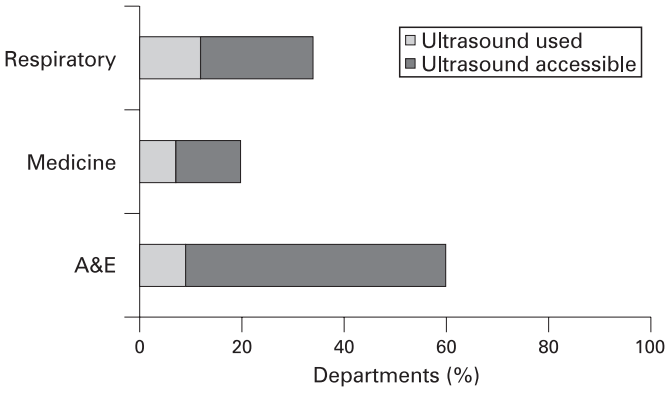
for use by clinical teams across the hospital. The aim of this project was to establish to what extent these are available and being used by A&E, medical and respiratory teams for guiding chest drain insertion.

A telephone survey was conducted, interviewing a junior doctor working in each of the A&E, medical admissions and respiratory departments in all training hospitals across the Thames region (this represents 168 clinical teams in 58 hospitals). This was performed over a one-week period in July 2008.

Portable ultrasound machines were accessible for use by A&E clinicians in 60% (33/55) of A&E departments. However, they were only used to assist with guidance of chest drains in five of these 33 departments (15%). The anatomical approach was suggested as the most common approach used in A&E (88%). A&E clinicians commented in 15 hospitals (27%) that they would refer the patients to the medical team rather than insert the drain themselves (see fig).



Abstract P164 Figure Accessibility and use of portable ultrasound for the placement of chest drains for pleural fluid.

Portable ultrasound machines were accessible for use by medical juniors in 20% (11/55) of hospitals; however, they were only used for drain insertion in four hospitals (with involvement of the respiratory team in all cases). In the remaining hospitals, if ultrasound was needed, input from the radiology department was required (93%). Only three departments (5%) had a policy to use ultrasound guidance for all cases.

In respiratory departments, doctors had access to portable ultrasound in 34% (20/58) of hospitals and this was utilised for drain placement in seven hospitals. Only four departments (7%) had a policy to use ultrasound guidance for all cases.

44 of 168 (26%) doctors could identify their training lead for chest drain insertion. In 77% of cases, this was a member of the respiratory team.

This survey documents that across the Thames region, the use of ultrasound in chest drain placement is not routine current practice. Even when portable ultrasound is available clinicians are still relying on an anatomical approach as first line.

P165 THE USE OF AUTOLOGOUS BLOOD PATCH FOR PLEURODESIS IN TREATMENT OF SECONDARY SPONTANEOUS PNEUMOTHORAX: A RETROSPECTIVE STUDY IN CYSTIC FIBROSIS PATIENTS

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Background: Pneumothorax is common in patients with cystic fibrosis (CF). It is often treated with autologous blood patch pleurodesis (ABPP) as it is well tolerated and has less impact on transplantation. However, its effectiveness has never been adequately assessed in the treatment of secondary spontaneous pneumothorax. In our study we look at CF patients with spontaneous pneumothorax who were treated with this procedure to determine the efficacy and any complications.

Methods: Retrospective analysis of patients with CF who presented with pneumothorax between January 1993 and January 2008. We looked for recurrence and any complications with the procedure.

Results: We found a total of 17 patients with pneumothorax who were treated with ABPP, three patients who only had intercostal chest drain and another two who were treated with talc pleurodesis. The time period for observation for recurrence varied from 6 months in the case of the most recent admission to 15 years. All 17 patients had large (>2 cm) symptomatic pneumothorax on admission. Of these, six (35.2%) patients had a recurrence of pneumothorax on the same side, four within 6 weeks, one 13 months and one patient 17 months later. One recurrent patient presented with tension pneumothorax and was treated with talc pleurodesis with no subsequent recurrence. The remaining five patients were treated with repeat blood pleurodesis and three had no further recurrence over the next 2 years, two had another recurrence and were then treated with talc pleurodesis with no further recurrence. There were no immediate complications in any of the patients and none developed pleural infection, which has been reported in previous studies. Of the three patients who were treated with chest drain alone, two had recurrence within 4 months and were treated with blood pleurodesis with no further recurrences over the next 2 years. The two patients who were treated with talc alone did not have any recurrence afterwards.

Summary: Autologous blood patch pleurodesis appears to be a well tolerated and safe procedure that is useful in patients who may later be candidates for lung transplantation. However, it does not appear to be as effective as talc in preventing a recurrence.

Understanding disease mechanisms in vitro/in vivo

P166 IDENTIFYING A GENE EXPRESSION SIGNATURE FOR PROGRESSIVE PULMONARY SARCOIDOSIS

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In recent years, DNA microarray technology has progressed from a means of identifying potential genes involved in disease causation to a technique that can be used to find subclasses in disease states and identify biological markers associated with disease outcome. In sarcoidosis, one of the key questions in clinical management is which subset of patients will progress to chronic disease and whether treatment in this group of patient will alter the natural history of the disease. There are currently no markers that can be applied at disease presentation to identify patients at risk of disease progression. It is thus also not possible to assess whether early treatment will improve outcome in this group. We question if a global gene expression pattern in the lungs might predict the subset of patients with pulmonary sarcoidosis that progresses to chronic disease. We hypothesise that in patients with progressive pulmonary sarcoidosis, a different set of genes are activated to drive the route of inflammation towards that of a fibrotic pathway. In the first part of the study, we examine the viability of gene profiling of RNA obtained from bronchoscopic transbronchial biopsies and if differences in gene expression pattern can be observed in the lungs from biopsy-confirmed sarcoid patients with asymptomatic disease and Scadding stage 1–2 chest *x* ray (CXR) changes compared with patients with persistent symptoms, Scadding stage 2–3 CXR changes and abnormal pulmonary function test (n = 8 patients, non-smokers, no treatment, excluding Loefgren's

syndrome, matched for gender and age). Labelled complementary RNA was hybridised to the gene 1.0 ST array chip using the Affymetrix oligonucleotide DNA microarray platform representing 29 000 well-annotated genes. These first results show distinctly different clusters of differentially regulated genes in the two subsets of patients, providing statistically significant gene expression signatures that differentiate these patients. These results provide the platform for further mechanistic investigation into the aetiopathogenesis of sarcoidosis and the basis for larger and longitudinal studies to test the utility of gene profiling in predicting disease progression in sarcoidosis.

P167 ADAPTIVE ANTIOXIDANT AND ANTI-INFLAMMATORY RESPONSES TO BIOMASS DERIVED PARTICULATE MATTER IN HEALTHY SUBJECTS

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Introduction and Objectives: Particle emissions arising from wood burning contribute significantly to the airshed in certain regions of Europe and North America and represent a chronic health problem in the developing world. In this study we performed controlled exposures of volunteers to wood smoke (WS) derived PM_{10} to assess their impact on the lung.
Methods: 19 healthy subjects were exposed in a double-blinded study to both WS (230 $\mu g/m^3$ PM_{10}) and filtered air for 3 h. Bronchial wash, bronchoalveolar lavage (BAL) and airway biopsies were obtained 24 h post-exposure. Inflammation and airway redox status were assessed using differential cell counts and quantification of low molecular weight antioxidants: glutathione, urate and ascorbate. To assess the impact of this challenge on the transcriptome RNA was extracted from BAL cells and analysed on Affymetrix U133 Plus 2.0 micro-arrays. The expression data were analysed using both dChip and Partek softwares to identify genes with significantly different expression in response to WS. Differentially expressed genes were validated by quantitative PCR and when possible confirmed at the protein level.
Results: No inflammation or evidence of antioxidant losses were observed in any of the sampled compartments 24 h after WS. Rather a significant increase in BAL glutathione was observed: 0.54 (0.32–0.74) versus 0.72 (0.46–1.19) μmol, $p<0.05$. To investigate this challenge further, WS-induced responses to the transcriptome of alveolar macrophages were examined in a panel of nine subjects from the original study. This micro-array analysis identified an upregulation of the anti-inflammatory gene Clara cell-specific 16 kDa protein (CC16) following WS, a result confirmed by PCR. Protein analysis demonstrated a similar increase in CC16 protein in BAL fluid: 104.0 (84.0–120.2) versus 124.1 (94.5–179.2) μmol, $p<0.05$.
Conclusions: These data suggest that the WS PM employed in this challenge are a relatively mild airway irritant, inducing a protective upregulation of the anti-inflammatory CC16 protein, as well as a mobilisation of GSH to the airway lining fluid in the healthy human lung. As these responses occurred in the absence of the induction of other well-established antioxidant and xenobiotic genes, these data throw light on the very early adaptive responses of the airway to irritant challenges.

P168 ROLE OF TUMOUR NECROSIS FACTOR α RECEPTOR SUBTYPES IN TUMOUR NECROSIS FACTOR-INDUCED MOUSE LUNG MICROVASCULAR ENDOTHELIAL CELL ACTIVATION

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Introduction and Objectives: Tumour necrosis factor (TNF) alpha is an important inflammatory mediator of acute lung injury (ALI). Cellular effects of TNF are regulated via two receptors, type I p55 and type II p75. However, the precise roles of these individual receptors during ALI remain unclear. In this study we investigated the role of TNF receptor subtypes in TNF-induced lung microvascular endothelium activation in vivo and in vitro.
Methods: Wild-type C57BL6 mice, or mice lacking p55 (p55KO), p75 (p75KO) or both TNF receptors (p55/p75KO) were challenged intravenously with 500 ng recombinant mouse TNF. After 2–4 h lungs were harvested and a single cell suspension obtained by mechanical disruption with enzymatic digestion. For in vitro experiments, primary lung endothelial cells were isolated from wild-type mice, pre-incubated for 1 h with neutralising antibody against p75 and treated with 10 ng/ml TNF for 4 h. For both in vivo and in vitro studies, cells were stained with fluorophore-conjugated antibodies for adhesion molecules PECAM-1 (for identification of endothelial cells), ICAM-1, VCAM-1 and E-selectin and their surface expression on endothelial cells determined using flow cytometry.
Results: TNF administration induced upregulation of all adhesion molecules in wild-type mice. Upregulation of VCAM-1 and E-selectin was effectively abolished in p55KO and p55/p75KO mice, whereas ICAM-1 upregulation was substantially (70–80%) attenuated. In p75KO mice, VCAM-1 upregulation was attenuated (mean fluorescent intensity 28 $\pm$ 6.4 for p75KO vs 47 $\pm$ 11 for wild-type; $p<0.01$; mean $\pm$ SD) and there was a trend towards attenuated E-selectin upregulation, but ICAM-1 was unaffected. Inhibition of ligand binding to the p75 receptor in vitro caused a significant ($p<0.05$) decrease of VCAM-1 and E-selectin expression (28% and 23%, respectively) but had no effect on ICAM-1 in lung endothelial cell culture.
Conclusions: These results demonstrated that the p55 receptor is critical for TNF-mediated upregulation of pulmonary microvascular adhesion molecule expression. In contrast, the p75 receptor contributes to the upregulation of VCAM-1 and E selectin, but not ICAM-1. The differential effects of the p55 and p75 receptor on the different adhesion molecules suggests a complex interplay between processes stimulated by TNF receptors in the pulmonary microvasculature during the progression of ALI.
Funding: This work was supported in part by Wellcome Trust.

P169 REDOX MODIFICATION OF DITHIOLS/DISULPHIDES REGULATES TUMOUR NECROSIS FACTOR ALPHA-CONVERTING ENZYME ACTIVITY ON PRIMARY HUMAN MONOCYTES

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Introduction: Tumour necrosis factor (TNF) and reactive oxygen species (ROS) are known to be important mediators in the pathophysiology of sepsis and acute lung injury. TNFα-converting enzyme (TACE) is the enzyme responsible for cleaving membrane TNF to its soluble form. We previously demonstrated the involvement of ROS in lipopolysaccharide-induced TACE activity upregulation in human monocytes, suggesting significant interplay between ROS and TNF via TACE.[1] An emerging concept in ROS signalling is the control of protein structure and function by dithiol-disulphide exchange, which is catalysed by redox-sensitive oxioreductases. Here we investigated whether dithiol-disulphide exchange could be the effector mechanism by which lipopolysaccharide upregulates TACE enzymatic activity.
Methods: Primary human monocytes were isolated from peripheral blood and purified by negative immunomagnetic bead selection. Monocytes (85–90% purity) were stimulated with lipopolysaccharide in the presence of thiol-modifying reagents. TACE enzymatic activity was measured by a novel fluorescence resonance energy transfer peptide-based assay[2] and TACE expression assessed by flow cytometry.

Results: Lipopolysaccharide stimulation of monocytes resulted in a large, rapid, membrane expression-independent increase in TACE activity (16 ± 3 vs 76 ± 9 fluorescence units (FU)/minute for 10^5 monocytes, p<0.001). This increase was prevented by pre-treatment of cells with inhibitors of dithiol-disulphide oxioreductases, ie, phenylarsine oxide (PAO) (87 ± 9% attenuation, p<0.001) or its cell-impermeable mimic, GSAO (98 ± 16%, p<0.001). The addition of diamide, a dithiol oxidising agent, abolished the lipopolysaccharide-induced increase in TACE activity (94 ± 9%, p<0.001), whereas treatment with dithiothreitol, a disulphide-reducing agent, by itself, upregulated TACE activity (32 ± 8 vs 100 ± 15 FU/minute, p<0.001).
Conclusions: These results strongly suggest that lipopolysaccharide-induced TACE activity upregulation involves dithiol/disulphide exchange. Inhibition of oxioreductases on the cell surface was able to prevent lipopolysaccharide-induced TACE upregulation, whereas dithiol oxidation mimicked this inhibition and disulphide reduction stimulated TACE activity. Therefore, we propose that lipopolysaccharide stimulation causes a reduction of critical disulphides (ie, cleavage of disulfide bonds) within TACE on the cell surface, resulting in a change in protein conformation to a more active form. This reaction is presumably mediated by a redox-sensitive disulphide exchange catalyst (eg, thioredoxin 1), which is activated by ROS generated following lipopolysaccharide stimulation.

1. **Scott** *et al*. *Thorax* 2007;**62**:A114.
2. **Alvarez-Iglesias** *et al*. *Lab Invest* 2005;**85**:1440–8.

P170 BIOMARKERS OF LUNG INJURY IN PLASMA AND EXHALED BREATH CONDENSATE AFTER ONE-LUNG VENTILATION FOR LUNG RESECTION

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Methods: Blood was taken preoperatively (T1), and postoperatively at 2–4 h (T2) and day 1 (T3) from eligible adults undergoing LR with OLV. EBC was collected from both lungs preoperatively, at 30-minute intervals during OLV and on resuming ventilation to both lungs. IL-6, IL-8, tumour necrosis factor (TNF) alpha, surfactant protein (SP)-D, receptor for advanced glycation end-products (RAGE), Krebs von den Lungen (KL) 6 and von Willebrand factor (vWF) levels were determined in plasma by ELISA. Intraoperative ventilatory parameters and the development of ALI were recorded.
Results: Thirty patients of mean age 58.6 years (range 23–80) were studied, none developed clinical ALI. Lobectomy was performed in 17 patients, pneumonectomy in one, and a lesser resection in 12. Ventilatory parameters during OLV, expressed as median (interquartile range), were: OLV time 147 minutes (121–196), plateau pressure 23 cmH_2O (18–26), tidal volume 5.3 ml/kg (4.5–6.4). IL-8 and TNFα levels were at or below the limit of detection at all time points. Other plasma biomarker levels are shown in the table. EBC pH did not change significantly during OLV. There was no correlation between biomarkers and ventilatory parameters.
Conclusions: IL-6, RAGE and vWF are significantly elevated after LR and OLV. However, plasma biomarkers of lung injury do not correlate with ventilatory parameters during OLV. This may be due to the relatively "protective" ventilatory strategy applied.
Funding: This work was supported by a British Lung Foundation Trevor Clay Memorial Grant.

Abstract P170 Table Plasma biomarkers preoperatively (T1), 2–4 h (T2) and 1 day post-operatively (T3) in patients undergoing lung resection.

Biomarker	T1	T2	T3
IL-6 (pg/ml)	0 (0–0)	187 (131–290)***	83 (36–151)***
KL-6 (U/ml)	268 (204–347)	217 (167–310)**	206 (140–265)***
RAGE (pg/ml)	929 (623–1083)	1369 (869–2159)***	765 (535–1076)
vWF (U/dl)	103 (88–158)	152 (123–193)**	185 (152–232)***
SP-D (ng/ml)	569 (276–886)	507 (306–813)	424 (176–705)***

**p<0.01; ***p<0.001 versus T1 (one-way analysis of variance, Dunn's multiple comparison test). Values are median (interquartile range). KL-6, Krebs von den Lungen; RAGE, receptor for advanced glycation end-products; SP-D, surfactant protein; vWF, von Willebrand factor.

1. **Kutlu CA,** *et al*. *Ann Thorac Surg* 2000;**69**:376–80.
2. **Moloney ED,** *et al*. *Am J Respir Crit Care Med* 2004;**169**:64–9.

P171 POLIDOCANIL-INDUCED TRACHEAL INJURY REPAIRS WITH RAPID EXPANSION OF RARE KERATIN 14-POSITIVE CELLS WITH TRANSDIFFERENTIATION TO A CILIATED EPITHELIUM IN 8 DAYS

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Background: Basal cells are proposed to be stem cells of the trachea and major airways. A small population are keratin 5 and 14 positive (K14). Little is known about K14-positive cell proliferation and lineage commitment in normal conditions due to the slow turnover of these cells. This study used a polidocanol-induced airway injury model to encourage proliferation of the tracheal stem cell pool.
Method: 6–8 week-old C57BL/6F1 mice received intratracheal instillation of 15 μl 2% polidocanol. Mice were recovered for 1, 2, 3, 8 and 28 days; three per time point. PBS treated mice served as controls. Mice were intraperitoneally injected with BrdU 100 mg/kg 1 h before being killed.
Results: In undamaged mice, tracheal K14-positive cells were rare (2.99% ± 1.23), and BrdU staining infrequent (0.79% ± 0.25). 24 h after polidocanil injury there were no detectable K14 or BrdU-positive cells with damaged areas simply showing denuded basement membrane with a few uncharacterised cells. At 48 h there was a rapid expansion of cells forming a simple epithelium, of which 100% were K14 positive and this was accompanied by an increase in BrdU-positive cells. At 72 h a stratified squamous epithelium formed with all cells remaining K14 positive. However, for the first time some cells were now K14 "dim" suggesting a down-regulation of K14. Other phenotypic markers (CCSP and acetylated tubulin (ACT) for Clara and ciliated cells, respectively) were negative. By 8 days the trachea had reformed a pseudostratified epithelium and expression of the ciliated cell marker ACT was extensive. BrdU decreased dramatically after the first 48 h (48 h 43.16% ± 10.27; 72 h 3.39% ± 2.38 and 8 days 3.25% ± 0.89). At 8 days cells were seen clearly to express both K14 and ACT, suggesting transdifferentiation or progenitor function of K14 cells. On day 28, K14 was expressed in basal cells only but their numbers were increased compared with undamaged controls (12.79% ± 6.77 vs 2.99% ± 1.23, p<0.01).
Conclusion: Our study demonstrates that the K14-positive cell population undergoes a dramatic expansion in mouse tracheal epithelium after airway injury and appears to act as the key cell in acute airway repair.

P172 ACTIVATION OF THE WNT/β-CATENIN PATHWAY IN MURINE TRACHEAL CELLS BLOCKS CILIATION AND INDUCES SQUAMATION IN AIR–LIQUID INTERFACE CULTURES

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Background: The canonical Wnt pathway plays a role in the epithelial cell fate decisions of several organs. We hypothesised that increasing β-catenin signalling would alter tracheal epithelial cell fate decisions. In the present study, we investigated the effect of activating the Wnt/β-catenin pathway in primary mouse tracheal epithelial cells (MTEC) in air–liquid interface culture (ALI).

Method: Fresh isolated MTEC were treated with the non-specific GSK-3b inhibitor LiCl (10 mmol) or the specific GSK-3b inhibitor SB 415286 (10 μmol or 30 μmol). NaCl and DMSO-treated cells were used as controls. On day 6, EMT was assessed morphologically and by expression of the myofibroblast marker, alpha smooth muscle actin (α-SMA). On day 14 after ALI, epithelial cell differentiation was assessed by immunofluorescence of K14 (basal cells), acetylated tubulin (ciliated cells) and Clara-cell-specific protein expression (Clara cells).
Results: Pre-ALI cell cultures were assessed by immunofluorescent staining. LiCl 10 mmol and treated cells were sparse, enlarged, had large nuclei and had increased α-SMA expression. Co-staining of K14 and α-SMA suggested basal cells were undergoing EMT. Li-treated cultures were not capable of undergoing ALI and instead leaked and died. SB-treated MTEC were assessed after 7 days at ALI. SB-treated ALI cultures were found to have inhibited or delayed ciliation (reduced cell numbers). Confocal microscopy demonstrated a failure of cilia formation rather than acetylated tubulin production (compared with DMSO-treated controls). SB-treated cells also formed areas of stratified epithelium.
Conclusion: SB treatment of MTEC ALI cultures induced a failure of ciliated cell differentiation and formation of a stratified squamous epithelium.

P173 NEUTROPHILS FROM PATIENTS WITH VENTILATOR-ASSOCIATED PNEUMONIA: PRO-INFLAMMATORY AND CYTOTOXIC INTERACTIONS WITH ALVEOLAR EPITHELIUM

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Introduction: Ventilator-associated pneumonia (VAP) is the commonest fatal hospital-acquired infection and increases morbidity and mortality. The disease is characterised by interactions between neutrophils and micro-organisms within the alveolar space. We hypothesised that peripheral blood neutrophils from patients with VAP would exhibit enhanced release of inflammatory mediators in an in-vitro model of neutrophil–alveolar epithelial cell interaction.
Methods: The model was created using A549 cells, which share characteristics with type II alveolar epithelial cells. These were co-cultured with neutrophils stimulated with lipopolysaccharide from *Escherichia coli* for 24 h and the supernatants assayed for chemokines and lactate dehydrogenase (as a maker of cytotoxicity). Controls with A549 cells alone, or with either unstimulated neutrophils or lipopolysaccharide were conducted simultaneously. Patients with clinically suspected VAP were recruited and VAP confirmed or refuted by quantitative culture of bronchoalveolar lavage fluid. Neutrophils from these patients, and age/sex-matched controls were retrieved by dextran sedimentation and Percoll gradient extraction. A subset of experiments was conducted with neutrophils separated from the A549 cells by membranes with 0.4 μm pores to determine contact dependency of the observed effects.
Results: 87 individuals were recruited, 15 patients with VAP, 51 patients without VAP and 21 age/sex matched controls. Neutrophils from patients with VAP were significantly more cytotoxic than those without VAP or controls (see table) and stimulated the release of significantly greater amounts of IL-8 (see table). Levels of MCP-1 and IL-6 were significantly elevated compared with controls, but these did not differ between the two patient groups. Separation of neutrophils and A549 cells by permeable membranes abolished these effects.

Abstract P173 Table

	VAP	Non-VAP	Matched volunteers	p Value (by ANOVA)
LDH (ratio to untreated A549)	3.96	2.00	1.2	0.007
IL-8 (ratio to untreated A549)	262	135	10	0.02

ANOVA, analysis of variance; LDH, lactate dehydrogenase; VAP, ventilator-associated pneumonia.

Conclusions: In an in-vitro model of pneumonia, neutrophils induced the release of a range of pro-inflammatory molecules including IL-8, IL-6 and MCP-1. These effects are dependent on stimulation of the neutrophils by lipopolysaccharide and also on membrane to membrane contact. Neutrophils from patients with VAP induced significantly more IL-8 production than critically ill patients without VAP, although both groups produce more than those from matched volunteers. In addition, neutrophils from patients with VAP were significantly more cytotoxic. These findings may help explain why VAP leads to increased morbidity and mortality among already critically ill patients.

P174 STRAIN-INDUCED PROSTANOID RELEASE BY HUMAN ALVEOLAR EPITHELIAL CELLS

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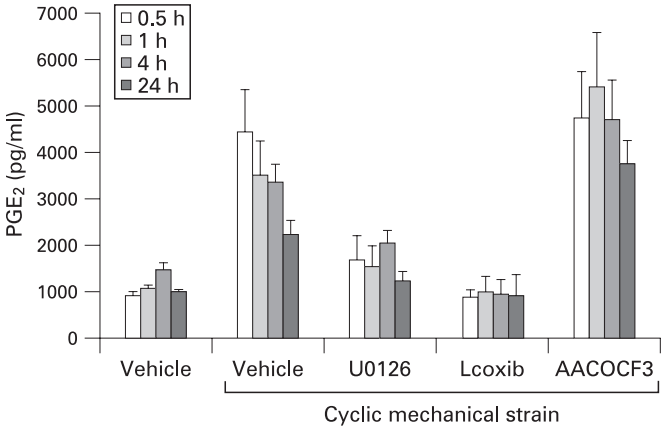
Background: Mechanical forces enhance the release of mediators that exacerbate lung damage and contribute to systemic inflammation and death—ventilator-associated lung injury (VALI). Prostanoids and prostaglandin (PG)E_2 in particular are produced in increased amounts by the lungs of patients and animal models of acute lung injury. Cyclic mechanical strain (CMS) increases prostanoid production from several cell types. For example, we have shown strain-induced, ERK1/2 pathway-dependent induction of cyclo-oxygenase-2 (COX-2) and activation of cytosolic phospholipase A_2 ($cPLA_2$) in human primary alveolar type 2 cell (hAT2) and in an acute murine VALI model that was associated with increased levels of PGE_2 in supernatant and lavage fluid, respectively.[1] Prostanoids may modulate multiple processes that contribute to lung injury and repair, including inflammation, wound healing, fibrosis and control of vascular tone.
Methods and Results: CMS was applied (Flexercell FX4000 apparatus: 0, 30% elongation, 2 h at 20 minutes) to hAT2 monolayers in vitro as a model of lung over-distension in the presence or absence of inhibitors of ERK1/2 (U0126 10 mmol), COX-2 (lumiracoxib 10 mmol) and $cPLA_2$ (AACOCF3 10 mmol). PGE_2 (ELISA, see fig) in supernatants collected after 0.5, 1, 4 and



Abstract P174 Figure Time course of PGE_2 production after cyclic mechanical strain.

24 h was increased by CMS in a manner dependent on COX-2 and the activation of the ERK1/2 signalling pathway, but not limited by $cPLA_2$ inhibition. COX-2 protein was detectable in unstimulated hAT2 by Western blotting but was upregulated after 12 h and 24 h but not 4 h. COX-1 expression was unaffected by CMS.
Discussion: CMS-induced PGE_2 production by hAT2 cells occurred rapidly and appeared to be mediated by COX-2 despite the maximal rate of production preceding COX-2 induction by several hours. Activation of the ERK1/2 pathway mediated $cPLA_2$ phosphorylation and COX-2 induction after CSM, but neither of these mechanisms can account for the inhibition of early PGE_2 production by U0126. Further investigation of these pathways may reveal novel targets for the treatment of patients with acute lung injury.
Funding: This work was supported by the British Lung Foundation.

1. **Pinhu L,** *et al*. *Thorax* 2007;**62**:S154.

P175 INHIBITORS OF GLUTATHIONE-S-TRANSFERASE INDUCE CELL DEATH IN LUNG EPITHELIAL CELLS

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Introduction: The glutathione S-transferase (GST) system plays an important role in cancer and chronic inflammatory conditions such as asthma. Evidence is also emerging that GST is a major determinant of acute lung injury (ALI). For example, GST μ and π polymorphisms were associated with increased primary graft failure following lung transplantation. However, the molecular mechanisms underlying the influence of GST in ALI remain unclear. We hypothesised that attenuated GST activity may promote lung cell injury via oxidative stress. Here we investigated the influence of pharmacological inhibitors of GST (ethacrynic acid (EA) and caffeic acid (CA)) on cultured mouse lung epithelial cells (MLE-12).
Methods: MLE-12 cells were exposed to EA or CA alone or in combination with oxidative stress inducers hydrogen peroxide (HP), a common lipid hydroperoxide tert-butyl-hydroperoxide (tBH) or hypoxia-reoxygenation (HR). Cell viability was assessed by the MTT assay. The role of oxidative stress in EA and CA responses was investigated by exploring the impact of the antioxidant N-acetylcysteine (NAC).
Results: Treatment of MLE-12 with EA resulted in a concentration-dependent reduction in cell viability over 5 h (97–44% of control MTT for 50–200 μmol EA, respectively). Similar findings were obtained following 24 h treatment with CA. Both HP and tBH caused concentration-dependent cell injury after 5 h, which was potentiated by subtoxic concentrations of EA. Whereas glucose deprivation and HR itself did not affect cell viability over 6 h, HR-induced cytotoxicity was evident in the presence of EA (76 ± 0.4 vs 97 ± 2.8 and 10 ± 0.8 vs 76 ± 0.4% control MTT for 50 and 100 μmol EA, respectively). The cytotoxic effects of both EA and CA were completely prevented by NAC administration.
Conclusion: Two chemically distinct GST inhibitors compromised the viability of lung epithelial cells and rendered the cells more susceptible to different forms of oxidative stress. Moreover, EA and CA-induced cytotoxicity were abolished by the antioxidant NAC. These findings suggest that GST activity is required for survival of cultured lung epithelial cells under stress conditions and may help to understand the novel roles of GST in clinically relevant acute lung injury syndromes.

Clinical tuberculosis

P176 THE STATE OF TUBERCULOSIS IN VANUATU

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Background: Tuberculosis is one of the top 10 causes of death in Vanuatu; however, reported rates of multiple drug resistance (MDR) and HIV are currently low. To date, no study has systematically examined the diagnosis, management and clinical outcome of tuberculosis in Vanuatu, a practice strongly advocated by the WHO.
Aim: To assess whether the diagnosis and management of tuberculosis in Vanuatu is in line with regional and WHO guidelines and evaluate the quality of routine surveillance data in order to advise future practice and policy.
Method: A retrospective review of clinical and laboratory records for all cases of tuberculosis in Vila Central Hospital, Vanuatu, from 1 April 2006 to 31 March 2007
Results: 51 cases of tuberculosis were identified: 26 female (51%), average age 28.7 years. This extrapolates to a population rate of 63.75/100 000 per year, reflecting a 7% rise since 2005. The majority of cases were pulmonary (28, 54.9%). Sputum smears were performed in 32 (63%) cases. Combination therapy was readily available. Three patients (6%) received the correct dose, regimen and duration of therapy. Inadequate surveillance for HIV and MDR-TB was observed. Only eight (15.6%) cases were tested for HIV, one (12.5%) was positive. No MDR-TB was identified.
Conclusions: National surveillance data may be inaccurate and the rising incidence in tuberculosis is likely to be underestimated. Given the high level of sexually transmitted diseases and the experience of other Melanesian islands, an HIV epidemic is likely. Existing public health systems and tuberculosis services are not meeting WHO standards and are ill-prepared to cope with such an eventuality.
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P177 HEALTH STATUS OF UK PATIENTS WITH ACTIVE TUBERCULOSIS STARTING THERAPY

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Introduction: Tuberculosis has re-emerged as a serious public health problem in the UK over the past two decades. Few studies, however, have looked at the impact of tuberculosis and treatment on patients' quality of life and functioning, and no data are available for the UK context.
Methods: Questionnaires were administered prospectively to patients in three London clinics at diagnosis, and one month into treatment for active tuberculosis. We assessed generic health-related quality of life (SF-36 and EuroQoL), symptoms and emotional impact (state–trait anxiety short-form (STAI-6), Center for Epidemiologic Studies depression scale (CESD) and worry items).
Results: To date, 42 baseline questionnaires have been returned. Most respondents (38 patients, 91%) were non-UK born, 13 (32%) were Indian and 12 (29%) black African, 18 (43%) were aged 30–45 years, and 12 were unemployed. 28 patients (68%) had pulmonary tuberculosis.

Symptoms frequently reported at diagnosis were tiredness (36 patients, 86%) and weight loss (29, 71%). The figure shows the proportion of patients reporting problems on the five EuroQoL domains plus their mean SF-36 and emotional scores. Higher SF-36 scores indicate better health status. Mean scores were below or just around 40 and the physical and mental component summary scores (36 and 40, respectively) were lower than the average observed for people with chronic illness. Higher STAI-6 and CESD scores indicate more symptomatology. The mean scores of 14 and 22, respectively, suggest the presence of anxiety and depression. Worries most frequently reported concerned respondents' own health (39, 93%), the health of their family (35, 83%) and infecting others (31, 80%).