase VEGF mRNA expression (Fig. 1). Five VEGF gene promoter region polymorphisms have been identified (14), and attempts have been made to associate gene polymorphisms with renal transplant rejection (164), Kawasaki disease (107), and with the risk of smoking-related chronic obstructive pulmonary disease (COPD) (160).

PIGF

PIGF is a homolog of VEGF and consists of three isoforms that arise via alternative splicing (PIGF-1, PIGF-2, and PIGF-3). This growth factor signals exclusively through VEGFR-1 (flt) and regulates the cross talk between VEGFR-2 and VEGFR-1 (21, 30, 79). PIGF stimulates vessel formation and maturation in vivo via effects on endothelial cells, monocytes, smooth muscle, or bone marrow mobilization of circulating precursors. Although both VEGF and PIGF bind to VEGFR-1, it appears that PIGF binding to VEGFR-1 enhances VEGF-dependent activation and phosphorylation of VEGFR-2, thus potentiating VEGFR-2-mediated cell signaling. PIGF regulates a number of genes (136) including Fli-1, neuropilin-2, and Egfl1 and activates the serine threonine kinase Akt (21). Of interest, bone morphogenetic protein-2 induces PIGF-1 in mesenchymal stem cells (136), recruits VEGFR-1+ stem cells from the bone marrow (79), and upregulates matrix metalloprotease (MMP)-9 and the release of soluble kit ligand.

VEGFRs

The VEGF family signals through three different receptors (81, 97, 99, 181, 182). VEGF-A binds to VEGFR-1 and VEGFR-2. These receptors interact and modify the biological effects of VEGF either positively or negatively, depending on the specific vascular bed, the experimental condition, and disease state.

Since its discovery by Terman et al. (181), VEGFR-2 or kinase domain receptor (KDR, human receptor) or fetal liver kinase-1 (the rodent ortholog receptor) has accounted for most VEGF effects on endothelial cells, such as cell proliferation, NO and prostacyclin production, angiogenesis, and vascular permeability (81, 182). VEGFR-2 (KDR) was first cloned from a human endothelial cell cDNA library due to its homology to the PDGF receptors, and expression of this receptor was detected in endothelial but not smooth muscle cells (97). The genetic locus of the KDR gene is human chromosome 4 (99). Ligand binding was found to be inhibited by heparinase and restored by addition of heparin (181). VEGFR-2 knockout mice are embryonic lethal, lacking both vasculogenesis and angiogenesis, indicative of a fundamental role of VEGFR-2 in

Table 1. **VEGF actions**

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Endothelium</th>
<th>Permeability (47, 152, 163)</th>
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<tbody>
<tr>
<td></td>
<td>Endothelium</td>
<td>Growth differentiation 9 (5, 43, 174)</td>
</tr>
<tr>
<td>Bone marrow precursor cells</td>
<td>Mobilization (3, 80)</td>
<td></td>
</tr>
<tr>
<td>Vascular smooth muscle cells</td>
<td>Inhibition of apoptosis (50, 70)</td>
<td></td>
</tr>
<tr>
<td>Type II alveolar epithelial cells</td>
<td>Surfactant metabolism (32)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Survival? (50, 140)</td>
<td></td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Maturation (42, 63)</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>Adhesion to endothelial cells (44)</td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>Recruitment (148)</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Chemotaxis (52)</td>
<td></td>
</tr>
</tbody>
</table>

Reference numbers in parentheses.

**Transcriptional control of VEGF gene**

Fig. 1. The complexity of VEGF transcriptional control. Not only is hypoxia involved, but also involved are cytokines, endotoxin, and estrogen. PI3K, phosphatidylinositol 3-kinase; HIF, hypoxia-inducible factor; HHV, human herpesvirus; STAT3, signal transducer and activator of transcription-3; GPCR, G protein-controlled receptor; V-SRC, viral SRC; C-Myc, an oncoprotein; LIF, leukemia inhibitory factor.
vascular biology (165). VEGF ligand binding to VEGFR-2 and cell signaling via the phosphatidylinositol 3-kinase/Akt pathway control endothelial cell survival (200). Activation of endothelial NO synthase via c-Src and phospholipase C γ1 (PLCγ1) and activation of prostacyclin synthase via MAPK lead to increased endothelial cell NO and PGI2 production, respectively (Fig. 2), which may contribute to endothelial cell survival. NO upregulation due to VEGF may also participate in the increased generation and mobilization of endothelial cell progenitor cells (3). The transcriptional control of the VEGFRs is complex. Hypoxia increases KDR (VEGFR-2) gene expression (8, 24, 189) perhaps via generation and action of TNF-α (155).

Nonendothelial cells also express VEGFR-2, including lung type II epithelial cells (51), hematopoietic progenitor cells (22), and cancer cells lines (205).

Waltenberger et al. (200) were the first to show that VEGFR-1 (Flt-1) and VEGFR-2 (KDR) transduce different VEGF-dependent cellular events. VEGFR-1 (Flt-1) is essential for the organization of the embryonic vasculature since embryos of VEGFR-1 knockout mice assemble endothelial cell tubes as abnormal vascular channels and die in utero due to lack of the structural organization of the vessel walls (57). Because VEGFR-1 relays poor growth-promoting signals and is weakly phosphorylated, early investigation of the roles of each VEGFR signaling indicated that VEGFR-1 might act as a silent receptor for VEGF, since its kinase activity is weak, and its downstream signaling is poorly delineated (200). VEGFR-1 might repress most of the endothelial cell effects of VEGF/VEGFR-2 signaling by serving as a decoy for VEGF (21), as documented by the excess endothelial cells in amniotic membrane vessels of embryos knocked out for VEGFR-1 but with intact VEGFR-2 (111). In addition, VEGFR-1, but not VEGFR-2, mediates the increase in monocyte adhesion to VEGF-treated endothelial cells (111) and induces expression of tissue factor by endothelial cells (128).

Recent evidence indicates that VEGFR-1 enhances VEGF-induced VEGFR-2 signaling during angiogenesis in several pathological conditions (21). Mice deficient in PIGF-1, which exclusively binds to VEGF-1, show normal vascular development but have an impaired angiogenic and edematogenic response during ischemia, inflammation, wound healing, and cancer (21). In fact, the combination of PIGF-1 and VEGF, but not PIGF-1 alone, strongly induced capillary sprouting in aortic rings of PIGF-1 mice. There is evidence that VEGFR-1 activation stimulates MMP-9 as mentioned. Metastatic seeding to the lung requires VEGFR-1-dependent activation of MMP-9 in the lung microcirculation. PIGF-1, in a VEGFR-1-dependent manner, enhances mobilization of hematopoietic stem cells during reconstitution of bone marrow after ablation via enhancement of cell motility (as observed with monocytes) and by VEGFR-1-mediated activation of MMP-9, causing release of c-kit (79).

The soluble variant of VEGFR-1 (or s-Flt) adds another layer of complexity to VEGF signaling (11, 89, 112). This soluble variant may decrease the bioavailability of VEGF (89) in pathobiological conditions such as preeclampsia (138).

### VEGF-Induced Cell Signaling

Upon ligand binding, VEGFRs undergo dimerization, assembly of a signaling complex and a signaling cascade ultimately leading to cell-specific effects. VEGF binding to VEGFR-1 leads to phosphorylation of tyrosine residue 1213 (whereas PlGF binding results in phosphorylation of residue 1309) (9). VEGFR-2 activation results in autophosphorylation of several tyrosine residues in the kinase insert domain, followed by binding of proteins containing the Src homology-2 domain with phosphotyrosines. VEGFR-2-triggered cell proliferation involves activation of the Src pathway and association with vascular endothelial cadherin. This association releases phosphorylated β-catenin to translocate to the nucleus and mediates lymphoid-enhancer factor-induced gene transcription. β-Catenin, and therefore the Wnt-pathway, plays a role in angiogenesis, as it increases VEGF gene and protein expression in endothelial cells and the phosphorylation of VEGFR-2 (169). VEGFR-2-mediated activation of the phosphatidylinositol 3-kinase/Akt pathway mediates endothelial cell survival (60), phosphorylates and inactivates caspase-9 (19) and Bad (37), and increases NO production by endothelial cells via NO synthase activation (43). Ligand binding to VEGFR-2 is followed by activation of focal adhesion kinases, p38 MAPK, and paxillin, thus enabling endothelial cell migration (36). Its interaction with src and Yes tyrosine kinases mediate VEGF-induced permeability (47).

### Role of VEGF in Lung Development and Fetal Distress Syndrome

The VEGF signaling pathway has been shown to play a critical role in embryonic vasculogenesis (Gebb S, Tuder RM, Voelkel NF, Abman SH, unpublished observations; 66), particularly during fetal lung development. Vessel development in the early lung determines lung structure maturation, and both undisturbed angiogenesis and vasculogenesis are necessary for
the successful building of the organ (82). The hypoxic environment of the developing lung favors HIF-1α-dependent gene expression (210), and HIF-2α also controls expression of the VEGF164 and VEGF188 isoforms in the developing lung (4).

Overall the roles of VEGF during lung development are likely complex and multilayered, including increased PAF expression and PKC activation. VEGF and VEGFR-2 expression can be demonstrated in branching tubular airways and in vascular mesenchymal cells during fetal development and in vitro in reconstituted cultures containing lung fetal epithelial and mesenchymal elements (66). Furthermore, isolated mesenchymal elements undergo regression and lack of growth with collapse of the branching pulmonary arteries (200). The coordinated building of airway epithelial and endothelial lung cell compartments requires a VEGF gradient, being produced in the tips of the growing airway tubular structures (82). The levels of fetal lung VEGF are of critical importance since lung VEGF overexpression, particularly when targeted to peripheral epithelial cells, causes lung dysmorphogenesis (4, 200), whereas a decrease in lung VEGF as a consequence of neutralization of VEGF or VEGF gene deletion using a Cre-Lox approach resulted in poor septal formation and an emphysematous pattern (70). Expression of the soluble VEGF121 form in the absence of the other isoforms of VEGF equally caused respiratory distress and poor lung development in mice (20). VEGF not only acts as a growth and morphogenetic factor for lung endothelial cells but also acts on type II pneumocytes. Type II cells express VEGF-2 (15, 31, 32), and VEGF enhances type II pneunocyte growth (133), although this effect may be indirect since VEGF165 did not induce cell growth of cultured fetal type II cells nor did it increase its surfactant production (157). Alveolar type II cells express KDR (51), and VEGF increases surfactant protein B and C VEGF-2 dependently (32). Not only does VEGFR inhibition lead to inhibition of angiogenesis and alveolarization in the developing rat lung (100), which persists into adulthood (123), but short-term VEGFR blockade also reduces the number of so-called blood islands and platelet endothelial cell adhesion molecule- and KDR-positive endothelial cells in the fetal rat lung explant preparation (Gebb S, Tuder RM, Voelkel NF, Abman SH, unpublished observations).

Consistent with the critical role of endothelial cells and VEGF during lung development, strategies to disrupt fetal and perinatal VEGF signaling result in respiratory distress and bronchopulmonary dysplasia (13). High doses of dexamethasone suppress VEGF levels (45) and VEGFR-2 expression (31) in the developing lung and cause emphysema in adult rats (27), as VEGF is apparently essential for both endothelial and epithelial cell growth in the lung. VEGF inhibition with a combined VEGFR-1 and VEGFR-2 blocker, SU-5416, led to lung immaturity (100) in rats, which persisted into adult life and caused pulmonary hypertension (123). These observations were confirmed in studies involving HIF-2α+/− mice, which showed defective surfactant production, alveolar sepal vessel deficit, and respiratory distress at birth that can be rescued with VEGF (32).

The increase in fetal lung levels of VEGF, VEGFR-1, and VEGFR-2 continues in the perinatal lung, reaching approximately twofold levels over those at postnatal day 6, and is paralleled in rodents by a concomitant increase in HIF-2α (91). However, during the critical period of perinatal lung adaptation to postnatal life, there is a requirement of normal VEGF lung levels and VEGFR-2 signaling. Overexpression of VEGF caused by tetracycline induction of a conditional VEGF promoter causes lung injury, with emphysematous lesions and hemorrhage (124). We have recently observed that VEGFR-2 neutralization with a monoclonal rat antibody during the first week of life causes alveolar injury with air space enlargement, which is temporary and is followed by recovery within the first month of life.

The realization of the importance of VEGF in fetal lung growth led to a vascular theory of bronchopulmonary dysplasia. Bronchopulmonary dysplasia results from injury to the alveolar cells, leading to respiratory distress (152). Bronchopulmonary dysplasia continues to be an important consequence of lung injury due to prematurity, mechanical ventilation, and hyperoxia treatment for lung distress during perinatal life. Bhatt et al. (13) showed decreased VEGF, VEGFR-1, and Tie-2 expression in lungs from infants dying with bronchopulmonary dysplasia. These findings were later confirmed by Lassus et al. (120), who also described decreased expression of VEGFR-1.

Hyperoxia is one of the main contributors to bronchopulmonary dysplasia. Hyperoxia decreases lung levels of VEGF (135). Kiekamp et al. (115) documented a reduction of VEGF in lungs of rats exposed to hyperoxia, associated with alveolar cell apoptosis and reduction of VEGFR-2 and VEGFR-1 expression. Hyperoxia (≥95% between postnatal days 6 and 14) dramatically reduced VEGF, VEGFR-1, and VEGFR-2 lung expression levels (91). Interestingly, inhibition of VEGFR-2, but not VEGFR-1, during the first week of life recapitulates the alveolar growth arrest seen with hyperoxia. However, in contrast to hyperoxia, these changes are reversible ( McGrath S and Tuder RM, unpublished observations). Hyperoxia may thus lead to persistent lung injury by means of a more widespread alveolar injury, permanently jeopardizing the lung cells ability to synthesize VEGF and/or respond via VEGFR-2.

PULMONARY HYPERTENSION

Pulmonary hypertension is associated with changes in vascular cell size (hypertrophy) and number (hyperplasia) involving all three cell types within the small precapillary pulmonary arteries, endothelial cells, smooth muscle cells, and adventitial fibroblasts. The importance of growth factors in pulmonary hypertension has not been completely elucidated since they may have disparate roles when comparing early vs. late disease and experimental vs. human pulmonary hypertension (208).

Subsets of patients with idiopathic pulmonary hypertension and severe pulmonary hypertension associated with congenital heart malformations, human immunodeficiency virus infection, liver disease, and collagen vascular disease present with abnormal proliferation of endothelial cells, forming plexiform lesions (190, 191, 199). Although germline mutations in bone morphogenetic protein receptor-2 and somatic inactivating mutations of transforming growth factor receptor-2 have been described in the disease (41, 118), angiogenic factors are likely instrumental in the abnormal growth of endothelial cells in pulmonary hypertension. VEGF is strongly expressed in the angioproliferative plexiform lesions in the lungs from patients with severe primary idiopathic and secondary forms of pulmonary hypertension (67, 84, 190), including children with vari-
ous forms of congenital heart diseases (67), persistent pulmonary hypertension of the newborn (121), and infants with pulmonary hypertension in the setting of congenital diaphragmatic hernias (167).

VEGF has been linked as potentially causative to the etiology of polyneuropathy, organomégalie, endocrinopathy, monoclonal gammopathy, and skin changes syndrome (POEMS syndrome)-associated pulmonary hypertension, as based on a case report (147), and in patients with human herpesvirus-8 infection and severe pulmonary hypertension (33). Here, the virus, regional ischemia, and cytokines could be responsible for VEGF overexpression.

In contrast to human angioproliferative disease (206), animal models reflect mostly the milder forms of pulmonary hypertension but allow the investigation of the role of a given growth factor in the initiation and progression of the disease (198). Table 2 lists the published results of pulmonary hypertension animal models where VEGF mRNA and/or protein levels had been measured in the lung or VEGF expression in the lung had been manipulated. Given VEGF-dependent endothelial cell PGI2 and NO production, one might predict that VEGF modulates hypoxic pulmonary vasoconstriction and pulmonary hypertension, a concept that has been verified experimentally. VEGF gene and protein expression is upregulated in the lung tissue after short- and long-term hypoxic exposure of animals (153). There is now a consensus that chronic hypoxia increases lung tissue VEGF expression and that VEGF is likely a modulator of chronic hypoxia-induced pulmonary vascular remodeling (154). In contrast, in the monocrotaline rat model of pulmonary hypertension, VEGF tissue expression appears to be decreased (153). VEGF overexpression protects against hypoxic (154) and monocrotaline-induced pulmonary hypertension (18). Interestingly, VEGF-B, in contrast to VEGF-A, enhances hypoxic pulmonary hypertension since knockouts were protected against elevation of pulmonary artery pressures and pulmonary vessel remodeling when compared with wild-type mice (202). A similar observation has been made with regard to PIGF knockout mice (21). This finding may be related to VEGF-B (like PIGF) binding to VEGFR-1 expressed by smooth muscle cells, inducing MMP-9, smooth muscle cell migration, and blood vessel remodeling (75). However, Louzier et al. (131) reported that VEGF-B knockout mice developed similar hypoxic pulmonary hypertension as wild-type mice and that VEGF-B overexpression protected against pulmonary hypertension in a NO synthase-independent manner.

Other pertinent information regarding the role of VEGF in pulmonary hypertension is found in Table 2 (6, 29, 61, 76, 153, 154, 189, 202).

Interestingly, mice carrying only a single functional copy of the HIF-1α or HIF-2α genes demonstrate impaired hypoxia-induced pulmonary remodeling and diminished pulmonary hypertension (16, 207); HIF-1α−/− animals have pulmonary arterial smooth muscle cells that are electrophysiologically different, indicating that hypoxia sensing and hypoxic vasorelaxation are HIF-1α dependent (207). Whether VEGF levels and signaling via KDR is altered in the lungs of HIF-1α−/− animals is unknown.

Last, we have described a rat model of severe pulmonary hypertension (178) caused by the combination of chronic VEGFR blockade by the small molecule tyrosine kinase inhibitor SU-5416 and chronic alveolar hypoxia. The pulmonary artery mean pressure in these animals is in excess of 60 mmHg, and precapillary arterioles are obliterated by proliferated endothelial cells. Because concomitant treatment of the animals with a broad-spectrum caspase inhibitor prevents the development both of severe pulmonary hypertension and of the vascular lesions (178), we postulate that vigorous endovascular proliferation is the consequence of significant initial endothelial cell apoptosis induced by SU-5416. Initial blockade of VEGFRs and VEGF signal transduction, which are critical for pulmonary endothelial cell survival, might have allowed for the subsequent emergence of an apoptosis-resistant, hyperproliferative vascular cell phenotype (191). Locally increased shear stress may contribute to this abnormal endothelial cell growth.

This model has been used by us to develop novel treatment strategies aimed at the severe angioproliferative component of pulmonary hypertension. Both bradykinin receptor II antagonist and simvastatin have so far been shown as effective treatments of pulmonary hypertension in this SU-5416/chronic hypoxia model (Taraseviene-Stewart L, Sercbavicius R, Choe KH, Cool C, Wood K, Tuder R, Burns N, Kasper M, Voelkel NF, unpublished observations). Of interest, the reduction by these agents in pulmonary artery pressure in this model was associated with partial reversal of the oblitative pulmonary artery lesions due to endothelial cell apoptosis (Taraseviene-Stewart L, Sercbavicius R, Choe KH, Cool C, Wood K, Tuder R, Burns N, Kasper M, Voelkel NF, unpublished observations).

Table 2. VEGF and pulmonary hypertension

<table>
<thead>
<tr>
<th>Pulmonary Hypertension Model</th>
<th>Animal Species</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Hypoxia</td>
<td>Rat (lung)</td>
<td>↑ VEGF mRNA protein</td>
<td>(153,189)</td>
</tr>
<tr>
<td></td>
<td>Rat (lung)</td>
<td>↑ KDR mRNA protein</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Rat adenovirus-VEGF overexpression</td>
<td>↑ VEGF mRNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mice VEGF-β-deficient</td>
<td>Pulmonary hypertension</td>
<td>(154)</td>
</tr>
<tr>
<td>Monocrotaline</td>
<td>Rat (lung)</td>
<td>Pulmonary hypertension</td>
<td>(202)</td>
</tr>
<tr>
<td></td>
<td>Rat (heart)</td>
<td>VEGF mRNA</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>Rat VEGF-transfected syngeneic smooth muscle cells</td>
<td>Prevention of progression of pulmonary hypertension</td>
<td>(18)</td>
</tr>
<tr>
<td>TFN-α overexpression</td>
<td>Mice</td>
<td>↓ VEGF mRNA</td>
<td>(61)</td>
</tr>
<tr>
<td>Intrauterine ductus arteriosus ligation</td>
<td>Sheep (late gestation)</td>
<td>↑ Pulmonary hypertension</td>
<td>(76)</td>
</tr>
</tbody>
</table>
LUNG INJURY

Acute lung injury and its more severe form, acute respiratory distress syndrome (ARDS), involve a disruption of the alveolar-capillary membranes, with local inflammation ultimately leading to alveolar flooding with serum proteins and edema fluid (28, 139). Infiltrating inflammatory cells such as monocytes and local matrix degradation might be sources of VEGF that can increase alveolar capillary permeability. Patients with ARDS showed increased plasma VEGF and increased production by peripheral blood mononuclear cells when compared with patients at risk, normal individuals, and ventilated patients (183). The increased plasma VEGF from ARDS patients might have mediated the increase in lung permeability since it accounted for 50% of the permeability activity of ARDS plasma on cultured endothelial cells. In contrast, lungs of patients with sepsis, also a trigger of acute lung injury, have significantly lower levels of VEGF isoforms 121 and 165 and VEGFR-2 (194). Because the latter study was performed with autopsied lungs, it is difficult to interpret whether the reduction in VEGF expression was present in vivo and whether it is part of the pathogenesis of septic shock.

EMPHYSEMA

Inflammation and protease/antiprotease imbalance are the concepts used to explain the pathogenesis of cigarette smoke-induced emphysema (166). However, these concepts have so far failed to explain the unique nature of alveolar septal destruction in emphysema and why other inflammatory lung diseases, for example, lobar pneumonia, do not cause destructive air space enlargement. The novel concept of disruption of a lung cellular and molecular maintenance program to explain emphysema was developed based on the finding that a decrease of VEGF and VEGFR-2 expression in the lung tissue was associated with emphysema and the presence of a large number of apoptotic alveolar cells (109). Subsequently, these studies were followed by several independent investigations confirming VEGF reduction in more severe forms of emphysema (104, 116). Kasahara et al. (108) showed that chronic inhibition of VEGF or VEGFR-1 were tested; both inhibited efferocytosis. To determine whether VEGF or its receptors influence efferocytosis, macrophages were treated with the broad VEGFR inhibitor SU-5416. In these experiments, SU-5416 inhibited efferocytosis of apoptotic Jurkat T cells by human monocyte-derived macrophages in a dose-dependent manner. Because macrophages synthesize VEGF, which may act in an autocrine fashion to stimulate VEGFR-1, neutralizing antibodies against VEGF and VEGFR-1 were tested; both inhibited efferocytosis. In vivo, SU-5416 also inhibited clearance of apoptotic thymocytes after intratracheal administration in mice. Therefore, VEGF may perform a dual role in the lung by regulating both apoptosis and efferocytosis, such that disruption of VEGF signaling may dysregulate lung homeostasis and contribute to the pathogenesis of emphysema.

Alterations of VEGF lung tissue expression or VEGF signaling have been demonstrated in two rat models of emphysema. Decreased lung tissue VEGF protein expression can be shown in the new model of autoimmune emphysema (179), and altered Akt phosphorylation occurs in lungs of rats with steroid-induced emphysema (27). Whether VEGFR blockade...
or VEGF depletion results in generation of reactive oxidants in the endothelial cells is unclear, as is the temporal sequence of apoptosis, oxidant stress, and activation of matrix metalloproteinases (188). However, there is experimental evidence that VEGF upregulates MgSOD (1) and that apoptosis results in oxidative stress (113). In the steroid emphysema model, MMP-9 is overexpressed and VEGF signaling is impaired in the lung (27).

LPS has been shown to induce emphysema in rats (172), and, interestingly, LPS induces apoptosis of human lung microvascular cells, which can be inhibited by VEGF (143).

Finally, overexpression of PIGF in mice has been shown to be associated with significant emphysema (186). PI GF is abundantly expressed in lung tissue, and it regulates the cross talk between VEGFR-2 and VEGFR-1. PI GF overexpression in these mice was associated with air space enlargement at 6 mo of age; VEGF mRNA was decreased in the lungs, and apoptotic events were frequent in type II cells (186).

ASThma

Because “asthma” is functionally defined as reversible airway obstruction associated with a variety of trigger factors, including exercise and aspirin sensitivity, this airway hyperactivity in genetically susceptible individuals has focused most of the interest on inflammation and immune responses in the bronchi. Several groups have in recent years measured VEGF protein levels in bronchoalveolar lavage fluid from patients with asthma or examined VEGF and VEGFR expression in bronchial biopsy specimens. Demoloy et al. (40) reasoned that VEGF “is a multifunctional cytokine which plays a role in chronic inflammation” and Hoshino et al. (92, 93) showed increased VEGF expression in CD34 cells, eosinophils, and macrophages. Asai et al. (7) showed increased VEGF sputum levels in asthmatic patients. Lee et al. (126) demonstrated that VEGF contributed to airway hyperreactivity in a murine model of toluene diisocyanate-induced asthma, and Kanazawa et al. (103) invoked a role of VEGF in exercise-induced asthma. It has been shown that VEGF causes VEGFR-1-dependent eosinophil chemotaxis (7) and that mast cells produce VEGF; T lymphocytes possess both VEGFR-1 and VEGFR-2 (25, 103), and T helper 2 (Th2) cytokines (IL-4, IL-5, IL-13) enhance VEGF production in airway smooth muscle cells (34). Indeed, VEGF could play an important role in asthma, causing hypervascularity of the airways (93) and mucosal edema; after all, VEGF is a potent permeability factor. Interestingly, β-adrenergic agonists increase the transcription of VEGF mRNA (58).

It is tempting to speculate that VEGF may play a role in the immune response in asthma (Th2 response and VEGF overexpression) and also in COPD/emphysema (Th1 response and decreased VEGF expression and impaired VEGFR signaling). If so, then asthma is associated with angiogenesis (93) and emphysema with impaired vessel maintenance (199). A “VEGF-centric” vascular hypothesis of chronic airway diseases would transcend current concepts of the pathobiology of asthma and COPD, introducing a link between VEGF and T cells, hypervascularity (angiogenesis), Th2 predominant chronic inflammation on one side, and emphysema, loss of alveolar septal capillaries, and a Th1 response on the other side of a spectrum of presentations.

LUNG CANCER

VEGF is overexposed in malignant tumors (10), including glioblastoma multiforme, and in lung cancer (110, 146). Recently, it has been appreciated that VEGF-C and VEGFR-3 are expressed in gastric and lung cancer tissues and that these proteins may be important as drivers of tumor lymphangiogenesis (150). Kaya and associates (110) reported that high serum VEGF levels in patients with lung cancer are associated with a poor prognosis. However, a meta-analysis by Nieder et al. (146) failed to confirm this finding, yet lung cancer macrophage VEGF-C expression correlated with the prognosis, and high tumor vascularity was associated with high VEGF and E-cadherin expression and low tumor cell differentiation (170). VGA1102, a novel VEGFR antagonist, has been shown to inhibit the growth of LC-6 human non-small cell lung cancer cells (193), and likewise AEE788, a combined EGF and VEGFR antagonist, inhibited VEGF-induced angiogenesis in tumor-bearing mice (185). Tumor-infiltrating T cells express VEGF (59, 132), suggesting that they can play a role in tumor vessel growth, and, in addition to chronic lymphocytic leukemia cells, autocrine VEGF may, via STAT proteins, enhance the resistance of these cells to apoptosis (50, 127).

SYNOPSIS

In the adult lung, VEGF is homeostatic, part of the “lung structure maintenance program,” which, to play its pleiotropic regulatory roles, must be at the right place in the right amount (174). Too much VEGF production and action may lead to pleural effusion (26) and contribute to the increased vascular permeability (159) in acute lung injury (106, 144).

Whereas VEGF as well as VEGFR-1 and VEGFR-2 proteins are highly overexpressed in the fibromyxoid lesions in bronchiolitis obliterans (119), Cosgrove et al. (35) showed lack of vasculature in the myofibroblastic foci associated with lack of VEGF expression in idiopathic pulmonary fibrosis, and administration of VEGF to immunosuppressed allografts improved the rate and density of allograft reepithelialization (74). This pleiotrophic angiogenesis and endothelial cell survival factor is of critical importance for lung development and postnatal lung tissue maturation (15, 39, 134) and plays a role in the pathogenesis of COPD/emphysema, acute lung injury, asthma, severe angio proliferative pulmonary hypertension, and in lung cancer. New data (Gebb S, Tuder RM, Voelkel NF, Abman SH, unpublished observations) demonstrated arrested branching morphogenesis in the rat fetal lung explant preparation within 24 h after addition of the combined VEGFR-1 and VEGFR-2 antagonist SU-5416; interestingly, not only was there a loss of vasculature, as shown by loss of eNOS staining, but a dose-dependent reduction in VEGFR-2 mRNA, shown by in situ hybridization and disappearance of VEGFR-2 (KDR) protein by Western blotting (Gebb S, Tuder RM, Voelkel NF, Abman SH, unpublished observations). This study not only illustrates the developmentally important role of VEGF but also the complex relationship between VEGFR signaling and receptor expression.

In addition to the control of angiogenesis and tumor angiogenesis, it is now becoming clear that VEGFRs are involved in macrophage functions, for example, in the phagocytic uptake of apoptotic cells (83), and also that VEGF and VEGFRs may play a major role in immune surveillance and immune modu-
litation (125). Lack of VEGFR signals causes loss of vasculature, even in skeletal muscles (175), whereas hyperactive VEGF signaling causes angioproliferation (86). However, as VEGF antagonists inhibit endothelial cell growth tissue dependently, paradoxically, induction of initial endothelial cell apoptosis may be followed by the evolution of apoptosis-resistant cells and subsequent hyperproliferation of these surviving endothelial cells (161).

Organ-specific endothelial cells (65) differ in their capacity to generate VEGF protein for their own (autocrine) survival and maintenance and, therefore, may, depend to, varying degrees, on contextual epithelial cell and inflammatory cell VEGF sources. The fact that lung microvascular endothelial cells produce and secrete large amounts of VEGF protein (171) may be teleologically explained, i.e., these cells produce so much VEGF because they “need” it for their survival. This might explain why lung microvascular endothelial cells are particularly vulnerable to VEGFR blockade and why VEGF is critical for the structural integrity of the lung.

It is probably not by accident that the regulation of production and stability of this important protein and its multiple signaling pathways are enormously complex, as is the transcriptional control and stability of the upstream transcription factor HIF-1α (173, 204). It is becoming clear that oxygen and oxidative stress are central in this fabric of regulatory mechanisms, since hypoxia also induces the expression of endoplasmic reticulum oxidoreduction-I-Lα (137), which controls VEGF secretion, and oxidative stress inactivates VEGF survival signaling in endothelial cells via the action of peroxynitrite (46).

Further complexity has been added recently to the interaction between VEGF and prostacyclin. Neagoe et al. (145) showed that VEGF-induced prostacyclin synthesis by endothelial cells requires a VEGFR-1/VEGFR-2 heterodimer, and Buchanan et al. (17) demonstrated that prostacyclin-induced VEGF production was cyclooxygenase-2 dependent. Thus, it is likely that, also in lung vessels, VEGF, prostacyclin, and NO are forever intricately linked and form the backbone of their biology (Fig. 2). Finally, since VEGF plays a critical role in the mobilization of precursor cells from the bone marrow (122), VEGF may well control precursor cell-dependent lung tissue repair and the number of bone marrow-derived mast cells, macrophages, and dendritic cells in the lung.

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REFERENCES


Invited Review

VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE LUNG


