Vascular endothelial growth factor and related molecules in acute lung injury

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1Thoracic Surgery Research Laboratories, Toronto General Research Institute, University Health Network, Toronto M5G 2C4; Departments of 2Critical Care Medicine and 3Cardiology and Division of Molecular and Cell Biology Research, St. Michael’s Hospital, Toronto M5B 1W8; and 4Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Mura, Marco, Claudia C. dos Santos, Duncan Stewart, and Mingyao Liu. Vascular endothelial growth factor and related molecules in acute lung injury. J Appl Physiol 97: 1605–1617, 2004; doi:10.1152/japplphysiol.00202.2004.—VEGFs and their receptors have been implicated in the regulation of vascular permeability in many organ systems, including the lung. Increased permeability and interstitial and pulmonary edema are prominent features of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Extrapolating data from other organ systems and animal experiments have suggested that overexpression of VEGF functions primarily as proinjurious molecules in the lung. Recent data, from animal models as well as from patients with ARDS, have shown decreased levels of VEGF in the lung. The role of VEGF and related molecules in ALI/ARDS is, therefore, controversial: what has become clear is that there are many unique features in the regulation of pulmonary vascular permeability and in VEGF expression in the lung. In this review, we explore a growing body of literature looking at the expression and function of VEGF and related molecules in different models of ALI and in patients with ALI/ARDS. Novel evidence points to a potential role of VEGF in promoting repair of the alveolar-capillary membrane during recovery from ALI/ARDS. Understanding the role of VEGF in this disease process is crucial for developing new therapeutic strategies for ALI/ARDS.

acute respiratory distress syndrome; pulmonary edema; angiopoietins; hypoxia; hyperoxia

ACUTE LUNG INJURY (ALI) AND ITS MOST SEVERE MANIFESTATION, acute respiratory distress syndrome (ARDS), are clinically defined as severe dysfunction of gas exchange and chest radiographic abnormalities following a predisposing injury, in the absence of heart failure (14). ARDS and ALI may occur following various inciting events, including serious illness, such as sepsis, trauma, or organ transplantation. Overwhelming intrapulmonary inflammation, endothelial and epithelial injury, and consequent reparative responses are key components of the evolving ALI and progression to ARDS (14, 91).

The hallmarks of ALI are increased capillary permeability, interstitial and alveolar edema, influx of circulating inflammatory cells, and formation of hyaline membranes. Increased permeability leads to pulmonary edema, a life-threatening condition resulting from an imbalance between forces driving fluid into the air spaces and biological mechanisms for its removal. The severity and outcome of ALI depend on the balance between alveolar epithelial and/or vascular endothelial injuries and their repair mechanisms. The importance of endothelial injury and increased vascular permeability to the formation of pulmonary edema in this disorder is well established (14, 91).

VEGF plays an important role in several organs by directly regulating vascular permeability to water and proteins. For example, in the brain, VEGF is responsible for hypoxia-induced vascular leakage and edema formation; inhibition of VEGF activity by a neutralizing antibody can block the hypoxia-induced increase in vascular permeability (117). The role of VEGF in the control of pulmonary permeability is, however, controversial. Systemic expression of VEGF has been shown to cause widespread multiorgan capillary leakage in an animal model, suggesting that the overexpression of VEGF plays a pivotal role in the development of pulmonary edema (67). However, recent animal studies and clinical data support a protective role for VEGF in ALI and ARDS patients (30, 133). Understanding the relationship between VEGF and pulmonary permeability in ALI/ARDS may lead to the development of novel therapeutic interventions for this syndrome.

In the lung, the regulation of pulmonary permeability and the expression of VEGF and VEGF-related molecules have many unique features. Consequently, it is not appropriate to simply extrapolate knowledge from other organ systems and apply them to the lung. Therefore, we have undertaken a systematic review of the literature, focusing on features pertaining to VEGF regulation and function in the lung and in particular its potential role in the pathophysiology of ALI/ARDS. In addition to reviewing the current state of knowledge, the objective of this paper is to further discuss controversial observations related to the role of VEGF, its related factors,
and their receptors in ALI/ARDS, offering alternative perspectives on this intricate system.

**BIOLOGY OF VEGF AND RELATED MOLECULES**

The VEGF family has several members, and each acts through specific receptors. The biology of VEGFs has recently been the focus of many excellent reviews (9, 10, 43). Interactions between VEGFs and angiopoietins (Ang) is very important in the regulation of angiogenesis and vascular permeability (83). For the purpose of this review, we will focus on the role of VEGF and related molecules that have been implicated to play in the lung. The characteristics and properties of VEGFs and Ang and the potential interplay between VEGF and related molecules and their receptors in the lung are summarized in Table 1 and illustrated in Fig. 1.

**VEGFs**

The human VEGF gene family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor (PIGF), all with multiple and diverse biological functions (43). The genes for VEGF family members also rely on alternative exon splicing to confer various isoforms for biological and functional specificity (109, 113).

The most studied molecule of the VEGF family is VEGF-A. The gene encoding human VEGF-A is organized into eight exons. Multiple protein isoforms are generated through alternative splicing of the pre-mRNA (136). Human VEGF-A isoforms include 121, 145, 165, 183, 189, and 206 amino acids (VEGF121, VEGF145, VEGF165, VEGF183, VEGF189, and VEGF206, respectively). VEGF121 is a soluble isofrom, whereas VEGF189, VEGF206, and 60–70% of VEGF165 are found in association with cells or sequestered in the extracellular matrix (43). Most VEGF-A isoforms contain a heparin-binding domain. VEGF189 and VEGF206 bind to heparin with high affinity (60). The absence of heparin-binding domain in VEGF121 results in a loss of mitogenic activity (73), as demonstrated by the observation that transgenic mice expressing exclusively VEGF120 (mouse VEGF is one amino acid shorter) die shortly after delivery, due to severe angiogenic defects (22). VEGF145 and VEGF183 are less frequent splicing variants (109, 113). Due to its bioavailability and biological potency, VEGF165 is the predominant isofrom of VEGF-A, and from hereon the abbreviation VEGF refers to VEGF165, except if otherwise specified.

The major site for VEGF-B expression is the heart (103). VEGF-B forms heterodimers with VEGF and may, therefore, modulate its signaling (103). A study on VEGF-B knockout mice suggested that VEGF-B may have a role in the development of chronic hypoxic pulmonary hypertension in mice by contributing to pulmonary vascular remodeling (142).

VEGF-C and VEGF-D induce growth of the lymphatic vasculature in vivo (63). They can also induce capillary endothelial cell (EC) migration and proliferation in culture (19, 95) and act as vascular permeability factors at higher concentrations (68, 115). VEGF-D shares 61% identity with VEGF-C (111). VEGF-C and VEGF-D mRNA are most abundant in the heart, lung, skeletal muscle, colon, and small intestine (95, 104). Furthermore, VEGF-C is highly expressed by activated macrophages (124).

### Table 1. Characteristics and properties of VEGF and related molecules in the lung and their potential role in acute lung injury.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sources</th>
<th>Receptor</th>
<th>Stimulating Factor</th>
<th>Potential Functions</th>
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<tr>
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<td>VEGFR-1</td>
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<td>EC proliferation</td>
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<td>Airway epithelial cells</td>
<td>VEGFR-2</td>
<td>Mechanical stretch</td>
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<td>NRP-1</td>
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<td>Neutrophils</td>
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<td>IL-6</td>
<td>Migration of monocytes</td>
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<td>(Heart)</td>
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<td>VEGF-C/D</td>
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<td>VEGFR-1</td>
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<td>Ang-1</td>
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<td>Hypoxia</td>
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<td>Interstitial cells</td>
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<td>VEGF-A</td>
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<tr>
<td>Ang-2</td>
<td>Airway epithelial cells</td>
<td>Tie-2</td>
<td>Hypoxia</td>
<td>↑ Vascular permeability</td>
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<td></td>
<td>ECs (at sites of active vascular remodeling)</td>
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<td>VEGF</td>
<td>Antiapototic for ECs</td>
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<td>Ang-3/4</td>
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<td>Interstitial cells</td>
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<td>HIF-1α</td>
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PIGF, placenta growth factor; Ang, angiopoietin; EC, endothelial cells; NRP, neuropilin; ROS, reactive oxygen species; TGF, transform growth factor; HIF, hypoxia-induced factor; ↑, increase; ↓, decrease.
VEGF-E was identified in the genome of Orf parapoxvirus and shares ~25% amino acid identity with mammalian VEGF-A (99). VEGF-E shows almost equal levels of mitotic activity on primary ECs and vascular permeability activity as VEGF (99).

PIGF is expressed in the placenta, heart, lung, thyroid, brain, and skeletal muscle (108). PIGF stimulates angiogenesis and induces vascular permeability when coinjected with VEGF (4). The absence of PIGF has negligible effects on vascular development, but loss of PIGF impaired angiogenesis, plasma extravasation, and collateral growth during ischemia, in wound healing, and cancer (21). PIGF, an isoform of VEGF-131, an isoform of PlGF, PlGF-2, is expressed in human umbilical vein ECs and placenta tissue and can bind with high affinity to species of PlGF and VEGF homodimers (20). A second species of PIGF, PIGF-2, is expressed in human umbilical vein ECs and placenta tissue and can bind with high affinity to heparin (58).

**VEGF Receptors**

The biological activity of VEGF is dependent on its interaction with specific receptors. Three VEGF receptors (VEGFRs) have been identified: VEGFR-1, VEGFR-2, and VEGFR-3 (43, 141). In addition, VEGF interacts with a family of coreceptors, the neuropilins (97, 114). Both VEGFR-1 and VEGFR-2 have seven extracellular immunoglobulin-like domains and a single tyrosine kinase transmembrane domain (118, 131) and are expressed on vascular ECs. VEGFR-1 is also expressed on activated macrophages, monocytes, placental trophoblasts, and renal mesangial cells (7, 24). VEGFR-1 immunoreactivity was found in bronchial epithelium and type II pneumocytes of adult mouse lungs (41). The finding of VEGFR-1 in the distal epithelium of human developing lung suggests a possible autocrine role for VEGF in alveolar epithelial cell proliferation and differentiation (17). A soluble, alternatively spliced form of VEGFR-1 has been shown to inhibit VEGF activity (72). VEGFR-2 immunoreactivity was detected in Clara cells of adult mouse lungs (41). VEGFR-3 is predominantly expressed in the endothelium of lymphatic vessels (64, 78).

The specific functions of VEGFR-1 are still under debate. Several lines of evidence suggest that VEGFR-1 may mediate vascular organization (45). Inactivation of the VEGFR-1 gene leads to a very severe disorganization of the vascular system (46). It has been shown that the migration of monocytes in response to VEGF is mediated by VEGFR-1 (8). VEGFR-2 is the major mediator of endothelial differentiation and proliferation (45). Studies using VEGF mutants that bind selectively to either VEGFR-1 or VEGFR-2 demonstrated that most of the angiogenic activities of VEGF as well as the effects of VEGF on permeability are mediated by VEGFR-2 (54). VEGF binds to and activates VEGFR-1 and VEGFR-2 (141). VEGF-B selectively binds to VEGFR-1, by competing with VEGF (64, 84, 102). VEGF-C and VEGF-D bind to VEGFR-2 and VEGFR-3 on ECs (104). VEGF-C and VEGF-D also regulate the lymphatic ECs via VEGFR-2 and VEGFR-3 (65). Interestingly, in mice, VEGF-D binds to VEGFR-3 but not to VEGFR-2 (5). VEGF-E selectively binds to VEGFR-2 but not to VEGFR-1 (93, 99). PIGF specifically acts on VEGFR-1 (106).

Treatment of cells with VEGF induces receptor heterodimerization; these heterodimers are functional signaling units (62). Similarly, VEGF-C stimulation of lymphatic ECs induces the formation of VEGFR-2/VEGFR-3 heterodimers (35). Differences in the phosphorylation-site pattern between homo- and heterodimeric VEGFRs suggest that the signal transduction properties and biological function are distinct for the heterodimerized receptors (35). PIGF can regulate inter- and intramolecular cross talk between VEGFR-1 and -2: VEGFR-1 activation by PIGF results in intermolecular transphosphorylation of VEGFR-2 and consequent amplification of VEGFR-2-driven angiogenesis (4). By binding to VEGFR-1, PIGF may also increase the proportion of VEGF available to activate VEGFR-2 and thus potentiate the VEGF-dependent angiogenesis. These observations suggest a “decoy” function for VEGFR-1, to prevent VEGF binding to VEGFR-2 and its
activity on vascular endothelium (106). A pivotal role for PIGF and VEGFR-1 in regulating VEGF-dependent angiogenesis under pathological conditions has been suggested (21).

Neuropilin-1 (NRP-1), a receptor for semaphorins/collapsins involved in axonal guidance, can act as an isoform-specific coreceptor for VEGF, VEGF-B, and PIGF-2. NRP-1 has a wide tissue distribution and may enhance the effectiveness of VEGFR-2-mediated transduction by presenting VEGF to VEGFR-2 (126). In contrast, there is no evidence of neuropilins signaling after VEGF binding (96). The embryonic lethality of NRP-1 null mice suggests a role for NRP-1 in the development of vascular system (70).

Ang

Ang are vascular growth factors that were originally discovered as ligands for the Tie-2 receptor, which belongs to a family of receptor tyrosine kinases that are selectively expressed within the vascular endothelium (33).

Ang-1 plays a critical role in vascular development by mediating reciprocal interactions between the endothelium and the surrounding matrix and mesenchyme (32, 128). In the adult, Ang-1 has been detected in the majority of tissues studied, and it is expressed by a variety of cells, including vascular smooth muscle cells and pericytes, which are in proximity to ECs (116). Ang-1 has also been identified in platelets and can be released from them at sites of vascular injury (81). Ang-1 has several isoforms; the full-length Ang-1 can promote angiogenesis by activating its receptor Tie-2 (32). Three alternatively spliced forms of Ang-1 have been identified and may act as dominant negative forms of the protein, conferring additional levels of functional regulation (33, 83).

Ang-1 has a naturally occurring antagonist, Ang-2, which blocks the ability of Ang-1 to activate its receptor (85). However, Ang-2 may also have a direct role in stimulating Tie-2 receptor signaling, and it has been reported to induce angiogenesis in vitro (130). At high concentrations, both Ang-1 and Ang-2 can act as survival factors for the ECs through the activation of the Tie-2 receptor (74). Ang-2 is critically involved in the postnatal vascular remodeling (3) and is selectively expressed by ECs at sites of vascular remodeling, where it appears to have an autocrine function (87).

Ang-2 promotes vascular leakage by antagonizing the actions of both Ang-1 and Ang-4 (69). Ang-3 and Ang-4 are mouse and human counterparts of the same gene locus; both bind to Tie-2 receptor, although Ang-3 appears to act as an antagonist, whereas Ang-4 appears to function as an agonist (140). Compared with Ang-1, Ang-4 has a restricted expression in the human adult, being prominent in the lung (69, 98).

Cellular Sources of VEGFs and Ang in the Lung

VEGF expression has been identified in perivascular cells of many organs. VEGF mRNA is most abundant in the lung, kidney, and spleen, where the transcript is localized primarily in epithelial cells and vascular smooth muscle cells (121). In situ hybridization has demonstrated that the major sources of VEGF, in both animal and human lungs, are mesenchymal and alveolar type II epithelial cells (66). VEGF released by alveolar epithelial cells may modulate specific functions of the adjacent vascular endothelium in a paracrine fashion (121). In addition, immunohistochemistry studies in healthy mice have localized VEGF in the airway and vascular smooth muscle cells (30). Activated bronchial airway epithelial cells also release VEGF (77). In mice, Ang-1, Ang-2, and Ang-4 expression is seen in airway epithelium, whereas Ang-1 and Ang-4 are also seen in alveolar interstitial cells (69). It has been suggested that type II epithelial cells may produce PIGF (138).

Gas exchange is the most important function of the lung. This depends on the “perfect” match between ventilation and perfusion. Because VEGFs and Ang are expressed on epithelial cells and their receptors mainly on ECs, this anatomic arrangement may direct the vasculogenesis during lung development. This anatomic arrangement may also facilitate angiogenesis during healing.

In healthy human subjects, the VEGF protein is highly compartmentalized to the lung with alveolar levels of VEGF protein 500 times higher than in plasma (66). The high levels of VEGF protein on the respiratory epithelial surface may function as a physiological “reservoir” (66). Under normal (nonstress) situations, VEGF may slowly diffuse across the alveolar epithelium to exert its biological function. However, under conditions of stress or injury such as in ALI, because of the anatomic proximity between epithelial cells and ECs along the alveolar-capillary membrane, VEGF may literally spill onto ECs, increasing vascular permeability and leading to interstitial and pulmonary edema (66).

Under normal conditions, alveolar macrophages produce little soluble VEGF and, therefore, are not likely to substantially contribute to intrapulmonary VEGF levels, but, in injured lungs, they may become a more important source of VEGF (133). Lung injury stimulates the migration of monocytes and neutrophils (37, 94). Neutrophils carry intracellular pools of VEGF within granules, which can be mobilized during degranulation (50). Altered VEGF production by inflammatory cells may, therefore, account for the increase in VEGF seen in ALI.

Regulation of Expression of VEGFs, VEGFRs, and Ang

Hypoxia. Mammals respond to hypoxia by a variety of mechanisms that function at multiple levels. Induction of VEGF is considered to be a local adaptation to hypoxia (89). In a murine model of systemic hypoxia, VEGF induction was highest in the brain, but also occurred in the kidney, testis, lung, heart, and liver (89). In situ hybridization analysis showed that a distinct subset of cells within each organ responded to the hypoxic stimulus with an increase in VEGF expression: glial cells and neurons in the brain, tubular cells in the kidney, and Sertoli cells in testis (89). In the lung, after hypoxic exposure, VEGF expression increased slightly but homogeneously in all regions of the lung, mostly in the alveolar epithelial cells (89).

Hypoxia-induced VEGF production is mediated by hypoxia-induced factor (HIF)-1α (119). HIF-1 is an oxygen-dependent transcriptional factor consisting of an α-subunit (HIF-1α) and a constitutively expressed β-subunit (HIF-1β). Under hypoxia conditions, HIF-1α acts as a master regulator of numerous hypoxia-inducible genes, by interacting with coactivators such as p300/ACB. HIF-1α binds to a core sequence of the hypoxia-responsive element in the promoters of hypoxia-responsive genes and induces their expression (79).

To distinguish the effects of hypoxia from other sequelae of ischemia, cultured cells were exposed to reduced O2 tension,
and induction of VEGF mRNA was demonstrated in a variety of cell types, including bovine pulmonary artery ECs (47, 82). In contrast to VEGF, PlGF seems to be moderately downregulated by hypoxia (108). Expression of VEGFR-1, but not VEGFR-2, was induced by hypoxia in ECs of the lung, heart, brain, kidney, and liver (89). The hypoxia-responsive element has been identified in the promoter of VEGFR-1 but not of VEGFR-2 (51). Ang-1 and Ang-2 expression is upregulated by both hypoxia and VEGF (100, 107). Ang-2 expression is downregulated by basic fibroblast growth factor (87).

Induction by cytokines and other inflammatory mediators. In addition to hypoxia, VEGF induction has been described in vivo and in vitro in response to several stimuli, including reactive oxygen species (27), glucose deprivation (122), inhibition of nitric oxide (61, 139), growth factors (e.g., transforming growth factor-β1) (15), and various inflammatory cytokines, such as TNF-α, IL-6, and IFN-γ (28, 48, 145) (Fig. 2, left). Transforming growth factor-β1 is widely expressed in airway epithelial cells and has been shown to strongly stimulate VEGF expression and release by non-ECs, which may contribute to neovascularization and repair of injuries to the lung endothelium (15). In vitro studies suggest that IL-6 (28, 145) and TNF-α (48) can upregulate VEGF expression. Both IL-6 and TNF-α have been implicated as mediators of increased vascular permeability and remodeling in several disorders (28). IL-6, like VEGF, is induced in response to hypoxia (145). The expression of IL-6 and VEGF is closely linked, suggesting that they may act synergistically to regulate vascular permeability. IFN-γ can also regulate the expression of VEGF mRNA in a cell type-specific manner (76, 137, 143). The expression of IFN-γ in response to inflammation and wound healing may be one of the signals that triggers the angiogenic process through the induction of VEGF expression. A brief exposure of human monocytes to LPS led to a significant upregulation of the VEGFR-1 mRNA level (8).

Endothelin (ET) can act via its ET-A receptor to stimulate the production of VEGF mRNA and protein (101, 127). The ET-mediated stimulation of VEGF production occurs via increases in the expression of HIF-1α, even under normoxic conditions (127).

Oxygen-independent induction of VEGF in the lung. The role of ischemia-reperfusion injury is central to the pathophysiology of many disorders, including myocardial infarction, peripheral vascular insufficiency, stroke, major trauma, hypovolemic shock, and sepsis. This is primarily due to the impaired microcirculation and ensuing tissue hypoxia, followed by reperfusion and reoxygenation. However, pulmonary ischemia is not necessarily associated with tissue hypoxia if the lung is inflated with oxygen while blood flow is impaired (12). Thus the vascular injury that occurs in the ischemic pulmonary vasculature may be independent of hypoxia. Becker and colleagues (11) discovered an oxygen-independent upregulation of VEGF using an isolated ferret lung model. The degree of increased VEGF expression during ventilated pulmonary is-

Fig. 2. Overall hypothesis of the role of VEGF in acute lung injury (ALI). Left: in the early stage of lung injury, different insults and proinflammatory cytokines stimulate the production and release of VEGF from type II cells, alveolar macrophages, and marginating neutrophils. Therefore, the epithelial-endothelial barrier is exposed to high concentration of VEGF, which may alter the state of adherens junction complexes (AJs) and cause vascular leakage and interstitial edema. Middle: during the development of lung injury, damage of type I and type II epithelial cells and the release of proteases from neutrophils decrease the VEGF concentration in the alveolar compartment. The loss of compartmentalization and the release of VEGF from other organs and circulating leukocytes may increase the serum concentration of VEGF. Right: during the recovery of lung injury, type I and type II cells are being repaired, and the VEGF production can increase again, which may contribute to the repair and angiogenesis by acting on VEGFR-2. The role of VEGFR-1 in these processes is unknown. ROS, reactive oxygen species.
chemia was independent of oxygen concentration in the ventilatory gas mixture. Interestingly, the expression of HIF-1α mRNA was increased to a similar degree during 180 min of ventilation, regardless of oxygen concentration, but the increase of HIF-1α protein was not significant during hyperoxic ischemia, even though it increased sixfold in hypoxic ischemic lungs (11). The mechanisms of this oxygen-independent VEGF expression are still unknown. Mechanical forces and cytokines may be involved in these processes. It has been shown that mechanical stretch increased expression of VEGF (55, 147) and VEGFR-2 (129), both in vitro and in vivo.

**Major Functions of VEGF and Related Molecules**

VEGF and related molecules have profound effects on the EC biology, by regulating cell proliferation, apoptosis, angiogenesis, vascular permeability, and monocytes recruitment. **Cell proliferation.** The ability of VEGF to induce proliferation of ECs from arteries, veins, and lymphatics has been demonstrated in animal models (80). Although ECs are the primary target of VEGF, a recent study showed that VEGF can also stimulate the production of surfactant by alveolar type II cells (29) and stimulates growth of lung airway epithelial cells in vitro (17).

**Apoptosis.** VEGF has been well characterized as an endothelial survival factor, and it prevents microvascular apoptotic cell loss (1, 53). VEGF survival signals in ECs are mediated by VEGFR-2 through the phosphatidylinositol 3-kinase/Akt signal transduction pathway (53). VEGF also promotes the expression of antiapoptotic proteins Bcl-2 and A1 in vascular ECs (52) and can specifically block extrinsic signal-induced apoptosis of ECs, mediated by TNF receptors and Fas (1). Prosurvival signaling by VEGF may, therefore, alter the threshold level of EC susceptibility to intrinsic and extrinsic inducers of apoptosis (1).

Tsao et al. (138) have recently reported pulmonary emphysema in PI GF-trangenic mice; overexpression of PI GF is associated with increased apoptosis of type II pneumocytes and reduced expression of VEGF. They further demonstrated that recombinant PI GF inhibits proliferation and promotes cell death of mouse type II pneumocytes in culture (138).

**Angiogenesis and vasculogenesis.** Angiogenesis, a process of generation of new blood vessels from preexisting vasculature, is accompanied in almost all states by increased vascular permeability (9). Uptregulation of VEGF expression is associated with pathological angiogenesis, which occurs in chronic hypoxia, tumor growth, and rheumatic and ophthalmic diseases (16, 89). VEGF expression is also critical for vasculogenesis during fetal development, as demonstrated by embryonic lethality of heterozygous VEGF-null mutants (42). Interactions between VEGF and Ang family members may be required for normal vascular development (16, 89). VEGF and Ang-1 have distinct roles in vascular development: VEGF is mitogenic and induces EC differentiation and migration, whereas Ang-1 may be necessary for stabilization of developing vascular networks. VEGFR-2 is critical for VEGF-mediated angiogenesis, both in the developing and in the adult animals (120).

**Vascular permeability.** The permeability-enhancing effects of VEGF underlie a significant role of this protein in acute inflammation. Increased vascular permeability in response to exogenous VEGF administration has been reported in skin (26), muscle (112), and gastrointestinal tract (123). Increased permeability and fenestration (112) have been found in large vessels (59), microvascular vessels (39), and ECs in culture (10). Moreover, systemic expression of VEGF has been shown to cause widespread multiorgan capillary leakage; this effect is attenuated by concomitant expression of Ang-1 (135).

Ang-1 and Ang-4 have been shown to modulate endothelial permeability of vessels by altering the state of the adherens junction complexes (AJCs) (34, 146). AJCs are endothelial-specific structures that regulate the permeability of mature vessels in response to inflammatory stimuli as well as EC growth patterns and angiogenesis during embryonic development (49). Ang-1 inhibits vessel leakage in response to VEGF or other proinflammatory agents, by stabilizing the AJCs (128, 134) and promoting vessel maturation through its antiapoptotic action (105). The same actions have been proposed for Ang-4, whereas Ang-2 promotes vascular leakage by antagonizing the actions of both Ang-1 and Ang-4 through competitive binding to the Tie-2 receptor (69).

**Monocyte recruitment.** Treatment of human monocytes with recombinant VEGF induces monocyte activation and migration. The chemotactic activity of VEGF is mediated by VEGFR-1 and is inhibited by a specific antiserum against VEGF, by heat treatment of VEGF, and by protein kinase inhibitors (8). PI GF may also act as a chemoattractant for inflammatory cells (65).

**ROLE OF VEGF AND RELATED MOLECULES IN ALI**

Numerous animal studies have alluded to various potential roles of VEGF and related molecules in ALI. It appears that the expression and function of these molecules are influenced by the variability of the injurious factors in different modeling settings. In-depth comparisons of similar and contrasting results will help us to understand the role of VEGF and related molecules in ALI.

**Overexpression of VEGF Increases Pulmonary Permeability**

Kaner et al. (67) demonstrated that the intrapulmonary overexpression of VEGF through intratracheal administration of an adenoviral-mediated vector resulted in high-permeability edema in murine lungs. Pretreatment with soluble VEGFR-1, which binds to VEGF and prevents it from activating its cognate receptors, blocked the development of edema. These data strongly indicate that VEGF and its receptors could participate in the regulation of pulmonary permeability and that elevated alveolar VEGF levels could lead to pulmonary edema (Fig. 2, left). However, the transient and overwhelming expression of VEGF mediated by adenoviral gene transfer may or may not represent the pathological process of ALI in clinical settings.

ET may contribute to the formation of pulmonary edema, particularly under hypoxic conditions, where it increases VEGF production via the ET-A receptor (23). The ET-B receptor, on the other hand, is thought to be internalized after ET binding, in turn acting to reduce circulating levels of ET protein (36). In ET-B receptor-deficient rats, lung HIF-1α and VEGF content was greater in both normoxia and hypoxia, compared with controls; accordingly, the pulmonary vascular leak was more pronounced (23). The inhibition of VEGF with a VEGF-Trap-soluble decoy receptor markedly reduced pul-
monary vascular protein extravasation in the hypoxic ET-B receptor-deficient rats, indicating that the increased expression of VEGF in the lung contributes to vascular leak (23). However, even with VEGF antagonism, albumin extravasation in the hypoxic ET-B-deficient rats was still greater than in normoxic control animals, suggesting that factors other than VEGF may also contribute to hypoxia-induced changes in vascular permeability (23).

LPS-Induced Lung Injury

Using an LPS-induced lung injury model, Karmpaliotis et al. (69) were able to illustrate the significance of understanding the balance between VEGF and Ang in determining the outcome and severity of lung injuries. In mice exposed to LPS for 1, 2, or 4 days, immunostaining for VEGF in the lung was markedly increased, associated with the influx of mononuclear cells and neutrophils in the alveolar compartment. This finding was paralleled by the increase in VEGF mRNA expression, development of inflammation, capillary leakage, and lung edema (69). In contrast, Ang-4 expression in epithelial cells decreased substantially over time. Increased immunostaining for Ang-2, which is usually associated with increased vascular permeability, was detected in the alveolar compartment, particularly in the mononuclear inflammatory cells (69). The pattern of VEGF and Ang-2 upregulation accompanied by Ang-4 downregulation emphasizes the importance of the balance between “proleakage” (VEGF and Ang-2) and “antileakage” (Ang-4) vascular growth factors in the regulation of capillary integrity.

On the other hand, in a rat model of ALI induced by alveolar instillation of *Pseudomonas aeruginosa* for 4 h, 24 h, and 5 days, VEGF protein and mRNA expression were decreased during the initial phase of ALI, compared with control rats (86). One possible explanation for the decrease in VEGF in this model is the bacterial-induced epithelial cell death. Direct injury of alveolar epithelial cells by bacteria may lessen the main source of VEGF in the lung by decreasing the number of cells producing VEGF (86).

Ischemia-Reperfusion-Induced Lung Injury

Exposure of the lungs to acute ischemia occurs with lung preservation for transplantation, and pulmonary edema is an early complication of this procedure. In clinical lung transplantation, VEGF levels were found to be decreased in the bronchoalveolar lavage (BAL) fluid from the lungs of patients with ALI, perhaps reflecting the epithelial damage after ischemia and reperfusion (92). In a rat model of lung transplantation, the decrease in VEGF protein expression after 6 h of hypothermic preservation (ischemia) and 50 min of reperfusion was associated with a reduction in pulmonary oxygenation capacity and was inversely related to the volume of type II cells, as evaluated with electron microscopy. These findings support the hypothesis that a decline in pulmonary VEGF protein expression after ischemia-reperfusion is a direct result of type II cell injury (40). This theory is consistent with the observation that increased epithelial cell apoptosis occurs after lung transplantation (44). It is noteworthy that, in a unilateral warm ischemia model, after ligation of the left pulmonary artery, both VEGF protein and VEGFR-2 in the left lung increased by 4 h and then returned to baseline by 24 h, whereas increased VEGF and VEGFR-2 mRNA expression was sustained throughout 24 h of unilateral ischemia (71). Therefore, the effects of ischemia and/or reperfusion on the VEGF system in the lung transplantation setting (prolonged hypothermic preservation of donor lung, followed by warming up and reperfusion) are different from what occurs at the body temperature.

Hyperoxia-Induced Lung Injury

Hyperoxic lung injury is characterized by widespread alveolar-epithelial and microvascular EC damage and necrosis. The resolution of these injuries is dependent on angiogenesis (31). Ekekezie and coworkers (38) demonstrated a decrease of VEGF mRNA and protein expression in lung homogenates from young piglets after exposure to hyperoxia for 5 days, which was accompanied by a paradoxical rise in lavageable lung VEGF protein fraction. This may be due to proteolytic cleavage and release of extracellular matrix-bound VEGF protein (38). Hyperoxia is indeed known to activate proteinases, including matrix metalloproteinases and urokinase type plasminogen activator (18, 57).

Ventilator-Induced Lung Injury

Excessive alveolar distension associated with mechanical ventilation (MV) can cause ALI, manifested by increased vascular permeability and alterations in lung tissue mechanics, and may promote a proinflammatory state that predisposes to multiple organ failure (125). A multicenter trial demonstrated that MV with lower tidal volumes (VT) significantly decreased mortality in patients with ARDS, compared with MV with higher, conventional VT values (2). A number of proinflammatory cytokines and chemokines are released into the circulation with high-VT ventilation, and mechanically ventilated patients with ARDS are at increased risk for the development of multisystem organ failure (110, 144).

Gurkan et al. (56) established an in vivo murine model to assess the differential effects of ventilator strategies on the development of ALI and systemic organ inflammation. After intratracheal aspiration of hydrochloric acid with high-VT ventilation (17 ml/kg), mice developed lung injury accompanied by increased levels of IL-6 and VEGFR-2 in the lung, liver, and kidney. However, no significant differences in pulmonary concentrations of VEGF were seen (56). The lack of change of VEGF in the lung has also been reported from open-chest rabbits exposed to MV with high-positive end-expiratory pressure (13). With the knowledge that lung epithelial cells represent the primary source of VEGF production in the lung, lack of increase of VEGF levels following acid aspiration and 4 h of MV may be a result of epithelial injury. Interestingly, high-VT ventilation of healthy mice led to increased VEGF and IL-6 concentrations in both liver and kidney, even in the absence of lung injury (56). MV in the lung, therefore, may induce the release of soluble factors that may stimulate VEGF expression in other organs. This phenomenon may contribute to ventilator-induced lung injury and related multiorgan failure.

Moreover, Choi and coworkers (25) demonstrated increased serum VEGF levels in response to high-VT (20 ml/kg) ventilation in rats, likely indicative of the loss of capillary permeability with subsequent loss of pulmonary compartmentalization. Alternatively, the increased VEGF levels in serum may be secondary to increased production of VEGF either by circulat-
VEGF AND ARDS

Pulmonary injury in ARDS causes disruption of both sides of the alveolar-capillary interface, with consequential hyperfiltration, alveolar flooding, and hypoxia. To investigate the two sides of the alveolar-capillary membrane, both vascular and alveolar compartments have been studied. Increased plasma levels of VEGF and a reduction of VEGF in BAL fluid were documented in 40 patients with ARDS compared with patients at risk for ARDS (132). In a separate study, Maitre et al. (86) analyzed BAL fluid from 19 patients with ARDS collected within the first 7 days from the diagnosis, compared with BAL fluid from patients without ARDS. This study corroborated the finding that ARDS is associated with a decrease in VEGF protein in the lung (86). Degradation of VEGF by proteases released from infiltrated neutrophils and other inflammatory cells in the alveoli may be responsible for VEGF decline in the lung. A continuous decrease of VEGF levels in supernatants of lysed neutrophils in cell culture experiments was observed and was related to the release of large quantities of proteases from neutrophils into the culture medium (75). Furthermore, a decrease in alveolar type II cellularity, due to apoptosis, has been observed during resolution of ARDS (6), which may reduce the production of VEGF in the alveolar space. Release of soluble Fas ligand has been suggested as a potential mechanism of apoptosis in ARDS (90). This may partially explain the decrease of VEGF, at least during the developing phase of ALI/ARDS (Fig. 2, middle).

On the other hand, in the early onset of ALI/ARDS, a widespread but patchy destruction of the alveolar epithelial membrane is observed (14). The consequent damage of pneumocytes may lead to increased release of VEGF from the lung to the plasma, which may partially explain the increase in VEGF in plasma of ARDS patients (133). In addition, ARDS represents only the pulmonary manifestation of a widespread endothelial injury in multiple organs. Approximately 50% of ARDS cases result from injury process occurring in organs remote from the lung (2). Stimulation of neutrophils with LPS and IL-13 from patients with ARDS or at risk resulted in increased VEGF production (133). Thus VEGF produced by inflammatory cells and other organs may also contribute to the increased VEGF levels in plasma (Fig. 2, middle).

During the progression of ARDS, patients with increasing levels of VEGF in epithelial lining fluid had better recovery (133). This observation is also intriguing: although VEGF can increase vascular permeability, there is evidence to support its protective role in the lung. When transgenic mice overexpressing IL-13 were exposed to hyperoxia, a protective role was noted, which was associated with increased production of VEGF in the lung. Furthermore, treatment with VEGF neutralization antibody decreased the survival of IL-13-overexpressing mice exposed to hyperoxia, suggesting that VEGF protects injured alveolar lining cells by interacting with either VEGFR-1 or VEGFR-2 (30). VEGF mRNA expression increased in alveolar epithelial cells during recovery from oxygen injury (88). This may be due to type II cell proliferation, as a reparative response to injury. Increased VEGF may further protect ECs from apoptosis and promote angiogenesis. In the same context, the observed reduction of VEGF in plasma of ARDS patients in the late phase could be due to the recovering of the alveolar epithelium and endothelium that restores the barrier to VEGF (133) (Fig. 2, right). Therefore, it remains to be established whether increased VEGF levels in the lung during the recovery phase of ARDS represent a marker of resolution of lung injury, or whether VEGF is actively involved in promoting lung repair of the alveolar-capillary membrane.

Summary and Speculation

The general pathological manifestation of ALI and ARDS is similar in different models and clinical settings, but the underlying mechanisms are very different. The expression and function of VEGF system in ALI vary, depending on the pathological conditions, timing, and degrees of endothelial and epithelial damage. It is possible that, in the early stage of lung injury, acute inflammatory response-induced VEGF release from alveolar epithelial cells and leukocytes increases the permeability of endothelial layer of the barrier and contributes to the formation of interstitial edema in the lung (Fig. 2, left). With the further development of pulmonary edema, the damage of alveolar epithelial layer may reduce the production of VEGF in the lung. Bacterial and viral infection, acid aspiration, and high concentration of oxygen may directly damage the lung structures and thus reduce the VEGF production (Fig. 2, middle). During the recovery period of ALI, increased VEGF from alveolar epithelial cells may function through their receptors to participate in the angiogenesis, an important component of lung repair (Fig. 2, right). These hypothetic explanations need to be tested in future investigations.

PERSPECTIVES

This review has highlighted the complexity of the VEGF system in ALI. In light of this insight, what will be the most important questions for future studies to determine the role of VEGF and related molecules in ALI/ARDS?

The Role of Alveolar Epithelium in VEGF-related Pulmonary Permeability

Although, in general, VEGF and related molecules are involved in the regulation of vascular permeability and angiogenesis, we should keep in mind the special features in the lung. In contrast to other organs in which the endothelium represents the main barrier to capillary leakage, the lung has a dual epithelial-endothelial barrier. Under normal conditions, the junctional complex of respiratory epithelium provides an effective barrier: preventing leakage of solutes and fluid into the lung. In contrast, paracellular pores through the endothelial surface allow free passage of hydrophilic solutes (69). Under pathological conditions, although the loss of integrity in the endothelial barrier is evidently a prerequisite for development of interstitial edema, pulmonary edema is a consequence of loss of integrity in the epithelium. As of now, most of the studies have focused on the role of VEGF in ECs, and the involvement of the VEGF system in the regulation of epithelium permeability has not yet been addressed. Furthermore, alveolar epithelial cells are not only a major source of VEGFs and Ang, but they also express VEGFRs (17, 41). Selective blockade of expression of VEGF and/or its receptors from...
epithelial cells would allow us to separately evaluate the effects of alveolar epithelial cells in VEGF-related ALI. To achieve this goal, specific molecular tools or genetically modified animal models would need to be developed.

**Regulation of VEGF and Related Molecules in the Lung**

The regulation of VEGF expression in the lung appears to be different from other organs. In the lung, ischemia is not necessarily associated with hypoxia; ischemic lung tissues or poorly perfused areas could be inflamed or ventilated with a high concentration of oxygen. Ex vivo ventilation studies suggested that oxygen tension may not be the most important regulator for VEGF expression in the lung (11). This oxygen-independent regulation of VEGF in the lung is unique and needs to be confirmed by other experimental approaches. Mechanical forces, derived from the MV, and inflammatory responses from the preexisting clinical conditions (sepsis, trauma, acid aspiration, bacterial or viral infection, etc.) may have profound effects on the expression of VEGF and related molecules in the lung. Animal models and cell culture studies should be designed to test these potential mechanisms, both under physiological conditions and during acute inflammation.

**The Complexity of VEGF System in ALI**

To date, most studies only focused on VEGF-A. The information regarding other VEGF family members, Ang, and their receptors during ALI/ARDS, is largely unknown. Based on this review of the literature, VEGF and its related molecules should be studied as a group or an intricate system. This is particularly important for future clinical studies. The development of bioinformatics and proteomics will enable the investigators to study these factors simultaneously.

**Models for ALI/ARDS, from Bench to Bedside**

Currently, one of the major limitations for ARDS studies is the lack of adequate animal models. Clinically, ARDS usually lasts several days and weeks. Experimental models as well as human studies tend to use time points for data collection that favor convenience. Further studies with careful experimental designs are necessary to determine the role of VEGF system in both the early and late phases of pathophysiological processes following a major inflammatory insult (132, 133).

The expressions of VEGF-related molecules and their functions are mediated through multiple signal transduction pathways, which might be cell type specific. Because most cellular and molecular studies to date have used ECs from nonpulmonary sources, the conclusions drawn from these studies have to be taken with caution. Ideally, these studies should be conducted by using pulmonary ECs and preferably from microvasculature. As the permeability in the lung is controlled by both epithelial and ECs, coculture or organotypic cultures should be developed to explore this unique feature.

Although the role of the VEGF system in ALI can be dissected at the cellular and molecular levels and integrated with data from animal models, clinical investigations still hold the key to our understanding of this complicated system in ALI/ARDS. ARDS represents a complex syndrome that includes many subpopulations of patients. Multicenter clinical studies will be required to collect more data on the expression and function of VEGF and related molecules according to the clinical settings. Combined efforts from basic scientists and clinicians will be necessary for the development of specific treatment strategies to better manage the pulmonary edema in patients with ALI/ARDS.

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