

OCCASIONAL REVIEW

Vascular endothelial growth factor (VEGF) in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): paradox or paradigm?

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Thorax 2006;61:621–626. doi: 10.1136/thx.2005.040204

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.

the role of VEGF in ALI/ARDS. We 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 1967.¹ It is more common than is perhaps appreciated with an estimated incidence of 75 per 100 000 in some studies.⁶ It is estimated to account for nearly 16 500 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.⁷ ARDS continues to have a significant mortality of more than 35% despite recent improvements in ventilator strategies and in sepsis management.⁸ 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.⁹ Although the underlying mechanisms and factors governing susceptibility remain unclear, ARDS is characterised by alveolar epithelial injury and increased vascular permeability.⁶ Markers of both epithelial and endothelial injury have been correlated with outcome.^{6 10–12} An additional factor is the potential to induce damage by mechanical ventilation itself.^{13 14} 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

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).¹ 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.^{2 3} 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.^{4 5} These levels in normal alveoli are significant, twice the concentration previously shown to induce permeability and mitogenesis (particularly angiogenesis) *in vivo*.³ 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

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Received 20 May 2005
Accepted
20 December 2005

VEGF

The superfamily of VEGF proteins consists of at least six members that are structurally and functionally related but with predominantly differing key roles.¹⁵ 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.^{2, 16} 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.¹⁷ It both induces vascular endothelial cell proliferation and promotes survival by induction of anti-apoptotic proteins bcl-2 and A1.^{18–21} VEGF increases microvascular permeability 20 000 times more potently than histamine.^{2, 3} Targets for VEGF bioactivity outside the vascular endothelium include macrophages, type II pneumocytes, and monocytes for which it may be chemotactic.^{22–26} It also has a vasodilatory function.²⁷

VEGF isoforms

Alternate splicing of the VEGF gene (6p21.3) transcript leads to the generation of several splice variants (isoforms) of differing sizes, the subscript relating to the number of amino acids present (VEGF₁₂₁, VEGF₁₄₅, VEGF₁₄₈, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉ and VEGF₂₀₆).^{28–30} VEGF₁₆₅ is the predominant isoform and most biologically active in the physiological state.³¹ 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.²⁹ Plasmin, the acute phase protein, can also cleave the isoforms to form PL-VEGF₁₁₀ and a recently identified isoform, VEGF_{165b}, is inhibitory in function and may not be detected in standard VEGF assays necessitating reappraisal of current VEGF data.³² The mechanisms by which splicing occurs and is regulated are poorly understood.

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).^{15, 31, 33} They were initially thought to be largely confined to vascular endothelium but have subsequently been detected elsewhere including activated macrophages, AE2 cells, and neutrophils.^{22–25, 34–36} 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.^{22, 31} 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.^{37, 38} VEGF-R2 knockout mice fail to develop blood islands or organised blood vessels resulting in early death.³⁹ VEGF-R2 also has a pro-survival function with anti-apoptotic effects on human umbilical venous endothelial cells (HUVECs).⁴⁰ In contrast, VEGF-R1 rarely induces cellular proliferation in cells lacking VEGF-R2.^{26, 41–44} 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.^{45, 46} 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.^{26, 42} Alternate splicing leads to a soluble form of VEGF-R1 (*sflt*) which can act as an inhibitor of VEGF activity.⁴⁷

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.^{48, 49} 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 VEGF₁₆₅ but not VEGF₁₂₁), and increases the effect of VEGF₁₆₅ by enhancing its binding to VEGF-R2.⁵⁰ This may also partially account for the greater mitogenic potency of VEGF₁₆₅ compared with the VEGF₁₂₁ 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.^{51, 52} In contrast, NRP-2^{−/−} are viable but do have absent or reduced lymphatic vessels and capillaries.^{53, 54}

VEGF polymorphism

Several functional human VEGF polymorphisms have been described.^{55–57} Significant interindividual variations in plasma VEGF levels and gene expression related to the presence of polymorphism have been reported.^{58–60} In one study a CT substitution at position 936 distal to the start of translation in the 3′-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.⁵⁵ No such changes in plasma levels were detected in another genetic association study, but this may have been due to different racial populations.⁶¹ 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.⁵⁵ 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.^{62–65} Indeed, the highest levels of VEGF mRNA are found in animal and human lung, which suggests that the alveolar epithelium is the predominant source.^{62, 66} 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.⁶⁷ VEGF-R1, NRP-1, and NRP-2 are all expressed in normal lung.^{25, 68} 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.^{15, 24}

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

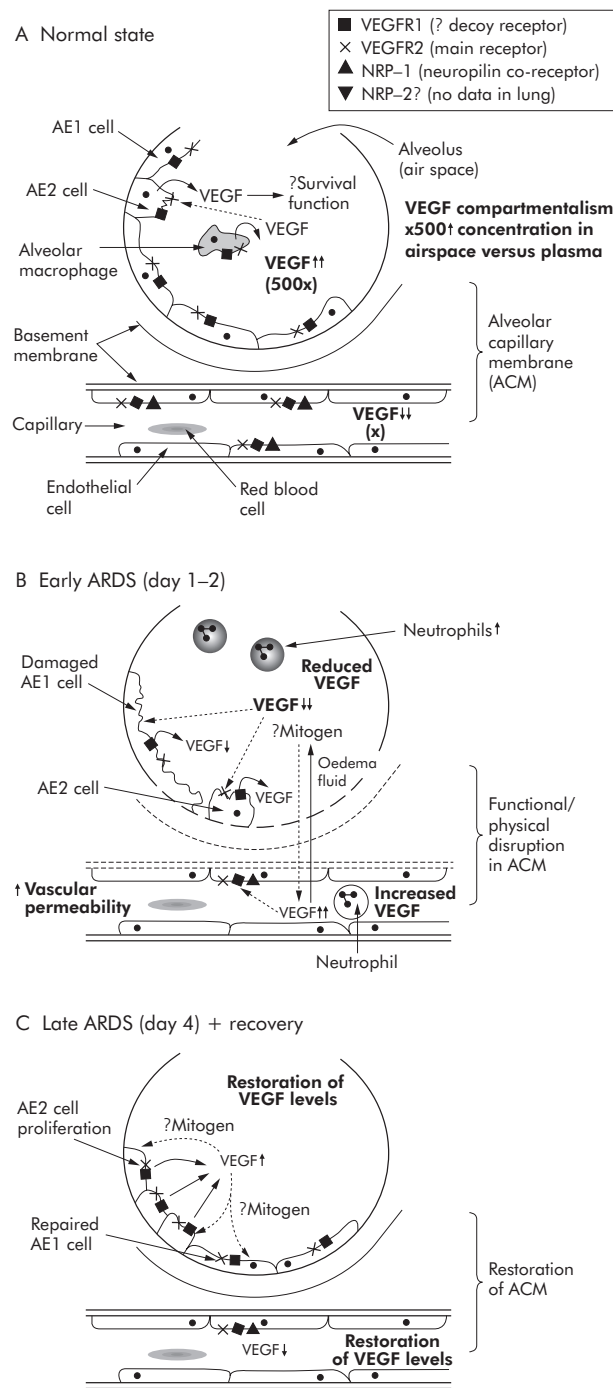


Figure 1 Diagrammatic representation of the human alveolar capillary unit in (A) normal healthy subjects, (B) subjects with lung injury, and (C) during the recovery phase.

pathological in the lung. Certainly, VEGF protein is secreted by alveolar cell-like cell lines in response to a number of pro-inflammatory stimuli potentially involved in ARDS, including lipopolysaccharide (LPS), neutrophil elastase and keratinolytic growth factor.⁶⁴ One influential early study of adenoviral VEGF₁₆₅ delivery to murine lung showed non-cardiogenic pulmonary oedema and increased pulmonary capillary permeability on the basis of histology, lung weight wet/dry ratios, increased albumin permeability, and Evans blue dye assay.⁶⁹ A subsequent study in VEGF₁₆₄ +/- mice targeted to respiratory epithelial cells demonstrated

pulmonary haemorrhage, endothelial destruction, and alveolar remodelling in an emphysema-like phenotype, although surfactant protein-B production was not affected which suggests that the primary effect may have been vascular disruption.⁷⁰ In more specific ALI/ARDS models such as LPS induced murine lung injury, intrapulmonary levels of VEGF increased following injury for 96 hours, mirroring an increase in bronchoalveolar lavage fluid protein and neutrophils with significant VEGF localisation to lung epithelium.⁷¹ In an acid induced murine model of lung injury, high tidal (injurious) volume ventilation strategies increased lung VEGF-R2 (the main signalling receptor for VEGF bioactivity) protein expression, but this was not reduced by a protective ventilatory strategy suggesting that, in this instance, the VEGF response may be secondary to more critical events.⁷² These data are consistent with a possible pathological role for VEGF in lung injury.

Studies suggesting a protective role for VEGF in the alveolar space

Increasing recognition of the presence of significant levels of VEGF in normal healthy lungs has led to a reconsideration of its role. Considerable evidence suggests that VEGF acts as an alveolar epithelial mitogen and stimulant. Exogenous VEGF acts as a growth factor on human fetal and neonatal murine pulmonary epithelial cells and is capable of restoring the A549 cell proliferation in an acid exposure model of injury, although this has not been a universal finding.^{24 73-75}

These studies would certainly suggest at least a developmental role for VEGF in the lung. A particularly significant contribution to this observation is the work of Compennolle *et al*⁷³ in which transgenic HIF-2 α deficient fetal mice (with consequent fatal respiratory distress syndrome in neonatal mice due to impaired surfactant production by type 2 pneumocytes) had low lung VEGF levels. Intrauterine or postnatal intratracheal delivery of VEGF₁₆₅ to the neonatal mice protected them against developing respiratory distress syndrome and increased surfactant production. In terms of models of lung injury per se, recovery of intrapulmonary VEGF levels to pre-injury levels has been noted following recovery in both hyperoxic rabbit and LPS rat models, and the presence of VEGF in the alveolar space appears to have a protective effect against hyperoxia in interleukin (IL)-13 transgenic mice.^{34 76 77} In similar hyperoxic neonatal and adult models the proportion of VEGF₁₈₉ decreases while the proportions of VEGF₁₂₁ and VEGF₁₆₅ increase, all returning to control values in recovery as measured by RT-PCR.⁶⁷ In a bacterial model of acute lung injury in rats reduced VEGF₁₂₁, VEGF₁₆₅ and VEGF₁₈₉ transcripts were noted.⁷⁶ Inhibiting VEGF activity by VEGF-R2 blockade (given subcutaneously) in rats leads to alveolar apoptosis and emphysema.⁷⁸ NRP-1 inhibition has also been shown to ameliorate VEGF induced permeability in a lung targeted VEGF overexpressing model.⁷⁹ NRP-2 is expressed in normal human lung bronchial epithelium.⁶⁸ The role of NRP-2 in an ARDS/ALI context has not been evaluated. An alternative approach of lung targeted ablation of the VEGF gene by adenoviral delivery in the adult mouse led to a persistent emphysema phenotype for at least 8 weeks. Interestingly, no inflammation or proliferation occurred but increased apoptosis was seen in these lungs.⁸⁰ These data support the suggestion that VEGF may have a pneumotrophic function and be an autocrine epithelial growth factor in the lung. It is already known to be a survival factor for the vascular bed via induction of anti-apoptotic proteins, but to date this has not been demonstrated in alveolar epithelium.²⁰ Such an autocrine role has been described on other specialised epithelial cells in the kidney.⁸¹

In summary, high levels of VEGF exist in normal human lung despite the lack of angiogenesis, oedema, or excess microvascular permeability occurring. Receptors are expressed in the air space, compatible with a biological (but as yet unclarified) physiological role for VEGF in the normal lung.

VEGF AND THE PULMONARY VASCULATURE

Pulmonary hypertension occurs in ARDS as well as endothelial injury and increased microvascular permeability.⁶ Studies looking at the effect of VEGF on the endothelial side of the alveolar capillary membrane are limited. This is in contrast to the extensive literature on the systemic vasculature and HUVECs where VEGF has been shown to act as a vasodilator in addition to its angiogenic role.⁸² VEGF is known to reduce transendothelial resistance of bovine lung microvascular endothelial cells for less than 60 minutes at concentrations of less than 10 ng/ml and to stimulate endothelial cell chemotaxis maximally at 10 ng/ml.⁸³ However, most published studies have looked at the vasculature overall and the potential development of pulmonary hypertension, rather than at the microvascular level.

VEGF may have protective effects on the pulmonary vasculature.^{84–85} In an ovine model of chronic intrauterine pulmonary hypertension, whole lung VEGF protein expression was downregulated with reduced VEGF expression in the airway epithelium, vascular endothelial and smooth muscle cells on immunohistochemistry.⁸⁶ Indeed, transgenic mice overexpressing tumour necrosis factor α (lung or systemic) display features of pulmonary hypertension associated with reduced VEGF and VEGF-R2 mRNA expression.⁸⁷ In contrast, VEGF overexpressing transgenic mice develop an abnormal vasculature and lethal phenotype.⁸⁸ Blocking VEGF activity in newborn rats with a VEGF-R2 inhibitor decreases arterial density and vascular growth as well as alveolarisation.⁸⁹ All these studies certainly support the known role of VEGF in vasculogenesis during embryonic and early life. Perhaps of more relevance to the ALI/ARDS scenario is the observation that, in hypoxic conditions inducing pulmonary hypertension, increased pulmonary arterial VEGF mRNA and protein are detected in both guinea pig and rat species.^{90–91} In the rat model these levels correlated with both the degree of vascular remodelling and the mean pulmonary artery pressure.⁹⁰ Intervention studies also favour a protective role against pulmonary hypertension. Administration of VEGFR tyrosine kinase inhibitor in newborn rats leads to pulmonary hypertension and abnormal lung structure.⁹² Administration of a specific VEGF₁₆₅ inhibitor leads to more significant features of pulmonary hypertension histologically and haemodynamically associated with decreased expression of endothelial nitric oxide synthase.⁸⁶ Furthermore, intratracheal adenovirally delivered VEGF₁₆₅ protects against hypoxic pulmonary hypertension in rats, possibly via increased endothelial nitric oxide production.⁹³ The apparent conflicting observational data may indicate that hypoxia is simply an overriding regulatory factor to VEGF bioactivity even if pulmonary hypertension has developed. The potential role of VEGF in primary pulmonary hypertension is another enormous issue which is not covered here.

HUMAN ALI/ARDS

To date, most observational studies of lung injury in humans show a reduction in intrapulmonary VEGF levels in the early stages of ARDS (fig 1B).^{76–94–95} This is consistent with a hyperoxic lung injury model in rabbits where alveolar epithelial expression of VEGF was reduced.³⁴ Several other investigators have found similar reductions in intrapulmonary VEGF levels in other forms of lung injury, including high altitude pulmonary oedema in adults, bronchopulmonary

dysplasia, persistent pulmonary hypertension of the newborn, idiopathic pulmonary fibrosis, and smokers.^{96–99} Recovery of intrapulmonary VEGF levels to pre-injury levels has been noted following recovery from both ALI in humans and high altitude pulmonary oedema (fig 1C).^{95–96} Potential mechanisms for these observations include physical disruption of the alveolar capillary membrane alone, reduced VEGF production, changes in isoform expression, and increased receptor density or splicing to the soluble form, but human data are limited. Changes in VEGF isoforms have been shown in lung tissue from critically ill patients with sepsis, reduced VEGF₁₂₁ and VEGF₁₆₅ mRNA compared with controls without sepsis (on immunohistochemistry and ELISA), but these patients may not necessarily have had ALI.¹⁰⁰ Intrapulmonary soluble VEGF-R1 has been found to be increased in ARDS.¹⁰¹ Indeed, in ex vivo experiments, VEGF₁₆₅ induced permeability in human pulmonary arterial endothelial cell monolayers is reduced by nearly 50% in the presence of 20 ng/ml soluble VEGF-R1 (*sflt*).¹⁰² However, in another study homogenates from early and late injured lungs had no difference in VEGF-R2 expression compared with controls, although AE2 cell proliferation and VEGF-R2 expression were noted in injured tissue and these data do not exclude a protective role for VEGF.⁹⁴ In contrast to these data, one observational clinical study failed to find a difference in serum VEGF levels in ARDS, although there were significant differences in methodology.⁷⁶

Changes in VEGF could also be related to polymorphism. The VEGF +936 CT polymorphism has recently been investigated in a cohort of controls, ventilated “at risk” patients, and patients with ARDS.¹⁰³ The polymorphic T allele occurred significantly more often in the ARDS group than in the other cohorts and was associated with a higher APACHE 3 score suggesting an association with susceptibility and severity, although the intrapulmonary effect is unknown.

CONCLUSIONS

The normal human lung contains significant amounts of the angiogenic factor VEGF without significant angiogenesis—an apparent paradox. Published data conflict. Many studies have suggested that VEGF may contribute to the development of non-cardiogenic pulmonary oedema. Other studies have proposed a more protective role on the alveolar epithelium following injury. In the in vitro and ex vivo studies of changes in VEGF concentrations, differences in the species and methods used may have contributed to apparent differences in findings. Variable animal models, the degree of endothelial injury in the overexpression models, and temporal variation in sample collection in these and in clinical studies also confound the data. Changes in splice variant and soluble receptor expression may also contribute, although published data are limited.

We suggest a paradigm for VEGF in the lung. We speculate that it may function as a pneumotrophic factor behaving in an autocrine fashion with its prime function in facilitating repair following lung injury: protecting and regenerating the epithelial surface yet contributing to the generation of pulmonary oedema across the underlying endothelium if disruption of the alveolar capillary membrane occurs as in ARDS. Functional VEGF polymorphisms may determine in part susceptibility to developing and severity of lung injury, but remain to be further investigated. Anti-VEGF therapy is already under investigation for lung cancer, vascular disease, pulmonary hypertension, and chronic inflammatory diseases.^{104–105} In the long term, treatment modulating the VEGF system may be of value in ARDS, but this will require a better understanding of its role in the lung and a better understanding of the regulatory mechanisms influencing VEGF bioactivity including changes in splice variants,

transcription factors, pro-inflammatory cytokines, and soluble receptors. Such a potent molecule is likely to have a physiological role which can be detrimental if activity is excessive, inappropriate, or uncontrolled. The challenge will be to limit the effects of such treatment to those desired, given the pleiotropic functions of VEGF in the body.

ACKNOWLEDGEMENTS

Secretarial support and graphics were provided by Sharon Standen.

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Supported by The Wellcome Trust (074702/Z/04/Z).

Competing interests: none.

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