hypercoagulable state secondary to hypoxia and/or heightened systemic inflammation. We investigated the effects of hypoxia on markers of coagulation and systemic inflammation in patients with COPD.

Methods: Twenty clinically stable patients with COPD, who were not receiving an inhaled corticosteroid, were recruited. Patients were randomly assigned to receive either medical air or 100% nitrogen through a 40% venturi mask at a flow rate of 10 l/minute for 2 h. All patients had spirometry prior to testing. Oxygen saturations, blood pressure and heart rate were measured throughout the study. Blood was sampled for thrombin anti-thrombin complex (TAT), prothrombin activation fragments 1+2 (F_{1+2}), von Willebrand factor (vWF), D-dimer and IL-6 at baseline and at 2 h. Non-parametric data were $\log_{10}$ transformed. Measurements at baseline and after 2 h testing were compared using the paired student t test.

Results: Patients (14 male), had a mean (SD) age of 68.8 years (8.3) and a mean (SD) FEV_1 of 1.75 litres (0.53). Patients in the hypoxia and control groups were similar in terms of age, gender, pack years smoked and severity of airflow obstruction. Baseline TAT, F_{1+2}, vWF, D-dimer and IL-6 levels and oxygen saturations were also similar between the groups. In the control group, there was no change in markers of coagulation or systemic inflammation over the 2-h period. In patients who underwent hypoxic challenge, there was an increase in $\log_{10}$ TAT ($p<0.001$), $\log_{10} F_{1+2}$ ($p<0.01$) and $\log_{10}$ IL-6 ($p<0.01$), whereas D-dimer and vWF levels were similar. Changes in serum IL-6 were related to changes in F_{1+2} ($r = 0.65$, $p<0.05$) but not TAT ($r = 0.39$, $p = 0.09$).

Conclusions: This single blind placebo-controlled pilot study demonstrates that a 2-h hypoxic challenge results in an increase in markers of coagulation and systemic inflammation, which are linked and which may explain the increased risk of VTE in COPD patients who are experiencing an acute exacerbation.

Abstract S51 Table

	Baseline	After 2 h	p Value
Oxygen saturations	94.4 ± 2.3	92.0 ± 3.5	0.096
TAT	10.2 ± 3.16	130.4 ± 3.6	<0.001
F_{1+2}	334.3 ± 2.47	1878.0 ± 3.93	0.01
D-dimer	173.1 ± 90.0	204.4 ± 89.6	0.10
vWF	124.2 ± 20.5	1167 ± 18.3	0.39
IL-6	3.17 ± 1.36	4.67 ± 1.40	0.002

F_{1+2}, prothrombin activation fragments 1+2; TAT, thrombin anti-thrombin complex; vWF, von Willebrand factor.

S52 ENDOTHELIAL DYSFUNCTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: IMPAIRED ENDOGENOUS FIBRINOLYSIS

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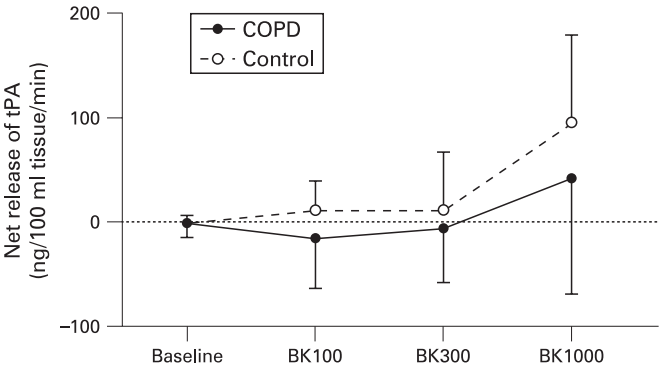
Introduction: Chronic obstructive pulmonary disease (COPD) is now established as a condition that not only affects the lungs, but also has systemic features. Cardiovascular disease is common in COPD, and contributes significantly to both morbidity and mortality. Dysfunction of the systemic vascular endothelium contributes to the formation and progression of atherosclerotic plaques. Stable and unstable coronary artery disease, smoking, air pollution and systemic inflammation have all been associated with impairment of endothelial function.

Hypothesis: Patients with COPD have impaired endothelial fibrinolytic and vasomotor function in comparison with age, sex and smoking-matched controls.

Methods: 17 male ex-smokers with and without COPD, with no history of cardiovascular disease were recruited. Forearm blood flow (FBF) in response to endothelial-dependent vasodilators (bradykinin and acetylcholine) and endothelial-independent vasodilators (sodium nitroprusside and verapamil) were measured using venous occlusion plethysmography. Bradykinin was used to stimulate endothelial tissue plasminogen activator (tPA) release. Venous blood was collected at baseline and following each infusion of bradykinin for tPA antigen.

Results: COPD patients were well-matched with controls regarding age and smoking history. Baseline blood flow was the same in COPD patients as in controls and remained so prior to each intra-arterial infusion. There were no differences in FBF responses to endothelial-dependent vasodilators between the two groups (COPD vs control: bradykinin, peak response, 16.0 vs 15.6 ml/100 ml of tissue/minute; $p = 0.55$; acetylcholine, peak response, 5.7 vs 6.5 ml/100 ml of tissue/minute; $p = 0.52$). There was no difference in FBF responses to endothelial-independent vasodilators between the two groups (sodium nitroprusside, peak response, 10.1 vs 9.6 ml/100 ml of tissue/minute; $p = 0.37$; verapamil, peak response, 11.5 vs 12.1 ml/100 ml of tissue/minute; $p = 0.67$). Bradykinin caused a dose-dependent increase in tPA antigen in both groups. Baseline net tPA antigen release was similar in each group, but bradykinin-stimulated release of tPA was reduced in the COPD group ($p = 0.035$) (see fig).

Conclusion: COPD is associated with impaired endothelial fibrinolytic function, which may contribute to the increased cardiovascular risk associated with this condition. There was no impairment of endothelial-dependent or independent vasomotor function.



Abstract S52 Figure Net tPA release.

Novel mechanisms in interstitial lung disease

S53 GALECTIN-3 REGULATES EPITHELIAL TO MESENCHYMAL TRANSITION IN LUNG EPITHELIAL CELLS

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Galectin-3 is a beta-galactoside binding animal lectin of approximately 30 kDa, which is highly expressed in fibrotic tissue of diverse aetiologies. Mice deficient in galectin-3 develop reduced fibrosis in several models of organ fibrosis in vivo. Galectin-3 is secreted by macrophages and is a potent mitogen for fibroblasts in vitro. In the chronic inflammatory milieu macrophages interact with other cell types including cells of mesenchymal origin (fibroblasts), which transdifferentiate into matrix-secreting myofibroblasts, with resultant scar formation and disruption of tissue architecture. Our previous work has demonstrated that fibroblasts deficient in galectin-3 fail to differentiate into myofibroblasts in

vitro and in vivo. In addition, myofibroblasts may arise from epithelial cells by a process of epithelial to mesenchymal transition (EMT). EMT of alveolar epithelial cells (AEC) has been widely observed in patients with interstitial pulmonary fibrosis (IPF). AEC convert into myofibroblasts following exposure to the profibrotic cytokine transforming growth factor beta (TGF-β) raising the possibility that epithelial cells may serve as a novel source of myofibroblasts in IPF. Primary epithelial cells from galectin-3 null mice show reduced differentiation into myofibroblasts in response to TGF-β in vitro, suggesting that galectin-3 may play a role in EMT in these cells. In the human lung epithelial cell line A549, inhibition of galectin-3 function with Bis-(3-deoxy-3-(3-methoxybenzamido)-β-D-galactopyranosyl)-sulfane a specific inhibitor of extracellular galectin-3 carbohydrate binding and siRNA-mediated depletion of galectin-3 and its membrane receptor CD98 inhibited TGF-β-mediated downstream signalling showing reduced activation of SMAD2/3. Inhibition of galectin-3/CD98 function reduced TGF-β-mediated expression of the myofibroblast marker alpha-smooth muscle actin and increased expression of the epithelial marker E-cadherin suggesting that galectin-3/CD98 regulates TGF-β-induced signalling and the development of EMT in alveolar epithelial cells. Therefore galectin-3 via its interaction with CD98 plays a key role in TGF-β-induced EMT in lung. Strategies to inhibit galectin-3 function may have potential as antifibrotic agents in IPF.

S54 REGULATION OF ALTERNATIVE MACROPHAGE ACTIVATION BY GALECTIN-3

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Macrophages stimulated with Th2 cytokines IL-4 and IL-13 have been classified as alternatively activated and are implicated in diverse disease pathologies such as asthma, organ fibrosis and granulomatous diseases. Alveolar macrophages from patients with idiopathic pulmonary fibrosis (IPF) exhibit an alternative macrophage phenotype, which might be a part of a positive feedback loop with lung fibroblasts perpetuating fibrotic processes. Galectin-3 is a carbohydrate-binding lectin present on macrophages. Disruption of the galectin-3 gene specifically restrains IL-4/IL-13-induced alternative macrophage activation in bone marrow-derived macrophages in vitro and in resident lung macrophages in vivo without affecting IFNγ/lipopolysaccharide-induced classic activation or IL-10-induced deactivation. Increased galectin-3 expression and secretion is a feature of alternative macrophage activation, IL-4 stimulates galectin-3 expression and release in parallel with other phenotypic markers of alternative macrophage activation. By contrast, classic macrophage activation induced by lipopolysaccharide inhibits galectin-3 expression and release. IL-4-mediated alternative macrophage activation is inhibited by siRNA targeted deletion of galectin-3 or its membrane receptor CD98 and by inhibition of phosphoinositol-3OH kinase. In addition, IL-4 induced alternative activation is blocked by Bis-(3-deoxy-3-(3-methoxybenzamido)-β-D-galactopyranosyl)-sulfane, a specific inhibitor of extracellular galectin-3 carbohydrate binding. These results demonstrate that a galectin-3 feedback loop drives alternative macrophage activation. Our previous studies have shown that mice deficient in galectin-3 display reduced fibrosis in several models of organ fibrosis. Mice treated intratracheally with bleomycin show increased expression of galectin-3 in broncoalveolar lavage fluid, which correlates with the onset of lung fibrosis. Pharmacological modulation of galectin-3 function may represent a novel therapeutic strategy in pathologies associated with alternatively activated macrophages such as IPF.

S55 THE BENEFICIAL EFFECTS OF ORAL COTRIMOXAZOLE (SEPTRIN) UPON PERIPHERAL BLOOD CHANGES IN USUAL INTERSTITIAL PNEUMONIA AND NON-SPECIFIC INTERSTITIAL PNEUMONIA, WHICH ARE LIKELY BIOMARKERS OF OXIDATIVE STRESS

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Introduction: Glutathione (GSH) is a major lung antioxidant with reduced levels described in interstitial lung disease (ILD) that are improved by N-acetylcysteine, a precursor of GSH. Gamma glutamyl transferase (γGT) is key in the catabolism of GSH and produces reactive oxygen species (ROS). In mice, the genetic absence of γGT protects the lung against bleomycin-induced fibrosis and therefore supports the proposed adverse effects of γGT in lung fibrosis. Red blood cells exposed to ROS easily undergo membrane lipid peroxidation with increases in mean cell volume (MCV). Lung oxidative stress will recruit monocytes (MΦ) via cytokines such as granulocyte macrophage colony-stimulating factor from the bone marrow. We have observed a consistant feature (not yet described) in cases of ILD in which MCV, γGT and MΦ are increased in new patients referred for treatment. Patients treated with oral septrin following our pilot study, have shown reductions in these parameters with clinical improvement by 3 months.

Method: 149 patients (age range 41–92 years) with new diagnosis of ILD had baseline blood samples and computed tomography scans classified. The groups were compared with an age and gender-matched control population (n = 160) measured in the same laboratory. Changes after oral septrin treatment for a minimum of 3 months have been looked at in 59 patients.

Results: Means ± SD: Comparisons with controls (unpaired t test). Pre and post-septrin treated (paired t test) (see table).

Conclusion: MCV, γGT & MΦ were significantly increased in ILD and may represent oxidative stress. These parameters were significantly reduced by septrin treatment suggesting reduced oxidative stress.

Abstract S55 Table

Parameters (normal range)	MCV 84–98 fl	p Value vs control	γGT 7–40 U/l	p Value vs control	MΦ <0.6 × 10^9/l	p Value vs control
Controls n = 160	89 ± 4.2		29 ± 2.2		0.35 ± 1.1	
UIP n = 104	94 ± 6.4	0.0001	58.6 ± 24	0.0001	0.6 ± 0.3	0.0001
NSIP n = 31	93 ± 7.7	0.0038	38.2 ± 26.6	0.0038	0.6 ± 0.2	0.0001
Mixed UIP/NSIP n = 14	94.5 ± 5.3	0.0001	44.8 ± 29	0.0001	0.7 ± 0.2	0.0001
Pre-septrin UIP n = 39	96.3 ± 5.2	Pre-post p value	55.4 ± 20	Pre-post p value	0.64 ± 0.25	Pre-post p value
Post-septrin UIP	91.5 ± 4.7	0.0001	29.7 ± 34	0.0001	0.32 ± 0.1	0.0001
Pre-septrin NSIP n = 20	92.5 ± 7.4		46.1 ± 21		0.72 ± 0.3	
Post-septrin NSIP	88.1 ± 5.4	0.0001	29.4 ± 31	0.0001	0.45 ± 0.2	0.0001

γGT, gamma glutamyl transferase; MCV, mean cell volume; MΦ, monocytes; NSIP, non-specific interstitial pneumonia; UIP, usual interstitial pneumonia.

S56 ASSOCIATION OF IgG RECEPTOR FcγRIIIB POLYMORPHISMS WITH IDIOPATHIC PULMONARY FIBROSIS

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Background: Engagement of neutrophil Fcγ receptors by IgG complexes may contribute to the pathogenesis of idiopathic pulmonary fibrosis (IPF).[1] Neutrophil FcγRIIIB (CD16B) occurs as two allelic variants (NA1 and NA2) with different binding affinities for particular IgG subclasses. We aimed to determine the association of these polymorphisms with IPF in a cohort of white UK patients.
Methods: We determined FcγRIIIB NA1/2 polymorphisms in 67 patients with IPF (diagnosed according to the ATS/ERS consensus classification) and in 110 disease-free controls using allele-specific PCR amplification.
Results: Significant skewing in the distribution of FcγRIIIB genotypes was observed between IPF patients and disease-free controls. In the IPF cohort there was higher frequency of the NA1/NA1 genotype (0.18 vs 0.04; $p = 0.003$) and lower NA2/NA2 genotype frequency (0.33 vs 0.52; $p = 0.002$). Overall, NA1 allele frequency was increased in IPF patients compared with controls (0.43 vs 0.26, $\chi^2 = 5.6$; $p = 0.018$). IPF was associated with presence of the NA1 allele (odds ratio (OR) 2.27; 95% CI 1.13 to 3.16, $p = 0.02$), whereas the presence of the NA2 allele may be protective against IPF (OR 0.19; CI 0.05 to 0.63, $p = 0.006$).
Conclusions: We have demonstrated an association between FcγRIIIB polymorphisms and IPF. Given the increased binding affinity of the NA1 isoform for IgG1 and IgG3, these findings support a role for IgG complexes in the pathogenesis of IPF.

1. **Dreisin RB,** Schwarz MI, Theofilopoulos AN, *et al*. Circulating immune complexes in the idiopathic interstitial pneumonias. *N Engl J Med* 1978;**298**:353–7.

S57 PRO-INFLAMMATORY CYTOKINES DYSREGULATE LUNG EPITHELIAL WOUND REPAIR VIA EPITHELIAL TO MESENCHYMAL TRANSITION

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Introduction: Epithelial to mesenchymal transition (EMT) has been implicated in fibrotic remodelling seen in conditions such as idiopathic pulmonary fibrosis and posttransplant obliterative bronchiolitis (OB). The role of inflammation in driving fibrosis remains a subject of debate. Previous work in our laboratory has shown that tumour necrosis factor alpha (TNFα) accentuated transforming growth factor beta (TGFβ) 1 driven EMT. We hypothesised that the pro-inflammatory cytokines IL-1β and IL-8 may also accentuate TGF-β1 driven EMT and contribute to the dysregulated repair in damaged lung tissues.
Aims: To evaluate the effect of IL-1β and IL-8 on TGF-β1 driven EMT in lung epithelium using a standardised epithelial wound model.
Materials and Methods: A549 cells and primary bronchial epithelial cells were cultured for 72 h with TGF-β1 ± IL-1β, IL-8 or TNFα and EMT assessed. Morphology was demonstrated with haematoxylin and eosin stain. Western blot analysis and confocal microscopy were used to study epithelial and mesenchymal protein expression. Pro-matrix metalloproteinase (MMP) production was assessed by gelatine zymography. Extracellular matrix deposition was examined using TCA protein precipitation. Confluent monolayers of A549 cells were injured with a standardised 1 mm wound and treated with TGF-β1 ± IL-1β, IL-8 or TNFα and the quality and rate of wound closure assessed by morphometry.
Results: TGF-β1 dramatically downregulates the expression of the epithelial markers cytokeratin-19 and E-cadherin (22% and 59%, respectively) and upregulates vimentin expression (60%), fibronectin expression (426%) and deposition (170%) and pro-MMP-9 secretion (270%) compared with control cells ($p<0.05$, $n = 3$). Co-treatment of the cells with TGF-β1 plus TNFα or TGF-β1 plus IL-1β significantly upregulated vimentin expression (29% and 41%, respectively), fibronectin expression (60% and 10%, respectively) and pro-MMP-9 secretion (81% and 39%) compared with TGF-β1 alone ($p<0.05$, $n = 3$). Co-treatment with TGF-β1 plus IL-8 had no significant effect compared with TGF-β1 alone ($p>0.05$, $n = 3$). At high concentrations (10 ng/ml) TGF-β1 inhibits wound closure. Co-treatment with TGF-β1 plus TNFα or TGF-β1 plus IL-1β causes accelerated wound closure; however, the quality of the wound repair is highly dysregulated.
Conclusion: IL-1β, but not with IL-8, accentuated TGF-β1 driven EMT in lung epithelial cells. Furthermore, the presence of the pro-inflammatory cytokines IL-1β or TNFα promotes dysregulated wound repair with fibrosis.

Pulmonary arterial hypertension: models and mechanisms

S58 MECHANICAL STIMULATION OF HUMAN LUNG MICROVASCULAR ENDOTHELIAL CELLS CAUSES REMODELLING OF THE ALVEOLAR-CAPILLARY MEMBRANE AND DYSPNOEA IN CHRONIC HEART FAILURE

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Introduction: Dyspnoea is a common and debilitating symptom of chronic heart failure (CHF). Patients with CHF have reduced pulmonary microvascular permeability and gas transfer that persists after effective treatment of pulmonary oedema, suggesting a structural abnormality of the alveolar-capillary membrane (ACM). Structural remodelling of the lungs in CHF is characterised by proliferation of myofibroblasts with excess matrix deposition in alveolar septae. Endothelin-1 (ET-1), a potent vasoconstrictor and mitogen, binding endothelin A receptor and endothelin B receptor on human fibroblasts stimulates collagen 1 synthesis. The plasma ET-1 concentration in CHF correlates with the degree of haemodynamic disturbance, dyspnoea and mortality. We propose that in CHF, pulmonary venous hypertension and resultant mechanical strain on the pulmonary microvasculature stimulates the release by endothelial cells of "fibrogenic" mediators including ET-1. This process contributes to ACM remodelling and dyspnoea in patients.
Methods: The expression of message for components of the ET-1 pathway was investigated in strained (static mechanical strain 20% elongation for 4 h, Flexercell 4000) monolayers of human lung microvascular endothelial cells (HLMVEC; Lonza, UK) and in whole lungs of rats with or without heart failure 16 weeks after myocardial infarction (MI) or a sham procedure.[1] Supernatants from HLMVEC strained in the presence phosphoramidon (0–5 mmol), an endothelial converting enzyme inhibitor were added to serum starved human fetal lung fibroblasts (HFL-1) to assess cellular proliferation (CyQUANT assay).
Results: Expression of ppET-1 by HLMVEC increased after SMS and in whole lungs of rats after MI, with a decrease in endothelin B receptor message also seen in lungs post-MI (fig). Supernatants from HLMVEC stretched in the absence but not in the presence of phosphoramidon (5 mmol), which abolished stretch-induced ET-1 release, increased proliferation by HFL-1 cells.
Conclusion: Mechanical strain stimulates the release of ET-1 by HLMVEC, which may cause local fibroblast proliferation. An increase in ET-1 message is also seen in whole rat lungs at 16 weeks post-MI.
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