than from Ad-lacZ (5.4×10⁵, P<0.05) or PBS mice (6.3×10⁵, P<0.05). Median elafin level in the Ad-elafin group was 117 ng/ml. We conclude that elafin gene transfer protects lung epithelium against HNE, and augments LPS-induced neutrophilia. Elafin's role in airway neutrophils while protecting pulmonary parenchyma, potentially suggesting novel therapeutic strategies.

**P4 THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT IN ASThma**

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Epithelial-mesenchymal interactions play important roles during lung development, repair and inflammation. These interactions appear to be locally regulated by a resident layer of myofibroblasts and fibroblasts in close proximity to the epithelium (the epithelial-mesenchymal trophic unit) [Evans et al, Am J Respir Cell Mol Biol 1993;8:188-92]. In asthma, there is an increase in number of subepithelial myofibroblasts and these are responsible for deposition of interstitial collagen fibers and thickening of the subepithelial basement membrane (SBM) [Brewer et al, Am J Respir Cell Mol Biol 1994;10:7-11]. We postulate that this increase in mesenchymal activity arises from altered communication with the bronchial epithelium, which is characteristically damaged in asthma.

Using increased epidermal growth factor receptor (EGFR) expression as a marker of injury, we found that the amount of EGFR detected in asthmatic bronchial epithelium increased in proportion with disease severity and correlated with SBM thickening (r=0.62, P<0.001). Although in vitro experiments confirmed the ability of EGFR to promote LPS-mediated repair of bronchial epithelial cell monolayers, previous biopsy studies have failed to show increased epithelial proliferation in asthmatic [Demoley et al, Am J Respir Crit Care Med 1994;150:214-7]. As these observations suggested that mitogenic signalling from the EGFR might be blocked in asthma, we used our in vitro model to study the relationship between EGFR inhibition and the production of profibrogenic growth factors. The EGFR-selective inhibitor, tyrphostin AG1478, reduced EGFR-mediated wound closure and this resulted in a 4-fold increase in release of TGFβ, a potent stimulus for myofibroblast collagen gene expression.

These observations suggest that epithelial damage causes a functional disturbance of the epithelial-mesenchymal trophic unit and that this may underlie the remodelling responses characteristic of chronic asthma.

**P5 THE TRANSCRIPTIONAL REGULATION OF INTERLEUKIN-8 (IL-8) FROM HYPOXIC HUMAN MACROPHAGES - A POTENTIAL ROLE IN THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)**

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Introduction. We have previously reported our finding of significantly elevated levels of IL-8 within the alveolar airspaces of patients at risk of developing ARDS [Lancet 1993; 343: 643-647]. These high IL-8 levels were detected as early as 85 mins following the initiating event and the macrophage identified as a potent source. Retrospective analysis of data from trauma patients revealed a correlation between reduced PaO₂/FiO₂ ratio on presentation to casualty and raised bronchoalveolar lavage IL-8 levels. We postulated that acute hypoxia may upregulate IL-8 synthesis in human macrophages.

Methods. Macrophages cultured from peripheral blood mononuclear cells from healthy volunteers were exposed hypoxia (PaO₂=5Kpa) or normoxia (PaO₂=20Kpa) for up to 2 hrs. Secreted IL-8 protein was measured by ELISA and mRNA expression by northern blotting. Activation of the IL-8 promoter-binding transcription factors AP-1, C/EBP and NFκB was assessed by electromobility gel shift assay (EMSA). Finally the relative specificity for hypoxia on IL-8 upregulation was addressed by measuring mRNA levels of related chemokines by multiplex RT-PCR. Results. Data is presented as mean±SEM. Hypoxia increases IL-8 protein secretion by 2 hrs compared to normoxic controls (0.9±0.28 vs 0.5±0.24 µml, P<0.02). Hypoxia increases IL-8 mRNA levels by 104±38% (p<0.05) as early as 60 mins post-exposure compared to normoxic controls. EMSA revealed activation of AP-1 by 90±34%, (p<0.05) and C/EBP by 70±18% (p<0.02) within 30 mins of hypoxia compared to normoxia. NFκB was not activated at these early time points. Multiple-probe RNase protection assay revealed that in contrast to the increase in IL-8, 2 hrs hypoxia significantly inhibited expression of the chemokines IP-10, MIP-1α, MIP-1β and MCP-1. Conclusions. In human macrophages short periods of hypoxia can upregulate IL-8 protein and mRNA. The transcription factors AP-1 and C/EBP are implicated in this activation. This rapid hypoxic upregulation is relatively specific to IL-8 and is not seen with several other chemokines. These findings may be relevant to our observation of elevated lung IL-8 levels in hypoxic trauma patients who subsequently develop ARDS. NH and SCID are supported by the Wellcome Trust.

**P6 SURFACTANT PROTEIN D IN HELICOBACTER PYLORI INFECTION**

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Background: Helicobacter pylori is a gram-negative bacteria which is one of the most common infectious pathogens in man. The outcome of infection is diverse and includes asymptomatic gastritis, duodenal ulcer, gastric ulcer and gastric malignancy.

Surfactant protein D (SP-D) is a collagenous glycoprotein, containing trimeric arrays of C-type lectin domains, which belong to a family of proteins implicated in innate immunity termed the ‘lectins’. These bind selectively to the surfaces of viruses, bacteria and fungi resulting in the aggregation of the micro-organisms, followed by enhanced phagocytosis. In humans SP-D has been shown to be synthesised in pulmonary tissue, specifically alveolar type II cells, and has also been detected in other sites including tears, amniotic fluid and fetal membranes where a speculative role of mucous barrier assembly and possible host defence is proposed.

Alms: The aim of this study was to determine whether SP-D is present in the gastric mucosa, whether SP-D binds and agglutinates H.pylori and whether H.pylori is functionally impaired by SP-D. In addition, binding of SP-D to lipopolysaccharide (LPS) was assessed quantitatively to compare the interaction of SP-D with LPS from different H.pylori strains.

Methods & Results: Expression of SP-D in gastric mucosa was demonstrated by immunohistochemical staining of gastric biopsy sections using polyclonal rabbit anti-SP-D. Expression was present at the luminal surface, foveolar region and gastric pits with maximal expression at the surface. Levels of expression were significantly increased in chemokines associated with H.pylori infection. H.pylori and agglutination of H.pylori by SP-D, in a lectin-specific manner was demonstrated by incubating the organisms with 10ug/ml SP-D in the presence or absence of calcium and in the presence or absence of mucin. Motility of H.pylori, assessed using a Holloway backtracker, showed a 50% reduction in curvilinear velocity. A competitive inhibition assay was developed using the binding of SP-D to mannose-coated microtitre plates. SP-D binding was measured using a biotinylated monoclonal anti-SP-D antibody with extravidin-HRP. LPS extracted from three H.pylori strains were shown to inhibit SP-D binding in a concentration dependent manner. The 50% inhibition concentrations varied between 9 and 90 ug/ml.

Conclusions: SP-D is expressed in gastric mucosa and the level is increased in H.pylori infection. SP-D binds and agglutinates H.pylori and inhibits the organism's motility. The ligand for SP-D binding is probably H.pylori. SP-D is expressed in human gastric mucosa and the level is increased in H.pylori infection. SP-D binds and agglutinates H.pylori and inhibits the organism's motility. The ligand for SP-D binding is probably H.pylori. SP-D binds and agglutinates H.pylori and inhibits the organism's motility.

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