Vascular endothelial growth factor increases pulmonary vascular permeability in cystic fibrosis patients undergoing lung transplantation∗, ∗∗

Katharina Krenn a, b, Walter Klepetko b, Shahrokh Taghavi b, Patrick Paulus a, Seyedhossein Aharinejad a, b, ∗

a Laboratory for Cardiovascular Research, Center for Anatomy and Cell Biology, Medical University of Vienna, Waehringerstr. 13, A-1090 Vienna, Austria
b Department of Cardiothoracic Surgery, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

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Abstract

Objective: Vascular endothelial growth factor (VEGF) is the prime regulator of angiogenesis and vascular permeability and its serum levels increase in cystic fibrosis (CF). The mechanisms of VEGF overproduction and its impact on CF lung pathology and pulmonary vascular permeability during lung transplantation are not fully understood. Methods: The expression of VEGF, its receptors, hypoxia inducible factor (HIF)-1α, β, angiopoietins, and endothelial cell marker CD31 were studied in lung biopsies of CF and COPD patients and controls, using real time reverse transcription (RT)-PCR and Western blotting. DNA binding activity of HIF-1 to VEGF-A promoter was assessed by electrophoretic mobility shift assay (EMSA) and wet-to-dry lung weight ratios as well as microvascular density (MVD) were determined. Serum VEGF-A concentrations in enzyme-linked immunosorbent assay (ELISA) and wet-to-dry weight ratios of donor lungs were monitored during transplantation in CF and COPD patients. Primary graft dysfunction (PGD) was diagnosed and graded according to the guidelines of the International Society for Heart and Lung Transplantation. Results: VEGF-A165 and Flt-1 mRNA expression (P < 0.05), VEGF-A (P < 0.05), and HIF-1α (P < 0.05) protein levels, DNA binding activity of HIF-1 to VEGF promoter (P < 0.001) and extravascular lung water content (P < 0.05) were increased in CF lungs versus controls, whereas MVD was unchanged. Before and during lung transplantation, VEGF-A serum concentrations were higher in CF versus COPD patients (P < 0.05) and 60 min following reperfusion donor lungs transplanted to CF patients had higher tissue water contents than in COPD patients (P < 0.05). PGD grade 3 occurred more frequently in CF (22.7%) versus COPD patients (4%). PGD grade 3 patients had significantly higher VEGF serum concentrations versus PGD grade 0–2 patients (P < 0.001). Conclusions: These data indicate that upregulated VEGF-A levels are most likely induced by enhanced HIF-1 binding to VEGF-A promoter, possibly contributing to elevated serum VEGF-A levels in CF. Furthermore, CF patients undergoing lung transplantation are possibly more susceptible to PGD because of increased VEGF-A expression that mediates increased lung graft vascular permeability.

Keywords: Lung transplantation; Cystic fibrosis; VEGF; HIF; Vascular permeability

1. Introduction

Cystic fibrosis (CF) is the most common life-shortening genetic disorder among Caucasian individuals [1]. The pulmonary manifestation of CF is characterized by the production of viscous mucus that obstructs the airways, underlying the subsequent inflammatory reactions and infections. The chronic inflammation, infection, and progres-
and diffuse pulmonary infiltration in chest radiographs without relation to infection, rejection, or surgical complications that occur within 72 h following transplantation. VEGF-A plays an important role in development of PGD because of its key role in regulating graft vascular permeability [8,9]. Elevated VEGF-A levels in end-stage CF patients, undergoing lung transplantation, may contribute to edema formation in the graft. The molecular mechanisms leading to VEGF overproduction in CF patients undergoing lung transplantation and its impact on vascular permeability of the lung graft are uncertain. This study addressed these issues.

2. Patients and methods

2.1. Patients

Twenty-two CF and 25 COPD patients admitted for double lung transplantation at the Medical University of Vienna between 2003 and 2005 were included in this prospective study (Table 1). Serum samples of the transplant recipient were obtained at the time of admission to hospital for lung transplantation, shortly before transfer to the operating room. Additionally, biopsies were taken from the peripheral upper lobe of the explanted recipient lung. During the transplant procedure, serum samples of the transplant recipient and tissue samples of the donor lung were taken at specific time points. Serum samples were obtained from recipients before reperfusion of the transplanted lung, and 15 and 60 min following reperfusion. Lung tissue biopsies were taken from the donor lung before and 60 min after reperfusion. All donors matched the standard criteria, and all were taken from the donor lung before and 60 min after reperfusion. All recipients before reperfusion of the transplanted lung, and at specific time points. Serum samples were obtained from recipients before reperfusion of the transplanted lung, and 15 and 60 min following reperfusion. Patients gave informed consent to be involved in the study which was approved by the institutional Ethics Committee. Lung tissue biopsies and serum samples of all patients and controls were snap frozen in liquid nitrogen, until further use as described below.

2.2. Assay

ELISA for VEGF-A (Quantikine, R&D Systems, Minneapolis, MN, USA) was done according to the manufacturer’s protocol. The minimal detectable VEGF level in the assay used was 9 pg/μl, and the microplate was precoated with monoclonal antibody raised against VEGF-A165 that also recognizes VEGF-A121, but does not cross-react with VEGF/PIGF heterodimers, as indicated by the manufacturer. Assay diluent was added onto each well of the microplate, and afterwards 100 μl standard or serum sample were added to each well. The wells were incubated for 2 h at room temperature, then aspirated and washed three times. VEGF antibody conjugated to horseradish peroxidase (200 μl) was added, and the mixtures were incubated for 2 h at room temperature followed by three washes. Then, 200 μl substrate solution (stabilized hydrogen peroxide, stabilized chromogen tetramethylbenzidine) was added to each well and incubated for 25 min at room temperature protected from light. The reaction was stopped by addition of 50 μl 1 mol/l sulfuric acid. The optical density was measured at 450 nm (microplate reader; Anthos, Salzburg, Austria).

2.3. Western blotting

Lung tissue biopsies were lyzed in solubilization buffer (10 mM Tris–HCl, 50 mM NaCl, 1% Triton X-100, 30 mM sodium pyrophosphate, 100 μM Na3VO4, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1X Complete™ — EDTA-free Protease Inhibitor Cocktail (Roche, Mannheim, Germany)). Insoluble material was removed by centrifugation (15000 rpm, 15 min, and 4°C). Tissue lysates (50 μg/lane) were separated by a 10% or 12% (according to protein size) SDS-PAGE prior to electrophoretic transfer onto 0.2 μm immuno-blot PVDF membrane for protein blotting (Bio-Rad Laboratories, Hercules, CA, USA). The blots were probed with polyclonal antibodies against VEGF-A (Neomarkers, Union City, CA, USA) and VEGF-C (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and monoclonal mouse antibodies against HIF-1α (Alexis Biochemicals, San Diego, CA, USA) and HIF-1β (Neomarkers) prior to incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Buckinghamshire, UK; Santa Cruz Biotechnology, and Jackson Immuno Research Laboratories, West Grove, PA, USA). Proteins were detected on the membrane using chemiluminescence (ECL, Amersham Biosciences) and specific protein bands were quantified using ImageQuant Version 5.0 software (Amersham Biosciences).

2.4. Real time RT-PCR

Total RNA was isolated from the homogenized recipient lung biopsies by a standard guanidinium thiocyanate-phenol-chloroform extraction [10]. cDNA was synthesized using avian myeloblastosis virus reverse transcriptase and 2 μg of total RNA primed with oligo dT-primer. After reverse transcription of RNA into cDNA, real time PCR was used to monitor gene expression using a LightCycler Instrument (Roche, Mannheim, Germany) according to the standard procedure [9]. Primers for real time PCR were designed using Prime software (Genetics Computer Group Package, Wisconsin, USA). The primer sequences used were: sense/antisense: VEGF-A165: 5′-AGCCCTTGGCCGCTTGGTCTCTA-3′/5′-GTGCTGCGCTTGGT-AGG-3′; VEGF-A189: 5′-AGCCCTTGGCCGCTTGGTCTCTA-3′/5′-GGGATTTGGCGCTTGGTCTCTA-3′; VEGF-B: 5′-TGCAAGACCTCATGATCCG-3′/5′-TGCTGGTCTCTACAGCACACT-3′; VEGF-C: 5′-CCCAAACTAACAATCT-3′/5′-CACAGGACATTTCCAG-3′; Flt-1: 5′-GTCACAGAAGAGATGAAGGT-3′/5′-CACAGT-

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CCGGCGACTTAGTGATT-3'; KDR: 5'-GCATCTCACTCGTTACAG C-3'/5'-CTTCTACATCTTTTACTCCC-3'; Ang1: 5'-TTCAGACCAC ACGGCTAC-3'/5'-TCTCCTCTTCTTCTCC-3'; Ang2: 5'-TGCA GTCACTACCTTC-3'/5'-CTCTACATCACGCCGT-3'; Tie2: 5'- TTCAACATTACACACTCC-3'/5'-GCTATAAGCACGATCTCC-3'; HIF-1α: 5'-CTGAGCCCTAATGTC-3'/5'-GTTGCCATTAGCAG TAGG-3'; HIF-1β: 5'-GGTCACAGAAGAGATGG-3'/5'-GGGAGA GAATAGCTGTTGGG-3'; CD31: 5'-GCTCTCTGTACATTGGCG-3'/ 5'-GAGGACCTTGAATCTCC-3'; β-Axin: 5'-TGCATCTCAAAA GCCAC-3'/5'-TCAAATGCTCTCAAGTCGTG-3'. PCR was per- formed using 'Hot Start' reaction mix (LightCycler — Fast Start DNA Master SYBR Green I). One microliter of cDNA was added to 19 μl of reaction mixture prepared according to the manufacturer’s protocol. The temperature profile included an initial denaturation for 10 min at 95 °C, followed by 37 cycles of denaturation at 95 °C for 15 s, annealing at different temperatures for 5 s, elongation at 72 °C (elonga- tion time depends on the length of the synthesized fragment, base pair divided by 25 yielded the time in seconds), and fluorescence monitoring at temperatures that depend on PCR-product length (82–90 °C). LightCycler3 Data Analysis Version 3.5.28 was used for PCR data analysis. The specificity of the amplification reaction was determined by performing a melting curve analysis. Standard curves for expression of each gene were generated by serial dilution of quantities of the signals was done by normalizing the signals of the different genes with the β-actin signal.

2.5. Electrophoretic mobility shift assay

Protein lysates were prepared as described above. EMSA probes were prepared by labelling a single stranded oligonucleotide with [γ-32P] ATP (Applied Biosystems, Boston, MA, USA), using a T4 polynucleotide kinase (New England Biolabs Inc., Beverly, MA, USA) and by annealing a complementary single stranded oligonucleotide. Labelled probes were purified using Sephadex-G50 spin columns. The sequence of the EMSA probe was for HIF 5'-CAG TGC ATA GTG CTG CTC CAA CAG GTC CTC TCC TCC C-3' sense and 5'-GGAG AGA GGA CCT GTT GGA GCC CAC GTA TGC ACT G-3' antisense. The probes were prepared by mixing 10 μl of 2× binding buffer (40 mM HEPES, 2 mM MgCl2, 8% Ficoll, 80 mM KCl, 0,2 mM EDTA, 0,2% NP-40), 1 μl salmon sperm DNA (1 μg/μl), 0,3 μl labelled double-stranded oligonucleotide, 20 μg protein extract. DNA binding complexes were separated by a 5% polyacrylamide-TBE gel run at 4 °C for 4 h at 180 V. Gels were dried, exposed using a PhosphoImager cassette (Amersham Biosciences) and transferred to a computer. The obtained images were analyzed off-line using Lucia G morphometry software (Nikon). Using VE-cadherin staining all capillaries were identified and marked by electronic dots and their density was evaluated in each field, yielding the MVD. The average MVD was then calculated for each section.

2.7. Wet-to-dry lung weight

As a measure of pulmonary edema in the explanted recipient lungs, wet-to-dry lung weight ratio was determined as described earlier [9]. The wet-to-dry lung weight ratio would be expected to increase with an increase in extravascular lung water.

2.8. Diagnosis and grading of PGD

Recipient blood gases and chest radiographs during the first 3 postoperative days were monitored for grading of PGD [7]. PGD was diagnosed and graded according to the ISHLT guidelines [7]. The lowest PaO2/FiO2 ratio occurring during the first 72 h following transplantation was used for PGD grading. Intra-operative extracorporeal membrane oxygenation (ECMO) was used alternatively to cardiopulmonary bypass (CPB) when single lung ventilation was not tolerated or in case of hemodynamic instability. Post-transplant ECMO was used as treatment for right ventricular failure or as treatment in patients with PGD grade 3. Patients on therapeutic postoperative ECMO or on mechanical ventilation with FiO2 > 0.5 on NO beyond 48 h from the time of transplant because of PGD were considered grade 3. Patients receiving oxygen per nasal canula or FiO2 < 0.3 within the first 72 h following transplantation were considered grade 0 or 1 depending on chest X-ray [7].

2.9. Statistical analysis

The mRNA and protein expression levels of all molecules as well as wet-to-dry lung weight ratios, MVD, and VEGF serum levels were compared between CF, COPD, and control patients or PGD grade 3 and grade 0-2 patients by analysis of variance. The correlation of VEGF tissue expression levels with PGD grades was examined by Spearman’s correlation coefficient. The simultaneous influence of pretransplant VEGF serum levels and diagnosis of CF versus COPD on PGD grade 3 risk was determined by multivariate logistic regression analysis. Data are expressed as mean value ± SD and statistical significance is set at P < 0.05.

3. Results

In explanted lung tissue of CF patients, VEGF-A165 mRNA levels were upregulated as compared with lung tissue of COPD patients and controls (P = 0.042 and 0.040, respectively), whereas mRNA levels of VEGF-A189, VEGF-B, and VEGF-C were not significantly changed (Fig. 1A). VEGF
receptor-1 (Flt-1) mRNA was upregulated in CF patients versus COPD patients and controls \((P = 0.039)\), although VEGF receptor-2 (KDR) mRNA expression was not significantly changed \((P = 0.043 \text{ and } 0.038, \text{ respectively; Fig. 1B})\). In correlation with mRNA expression levels, VEGF-C protein expression was not changed in CF versus COPD patients and controls \((P = 0.043 \text{ and } 0.038, \text{ respectively; Fig. 1B})\). The mRNA expression of angiopoietin (Ang1) and Ang2 and their receptor Tie2 showed no significant change in CF or COPD lung tissues versus controls \((P = 0.043 \text{ and } 0.038, \text{ respectively; Fig. 1C})\). The expression of the HIF-1 transcription factor subunits HIF-1α and HIF-1β was not changed at mRNA level in CF or COPD patients versus controls \((P = 0.037 \text{ and } 0.038, \text{ respectively; Fig. 2A})\). However, HIF-1α protein expression was significantly increased both in CF and COPD lung tissues versus controls \((P = 0.037 \text{ and } 0.038, \text{ respectively; Fig. 2B})\). In EMSA, increased binding activity of HIF-1 to the HIF binding site (HBS) of the VEGF promoter was observed in CF and COPD lung tissue samples versus controls \((P = 0.0005 \text{ and } 0.0008, \text{ respectively; Fig. 2C})\). To determine the main pathophysiological effects of VEGF excess production in the CF lung, angiogenesis was assessed by examination of CD31 mRNA expression and microvascular density; and the vascular permeability was monitored by measuring the wet-to-dry weight ratio in lung tissue samples. The mRNA expression levels of endothelial cell marker CD31 were not significantly changed in CF or COPD lung tissue \((P = 0.0005 \text{ and } 0.0008, \text{ respectively; Fig. 3A})\). Similarly, the microvascular density was not significantly changed in the CF or COPD lung tissue \((P = 0.004 \text{ and } 0.009, \text{ respectively; Fig. 3B})\). However, wet-to-dry lung weight ratio was elevated in CF and COPD patients as compared with controls \((P = 0.004 \text{ and } 0.009, \text{ respectively; Fig. 3D})\). Pretransplant VEGF serum concentrations were significantly higher in CF patients than in COPD patients \((P = 0.023; \text{ Fig. 4A})\). VEGF serum levels before lung graft reperfusion and at 15 and 60 min following reperfusion were
significantly elevated in CF versus COPD patients ($P < 0.05$; Fig. 4Ab-d). Wet-to-dry lung weight ratios of all donor lungs were similar at the end of warm ischemia (Fig. 4Ba). However, 60 min following reperfusion the wet-to-dry lung weight ratios of donor lung grafts transplanted to CF patients did not significantly change, while the wet-to-dry weight ratios of lung grafts in COPD patients decreased significantly ($P = 0.031$; Fig. 4Bb). PGD grade 3 occurred in 5 of 22 CF patients (23%), whereas PGD grade 3 was only observed in 1 of 25 COPD patients (4%; Table 2). Of 22 CF patients, 15 were transplanted using ECMO support. No CF patient was transplanted using cardiopulmonary bypass. Of 25 COPD patients, 2 required intra-operative ECMO and 1 was

![Fig. 2. (A) mRNA expression levels of HIF-1$\alpha$ and HIF-1$\beta$ in control, CF, and COPD lung biopsies. (B) Quantification of protein levels of HIF-1$\alpha$ and HIF-1$\beta$. HIF-1$\alpha$ protein is elevated in CF and COPD versus control lung biopsies; HIF-1$\beta$ protein is unchanged. (C) Quantification of HIF-1 DNA binding activity to VEGF promoter in EMSA and a representative EMSA image of HIF-1$\alpha$. HIF-1$\alpha$ DNA binding activity is increased in lung tissue of CF and COPD patients versus controls. Results are expressed as mean $\pm$ SD. (*) significantly different from controls ($P < 0.05$, B; $P < 0.001$, C).](https://academic.oup.com/ejcts/article-abstract/32/1/35/451924)

![Fig. 3. (A) mRNA expression of CD31 in control, CF, and COPD lung biopsies. (B) MVD in control, CF, and COPD lung tissue sections expressed as number of vessels per microscopic field. (C) Representative immunohistochemistry images, each showing two VE-cadherin positive vessels in a control, CF, and COPD lung. (D) The wet-to-dry lung weight ratio is significantly increased in CF and COPD versus control lungs. Results are expressed as mean $\pm$ SD. (*) significantly different from controls ($P < 0.05$).](https://academic.oup.com/ejcts/article-abstract/32/1/35/451924)

![Fig. 4. (A) VEGF serum levels in CF versus COPD patients: (a) pretransplant, (b) before reperfusion of the first transplanted lung, (c) 15 min after reperfusion of the donor lungs, and (d) 60 min after reperfusion of the donor lungs. (B) Wet-to-dry lung weight ratios of lung grafts transplanted to CF versus COPD patients: (a) before reperfusion and (b) 60 min after reperfusion. (C) Pretransplant VEGF serum levels of patients in PGD grade 3 are significantly higher than in patients in PGD grade 0–2 ($P < 0.001$). Results are expressed as mean $\pm$ SD. (*) significantly different ($P < 0.05$) from COPD patients (A), lung grafts of CF patients (B), or PGD grade 0–2 (C).](https://academic.oup.com/ejcts/article-abstract/32/1/35/451924)

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<td>Cystic fibrosis, n</td>
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<td>3</td>
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25 COPD patients (4%; Table 2). Of 22 CF patients, 15 were transplanted using ECMO support. No CF patient was transplanted using cardiopulmonary bypass. Of 25 COPD patients, 2 required intra-operative ECMO and 1 was
transplanted using cardiopulmonary bypass. Pretransplant VEGF serum levels were significantly higher in patients with PGD grade 3 compared with PGD grade 0–2 ($P = 0.005$; Fig. 4C). Tissue VEGF expression on mRNA and protein level correlated slightly with PGD grade, however, without reaching statistical significance. Interestingly, when assessing the simultaneous impact of diagnosis (COPD or CF) and pretransplant VEGF serum levels on PGD risk (PGD grade 0–2 versus grade 3) in a multivariate logistic regression analysis, it turned out that serum VEGF ($P = 0.013$) was a slightly better predictor of PGD than diagnosis of CF ($P = 0.112$).

4. Discussion

VEGF-A has been shown to mediate increased vascular permeability in donor lung grafts, and it may be involved in development of graft edema in PGD [8,9]. Hypoxia and pro-inflammatory cytokines induce the expression of VEGF-A by the transcription factor HIF-1 that binds to the cis-acting HBS in 5′-flanking region of VEGF gene [5]. HIF-1 originates from heterodimerization of its subunits HIF-1α and HIF-1β. HIF-1 activity is mainly regulated by increased production and stabilization of HIF-1α protein. Hypoxia enhances stabilization of HIF-1α protein in various tissues, leading to increased DNA-binding activity of HIF-1 to the VEGF promoter [11]. In this study, increased binding of HIF-1 to VEGF promoter was observed in CF as well as in COPD lung tissue. However, increased VEGF expression as a result of HIF-1 activation was only observed in CF lung tissue. In line with this, VEGF expression has been described to be downregulated in COPD patients with predominant emphysema [12]. These findings indicate that the mechanistic association between HIF-1 activation, VEGF overexpression, and increased vascular permeability can only be postulated in CF, not in COPD. Elevated VEGF serum levels have been demonstrated in CF patients with pulmonary exacerbations and correlated with the decrease in FEV1 [3]. Here we show that in addition to increased tissue VEGF expression levels, end-stage CF patients have elevated VEGF serum concentrations. Increased pulmonary VEGF protein levels in CF may contribute to elevated circulating VEGF levels in CF patients. In broncho-alveolar lavage fluid of CF patients, elevated concentrations of α2-macroglobulin, a marker of vascular leakage through the alveo-capillary membrane, have been reported [13]. In the present study, we found increased wet-to-dry lung weight ratios in CF and COPD lung tissues versus controls, indicating increased extravascular lung water content. However, the mechanisms leading to increased vascular permeability are most likely different in CF and COPD patients. While increased vascular permeability is associated with increased VEGF expression in CF patients, VEGF levels are unchanged in COPD patients versus controls. Furthermore, CF patients carry a higher risk of PGD following lung transplantation compared with COPD patients [14; and this study]. This implies that elevated pulmonary tissue and serum VEGF levels in CF patients possibly increase the risk of edema formation in the graft. In line with this, CF patients in this study showed a higher donor lung tissue water content at 60 min following reperfusion compared with COPD patients. In contrast, the mechanism leading to increased vascular permeability in COPD lung tissue shown in this study is probably tissue-based, eliminated by explantation of the recipient lung, and has no impact on the vascular permeability in the lung graft. The mechanistic link between VEGF expression and pulmonary edema has also been established in earlier studies on overexpression of VEGF in mouse lung [15,16]. These studies have shown that pulmonary VEGF overexpression leads to edema, hemorrhage, and macrophage accumulation. In the present study, VEGF serum levels in both patient groups decreased before reperfusion of the first transplanted lung graft compared with pretransplant values, although CF patients still had higher VEGF levels than COPD patients at this time point. Whether VEGF serum levels are still high enough to induce increased vascular permeability in the lung graft upon reperfusion or which role is played by local VEGF expression in the graft remains uncertain. However, increased tissue water content has been found in donor lungs transplanted to CF patients in our study and experimental studies point in the direction that VEGF is directly involved in edema formation following ischemia-reperfusion [17]. Furthermore, it cannot be ruled out that other yet unknown factors participate in the pathogenesis of lung graft edema, and that circulating VEGF may just be a marker or a trigger for increased pulmonary allograft vascular permeability in patients at risk.

Opposed to previous reports [2], we found no evidence of enhanced angiogenesis in CF lungs, suggesting a predominant role for VEGF-A in the promotion of vascular permeability rather than angiogenesis. Unchanged expressions of VEGF-B and VEGF-C in explanted CF lungs suggest that these factors do not contribute to CF lung pathophysiology. Promoter studies have discovered a HBS on the Flt-1 promoter, whereas KDR is not regulated by hypoxia [18]. Therefore, the elevated mRNA level of Flt-1 in CF pulmonary tissue may be caused by the action of HIF-1. However, in COPD lung tissue, which also showed increased HIF-1 DNA-binding activity, no Flt-1 mRNA increase was detected. Additionally, Flt-1 overexpression may be related to monocytes and mediate monocyte chemotaxis in response to elevated VEGF levels in CF [19]. Taken together, we cannot exclude that VEGF expression in CF pulmonary tissue may be additionally induced by other factors than HIF-1, e.g. IL-6 [20] or activation of neutrophils [21], and that these factors may be responsible for the discrepancy in the molecular profile between COPD and CF patients that was detected in spite of activation of HIF-1 in both patient groups. Ang1 and Ang2, acting through their receptor Tie2, promote angiogenesis and vascular remodeling in concert with VEGF [22]. As Ang1 and 2 and Tie2 mRNA expressions are not changed in CF or COPD, it is unlikely that they play a role in pulmonary tissue remodeling in these diseases.

One limitation of mRNA expression analysis in lung tissue is that the biopsy samples may contain a heterogenous tissue composition, so that one sample may contain more bronchial tissue while another may contain more blood vessels. To minimize this effect, all biopsies were taken at the same localization from the peripheral upper lobe. However, some of the relatively high standard deviations might be still the result of tissue heterogeneity.

Increased VEGF expression in end-stage CF patients undergoing lung transplantation may constitute a novel
recipient factor contributing to increased graft vascular permeability following reperfusion. Interestingly, in multivariate analysis increased serum VEGF was a more significant predictor of PGD grade 3 than diagnosis of CF, indicating that the correlation between increased VEGF serum levels and PGD may be particularly striking in CF patients, but not disease specific. Furthermore, the association of increased VEGF serum levels with PGD was more significant than the association of tissue VEGF expression with PGD, leading to the conclusion that increased graft vascular permeability is mediated rather by elevated VEGF levels in the intra-vascular compartment than in parenchymal recipient lung tissue comprising alveolar, airway, and vascular structures. In summary, our findings favor a role of VEGF in development of lung graft edema in CF patients undergoing lung transplantation.

References

Appendix A. Conference discussion

Dr R. Schmid (Bern, Switzerland): Do you have any treatment modalities to reduce VEGF pretransplant? And how was the evolution of VEGF after transplantation? Did you do any measurements after the transplantation?

Dr Krenn: Concerning treatment, maybe it would be an option in the future to have a short-acting VEGF antagonist; however, that’s not the case so far. Concerning the VEGF serum levels post-transplant, we have measured these in some patients, and the tendency is that they go down after transplantation, they diminish. I can say so far that the decrease in VEGF serum levels is greater in patients with grade 3 PGD, but there is not much data at the moment.

Dr A. Turna (Istanbul, Turkey): Did you look at any possible correlation between any immunological parameters and VEGF levels? What do you think about the source of the VEGF, from endothelial or any immunological source, like T lymphocytes? Do you have any speculation on that?

Dr Krenn: We did a correlation between VEGF serum levels and CRP as a marker of inflammation, but there was no correlation. My speculation about the source of the VEGF, from endothelial or any immunological source, like T lymphocytes? Do you have any speculation on that?