

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.

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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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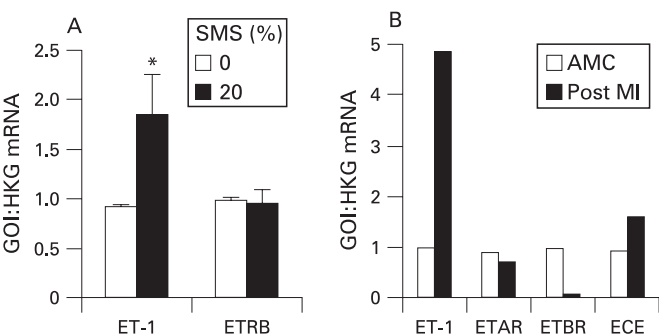
Introduction: Dyspnoea is a common and debilitating symptom of chronic heart failure (CHF). Patients with CHF have reduced pulmonary microvascular permeability and gas transfer that persists after effective treatment of pulmonary oedema, suggesting a structural abnormality of the alveolar-capillary membrane (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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Abstract S58 Figure (A) Relative expression of pre-proEndothelin-1 (ppET-1) and Endothelin B receptor (ETBR) mRNA compared to 2 housekeeping genes (HKG) in HLMVEC after static mechanical strain (SMS); (B) Relative expression of ppET-1, ETRB, Endothelin receptor A (ETRA) and Endothelin Converting Enzyme (ECE) mRNA in male Wistar rats 16 weeks after myocardial infarction (post MI) or sham operation (AMC); GOI- Gene of Interest; * $p < 0.05$ Mann-Whitney.

S59 THE OSTEOPROTEGERIN-TNF-RELATED APOPTOSIS-INDUCING LIGAND AXIS IN PULMONARY ARTERIAL HYPERTENSION

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Pulmonary arterial hypertension (PAH) is a devastating disease characterised by the narrowing and occlusion of small pulmonary arteries due to intimal hyperplasia. We have recently reported that osteoprotegerin, a member of the tumour necrosis factor (TNF) superfamily, is upregulated in serum and lesions of patients with idiopathic PAH. We have also shown that signals from BMP-R2, 5-HT and IL-1 converge upon and upregulate osteoprotegerin expression and that osteoprotegerin induces both proliferation and migration of PA-SMC in vitro, suggesting that osteoprotegerin plays a central role in the pathogenesis of PAH. Interestingly, osteoprotegerin is known to interact with TNF-related apoptosis-inducing ligand (TRAIL) and there is emerging evidence to suggest that TRAIL also plays an important role in vascular tissues. We hypothesised that TRAIL expression is also dysregulated in PAH and that this induces the proliferation and migration of PA-SMC, consistent with a role in the pathogenesis of PAH. TRAIL exists as a type II TNF family transmembrane protein. The extracellular domain can be proteolytically cleaved from the cell surface to act as a soluble cytokine (sTRAIL) capable of binding to five TRAIL receptors, (TRAIL-R1-4) and osteoprotegerin. We found that TRAIL expression was increased in lesions from patients with idiopathic PAH, but that the circulating levels of sTRAIL were significantly lower in idiopathic PAH patient serum compared with controls, leading to a significant difference in the osteoprotegerin:TRAIL ratio between the groups. In vitro, recombinant TRAIL was found to induce both proliferation and migration of human PA-SMC in a dose-dependent manner, a response requiring the phosphorylation of extracellular signal regulated kinase 1/2. Interestingly, the combination of the optimal concentrations of osteoprotegerin and TRAIL prevented both proliferation and migration in PA-SMC, suggesting that the relative concentration of these molecules is important in terms of cell phenotype. Although further work is clearly required, including the study in other groups of PAH patients, these data suggest that the combined increase in circulating osteoprotegerin and the decrease in circulating sTRAIL may play an important role in disease pathogenesis. The ratio of osteoprotegerin:TRAIL may provide a new biomarker for tracking disease progression.

S60 LYMPHOCYTE PROFILES IN IDIOPATHIC AND CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

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Introduction: Autoimmunity is thought to contribute to the pathobiology of pulmonary arterial hypertension (PAH), although the mechanisms remain unclear. Evidence in support of immune mechanisms include the high prevalence of anti-nuclear antibodies,¹ the strong association with certain autoimmune diseases such as scleroderma,² and evidence from animal models.³ We hypothesised that patients with idiopathic PAH may demonstrate differences in lymphocyte profiles compared with normal or disease controls.

Methods: Flow cytometry was used to assess the lymphocyte subsets in patients with idiopathic PAH (n = 10), heritable PAH with bone morphogenetic protein receptor-2 (BMPR2) mutations (n = 3), chronic thromboembolic pulmonary hypertension (CTEPH; n = 8) and healthy age and sex-matched controls (n = 8). All cases were clinically stable and patients with known autoimmune disease, ongoing infection or malignancy were excluded. In addition, explanted lung tissue from PAH patients (n = 20) undergoing transplantation were immunostained for lymphocyte markers to immunophenotype lymphocyte subsets.

Results: These are summarised in the table. Values are expressed as percentages of circulating cells. The percentage of circulating CD4+CD25+ cells was higher in idiopathic PAH but did not quite reach significance (p = 0.07). When this was narrowed down to the T-regulatory subset (CD4+CD25+CD127-) the BMPR-2 patients displayed a significant increase. There was also a significant decrease in CD19+ B cells in the idiopathic PAH patients. There was no difference in circulating percentages of CD3+ lymphocytes, CD3+CD4+ T helper cells, CD3+CD8+ cytotoxic T cells or CD56+ natural killer cells. Immunohistochemical staining has also demonstrated the presence of frequent CD8+ cells and occasional T-regulatory cells surrounding the vascular lesions associated with PAH.

Conclusions: The T-regulatory subset is increased in patients with heritable PAH with BMPR-2 mutations. This is not demonstrated in CTEPH. Further investigation is required to see if this is a pathological or a compensatory mechanism.

Abstract S60 Table Lymphocyte profiling in different populations of pulmonary hypertension

Lymphocyte subset	Controls Mean (SD)	IPAH Mean (SD)	IPAH + BMPR2 Mean (SD)	CTEPH Mean (SD)
CD3+	73.8 (5.4)	75.3 (6.2)	76.7 (4.7)	71.3 (11.9)
CD3+CD4+	49.6 (5.7)	47.0 (6.3)	50.9 (4.3)	50.8 (8.7)
CD3+CD8+	22.1 (5.1)	25.2 (5.3)	23.5 (5.5)	19.0 (7.8)
CD4+CD25+	17.8 (14.3)	32.5 (24.4)	32.1 (11.6)	20.9 (7.5)
CD4+CD25+CD127-	5.2 (1.5)	6.5 (2.3)	7.9 (2.5)*	4.4 (1.8)
CD56+	8.2 (3.4)	8.7 (4.0)	7.0 (3.1)	12.8 (8.1)
CD19+	15.8 (4.3)	12.0 (3.6)*	12.3 (1.1)	12.2 (6.2)

* $p < 0.05$, compared with healthy controls. BMPR2, bone morphogenetic protein receptor-2; CTEPH, chronic thromboembolic pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension.

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2. Mukerjee D, et al. Prevalence and outcome in systemic sclerosis associated pulmonary arterial hypertension. *Ann Rheum Dis* 2003;**62**:1088-93.
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S61 CHARACTERISATION OF PULMONARY VASCULAR REMODELLING IN A MOUSE MODEL OF SCHISTOSOMIASIS

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Introduction: Schistosomiasis is considered to be the commonest worldwide cause of pulmonary hypertension. At present there is no established animal model to study the pathobiology of this important condition. We sought to develop and characterise a mouse model that recapitulates the pulmonary vascular pathology seen in patients with schistosomiasis.

Methods: Mice (C57/BL6) were infected percutaneously with a high dose (~75–100 cercariae) or low dose (~30 cercariae) of *Schistosoma mansoni* and the development of lung and liver pathology was studied in the subacute (high dose) and chronic (low dose) settings. We determined the degree of right ventricular hypertrophy from the ratio of the weight of the right ventricle to that of the left ventricle plus septum. Liver and lung egg counts were performed at specified time points. Pulmonary vascular remodelling was assessed by morphometry following immunohistochemical staining with antibodies for smooth muscle alpha actin and the endothelial marker, von Willebrand factor.

Results: In the subacute, high dose setting mice showed few eggs in the lungs and no evidence of pulmonary vascular remodelling. In contrast, chronically infected animals had a much greater lung egg burden and developed marked pulmonary vascular remodelling, accompanied by perivascular inflammation from 12 weeks onwards. In addition, we observed the presence of plexiform-like lesions in these mice. Lung egg burden positively correlated with both liver egg burden and the degree of right ventricular hypertrophy in the chronic group.

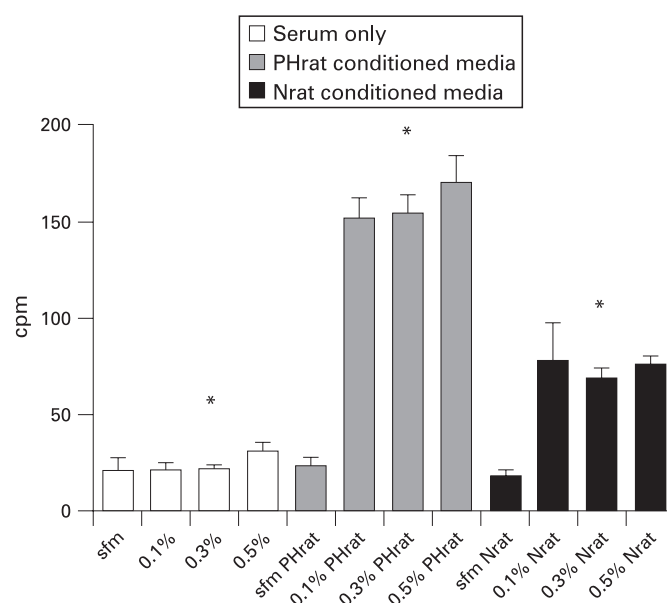
Conclusions: This study provides evidence for extensive pulmonary vascular remodelling in a mouse model of pulmonary schistosomiasis, including the formation of plexiform-like lesions. The degree of remodelling and right ventricular hypertrophy was related to the lung egg burden. This experimental model will be of use to study the pathobiology and treatment of pulmonary hypertension associated with schistosomiasis.

S62 PULMONARY ARTERY FIBROBLASTS DRIVE SMOOTH MUSCLE CELL PROLIFERATION IN A PULMONARY HYPERTENSIVE RAT MODEL

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Introduction: The cause of cellular proliferation leading to vascular remodelling in pulmonary arterial hypertension (PAH) is unknown. We have shown that exposure of pulmonary artery fibroblasts (PAF) to acute hypoxia releases a diffusible factor(s), which causes proliferation of pulmonary artery smooth muscle cells (PASMC) via a p38MAPK (MAPK)-dependent mechanism. We set out to determine if these same mechanisms hold true in a chronic hypoxic pulmonary hypertensive rat model in which pulmonary vascular remodelling is established.

Methods: Primary cell cultures of PAF and PASMC were isolated from rats exposed to hypobaric (550 mBar) hypoxia for 14 days (PHrat) and controls (Nrat) at atmospheric pressure. PAF from both controls and PHrat were treated with low dose fetal calf serum (0.1–0.5%) for 24 h and the supernatant was then transferred to quiescent PASMC in 24-well plates for a further 24 h. All experiments were performed in normoxia. [³H]-Thymidine assays were used to determine cellular proliferation. Total and phospho-p38MAPK levels in the PAF were assessed by Western blotting. The effect of blocking p38MAPK on the release of mitogenic factors was investigated by incubating the PAF with SB-205380, a specific p38MAPK inhibitor.



Abstract S62 Figure SMC proliferation with conditioned media.

Results: There was no proliferative response of the SMC to the low dose serum applied directly to the PASMC (mean cpm 21.5; SD \pm 3.41) (see fig). Although there was some proliferation of PASMC from the supernatant of Nrat PAF, there was a twofold greater proliferation observed with the addition of conditioned media across all ranges of serum from the PHrat PAF (74.4 SD \pm 9.4 vs mean cpm 158.6 SD \pm 11.4; $p < 0.005$). The addition of media obtained from PAF incubated with SB203580 to PASMC abrogated the increased proliferation (data not shown). Western blot confirmed that active phospho-p38 MAPK was increased in the 0.1% serum stimulated cells and absent in the SB203580 treated cells. Total p38 MAPK was the same in all conditions.

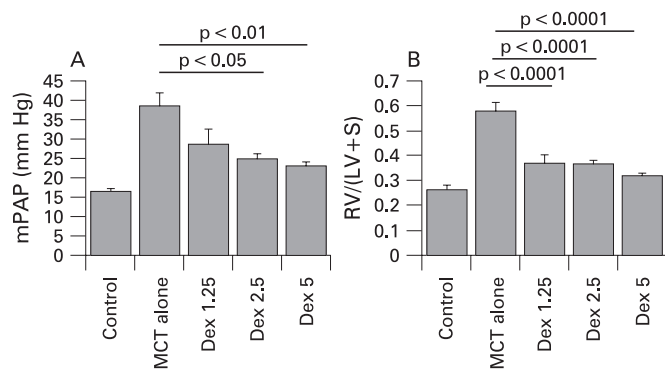
Conclusions: We have shown that fibroblasts from a PAH animal model release yet unidentified mitogenic factor(s) in normoxic conditions, which drives the proliferation of PASMC and is dependent on p38MAPK. Fibroblasts may play an important role in the remodelling process of PAH by inducing proliferation of other pulmonary vascular cell types.

S63 DEXAMETHASONE IMPROVES HAEMODYNAMICS IN ESTABLISHED INFLAMMATORY PULMONARY ARTERIAL HYPERTENSION IN A RAT MODEL

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Introduction and Objectives: There is increasing evidence for the contribution of inflammation to pulmonary vascular remodelling in pulmonary arterial hypertension (PAH), but little on the role of anti-inflammatory therapies in its treatment. Our group and others have shown that dexamethasone can prevent PAH in rats when co-administered with monocrotaline to induce PAH. Monocrotaline is known to induce PAH by 2 weeks and we wished to test the hypothesis that dexamethasone would reverse established PAH at this stage, making it more relevant to human disease.

Methods: Male wistar rats (100 g) were divided into five groups: controls receiving no monocrotaline, those receiving monocrotaline alone and those receiving monocrotaline then dexamethasone 1.25 mg/kg or 2.5 mg/kg alternate days (Dex1.25, Dex2.5) or 5 mg/kg daily (Dex5). Monocrotaline (60 mg/kg subcutaneous injection) was administered on day 1 and dexamethasone was given by intraperitoneal injection from days 15 to 28. At day 28, all



Abstract S63 Figure

rats were anaesthetised (35 mg/kg ketamine, 4 mg/kg xylazine and 0.5 mg/kg acepromazine) and haemodynamics measured using a 3.5 French umbilical vessel catheter. The ratio of right to left ventricular and septal weight was recorded (RV/LV+S).

Results: At day 28, the monocrotaline-alone rats had severe PAH with mean pulmonary arterial pressure (mPAP) (40.8 ± 6.4 mm Hg vs 16.4 ± 1.5 mm Hg, $p < 0.001$), right ventricular systolic pressure (RVSP) (94.3 ± 7.8 mm Hg vs 35.2 ± 2.4 , $p < 0.001$) and RV/LV+S (0.6 ± 0.1 vs 0.26 ± 0.06 , $p < 0.001$) compared with controls. PAH

was reversed in all the groups treated with dexamethasone with a suggestion of a dose-response effect, with mPAP falling to 28.7 ± 11.9 ($p = 0.07$ compared with monocrotaline alone), 24.9 ± 4.7 ($p < 0.05$) and 23.1 ± 3.5 ($p < 0.01$) in Dex1.25, Dex2.5 and Dex5, respectively (fig A). RVSP was also significantly lower in all three groups. Right ventricular hypertrophy as assessed by RV/LV+S was also reversed by dexamethasone compared with monocrotaline alone ($p < 0.001$ in all groups, fig B). None of the measurements in the Dex5 group were statistically different to controls. There was a significant improvement in survival between all the dexamethasone groups when compared with monocrotaline alone (log rank test $p < 0.001$).

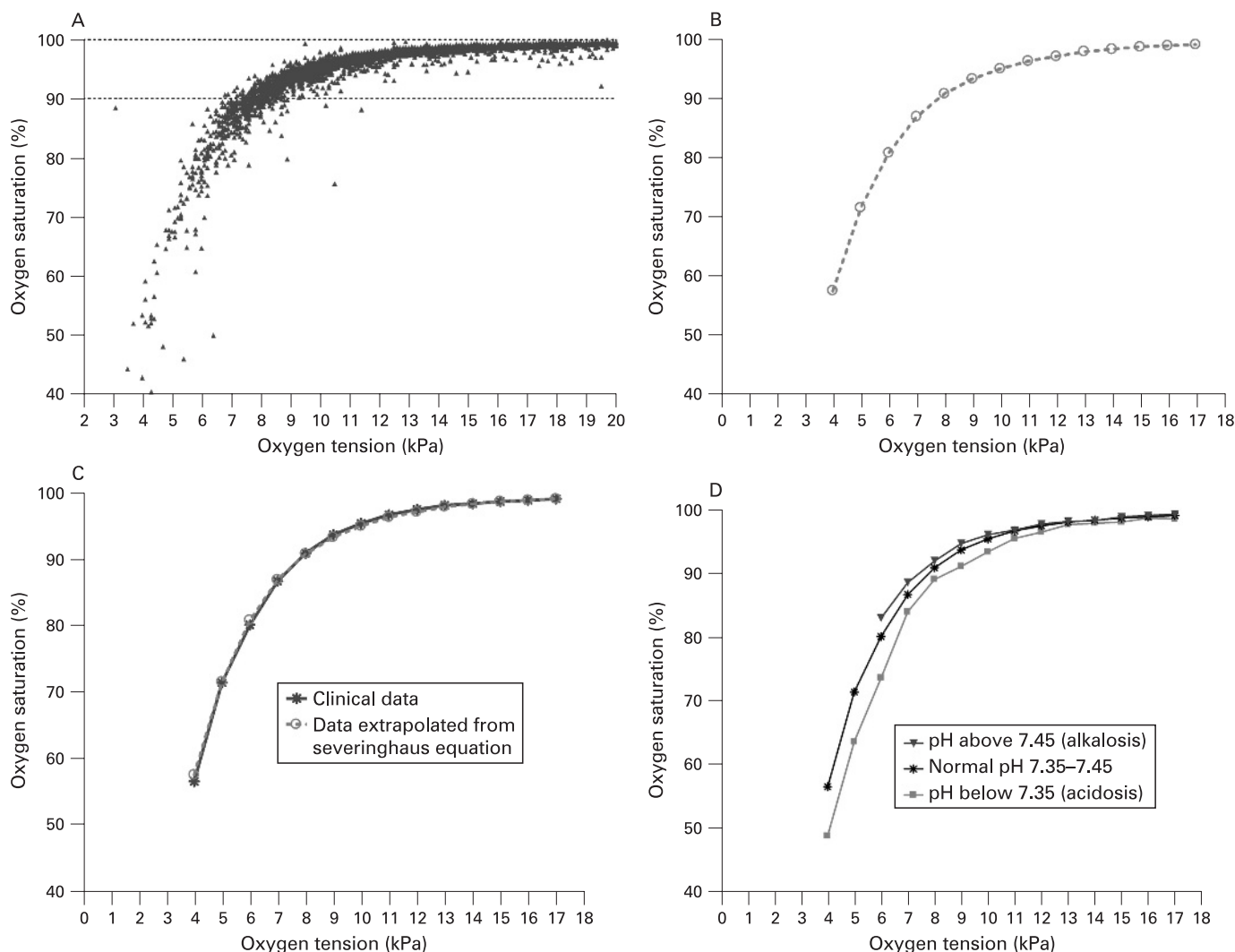
Conclusions: These results suggest that monocrotaline-induced PAH can be reversed by steroids, raising the possibility that an anti-inflammatory strategy may be beneficial in some cases of pulmonary hypertension.

Respiratory physiology

S64 CLINICAL VALIDATION OF THE SEVERINGHAUS OXYGEN DISSOCIATION CURVE

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Background: Severinghaus has described a widely used mathematical model to calculate the oxygen dissociation curve for human blood. (*J Appl Physiol* 1979;**46**:599). This model is based on the



Abstract S64 Figure (A) Saturation and oxygen tension of 3524 blood gas specimens; (B) Dissociation curve calculated from Severinghaus equation; (C) Clinical data compared with Severinghaus equation; (D) Clinical samples at normal, low and high PH.