RELATIONSHIP BETWEEN LUNG TISSUE EXPRESSION AND PLASMATIC LEVEL OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

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Abstract

PURPOSE: To investigate the vascular endothelial growth factor (VEGF) expression in the lung tissue of acute respiratory distress syndrome (ARDS) patients and also the VEGF plasmatic levels of these patients.

METHODS: We realized a prospective study including 10 patients diagnosed with ARDS. Lung specimens from ARDS patients were obtained by bronchoscopy or by autopsy in case of the deceased patients. The controls were 10 patients deceased from other causes than ARDS from whom necroptic pulmonary tissue samples. The determination of VEGF expression in the pulmonary tissue was performed using specific monoclonal antibodies VEGF, clones VG1 and JH 121. The controls were 10 patients deceased from other causes than ARDS from whom necroptic pulmonary tissue samples.

RESULTS: The ARDS etiology of the studied patients was mainly extrapulmonary (7 cases out of 10). Patients who died because of ARDS had a VEGF pulmonary expression significantly decreased compared to non-ARDS patients: 8.5 (4.1–9.9) versus 28.7 (9.5–48.6) (p < 0.001) (Fig. 1). The seric VEGF levels of ARDS patients were raised [230 pg/ml] compared to non-ARDS patients ([131 pg/ml] (p < 0.001). Alveolar macrophages were immunopositive in both groups. No significant statistical differences were noted between the two groups with regard to age, gender, period of ARDS condition, number of ICU days.

CONCLUSION: A decreased alveolar type II cells, induced by apoptosis was noticed in ARDS evolution, therefore reducing the VEGF production in the alveolar space and also contributing to the decrease in lung perfusion, but also to the consecutive increase of VEGF plasmatic level.

CLINICAL IMPLICATIONS: The over-expression of pulmonary VEGF, leads to increased vascular pulmonary permeability and consequently pulmonary oedema. Nevertheless, the VEGF expression in human alveolar epithelial cells also facilitates neovascularization, contributing to endothelial injury repair. Low VEGF pulmonary levels were associated with the severity of ARDS, whereas high VEGF levels were associated with the recovery from ARDS, signalling the role of VEGF in the pulmonary injury repairing process.

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Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI), remains a devastating condition with a high mortality. It is characterised by alveolar injury and increased pulmonary vascular permeability. Vascular endothelial cell growth factor (VEGF) was identified by its properties to increase permeability and act as a cellular growth factor, hence its potential for a key role in the pathogenesis of ALI/ARDS. This review describes the basic biology of VEGF and its receptors as an essential prerequisite to discussing the available and sometimes paradoxical published data, before considering a paradigm for the role of VEGF in the human lung.

A healthy alveolar capillary membrane is essential for the gas exchange function of the human lung. Injury and loss of this tissue contributes to the pathology of many forms of lung disease of which the archetypal example would be the most extreme form of acute lung injury (ALI)—namely, acute respiratory distress syndrome (ARDS). An understanding of the mechanisms involved in the injury and repair of this tissue would have significant impact on the clinical management and treatment of this and many other lung conditions. Vascular endothelial growth factor (VEGF) was originally identified by its properties as both a permeogen and a mitogen, key elements in the function of the alveolar capillary membrane, leading to interest in its role in many forms of lung disease, particularly ARDS. Intriguingly, in healthy human subjects VEGF protein levels are highly compartmentalised, with the directly oxygenated alveolar levels 500 times higher (2 nM) than plasma levels, despite VEGF production being closely associated with a hypoxia response element. These levels in normal alveoli are significant, twice the concentration previously shown to induce permeability and mitogenesis (particularly angiogenesis) in vivo. However, in healthy lung these processes are extremely restricted. These data suggest an important persistent or additional function of VEGF within the human lung that has not yet been characterised, which is normally tightly regulated and which goes awry in ALI/ARDS.

Current in vitro work, animal models, and clinical studies are somewhat conflicting as to the role of VEGF in ALI/ARDS. The attempt to resolve these apparent conflicts in the available data by proposing a unifying hypothesis for the role of VEGF in injured lung pertinent to ALI/ARDS—namely, that VEGF protects the alveolar epithelium with a role in repair following lung injury, but causes fluid flux across the exposed endothelium if the alveolar capillary membrane is functionally breached.

**ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)**

ARDS, the most extreme form of ALI, was first described in 1967. It is more common than is perhaps appreciated with an estimated incidence of 75 per 100 000 in some studies. It is estimated to account for nearly 15 500 deaths annually in the USA, roughly equal to the number of deaths due to HIV and emphysema, increasing to 74 500 if ALI is considered overall. ARDS continues to have a significant mortality of more than 35% despite recent improvements in ventilator strategies and in sepsis management. A host of conditions, including sepsis, trauma, aspiration, massive blood transfusion and burns, both direct and indirect insults, predispose to ARDS. However, exposure to a given “insult” does not guarantee that ARDS will follow; for example, there is a 40–60% risk of ARDS following Gram negative sepsis. Although the underlying mechanisms and factors governing susceptibility remain unclear, ARDS is characterised by alveolar epithelial injury and increased vascular permeability. Markers of both epithelial and endothelial injury have been correlated with outcome. An additional factor is the potential to induce damage by mechanical ventilation itself. Survival from ARDS requires resolution of these features and renewed integrity of the alveolar capillary membrane.

**BIOLOGY OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)**

To appraise and understand the published evidence in this area, it is essential to have some understanding of the basic biology of VEGF.

**Abbreviations:** AE, alveolar epithelial; ALI, acute lung injury; AP, activator protein; ARDS, acute respiratory distress syndrome; FLT, fms-like tyrosine kinase; HUVEC, human umbilical venous endothelial cell; LPS, lipopolysaccharide; NRP, neuropilin; VEGF, vascular endothelial growth factor; VEGF-R1, VEGF-R2, vascular endothelial growth factor receptor 1 and 2
VEGF

The superfamily of VEGF proteins consists of at least six members that are structurally and functionally related but with predominantly differing key roles. This review is confined to the importance of VEGF-A, termed VEGF throughout the text. These properties have led to investigation of this molecule in cancer, vascular diseases, chronic inflammatory disorders, and ALI as well as many other lung diseases including asthma, emphysema, pulmonary fibrosis, lung cancer, and pulmonary hypertension. VEGF is a 34–46 kDa glycoprotein that was first isolated from tumour cells but other cellular sources include macrophages, smooth cells, and epithelial cells. It is a potent angiogenic factor and critically regulates vasculogenesis such that embryos lacking a single VEGF allele have a lethal phenotype due to abnormal vascular development including that of the lung. It both induces vascular endothelial cell proliferation and promotes survival by induction of anti-apoptotic proteins bcl-2 and A1. VEGF increases microvascular permeability 20 000 times more potently than histamine. Targets for VEGF bioactivity outside the vascular endothelium include macrophages, type II pneumocytes, and monocytes for which it may be chemotactic. It also has a vasodilatory function.

VEGF isoforms

Alternate splicing of the VEGF gene (6p21.3) transcript leads to the generation of several splice variants (isoforms) of differing sizes, the subrecipe relating to the number of amino acids present. The predominant isoform is and most biologically active in the physiological state. The longer isoforms are cell associated (exons 6 and 7 have heparin binding activity allowing binding to the extracellular matrix) compared with the shorter diffusible isoforms. Plasmin, the acute phase protein, can also cleave the isoforms to form PL-VEGF, VEGF and VEGF. VEGF is the predominant isoform and most biologically active in the physiological state. The longer isoforms are cell associated (exons 6 and 7 have heparin binding activity allowing binding to the extracellular matrix) compared with the shorter diffusible isoforms. Plasmin, the acute phase protein, can also cleave the isoforms to form PL-VEGF, VEGF, and VEGF. VEGF is the predominant isoform and most biologically active in the physiological state.

VEGF-R1 and VEGF-R2

All VEGF isoforms bind to the tyrosine kinase receptors, VEGF receptor 1 (VEGF-R1) and VEGF receptor 2 (VEGF-R2). They were initially thought to be largely confined to vascular endothelium but have subsequently been detected elsewhere including activated macrophages, AE2 cells, and neutrophils. Hence, VEGF is capable of having its effect on both sides of the alveolar capillary membrane on both the epithelial and endothelial surfaces. There is evidence that the signal transduction cascades for VEGF-R1 and VEGF-R2 are different and, although VEGF-R1 has greater affinity for VEGF, VEGF-R2 is tyrosine phosphorylated much more efficiently upon ligand binding. VEGF-R2 is regarded as the main signalling receptor for VEGF bioactivity (angiogenesis, proliferation and permeability) and can cause proliferation in cells lacking VEGF-R1. VEGF-R2 knock-out mice fail to develop blood islands or organised blood vessels resulting in early death. VEGF-R2 also has a pro-survival function with anti-apoptotic effects on human umbilical venous endothelial cells (HUVECs). In contrast, VEGF-R1 rarely induces cellular proliferation in cells lacking VEGF-R2. This has led to the suggestion that VEGF-R1 may function mainly as a decoy receptor, although this is still contentious. Nevertheless, VEGF-R1→→ mice die between days 8.5 and 9.5 in utero from excessive proliferation of angioblasts, supporting a negative regulatory role on VEGF by VEGF-R1 at least during early development. In addition, targeted deletion of the tyrosine kinase domain but not the VEGF binding domain on VEGF-R1 does not cause death or obvious vascular defects, although it is required for some other functions such as monocyte chemotaxis. Alternate splicing leads to a soluble form of VEGF-R1 (sflt) which can act as an inhibitor of VEGF activity.

Neuropilins (NRP-1, 2)

By contrast, the neuropilins (NRP-1, NRP-2) are isoform-specific VEGF binding sites of different size and affinity to VEGF-R1 and VEGF-R2. They are expressed by endothelial cells in many adult tissues but lack the intracellular component containing tyrosine kinase activity and are regarded as VEGF co-receptors, being unable to signal themselves without the involvement of VEGF-R2. NRP-1 is isoform-specific, recognising exon 7 of VEGF (binding VEGF but not VEGF), and increases the effect of VEGF by enhancing its binding to VEGF-R2. This may also partially account for the greater mitogenic potency of VEGF compared with the VEGF isoform. Studies also support a role for NRP-1 in vasculogenesis and angiogenesis. NRP-1 knockout and over expressing mice both die prematurely from vascular defects. In contrast, NRP-2→→ are viable but do have absent or reduced lymphatic vessels and capillaries.

VEGF polymorphism

Several functional human VEGF polymorphisms have been described. Significant interindividual variations in plasma VEGF levels and gene expression related to the presence of polymorphism have been reported. In one study a CT substitution at position 936 distal to the start of translation in the untranslated region of the VEGF gene on chromosome 6 was associated with a 75% reduction in plasma levels in both heterozygotes and homozygotes in a Caucasian population. No such changes in plasma levels were detected in another genetic association study, but this may have been due to different racial populations. This polymorphism results in altered binding of the transcription factor activator protein 4 (AP-4), although whether the abolition of the AP-4 binding site is of specific relevance to the reduction in VEGF protein expression remains unclear. The effect of the CT genotype on intrapulmonary levels remains unknown at the current time.

VEGF AND THE ALVEOLAR SPACE

Studies of ARDS/ALI need to consider both sides of the alveolar capillary membrane (fig 1A). Isolated cellular studies of epithelial or microvascular endothelial cells give additional insight to animal models and clinical studies, as discussed below. In vitro studies have demonstrated an abundance of VEGF in lung tissue, especially in alveolar epithelium, including the A549 cell line and primary human cultured type II pneumocytes. Indeed, the highest levels of VEGF mRNA are found in animal and human lung, which suggests that the alveolar epithelium is the predominant source. Although the embryonic role of VEGF is undoubted, in all species studied to date adult lungs contain higher amounts of VEGF mRNA transcript than the developing lung. Changes in relative isoforms have also been observed with maturity, suggesting an ongoing role. VEGF-R1, NRP-1, and NRP-2 are all expressed in normal lung. Primary human type 2 alveolar epithelial (AE2) cells are known to express VEGF-R2, the main functioning VEGF receptor, which would facilitate an autocrine role in the air space for VEGF in addition to its well known paracrine effects on the vascular bed.

Studies suggesting a pathological role for VEGF in the alveolar space

The properties of VEGF described previously have led many workers to the hypothesis that VEGF would be solely...
Vascular endothelial growth factor and related molecules in acute lung injury

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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 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 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 (PlGF), 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). Due to its bioavailability and biological potency, VEGF165 is the predominant isoform 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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<tbody>
<tr>
<td>VEGF-A</td>
<td>Alveolar type II cells</td>
<td>VEGFR-1</td>
<td>Hypoxia</td>
<td>EC proliferation</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>Airway epithelial cells</td>
<td>VEGFR-2</td>
<td>Mechanical stretch</td>
<td>↑ Vascular permeability</td>
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<td>NRP-1</td>
<td>ROS</td>
<td>Angiogenesis</td>
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<td>VEGF-A</td>
<td>Macrophages</td>
<td></td>
<td>Glucose deprivation</td>
<td>Vasculogenesis</td>
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<td>VEGF-A</td>
<td>Neutrophils</td>
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<td>TGF-β1</td>
<td>Antiapototic for ECs</td>
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<td>VEGF-A</td>
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<td>IL-6</td>
<td>Migration of monocytes</td>
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<td>VEGF-A</td>
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<td>Endothelin</td>
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<td>(Heart)</td>
<td>VEGFR-1</td>
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<td>Pulmonary vascular remodeling</td>
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<td>VEGF-B</td>
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<td>NRP-1</td>
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<td>VEGFR-3</td>
<td>Proinflammatory cytokines</td>
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<td>PIGF</td>
<td>Alveolar type II cells</td>
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<td>PIGF</td>
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<td>Ang-1</td>
<td>Airway epithelial cells</td>
<td>Tie-2</td>
<td>Hypoxia</td>
<td>↓ Vascular permeability</td>
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<td>Ang-1</td>
<td>Interstitial cells</td>
<td>VEG-A</td>
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<td>Vasculogenesis</td>
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<td>Ang-2</td>
<td>Airway epithelial cells</td>
<td>Tie-2</td>
<td>Hypoxia</td>
<td>Antiapototic for ECs</td>
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<td>Ang-2</td>
<td>ECs (at sites of active vascular remodeling)</td>
<td>VEGF</td>
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<td>↑ Vascular permeability</td>
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<td>Ang-3/4</td>
<td>Interstitial cells</td>
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<td>↓ Vascular permeability</td>
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**Notes:**
- Ang, angiopoietin; EC, endothelial cells; NRP, neuropilin; ROS, reactive oxygen species; TGF, transform growth factor; HIF, hypoxia-induced factor; ↑, increase; ↓, decrease.

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**References:**

9. PlGF, placenta growth factor; Ang, angiopoietin; EC, endothelial cells; NRP, neuropilin; ROS, reactive oxygen species; TGF, transform growth factor; HIF, hypoxia-induced factor; ↑, increase; ↓, decrease.

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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-A (99). VEGF-E shows almost equal levels of mitotic activity on primary ECs and vascular permeability activity as VEGF (99).

VEGF-E was predominantly expressed in the endothelium of lymphatic vessels (64, 78).

The specific functions of VEGF-R1 are still under debate. Several lines of evidence suggest that VEGF-R1 may mediate vascular organization (45). Inactivation of the VEGF-R1 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 VEGF-R1 (8). VEGF-R2 is the major mediator of endothelial differentiation and proliferation (45). Studies using VEGF mutants that bind selectively to either VEGF-R1 or VEGF-R2 demonstrated that most of the angiogenic activities of VEGF as well as the effects of VEGF on permeability are mediated by VEGF-R2 (54).

VEGF binds to and activates VEGF-R1 and VEGF-R2 (141). VEGF-B selectively binds to VEGF-R1, by competing with VEGF (64, 84, 102). VEGF-C and VEGF-D bind to VEGF-R2 and VEGF-D on ECs (104). VEGF-C and VEGF-D also regulate the lymphatic ECs via VEGF-R2 and VEGF-R3 (65). Interestingly, in mice, VEGF-D binds to VEGF-R3 but not to VEGF-R2 (5). VEGF-R2 selectively binds to VEGF-R2 but not to VEGF-R1 (93, 99). PIGF specifically acts on VEGF-R1 (106).

VEGF receptors

The biological activity of VEGF is dependent on its interaction with specific receptors. Three VEGF receptors (VEGF-Rs) have been identified: VEGF-R1, VEGF-R2, and VEGF-R3 (43, 141). In addition, VEGF interacts with a family of coreceptors, the neuropilins (97, 114). Both VEGF-R1 and VEGF-R2 have seven extracellular immunoglobulin-like domains and a single tyrosine kinase transmembrane domain (118, 131) and are expressed on vascular ECs. VEGF-R1 is also expressed on activated macrophages, monocytes, placental trophoblasts, and renal mesangial cells (7, 24). VEGF-R1 immunoreactivity was found in bronchial epithelium and type II pneumocytes of adult mouse lungs (41). The finding of VEGF-R1 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 VEGF-R1 has been shown to inhibit VEGF activity (72). VEGF-R2 immunoreactivity was detected in Clara cells of adult mouse lungs (41). VEGF-R3 is predominantly expressed in the endothelium of lymphatic vessels (64, 78).

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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 VEGF-R2/VEGF-R3 heterodimers (35). Differences in the phosphorylation-site pattern between homo- and heterodimeric VEGF-Rs 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 VEGF-R1 and -2: VEGF-R1 activation by PIGF results in intermolecular transphosphorylation of VEGF-R2 and consequent amplification of VEGF-R2-driven angiogenesis (4). By binding to VEGF-R1, PIGF may also increase the proportion of VEGF available to activate VEGF-R2 and thus potentiate the VEGF-dependent angiogenesis. These observations suggest a “decoy” function for VEGF-R1, to prevent VEGF binding to VEGF-R2 and its
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 margaining neutrophils. Therefore, the epithelial-endothelial barrier is exposed to high concentration of VEGF, which may alter the state of adherens junction complexes (AJCs) 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.
ing inflammatory cells and/or by cells in other organs (Fig. 2, middle).

**VEGF AND ARDS**

Pulmonary injury in ARDS causes disruption of both sides of the alveolar-capillary interface, with consequential hyperinfiltration, 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, Maître 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 alveol 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 pathophysiological conditions, timing, and degrees of epithelial and endothelial 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

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Invited Review

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VEGF AND LUNG INJURY


Decreased VEGF concentration in lung tissue and vascular injury during ARDS

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ABSTRACT: Endothelial injury is an important prognostic factor in acute respiratory distress syndrome (ARDS). Decreased production of vascular endothelial growth factor (VEGF) in ARDS may favour vascular lesions, since VEGF promotes endothelial survival by inhibiting apoptosis.

This study sought to document low VEGF levels in lung tissue from ARDS patients, to determine whether the cause was injury to alveolar type II cells (the main pulmonary source of VEGF) and to evaluate the vascular consequences. Lung specimens were obtained by open biopsy or autopsy from 29 patients with severe ARDS (two survivors) and five controls.

As compared with controls, homogenates of lung tissue from ARDS patients contained less VEGF (median (interquartile range) ARDS 8.2 (4.7–12.2) versus controls 28.4 (9.9–47.1) ng g⁻¹ protein). Increased immunostaining with surfactant protein B was seen in ARDS lungs. Extensive cellular apoptosis (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling staining), including endothelial and alveolar type II cells, was demonstrated, and vascular bed density (CD31 immunostaining) decreased in ARDS lungs as compared with controls. VEGF levels were negatively correlated to apoptotic endothelial cell counts.

In conclusion, decreased vascular endothelial growth factor levels in lung tissue may participate in the decrease in lung perfusion in acute respiratory distress syndrome.

KEYWORDS: Alveolar type II cells, endothelial apoptosis, lung tissue

The detailed morphological description of acute respiratory distress syndrome (ARDS) by BACHOFEN and WEBEL [1] includes a decrease in lung capillary density. Conceivably, the resulting decrease in lung perfusion may lead to an increase in alveolar dead space, which has been recently shown to be a major prognostic factor during ARDS [2]. The pathophysiology of vascular lesions in ARDS is a challenging issue that has received little attention [3]. Recently, HAMACHER et al. [4] have demonstrated that bronchoalveolar lavage fluids of ARDS patients exhibit an ex vivo pro-apoptotic activity against pulmonary microvascular endothelial cells [4]. Conversely, a decrease in survival factors (anti-apoptotic) of the endothelium may be an alternative hypothesis. Low levels of vascular endothelial growth factor (VEGF) have been found in the lungs of patients with early ARDS [5, 6]. This VEGF decrease may contribute to the genesis of vascular injury, inasmuch as VEGF has been demonstrated to be a major survival factor for endothelium [7]. VEGF (or VEGF-A) is a highly conserved, dimeric, heparin-binding glycoprotein (molecular weight 46 kDa). At least four different VEGF transcripts resulting from alternate splicing of a single gene have been identified in human cells. VEGF121 and VEGF165 are secreted in a soluble form, whereas VEGF189 and VEGF206 remain cell-surface associated or are primarily deposited in the extracellular matrix. VEGF seems to specifically affect endothelial cell growth, survival and permeability. In the lung, VEGF is expressed primarily by epithelial cells and macrophages. VEGF is also produced by and stored in both human platelets and polymorphonuclear neutrophils. The biological activity of VEGF is dependent on interaction with specific receptors (VEGF-R1, 2, and 3), which are expressed not only by endothelial cells, but also by activated macrophages and alveolar type II epithelial cells. Endothelial survival is mediated via VEGF-R2/kinase insert domain-containing receptor (KDR) [8]. The aim of this study was two-fold: to determine whether the decrease in lung VEGF is related to epithelial injury, and whether it may be associated with increased endothelial apoptosis and decreased capillary density.
MATERIALS AND METHODS
Selection of acute respiratory distress syndrome and control patients
ARDS was defined as recommended by the international American-European consensus conference [9]. Lung specimens from ARDS patients were obtained by open lung biopsy or autopsy at the Sainte Marguerite Hospital, Marseille, France, and frozen immediately at -80°C for subsequent examination. Open lung biopsies were performed for clinical reasons, as detailed elsewhere [10]. The controls were patients undergoing thoracic surgery to treat nonsmall cell lung cancers, in whom lung tissue (normal appearance on microscopic examination) was sampled at a distance from the tumour area and processed as for the ARDS patients. The institutional review board for human studies (Sainte Marguerite Hospital, Marseille, France) approved the study protocol.

Histopathological examination
Sections that were 5 μm in thickness were cut from frozen lung tissue. All lung samples were stained (haemalum/eosin/saffron) for standard histopathological analysis in a blind fashion by a single histopathologist (F. Lange). Patients with ARDS were classified as having early or late disease, based on symptom duration at lung sampling (<5 days or ≥5 days) and on the presence of standard histological criteria for early or late ARDS [11]. Presence of an inflammatory exudate and hyaline membranes in the alveoli, denudation of the basement membrane, and oedema of the alveolar wall characterised the early exudative phase. The late fibroproliferative phase was characterised by type II pneumocyto proliferation associated with myofibroblast proliferation and/or fibrosis. This classification, based on both clinical and histological criteria, was used because it is more reliable than either set of criteria used alone; acute lung injury and resulting injury of the alveolar capillary wall can occur before the criteria for ARDS are met, and histological lesions are known to be patchy, with coexistence of early-stage and late-stage features in adjacent lung regions.

Protein expression in lung tissue homogenates
Quantitative determinations of VEGF and VEGF-R2/KDR protein expressions in tissue samples were performed using ELISAs, as recommended by the manufacturer (R and D Systems, Minneapolis, MN, USA). The VEGF ELISA kit recognises both VEGF121 and VEGF165 (soluble forms). Samples were solubilised by homogenisation in lysis buffer containing 20 mM hydroxyethyl piperazine ethane sulfonic acid, and 3-(3-cholaminopropyl diethylammonio)-1- propane sulfonate for VEGF-R2 measurement. Protein content was determined using the method of Bradford (Bio-Rad, Richemond, CA, USA). Vascular endothelial growth factor receptor 2
VEGF was detected using a specific mouse monoclonal antibody (MAB293; R and D Systems), and VEGF-R2 was detected using a mouse monoclonal antibody directed against the extracellular domain of the receptor (KDR-1; Sigma). Negative controls were run with nonimmune mouse immunoglobulin (Ig)G2B (R and D Systems) and IgG1 (Sigma). Tissue sections were placed in 1% paraformaldehyde and incubated in hydrogen peroxide (3% in PBS) to quench endogenous peroxidase. After rinsing, nonspecific antigenic sites were blocked by incubation with 2% normal goat serum (Sigma) in PBS with 0.05% Tween 20 for 30 min at room temperature. The slides were then incubated with anti-human SP-B antibody (at a dilution of 1:1,000) or negative control serum in a moist chamber for 2 h (room temperature). Subsequently, the slides were washed in PBS with 0.05% Tween 20, and then incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Catalog Laboratories, Burlingame, CA, USA) and revelation substrate (diaminobenzidine (DAB) chromogen) in the dark for 7 min. Tissue sections were counterstained with haematoxylin. All tissue sections were examined using a light microscope (final magnification 400 ×), and the number of positive cells was counted in 400 fields taken at random. Since the degree of tissue distension can vary across lung specimens, to ensure that the surface area assessed was the same in all specimens, the same number of alveoli in each tissue sample was evaluated.

Proliferating cell nuclear antigen
Proliferating nuclei were identified by staining for proliferating cell nuclear antigen (PCNA) with monoclonal antibody PC10 (PCNA staining kit; Zymed, San Francisco, CA, USA).

Vascular endothelial growth factor and vascular endothelial growth factor receptor 2
Endothelial cell nuclear antigen
Apoptosis was assessed by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) method. Briefly, apoptotic fragments were detected by adding digoxigenin-labelled nucleotides with terminal deoxynucleotidyl transferase using the APOPDETECT Plus Peroxidase in situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD, USA). The slides were deparaffinised and incubated in hydrogen peroxide (3% in PBS) to quench endogenous peroxidase. After rinsing, nonspecific antigenic sites were blocked by incubation with 2% normal goat serum (Sigma) in PBS with 0.05% Tween 20 for 30 min at room temperature. The slides were then incubated with anti-human SP-B antibody (at a dilution of 1:160) and negative control serum, in a moist chamber for 2 h (room temperature). The slides were then incubated with biotinylated goat anti-rabbit Ig (Sigma) as a secondary linking antibody, and labelled with peroxidase-conjugated streptavidin (Sigma). DAB chromogen was used to visualise the antibody, and tissues were counterstained with haematoxylin.

Immunohistochemistry

Apoptosis
Alveolar type II epithelial cells were detected using a specific rabbit polyclonal antibody directed against surfactant protein (SP)-B (Chemicon, Temecula, CA, USA), and negative controls were run with nonimmune rabbit serum (Sigma, St. Louis, MI, USA). The current authors chose to assess SP-B because this protein is more specific of type II alveolar epithelial cells than SP-A, and SP-C is known to be downregulated in animal models of acute lung injury. Tissue sections were placed in methanol at -20°C, and then incubated in hydrogen peroxide (3% in PBS) to quench endogenous peroxidase. After rinsing, nonspecific antigenic sites were blocked by incubation with 2% normal goat serum (Sigma) in PBS with 0.05% Tween 20 for 30 min at room temperature. The slides were then incubated with anti-human SP-B antibody (at a dilution of 1:1,000) or negative control serum in a moist chamber for 2 h (room temperature). Subsequently, the slides were washed in PBS with 0.05% Tween 20, and then incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Catalog Laboratories, Burlingame, CA, USA) and revelation substrate (diaminobenzidine (DAB) chromogen) in the dark for 7 min. Tissue sections were counterstained with haematoxylin. All tissue sections were examined using a light microscope (final magnification 400 ×), and the number of positive cells was counted in 400 fields taken at random. Since the degree of tissue distension can vary across lung specimens, to ensure that the surface area assessed was the same in all specimens, the same number of alveoli in each tissue sample was evaluated.

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Vascular endothelial growth factor and ARDS

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Kit (Qbiogen Inc., Carlsbad, CA, USA), according to the manufacturer’s instructions. Nuclei of apoptotic cells appeared brown and granular. To quantify the extent of microvascular endothelial apoptosis, co-staining with an anti-human mouse monoclonal antibody to CD31 (WM-59; PharMingen, San Diego, CA, USA) was used, which recognises the endothelial surface marker platelet/endothelial cell adhesion molecule 1. TUNEL sections were incubated with 2% normal goat serum (in PBS with 0.05% Tween 20), and, subsequently, with the primary anti-CD31 antibody (1:100,000 dilution, in PBS with 1% BSA) overnight at 4°C. Mouse nonimmune IgG1 (Sigma) was used as the negative control. Biotinylated goat anti-mouse antibodies (Sigma) were applied for 30 min at room temperature, followed by avidin-biotin peroxidase (Sigma) complexes for 30 min (room temperature). True Blue Peroxidase substrate was used as the final chromogen (KPL Laboratories, Gaithersburg, MD, USA). No counterstaining was used, to avoid possible interference with the specific dark-blue immunostaining of the endothelial cell surface. Double staining was considered positive when specific cells displayed a brown nucleus with a surrounding blue-to-black membrane-immunoreactive pattern. Positive cells were counted in 10 randomly selected areas (final magnification 400 ×).

Image analysis
A charge-coupled device Iris camera (CDD Iris; Sony France, Paris, France), coupled with an optical microscope (Leitz Laborlux, Wetzlar, Germany), was used to view the sections and to digitise colour images to a PC host computer. CD31 and TUNEL staining were estimated with Perfect Image software (ClaraVision, Orsay, France), which differentiates colours based on their red–green–blue proportions with a ±10% variation. At least 10 fields per section were analysed, with approximately the same number of alveoli. Results are given as the ratio (%) of the blue- (CD31) or brown- (TUNEL) stained surface area over the total field surface area (CD31 or TUNEL staining relative to total field surface).

Statistical analysis
Results are presented as medians (interquartile range) in the text. Data are presented as box-plots indicating the median and the 10th, 25th, 75th and 90th percentiles. Differences between groups were estimated using the Kruskal-Wallis test with post hoc Mann-Whitney analysis. To assess the possible influence of VEGF on pulmonary microcirculation, the relationships linking VEGF levels to endothelial quantification and apoptosis were assessed by Spearman’s rank correlation. p-Values < 0.05 were considered statistically significant.

RESULTS
Patient characteristics
A total of 29 ARDS patients were studied (20 males and nine females; median age 64 yrs (50–73)). Their median acute physiology and chronic health evaluation II score at admission was 16.9 (13.6–23.6). Open lung biopsy was performed as a diagnostic procedure in 15 patients and immediately after death in 14 patients.

At the time of lung tissue sampling, the arterial oxygen tension/inspiratory oxygen fraction ratio was <200 mmHg in 22 patients and 200–300 mmHg in seven patients. The cause of ARDS was direct lung injury in 17 patients (related to infection in 11 and to inhalation in six) and indirect lung injury in 12 patients (nonpulmonary sepsis in seven, nonseptic shock in four, and pancreatitis in one). The six patients who died within 5 days after ARDS onset were classified as having acute ARDS, whereas the remaining 23 patients were classified as having late ARDS; histological findings confirmed the classification in every case. Survival was only 7% (two out of 29) in this selected population.

The control group was composed of five patients (all males; median age 65 years (54–70)).

Vascular endothelial growth factor expression in lungs from acute respiratory distress syndrome and control patients
VEGF levels in the lung homogenates were lower in the ARDS patients than in the controls: 8.2 (4.7–12.2) versus 28.4 (9.9–47.1) ng·g⁻¹ protein, respectively (p = 0.03). There was no difference between the early and late ARDS subgroups (fig. 1). Results were similar when VEGF concentrations were normalised per gram of lung weight. In the controls, immunostaining for VEGF (fig. 2) labelled the bronchiolar cells and alveolar macrophages and strongly labelled some alveolar type II epithelial cells. Alveolar type I epithelial cells were negative. In the ARDS patients, staining was highly heterogeneous. Bronchiolar and alveolar epithelial cells were negative in some areas and positive in others, even within a tissue section. In early ARDS tissue samples, inflammatory cells were also positive.

Vascular endothelial growth factor receptor 2 expression in lungs from acute respiratory distress syndrome and control patients
There was no significant difference between VEGF-R2 concentrations measured by ELISA in lung homogenates from ARDS and control patients (116 (78–157) versus 156 (131–191) ng·g⁻¹ protein). No difference was found between early and late ARDS patients.

Immunostaining for VEGF-R2 was noted in endothelial cells in the control group; however, not all endothelial cells were
Vascular endothelial growth factor gene polymorphism and acute respiratory distress syndrome

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Acute respiratory distress syndrome (ARDS), the most extreme form of acute lung injury, continues to have a significant mortality of at least 35% of patients despite improvements in the management of sepsis and ventilatory support. Death in these patients is usually secondary to the physiological derangement of multiorgan rather than respiratory failure per se. Non-cardiogenic pulmonary oedema is a characteristic feature of ARDS. The potent effects of vascular endothelial growth factor (VEGF) on vascular endothelium as both a pericellular and mitogen have led to investigation of its potential role in the development of ARDS. In the early stages of this condition we have previously reported that plasma VEGF levels rise and intrapulmonary levels fall with normalisation of both during recovery. These changes in intrapulmonary VEGF have also been noted by other investigators in ARDS and high altitude pulmonary oedema. In normal subjects VEGF is compartmentalised with levels within the alveolar space 500 times that detected in the plasma, suggesting a physiological role which becomes disrupted when injury of the alveolar capillary membrane occurs and ARDS ensues.

VEGF is among those polymorphic genes with a potential role in ARDS, genetic polymorphism being a potential explanation for the low incidence of ARDS within the large numbers of subjects “at risk” of developing this syndrome. A CT substitution at position 936 distal to the start of translation in the +untranslated region of the VEGF gene on chromosome 6p21.3 has been associated with reduced plasma levels in both CT and TT genotypes in normal subjects. However, it is unclear how this relates to intrapulmonary VEGF, the probable major site of production. This substitution results in altered binding of the transcription factor activating enhancer binding protein 4 (AP-4), although whether the abolition of the AP-4 binding site results directly in the reduction in VEGF protein expression remains unknown. The role of this polymorphism has been studied in both COPD and sarcoidosis; no association was found with the former and a negative association with the latter. However, no study to date has examined the relationship between this allele and ARDS or any other critical illness.

We hypothesised that the VEGF+936 CT polymorphism would contribute to genetic susceptibility to ARDS. We have examined the association between the VEGF+936 genotype and the development and severity of ARDS, using ventilated “at risk” controls and normal healthy subjects for comparison to ensure any association was with ARDS per se rather than critical illness.

METHODS

Subjects
A total of 137 normal subjects and 220 ventilated patients were prospectively included in this single centre study. ARDS patients fulfilled the 1994 American-European Consensus Conference definition at any time during their intensive care admission (n = 117). “At risk” patients were ventilated and had similar degrees of physiological disturbance to those with ARDS using previously described criteria but did not fulfil the ARDS criteria at any time during their intensive care admission. Patients with trauma were considered to be at risk for ARDS if they were intubated or on mask continuous positive airway pressure (CPAP) and had either two or more of the following: multiple fractures (two or more fractures of femur, tibia, humerus, or stable pelvis); unstable pelvic fracture; pulmonary contusion; or massive transfusion (>15 units in 24 hours). Patients with suspected sepsis were considered to be at risk for ARDS if they had: (1) two or more of the following: temperature ≥39°C or ≤36°C; white blood cell count >14×10⁹/l or <4×10⁹/l, a positive blood culture, or a known or strongly suspected source of infection; and (2) two or more of the following: systemic vascular resistance <800 dyne.s/cm²; unexplained hypotension (systolic blood pressure <90 mm Hg for more than 1 hour); ongoing metabolic acidosis with anion gap >20 mmol/l; inotropic support to maintain systolic blood pressure >90 mm Hg; or a
Figure 1 (abstract P53)

Oleic acid infusion (0.20 mL/kg)

Supine  |  Sup-Ilop  |  Prone  |  Pron-Ilop
T3      |  T4        |  T5      |  T6

Basal  |  ARDS  
T1      |  T2      

Supine  |  Sup-Ilop  |  Prone  |  Pron-Ilop
4 cmH2O PEEP |  8 cmH2O PEEP |  Ilop  |  Ilop

Ilop, iloprost; Pron, prone; Sup, supine.

Different title

P53
Effects of inhaled iloprost on acute respiratory distress syndrome in prone and supine positions

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Introduction In several studies it has been shown that inhaled pulmonary vasodilators (NO and iloprost) can decrease the pulmonary hypertension and also improve the oxygenation during acute respiratory distress syndrome (ARDS) [1]. We investigated the effects of prone and supine positioning on the effects of inhaled iloprost in an animal-ARDS model.

Methods After approval of the animal ethics committee, 10 pigs were anesthetized and intubated. Invasive systemic and pulmonary arterial catheterizations were performed (T1). ARDS was induced in all animals with the infusion of oleic acid (0.15 to 0.30 mL/kg). The study design is shown in Figure 1. Hemodynamic and respiratory parameters and ventilation parameters were measured; arterial and mixed venous blood samples were drawn; and were recorded in T1 to T6. Pigs were ventilated in volume-controlled ventilation mode with FiO2 100%, with 4 cmH2O positive end-expiratory pressure (PEEP) in the beginning and with 8 cmH2O PEEP after induction of ARDS. Statistical analysis was made with Student’s t-test, repeated measures of ANOVA (with Tukey as the post-hoc test) and paired t-tests. P<0.05 was significant.

Results There was no significant difference between the sequences. Iloprost decreased the mean pulmonary arterial pressure in both supine (37 vs. 31 mmHg) and prone (38 vs. 29 mmHg) positions significantly, but there was no significant difference between both positions. Prone position was associated with an improvement in oxygenation compared with supine position both with or without iloprost application. There was no spillover effect of iloprost.

Conclusions Iloprost decreased pulmonary arterial pressures in both positions. On the other hand, the prone position improved oxygenation. The decrease in pulmonary arterial pressures and improvement in oxygenation was better in prone position + iloprost; however, these findings were not statistically significant.

Reference

P54
Decreased vascular endothelial growth factor expression in lung tissue during acute respiratory distress syndrome

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Introduction Endothelial injury is an important prognostic factor in acute respiratory distress syndrome (ARDS) [1, 2]. Vascular endothelial growth factor (VEGF) plays a critical role in endothelial destruction and angiogenesis [3]. The expression of VEGF in ARDS varies, depending on epithelial and endothelial damage [4, 5]. The objective of this study was to investigate the expression of VEGF in lung tissue from ARDS patients.

Methods Lung specimens were obtained by autopsy from patients with severe ARDS and were compared with a control group of non-ARDS patients autopsied. All lung samples were stained for standard histopathological analysis and for immunohistochemical methods using a specific mouse monoclonal antibody.

Results Compared with expression in non-ARDS control individuals, pulmonary expression of VEGF was significantly decreased (P<0.001) in ARDS patients. Alveolar macrophages were similarly immunopositive in both groups. No differences were noted with regard to the individual patient’s characteristics (age, gender, period of ARDS condition, number of ICU days).

Conclusions A decrease in alveolar type II cellularity, due to apoptosis, has been observed during ARDS that may reduce the production of VEGF in the alveolar space and may participate in the decrease in lung perfusion.

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References
Introduction Mechanical ventilation (MV) induces an inflammatory response that contributes to lung injury such as in acute lung injury or acute respiratory distress syndrome. The efferent vagus nerve can limit the inflammatory response via the α7 nicotinic acetylcholine receptor (α7nAChR), the so-called cholinergic anti-inflammatory pathway. The aim of this study was to evaluate the effect of the selective α7nAChR agonist GTS-21 on pulmonary and systemic inflammation and lung injury induced by MV using clinically relevant ventilator settings.

Methods C57BL6 mice (n = 40) were intraperitoneally injected with 8 mg/kg GTS-21 or placebo, after which they were mechanically ventilated for 4 hours (tidal volume 8 ml/kg; positive end-expiratory pressure 1.5 cm H2O; FiO2 0.45). Untreated, not mechanically ventilated mice were used as controls. Arterial blood gases were obtained at the end of the experiment and TNFα levels in GTS-21-treated mice were unaffected by GTS-21. MV strongly increased TNFα (53.9 ± 12.5 vs. 79.1 ± 5.6 pg/mg protein; P = 0.04). Similarly, in lung homogenates a distinct trend was observed towards lower TNFα levels in GTS-21-treated mice (53.9 ± 12.5 vs. 79.1 ± 5.6 pg/mg protein; P = 0.06). IL-10 levels were unaffected by GTS-21. MV strongly increased TNFα mRNA expression in lungs of placebo animals (21-fold compared with controls); this was significantly lower in GTS-21-treated mice (11-fold compared with controls; P = 0.02). IL-10 mRNA expression was similar in GTS-21-treated and placebo animals. MV strongly increased TNFα (53.9 ± 12.5 vs. 79.1 ± 5.6 pg/mg protein; P = 0.04). Similarly, in lung homogenates a distinct trend was observed towards lower TNFα levels in GTS-21-treated mice (53.9 ± 12.5 vs. 79.1 ± 5.6 pg/mg protein; P = 0.06). IL-10 levels were unaffected by GTS-21. MV strongly increased TNFα mRNA expression in lungs of placebo animals (21-fold compared with controls); this was significantly lower in GTS-21-treated mice (11-fold compared with controls; P = 0.02). IL-10 mRNA expression was similar in GTS-21-treated and placebo animals.

Conclusions MV with clinically relevant ventilator settings results in activation of the immune system. GTS-21 inhibits proinflammatory cytokine production while not affecting the anti-inflammatory cytokine IL-10. The reduced alveolar–arterial gradient in GTS-21-treated animals indicates attenuation of lung injury. In conclusion, limiting the inflammatory response appears to reduce lung injury, and therefore the cholinergic anti-inflammatory pathway may represent new treatment options for MV-induced lung injury.

P56
Usefulness of soluble E-selectin in the clinicopathologic assessment of acute lung injury/acute respiratory distress syndrome
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Introduction A retrospective observational study was conducted to evaluate whether the plasma level of soluble E-selectin [1] might be a specific pathologic marker of acute lung injury/acute respiratory distress syndrome (ALI/ARDS).

Methods The data of 52 critically ill patients admitted to the ICU with systemic inflammatory response syndrome and initiated on mechanical ventilation were retrospectively evaluated.

Results The plasma levels of soluble E-selectin determined within 24 hours of admission were significantly correlated with the Sequential Organ Failure Assessment scores determined within 24 hours of admission. Furthermore, the scores for both respiratory failure (evaluated by the PaO2/FiO2 ratio) and liver dysfunction (evaluated by the serum bilirubin value) in the Sequential Organ Failure Assessment scoring system were significantly correlated with plasma levels of soluble E-selectin. In relation to respiratory failure, the plasma level of soluble E-selectin was higher in patients with ALI/ARDS than in those without (Figure 1), and receiver operating characteristic analysis revealed that this parameter might be a specific marker of ALI/ARDS (Figure 2).

Conclusions Soluble E-selectin might be specific and useful marker for the clinicopathologic assessment of ALI/ARDS in critically ill patients with systemic inflammatory response syndrome. However, further investigation is clearly needed to determine whether soluble E-selectin can indeed predict the development of ALI/ARDS.

Reference

Figure 1 (abstract P56)
VASCULAR ENDOTHELIAL GROWTH FACTOR IS AN INDEPENDENT PROGNOSTIC MARKER IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME?

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Introduction: Vascular endothelial growth factor (VEGF) is a potent angiogenic and endothelial factor which is abundantly expressed in the normal lung.

Hypothesis: The purpose of this study was to assess the VEGF levels in lung tissue and plasma from acute respiratory distress syndrome (ARDS) patients, to determine if this factor could be an independent marker of outcome in patients with ARDS.

Methods: Plasma and tissue samples were prospectively collected from 20 patients who met the American—European Consensus Conference definition for ARDS (1) within 6 hours after intubation (VEGF plasmatic and tissue samples) and at the day of extubation (VEGF plasmatic) or postmortem (lung tissue). Plasmatic marker of angiogenesis (VEGF 165) were measured by ELISA. Lung specimens were obtained by bronchoscopic biopsy or by open necroptic biopsy. All lung samples were stained for standard histopathological analysis and for immunohistochemical methods using a specific mouse monoclonal antibody. Biomarker levels were compared between survivors (n=12), non-survivors (n=8) and control group (n=10).

Results: In the early stages of ARDS, plasma VEGF levels rise (non-ARDS patients; mean value: 141 pg/ml, SD: 47.19, ARDS patients; mean value: 230 pg/ml, SD: 64.80. p<0.001) and intrapulmonary levels fall (non-ARDS patients; mean value: 30.7%, SD: 8.95. ARDS patients; mean value: 8.46%, SD: 1.70; p<0.001), with normalization of both during recovery compared with plasmatic level and expression in non-ARDS control (Fig. 1).

Conclusions: These findings show that the initial phase of ARDS is associated with a decrease in VEGF in the lung and with an increase in the plasma. This downregulation may represent a protective mechanism aimed at limiting endothelial permeability and may participate in the decrease in capillary number that is observed during early ARDS. Persistent elevation of plasmatic VEGF over time predicts poor outcome.

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VEGF levels in the alveolar compartment do not distinguish between ARDS and hydrostatic pulmonary oedema

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ABSTRACT: Although overexpression of vascular endothelial growth factor (VEGF) 165 in the lung causes pulmonary oedema, its role in human acute lung injury (ALI) is unclear. VEGF levels are reported to be lower in bronchoalveolar lavage from ALI patients compared with normals, but these studies did not include a comparably ill control group with noninflammatory pulmonary oedema.

The current authors hypothesised that VEGF levels in pulmonary oedema fluid would be lower in ALI patients compared with control patients with severe hydrostatic pulmonary oedema. VEGF was measured in pulmonary oedema fluid and plasma from 56 patients with ALI and 46 controls with severe hydrostatic pulmonary oedema.

Pulmonary oedema fluid levels of VEGF did not differ between patients with hydrostatic oedema (median 799 pg mL\(^{-1}\), interquartile range (IQR) 226–2,281) and ALI (median 507, IQR 0.8–1,031). Plasma levels were also the same (median 20.5 pg mL\(^{-1}\), IQR 0–152 versus 4.8, IQR 0–99.8). There was no association between plasma or oedema fluid VEGF levels and outcomes including mortality.

Vascular endothelial growth factor levels in pulmonary oedema fluid were depressed both in acute lung injury and hydrostatic pulmonary oedema. The decrease in air space concentrations of vascular endothelial growth factor in acute lung injury may not be a function of the degree of lung injury, but rather may result from alveolar flooding.

KEYWORDS: Acute pulmonary oedema, acute respiratory distress syndrome

Vascular endothelial growth factor (VEGF) is an endothelial-specific mitogen that can induce endothelial permeability. There are four VEGF products of alternative splicing, VEGF 121, VEGF165, VEGF189 and VEGF206 [1]. VEGF121 and VEGF165 are secreted and have both mitogenic and permeability-inducing properties. VEGF165 is the predominant form in humans. Acute overexpression of VEGF165 in the lung causes pulmonary oedema and increased lung vascular permeability [2]. However, the role of VEGF in the pathogenesis of human acute lung injury (ALI) is unclear. Furthermore, the factors that modulate the release of VEGF in the lung are not well defined.

The normal lung is rich in VEGF [3]. The primary source of VEGF is alveolar epithelial cells [4] and levels of VEGF in the epithelial lining fluid are 500-fold higher than plasma levels [5]. In experimental studies, exposure to hypoxia decreased VEGF expression in the rat lung [6]. Conversely, exposure to hypoxia stimulates VEGF expression in alveolar epithelial cells in both in vitro and in vivo models [7]. These findings suggest that VEGF levels might be elevated in the acutely injured alveolus as a response to hypoxia.

However, clinical studies have not supported this hypothesis. Several groups have measured VEGF in the plasma and bronchoalveolar lavage fluid (BAL) from patients with ALI and acute respiratory distress syndrome (ARDS). Levels of VEGF in the plasma were higher in ARDS patients compared with normal controls [8], but levels of VEGF in BAL were lower in acute ARDS compared with normal controls, mechanically ventilated controls without pulmonary oedema or at-risk patients. Furthermore, as the acute lung injury resolved, levels of VEGF in BAL rose [9, 10]. One possible explanation for this finding is that alveolar epithelial injury is the primary modulator of alveolar levels of VEGF in the acutely injured lung. However, a comparably ill