Vascular Endothelial Growth Factor in Acute Lung Injury and Acute Respiratory Distress Syndrome

S. Barratt^{a, c} A.R. Medford^{b, c} A.B. Millar^{a, c}

^aAcademic Respiratory Unit and ^bNorth Bristol Lung Centre, ^cSouthmead Hospital, University of Bristol, Bristol, UK

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Abstract

Acute respiratory distress syndrome (ARDS) is the most severe form of lung injury, characterised by alveolar oedema and vascular permeability, in part due to disruption of the alveolar capillary membrane integrity. Vascular endothelial growth factor (VEGF) was originally identified as a vascular permeability factor and has been implicated in the pathogenesis of acute lung injury/ARDS. This review describes our current knowledge of VEGF biology and summarises the literature investigating the potential role VEGF may play in normal lung maintenance and in the development of lung injury.

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Acute Respiratory Distress Syndrome

The acute respiratory distress syndrome (ARDS) is a form of acute, diffuse, inflammatory lung injury that occurs following damage to the integrity of the alveolar capillary membrane (ACM) and is characterised by the accumulation of proteinaceous non-cardiogenic alveolar oedema, resulting in refractory hypoxaemia. Acutely, the morphological hallmarks are diffuse alveolar damage,

with interstitial and alveolar oedema, inflammation and the formation of hyaline membranes [1].

Since the initial description of ARDS by Ashbaugh et al. [2], progress has been made in understanding the pathogenesis of acute lung injury (ALI) [3, 4], but little progress has been made in the development of novel therapies. In spite of this, reports suggest that both the incidence and mortality of hospitalised patients are gradually declining, with current mortality rates estimated at 17–23% [5, 6]. The reason for this decline in mortality is likely to be multi-factorial, e.g. the introduction of policies to reduce hospital-acquired infections, limiting the use of blood transfusions and the widespread implementation of low-tidal volume, lung-protective ventilatory strategies [5, 6].

Survival from ARDS is thought to require renewed integrity of the ACM through the co-ordinated response of several complex molecular processes, including alveolar epithelial type (AE) 2 cell proliferation. Re-absorption of the oedema and clearance of alveolar protein must follow, with eventual normalisation of the alveolar structure, although deficits in respiratory function may persist.

Luyt et al. [7] recently compared long-term outcomes of survivors of pandemic H1N1 influenza-associated severe ARDS using extracorporeal lung assistance need as a surrogate ARDS severity marker. One year after intensive care unit discharge, 50 and 40% of patients with and without extracorporeal lung assistance, respectively, reported significant exertional dyspnoea, with 75 and 64% of patients demonstrating decreased diffusion capacity

despite minor CT abnormalities with near-normal and comparable lung function tests.

Some patients do not resolve their lung injury, however, and progressive fibrin deposition occurs with the development of lung fibrosis. This fibroproliferative phase of ARDS may occur as early as within the first 24 h of the predisposing insult [8, 9]. The biological processes that determine why a patient may survive with complete radiological resolution, survive with residual pulmonary fibrosis or succumb to this syndrome are not currently fully understood.

Vascular endothelial growth factor (VEGF) is a glycoprotein originally isolated as a permeability factor [10]. Whilst being critically important to organogenesis [11, 12], high levels of VEGF persist in the lungs in adulthood; indeed, of all the organs, the lungs are the most predominant source of VEGF in adults [13, 14]. These factors have led many, including our group [15], to consider that VEGF may play a role in both normal lung maintenance and in the pathogenesis of ARDS.

This review summarises our current understanding of VEGF biology, how it may contribute to the maintenance of the healthy lung and finally how changes in VEGF bio-availability may contribute to the pathogenesis of ARDS.

VEGF Biology

VEGF-A (VEGF hereafter) is a 34- to 46-kDa disulphide-linked dimeric glycoprotein belonging to a superfamily of VEGF-related proteins that includes VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF) [16].

VEGF was originally isolated as a permeability factor [10] but was subsequently shown to have additional potent mitogenic and angiogenic properties [10, 17, 18]. It is essential for normal organogenesis and angiogenesis in both humans and animals, where deletion of a single allele has been associated with lethality in mice [11, 12].

Six human VEGF mRNA variants exist (VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉ and VEGF₂₀₆), which are formed by alternative splicing of VEGF mRNA at exons 6 and 7. The isoforms subsequently formed, collectively termed the VEGF_{xxx} isoforms, differ in the number of amino acids in the resulting protein (denoted by the subscript number) [19]. The isoforms have varied heparin-binding affinities: VEGF₁₂₁ lacks both exons 6 and 7 and is the most freely diffusible isoform. VEGF₁₈₉ and VEGF₂₀₆ are almost completely sequestered in the extracellular matrix where they may act as a reservoir of

VEGF, released by heparinases or proteolytic enzymes, such as plasmin [20, 21]. VEGF₁₆₅ is the most extensively studied isoform and is considered to be the most biologically active. Lacking exon 6 but not exon 7, it has intermediate heparin-binding properties with 50% being freely diffusible [20].

A second family of VEGF isoforms is thought to exist, the VEGF_{xxx}b isoforms, formed by differential splicing of the VEGF gene, 66 bp distal to the conventional splice acceptor site in exon 8 (termed exon 8b). The resulting proteins correlate with the expected molecular masses for common VEGF_{xxx} isoforms but differ by an alternative terminal 6 amino acids: Ser-Leu-Thr-Arg-Lys-Asp (SLTRKD) instead of Cys-Asp-Lys-Pro-Arg-Arg (CDKPRR) in VEGF_{xxx} isoforms [22]. The carboxyl-terminal domain of VEGF has been shown to be necessary for determining mitogenic potency [23] and as such VEGF₁₆₅b, the most widely studied of the VEGF_{xxx}b isoforms, has been shown to be functionally different from VEGF₁₆₅. It inhibits VEGF₁₆₅-induced endothelial cell (EC) proliferation and migration in vitro, arterial vasodilatation ex vivo and angiogenesis in vivo [22, 24]. For these reasons, the VEGF_{xxx}b isoforms have been termed 'inhibitory or anti-angiogenic' isoforms.

Whilst VEGF is the most widely studied molecule of the VEGF gene family, other family members may form heterodimer complexes with VEGF [25], which can then competitively bind to VEGF receptors (VEGFRs) to modulate signalling [25, 26].

VEGF-B is predominantly expressed in the heart [27] and exerts its biological effects through VEGFR1 [25, 28] and neuropilin (NRP) 1 [29]. Unlike other VEGF family members, it does not appear to have a prominent role in developmental angiogenesis [11, 12, 30], but rather in blood vessel survival and growth under pathological conditions [31]. It has also been reported to contribute to the pulmonary vascular remodelling observed in the development of chronic hypoxic pulmonary hypertension in mice [32], although this was not confirmed by other studies [33].

Sharing sequence homology [34], VEGF-C and VEGF-D are both primarily associated with angiogenesis and lymphangiogenesis [35], signalling through VEGFR-2 and VEGFR-3 [26, 36]. At high concentrations, both VEGF-C and VEGF-D are also reported to act as vascular permeability factors [37]. Furthermore, tumour expression of VEGF-C and VEGF-D has been correlated with clinico-pathological features of various human cancers, including metastatic spread [38].

VEGF-E is an Orf virus-encoded VEGF, which binds specifically to VEGFR-2 [39]. Although this molecule has

Fig. 1. Schematic diagram of the VEGF receptors and co-receptors. VEGFR1 and VEGFR2 are structurally related tyrosine kinase receptors that bind VEGF-A with different affinities. They are composed of 7-immunoglobulin-like domains (circles), a single transmembrane spanning domain, a split intracellular kinase domain (rectangles) and a C-terminal tail carrying several tyrosine kinase residues involved in downstream signalling. The NRP1 and NRP2 receptors consist of 5 extracellular domains. B1/B2 domains (circles) bind directly to VEGF molecules. They have a short intracellular cytoplasmic tail and thus were historically thought to lack intrinsic catalytic function.

not been widely studied, evidence suggests that it can act as an EC mitogen and permeability factor with similar potency to that of VEGF₁₆₅ [39].

PlGF is expressed in several tissues, including the lung [40], and specifically binds to VEGFR1 [41]. Its role as an angiogenic and permeability factor within the systemic vasculature is well established [42]. Studies have also suggested a potential role for PlGF in the pathogenesis of emphysema [43] and in the pulmonary adaptive response to hypoxia [44].

VEGFR1 and VEGFR2

VEGF binds to three highly related tyrosine kinase receptors, VEGFR1, VEGFR2 and VEGFR3 characterized by 7 immunoglobulin-like extracellular domains with an intracellular split-kinase region (fig. 1). VEGFR3 expression is predominantly restricted to the lymphatic system [45] and is therefore not discussed further in this review.

VEGFR2 or kinase domain region (KDR)/fetal liver kinase-1 (Flk-1) is a 210- to 230-kDa glycoprotein that is considered by many as the main signalling receptor for VEGF bioactivity [46, 47]. Traditionally, VEGFR2 was thought to be exclusively expressed by ECs with highest expression levels during embryonic vasculogenesis and angiogenesis [48, 49], where it is critical to normal vascular development [50]. Subsequently, it has been shown that VEGFR2 is expressed on both sides of the ACM by a variety of cells, including lung macrophages [16] and AE2 cells [51].

The related VEGFR1 [or Fms-like tyrosine kinase 1 (Flt-1) in the mouse] is a 180- to 185-kDa receptor tyro-

sine kinase glycoprotein [52] that binds to VEGF at sites distinct to those of VEGFR2 [23].

It was initially believed that VEGFR1 was largely confined to vascular endothelium throughout development and in adulthood [53], but has subsequently been detected in a wide range of non-ECs both in the lung and elsewhere in the body, such as in glomerular ECs [54], monocytes, macrophages [55] and AE2 cells [56].

The precise role of VEGFR1 and its alternatively spliced, soluble form (sFlt1), remains unclear, but an abundance of evidence supports a negative regulatory role on VEGF bioactivity, acting as a decoy or silent receptor to sequester VEGF from VEGFR2 binding [57, 58]. Supporting this theory, both VEGFR1 and sFlt-1 bind to VEGF with higher affinity than VEGFR2 [53, 59] but induce only weak intracellular tyrosine kinase activity [60].

However, more recent evidence disputes the theory of VEGFR1 as a silent receptor. Zhang et al. [61] suggest that VEGFR1 activation is essential for growth and survival of human dermal ECs, whilst Kanno et al. [62] have implicated VEGFR1 in EC actin reorganization and migration.

Co-Receptors: NRP1 and NRP2

NRP1 and NRP2 are 130-kDa proteins that were originally identified as receptors for semaphorins: important growth factors for axonal guidance in neural development [63–65]. Subsequently, it was found that the NRPs also function as VEGF isoform-specific receptors, binding VEGF₁₆₅ with the highest affinity [66].

The primary structure of the NRPs is well conserved (44% primary structure homology) [64] (fig. 1). Importantly, however, they differ at the point where VEGF binds to the receptor (within the b1 domain) and consequently VEGF binds to NRP2 with approximately a 50-fold lower affinity than to NRP1 [67].

Both NRP1 and NRP2 receptors have been identified on several cell types, including on both sides of the ACM: AE2 cells [68], adult ECs [66], by several tumour cell lines [69], stem cells [70], neuronal cells [71] and by lung cancer cells [72].

NRPs have a short intracellular domain and as such complex formation with a co-receptor has been deemed necessary for signal transduction [73, 74]. Functioning as a co-receptor, NRP1 has been shown to augment the binding of VEGF₁₆₅ to VEGFR2 when co-expressed on the same cell, enhancing VEGF₁₆₅-mediated proliferation and migration [66]. More recent data suggest, however, that NRP1 may function independently in the maintenance of the normal alveolar structure [75] as NRP1-deficient mice display *de novo* pulmonary abnormalities. Furthermore, enhanced airspace enlargement and epithelial apoptosis were observed in response to chronic cigarette exposure compared to control mice.

VEGF in Lung Maintenance

As previously mentioned, of all the organs, expression of VEGF mRNA in adulthood is highest in the lung in both animals and in humans [13, 14]. In the healthy human lung, VEGF levels are highly compartmentalised, with alveolar protein levels 500 times higher (2 nM) than plasma levels [76]. These levels are also twice those previously shown to induce permeability and mitogenesis *in vivo* [77], and yet in the normal lung these processes are extremely limited. This implies a persistent role for VEGF in the adult lung that extends beyond its critical role in development.

The alveolar epithelium is considered to be the predominant source of VEGF in the lung [14, 56, 76], although other cells, such as smooth muscle cells, macrophages, and ECs, also express VEGF [78, 79]. Likewise, VEGFR and co-receptors are expressed on both sides of the ACM [68].

Although AE2 cells are thought to be the main source of VEGF [76], the physiological role of VEGF generated from this source remains unclear [76]. It has been proposed that it may function as a survival factor for both epithelial cells [80, 81] and ECs [82] in the normal lung,

possibly acting in an autocrine fashion on epithelial cells [81] and modulating the functions of the adjacent vascular endothelium in a paracrine manner [83]. The VEGF survival signal in both ECs and epithelial cells is in part mediated through the phosphatidylinositol 3-kinase/Akt signal transduction pathway [81, 82].

In a model of mechanical alveolar cell over-distension, VEGF also influenced epithelial cell survival [84]. In this model, high-amplitude mechanical stretch induced secretion of VEGF from rat AE2 cells grown *in vitro* which at high concentrations inhibited stretch-induced apoptosis of these cells.

Whilst the ability of VEGF to induce proliferation of the systemic vasculature is well established [85], studies have also suggested that VEGF can stimulate growth of AE2 cells *in vitro* [86] and surfactant production [87].

Furthermore, VEGF promotes EC expression of the anti-apoptotic proteins BCL-2 and A1 [88], whilst additionally blocking EC apoptosis mediated through the surface 'death receptors' TNF receptors and Fas [89]. To date, few studies have investigated the effect of VEGF on lung epithelial cells, but limited evidence from *in vitro* studies suggests that at high concentrations VEGF may also inhibit AE2 apoptosis [84, 86].

Animal studies using adenoviral vector-mediated, lung-targeted ablation of the VEGF gene resulted in apoptosis of alveolar septal wall cells and the development of an emphysema phenotype in mice, providing corroborative evidence that VEGF in AE2 cells contributes to alveolar epithelial cell survival and lung maintenance [90]. Likewise, targeted deletion of VEGF in distal lung epithelial cells (SPC-VEGF-KO mice) also resulted in the development of emphysema-like pathological findings with an increase in activated caspase-3-positive AE2 cells [91].

VEGFR2 signalling appears central to lung maintenance *in vivo*, as chronic VEGFR inhibition resulted in alveolar septal cell apoptosis and emphysema, associated with a reduction in VEGFR2 expression and phosphorylation [92, 93]. Furthermore, mesenchymal stem cell (MSC) administration improves emphysema induced by cigarette smoke exposure in rats, mediated in part through up-regulation of VEGF and VEGFR2 [94].

Whilst these studies suggest that VEGF is important for lung maintenance, it is apparent that expression levels are tightly regulated in the normal lung since conditional over-expression of VEGF in respiratory cells also causes an emphysema-like phenotype in adult mice [95]. Moreover, lung-targeted VEGF₁₆₅ transgenic mice develop pulmonary neovascularisation and increased vascular permeability [96].

These studies suggest that following development VEGF assumes a role in normal lung homeostasis and in these circumstances the respiratory epithelium may provide a potential physiological reservoir of VEGF. A healthy ACM, compartmentalising VEGF, may be crucial to this process [76]. If the integrity of the ACM is breached, as occurs in ALI/ARDS, VEGF may reach the EC at high levels, inducing increased vascular permeability and pulmonary oedema [15, 76].

Role of VEGF in ALI/ARDS

To date, the majority of research exploring the role of VEGF in the lung has focused on the VEGF-A molecule. There are numerous apparently conflicting reports of VEGF acting as a pathological or protective factor in ALI/ARDS, which are discussed below.

VEGF-C has been implicated in the modulation of innate and adaptive immune responses in the development of chronic inflammation associated with experimental obliterative airway bronchiolitis [97], but investigation into its potential role in ALI/ARDS has not been addressed.

VEGF Genetic Polymorphisms in ALI/ARDS

There is growing evidence to suggest that genetic factors may play a role in the development of lung injury. We have previously reported on the presence of a genetic polymorphism (VEGF +936), associated with lower broncho-alveolar lavage fluid levels of VEGF, which is frequently present in patients with ARDS and associated with higher APACHE III scores [98, 99].

In a case-control study, this VEGF +936 polymorphism was also present at significantly higher frequency in Chinese patients with ARDS than in controls (odds ratio = 3.85, $p = 0.04$) and was associated with increased mortality (odds ratio = 5.72, $p = 0.03$) [100].

Other genetic variations in the VEGF pathway have also been implicated in the development of ALI/ARDS. Single nucleotide polymorphisms (SNPs) in VEGFR1 and in downstream mediators of the VEGF signalling pathway (e.g. RAF1 and NRAS) were consistently associated with the risk of pulmonary complications, which included ALI and ARDS, following lobectomy, but the exact functional significance of these SNPs has yet to be determined [101].

VEGF genetic polymorphisms (SNPs rs10434 and rs3025028) have also been implicated in the genetic determination of airway function from birth to adulthood.

The functional significance of SNP rs3025028 polymorphism has also been linked to alterations in ratios of plasma VEGF_{165b} to VEGF_{165a} in several cohorts [102].

VEGF in the Exudative Phase of ALI/ARDS

Early ARDS is characterised by increased vascular permeability with exudation of proteinaceous fluid and migration of inflammatory cells from the vascular compartment into the interstitium and alveolar space.

Human and Cellular Studies

To date, the majority of human studies have shown a reduction in intrapulmonary VEGF in early ARDS with an associated rise in VEGF plasma levels [103–105] and normalization of both during recovery [103, 105].

The observed reduction in intrapulmonary VEGF in early ARDS may be explained by a number of hypotheses. Firstly, as a prominent source of VEGF, direct injury to and clearance of AE2 cells may significantly reduce the cellular production of VEGF. In support of this hypothesis, a reduction in AE2 cellularity due to apoptosis has been observed in ARDS [106], which may be mediated in part through the release of Fas ligand [107]. Contradicting this theory, however, Tudor et al. [108] reported a decrease in VEGF mRNA after intraperitoneal injection of lipopolysaccharide (LPS) in rats, a model in which epithelial injury does not occur.

It has also been speculated that these changes may be due to the degradation of VEGF by proteases and inflammatory cells in the alveolar space [105, 109].

Another possible explanation for the intrapulmonary reduction in VEGF includes differential splicing of the VEGF gene, producing less soluble and more membrane-bound isoforms. We have explored this hypothesis and have shown a lower ratio of soluble/cell-associated isoforms in early ARDS compared to normal and late ARDS patients, and this was also true in murine lung injury [110]. For instance, the soluble VEGF₁₂₁ isoform is unable to form complexes with membrane-bound heparin sulphate proteoglycans [20]. These proteoglycans are considered cofactors for VEGF signalling that are thought to stabilise the ligand/receptor complex, delaying internalisation and degradation of activated VEGFRs and thus potentiating downstream signalling [111]. Alterations in the balance of expression of VEGF isoforms may therefore affect biological responses.

Finally, increased binding of VEGF to ‘decoy’ receptors in early ARDS may also provide an explanation for

the observations reported in the human lung. In support of this hypothesis, Perkins et al. [112] detected significant quantities of intrapulmonary soluble VEGFR1 protein in early ARDS compared to late ARDS or normal patients.

The observed increase in plasma VEGF levels may be partially explained by the release of VEGF from the intrapulmonary compartment, along a transepithelial gradient, following destruction of the ACM integrity. Moreover, ARDS represents only the pulmonary manifestation of widespread endothelial damage [34].

Activated macrophages [103] and neutrophils [113] also become a more important source of VEGF in ALI/ARDS and may contribute to the increased levels of VEGF observed. VEGFR-1 has been identified on monocytes/macrophages, the activation of which led to monocyte activation and chemotaxis. The authors hypothesised that apical AE2 cell VEGF secretion may play an additional role in the recruitment of immune cells into the alveolar space [114].

Animal Studies

One of the first studies attempting to understand the underlying mechanism of this increased vascular permeability demonstrated, using an adenoviral gene vector, that lung-targeted over-expression of human VEGF₁₆₅ in mice resulted in pulmonary oedema and increased vascular permeability, suggesting a significant pathological role of VEGF in ARDS [115]. Furthermore, this effect could be attenuated by treatment with biological inhibitors of VEGF activity, both soluble VEGFR1 [115] and the anti-VEGF antibody bevacizumab [116]. Similarly, pre-treatment of rats with adenovirus-encoding soluble VEGFR2 prevented ischemia-reperfusion-induced lung injury [117].

In homology with these findings, inhibition of VEGF activity with a soluble VEGF trap decoy receptor reduced vascular protein leak of hypoxic endothelin-B receptor-deficient rats [118]. In these animals, vascular protein leak did not return to control levels, however, suggesting that VEGF activity is not the sole factor contributing to hypoxia-induced changes in vascular permeability.

Corroborative evidence that VEGF contributes to the initial exudative phase of ARDS was provided by Karmaliotis et al. [119], who demonstrated increased immunostaining for VEGF in the lung, associated with the influx of mononuclear cells and neutrophils in the alveolar compartment, using an LPS-induced lung injury model. This finding was paralleled by an increase in VEGF mRNA expression, development of inflammation, capillary leakage and lung oedema [119].

Using an ovine smoke inhalation and pneumonia model of lung injury, Lange et al. [120] attempted to evaluate the time course of changes in VEGF expression. Linear increases in transvascular fluid flux and lung water content were observed following induction of lung injury. Transient increases in microvascular permeability were observed 12 h after lung injury, occurring concurrently with peak levels of total lung VEGF protein levels (measured by immunoblotting).

Increased VEGF and VEGFR2 expression in the lung has also been reported in an animal model of sea water immersion after open chest trauma-induced ALI [120–122].

Moreover, in an *in vivo* mouse model of high tidal volume, mechanical ventilation [124], a ventilation strategy previously associated with increased lung injury [123], increased microvascular permeability, neutrophil influx, epithelial apoptosis and VEGF mRNA and protein production were observed, which were attenuated by knock-down of VEGF by short interfering RNAs.

The chronological expression of VEGF has also been studied in an LPS-induced model of lung injury [122]. In contrast to those studies previously discussed, VEGF protein expression in pulmonary tissue, as determined by ELISA and immunoblotting, was down-regulated in a time-dependent manner in response to injury. Furthermore, pulmonary levels of its angiogenic mediator, VEGFR2, and two important downstream signalling molecules (phosphorylated Akt and endothelial nitric oxide synthase) were also down-regulated in a similar manner. In contrast, plasma levels of VEGF and VEGFR1 increased following LPS administration (peaking at 6 h). Blockade of VEGFR1 led to partial resolution of pulmonary VEGF levels and attenuated some features of ALI, suggesting that VEGF may play a pathological role through VEGFR1 in LPS-induced ALI.

In summary, numerous reports suggest a pathological role for VEGF in ALI/ARDS. Reports apparently conflict on whether VEGF expression is increased or decreased within the lung, and this may be explained in part due to the numerous models of lung injury used, in addition to different methodologies and end points.

VEGF in the Fibroproliferative Phase of ALI/ARDS

The fibroproliferative phase of ALI/ARDS is characterised by AE2 cell proliferation in an attempt to repair the denuded epithelium [125] and migration of fibroblasts into the interstitium and intra-alveolar space with

deposition of collagen-rich extracellular matrix [125]. Evidence suggests that fibroproliferation is an early response to lung injury, occurring in parallel with the exudative phase [8].

Human and Cellular Studies

VEGF₁₆₅ induces the proliferation of AE2 cells in culture, an effect that can be inhibited by VEGF_{165b} [126], thus factors that alter VEGF gene splicing may affect the fibroproliferative response in ARDS. The effect of VEGF on AE2 proliferation is discussed further in the section: 'VEGF in Resolution/Repair from ARDS'.

Animal Studies

The role of VEGF in the development of fibrosis in ARDS has not specifically been addressed, although numerous studies of idiopathic pulmonary fibrosis exist, supporting a possible pro-fibrotic role. Using animal models of pulmonary fibrosis, VEGFR2 inhibition attenuated pulmonary collagen deposition and TGF- β_1 signalling [127], whilst anti-VEGF therapy in the form of sFlt-1 resulted in a reduction in lung collagen deposition with additional anti-inflammatory and anti-angiogenic effects [128]. In contrast, targeted myeloid cell-specific deletion of VEGF aggravated bleomycin-induced pulmonary fibrosis [125], whilst mice with EC-specific VEGF deletion developed de novo pulmonary fibrosis that preceded multi-organ failure from systemic vascular pathologies [126].

There are apparent conflicting findings as to the role of VEGF in animal models of pulmonary fibrosis. Furthermore, the underlying pathogenesis of fibrosis in ARDS may differ compared to other diseases/disease models and thus it is difficult to draw conclusions about ALI/ARDS from these studies. Research is required to explore this area in more detail [129, 130].

VEGF in Resolution/Repair from ARDS

Resolution of ARDS requires termination of the fibroproliferative response, a functioning ACM to facilitate clearance of pulmonary oedema and removal of inflammatory cells and MSCs [125].

Human and Cellular Studies

Observational human studies suggest a role for VEGF in the recovery from ARDS. It has been observed that in patients with ARDS, elevated plasma levels of VEGF become reduced in those who recover, whilst intrapulmonary VEGF levels that are reduced in early ARDS rise in

recovery [103]. Furthermore, in human ARDS lung samples, VEGF levels were negatively correlated with apoptotic EC counts [131]. Differential temporal up-regulation of VEGFR1, VEGFR2 and NRP1 in human ARDS provides corroborative evidence of functional regulation of VEGF bioactivity via VEGFR2, consistent with a protective role of VEGF in recovery from lung injury [68].

The presence of VEGF_{165b} has also been demonstrated in healthy human lung tissue, with reduced levels of pulmonary expression reported in ARDS [126]. In vitro experiments have indicated that VEGF_{165b} inhibits the proliferative effect of recombinant VEGF₁₆₅ protein on both human primary ECs and AE2 cells [126]. Taken together, these results also suggest a possible protective role for the VEGF_{xxx}b family in fibroproliferation/repair after lung injury.

Destruction of the vascular endothelium is characteristic of the hyperoxic model of lung injury and recovery requires proliferation of ECs. Using this model, AE2 cells were shown to express increased VEGF mRNA during recovery [132], further implicating VEGF in the regulation of ACM repair following injury.

Animal Studies

Over-expression of IL-13 in transgenic mice conferred survival benefit from hyperoxia-induced lung injury, associated with isoform-specific (120- and 188-amino acid isoforms) increases in broncho-alveolar lavage fluid VEGF [133].

Likewise, transgenic overexpression of VEGF₁₆₅ conferred cytoprotection against hyperoxic lung injury, which in part was mediated through enhanced production of A1 [134].

It remains to be established, however, whether enhanced VEGF expression in the fibroproliferative/recovery phases of ARDS/ALI represents a marker of resolution or is actively participating in the repair process [80, 84, 87, 92, 94, 133, 135].

VEGF Has Both a Pathological and a Protective Role in the Lung in Health and Disease

An elegant study by Mura et al. [91] attempts to draw together the role of VEGF in health and disease. In this study, conditional knockout of VEGF in mouse lung AE2 cells led to the development of emphysema in 28- to 32-week-old animals. Conditional targeting did, however, not significantly affect lung development from the embryonic to the young adult stage, suggesting that

(For legend see next page.)

VEGF is critical in the maintenance of normal alveolar structures, especially in older individuals. In an in vivo model of extrapulmonary ARDS (intestinal ischemia-reperfusion) using young adult transgenic mice (7–10 weeks), features of attenuated lung injury were demonstrated compared to wild-type controls, in keeping with the known role of VEGF as a vascular permeability factor. Interestingly, increased caspase-3-positive AE2 cells were also detected following intestinal ischemia-reperfusion. Thus, results from targeted blockade of VEGF in AE2 cells point to a complex role for VEGF in ALI/ARDS

as an important factor contributing to the acute inflammatory response and pulmonary oedema on the one hand, and as a protective factor for the alveolar epithelial barrier on the other.

VEGF Therapy in ALI/ARDS

This review has highlighted a potential pathological role for VEGF in the development of pulmonary oedema associated with ALI/ARDS. Anti-VEGF therapies in

Fig. 2. Schematic diagram of the human alveolar unit in health (a), in early ARDS (1–2 days; b) and in late ARDS and recovery (c).

the form of monoclonal antibodies, e.g. bevacizumab (Avastin) and tyrosine kinase inhibitors, e.g. sunitinib (Sutent), have already been developed for the treatment of certain cancers [136–138] and age-related macular degeneration [139]. These therapies have a significant adverse effect profile following systemic administration, including hypertension, proteinuria, cardiac ischaemia, cerebral thrombosis and gastrointestinal perforation [140].

Clinical trials are required to establish the efficacy of drugs targeting VEGF/VEGFR in ARDS. Unfortunately, a planned phase 2 clinical trial studying the efficacy of bevacizumab in preventing ARDS (NCT01314066) has recently been withdrawn, prior to enrolment, due to inadequate funding.

Modulation of the fibroproliferative response provides an additional potential therapeutic target for ALI/ARDS. It is possible to speculate that pro-angiogenic therapy may stimulate the repair of the ACM and alleviate the pulmonary oedema associated with ALI/ARDS.

In keeping with this theory, Compennolle et al. [87] demonstrated a beneficial effect of VEGF administration during the respiratory distress syndrome in neonatal mice that was mediated through VEGFR2. Additionally, Corne et al. [133] found that mice transfected with IL-13 during hyperoxia-induced lung injury had an increase in survival that was mediated in part by an increase in VEGF synthesis [133]. Over-expression of

VEGF in mice has, however, also been associated with increased mortality linked to pulmonary haemorrhage [95].

Trials of adenoviral [141] and plasmid [8] vectors expressing VEGF have failed in ischaemic heart disease due to side effects and lack of efficacy.

Our current understanding of the complex role VEGF may play in lung maintenance, injury and repair is incomplete. Studies presented in this review have implicated VEGF as both a pathological and protective factor in the exudative and fibroproliferative phases of ARDS, which occur concurrently. VEGF also plays an important physiological role in various other organ systems and has potential pleiotropic effects [8, 141]. The greatest challenge in developing future modulatory VEGF therapy for ALI/ARDS would therefore be in its delivery in a controlled yet targeted manner.

Conclusions

Significant amounts of VEGF exist in the normal lung in the absence of significant mitogenesis, angiogenesis or vascular leakiness. There are apparent contradictory data on the role of VEGF in lung maintenance and in ALI/ARDS that may be explained in part due to methodological differences in study design. It highlights the difficulty of drawing conclusions from studies that use different

animal models of ALI and sample different biological compartments at different time points.

Furthermore, in both humans and animals, ALI/ARDS is a heterogeneous condition that has rapidly evolving phases, in which VEGF may play different or even opposing roles. Questions regarding the exact role of VEGF on the epithelial-endothelial barrier and the potential role for VEGF in the evolving stages of ARDS are being explored but remain unanswered.

To date, VEGF-A has been the most widely studied member of the human VEGF gene super-family. This review has highlighted the paucity of information regarding the role of other VEGF family members in ALI/ARDS. Whilst dissection of the underlying cellular and molecular processes is crucial, exploring the intricate interplay of the other family members in this complex syndrome is also paramount.

We have previously speculated that VEGF may function as a protective factor in the normal lung facilitating lung repair following injury through epithelial regeneration and yet become pathological, contributing to the development of pulmonary oedema across the underlying endothelium if the integrity of the ACM is breached [15]. Data to support this paradigm are accumulating [91] (fig. 2).

In the long term, a greater understanding of VEGF biology and the regulation of VEGF bioavailability in health and disease is required before treatments modulating VEGF may be used in ALI/ARDS.

Financial Disclosure and Conflicts of Interest

None to declare.

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