implicated in activation of fibroblasts and could contribute to fibrogenic responses in lung disease. However, further studies are required to confirm relative importance within the family and reveal mechanisms of action.

S121 INDIVIDUAL CELL TRACKING IN A TRANSGENIC ZEBRAFISH INFLAMMATION MODEL REVEALS THE FATES OF INFLAMMATORY NEUTROPHILS DURING INFLAMMATION RESOLUTION

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Removal of inflammatory neutrophils from sites of inflammation can occur by a number of routes; into exudates, by apoptosis followed by macrophage clearance and by retrograde chemotaxis. The relative contribution of these disposal mechanisms in vivo has been hard to define, and the lifespan of an in vivo tissue neutrophil has been hard to directly measure. We have generated transgenic zebrafish expressing the fluorescent photo-convertible protein, Kaede, in neutrophils.

Objective To label individual inflammatory neutrophils and track their fate during inflammation resolution in vivo.

Method Individual neutrophils were marked by photoconverting the Kaede protein using 405 nm laser light restricted to the individual cell profiles. Known numbers of neutrophils were photoconverted and visualised over 48 h. In subsequent experiments, an inflammatory reaction was induced by sterile tail transection of transparent zebrafish larvae. Kaede labelled neutrophils are recruited to the site of injury where they can be photoconverted and followed using time lapse video microscopy.

Results By counting the number of remaining photoconverted neutrophils over time, the half-life of a neutrophil was calculated. Our data suggest the lifespan of a zebrafish neutrophil in the tissues is 117.7 (CI 95.67 to 157.8) h, a figure comparable to that inferred for human tissue neutrophils. Timelapse videos reveal a population of neutrophils that migrate away from the site of injury, undergoing retrograde chemotaxis. Whilst neutrophils can migrate away from the site of injury, they are not completely free to do so. The apparent restriction on their behaviour may be due to the presence of a persisting chemical gradient or may reflect an intrinsic feature of neutrophil behaviour.

Conclusions These data demonstrate the power of this model to inform our understanding of phagocyte behaviour and interaction in vivo.

S122 THE M1 MACROPHAGE PHENOTYPE ACCENTUATES TGF-β1 DRIVEN EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) VIA THE SECRETION OF TNFα

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Introduction Obliterative Bronchiolitis (OB) is characterised by fibrotic obliteration of small airways which adversely affecting graft function and survival after lung transplantation. It has been shown in vitro and in vivo that primary bronchial epithelial cells (PBEC) from the transplanted lung can undergo epithelial to mesenchymal transition (EMT) and this process may contribute to the development of OB. We have shown that activated macrophages can disrupt epithelial wound repair by accentuating TGF-β1-driven EMT. We hypothesised that this effect might be limited to macrophages with an M1 phenotype and that their secretory products might be a target for limiting the inflammatory accentuation of EMT.

Methods and materials The THP-1 monocytic cell line was stimulated with clinical isolates of *Pseudomonas aeruginosa* (PA) and the effect of the activated cells or conditioned media on TGF-β1-driven EMT assessed in PBEC (Western blotting, confocal microscopy). In addition, THP-1 cells were differentiated to an M1 phenotype by treatment with IFNγ and an M2 phenotype with IL-4/IL-13 and cytokine release (ELISA) and their effect on TGF-β1 driven EMT assessed. The effect of blocking TNFα secreted from activated THP-1 cells on EMT was assessed using an anti-TNFα antibody.

Results Treatment with TGF-β1+activated THP-1 cells had no effect on EMT marker expression ($p>0.05$ n=6). However, co-treatment with TGF-β1+conditioned media from activated THP-1 cells dramatically accentuated TGF-β1-driven EMT ($p<0.05$ n=6). M1 differentiated THP-1 cells released 8.4-fold more TNFα and 8.1-fold more IL-1β than M2 cells ($p<0.05$, n=3). Conditioned media from M1, but not M2, cells dramatically accentuated TGF-β1 driven EMT ($p<0.05$ n=6). Blocking TNFα in the conditioned media from THP-1 cells significantly inhibits the decrease in E-cadherin (39%±4%) and the increase in vimentin (59%±18%) and fibronectin (72%±14%) expression ($p<0.05$, n=5).

Conclusion The secretory products of M1, but not M2, macrophages significantly accentuate TGF-β1 driven EMT. TNFα appears to be a major constituent of this accentuating action. This raises the possibility that either TNFα targeted therapies or modulation of macrophage phenotype may inhibit the inflammatory accentuation of EMT in airway epithelium.

S123 MONONUCLEAR INFLAMMATION AND DISRUPTION OF NORMAL ALVEOLAR STRUCTURE FOLLOWING DELETION OF Gαq/11, BUT NOT Gα12/13, IN TYPE II ALVEOLAR EPITHELIAL CELLS

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Activation of latent TGFβ by the epithelially restricted αvβ6 integrin is induced by activators of the RhoA signalling pathway and is critical in the pathogenesis of lung injury and fibrosis. The G-proteins, Gα12 and Gα13 are known to activate RhoA and we have previously shown that the αvβ6 integrin can mediate TGFβ activation via Gαq and RhoA. To establish the role of these G-proteins in both normal lung development and following lung injury, we generated mice with a targeted deletion of Gαq/11 or Gα12/13 in SpC-positive Type II alveolar epithelial cells. SpC-Cre mice were crossed with either Gαq(flox-flox)/11(-/-) or Gα12(-/-)/13 (flox-flox) mice and the lungs analysed histologically at 6 and 8 weeks after birth. At 6 weeks, lungs from mice with a homozygous deficiency in SpC-Gαq/11 contained focal inflammatory infiltrates consisting primarily of mononuclear leukocytes. Inflammation was associated with the localised disruption of normal alveolar architecture and the appearance of abnormal Type I and Type II alveolar epithelial cells, identified by SpC and T1α immunohistochemistry, within in the alveolar airspaces. Furthermore, immunohistochemical analysis of phosho-Smad2 levels in these lungs detected increased staining in the inflammatory foci within the homozygous SpC-Gαq/11 knockout lungs. At 8 weeks, the inflammatory foci were more numerous and lung architecture was severely disrupted with multiple abnormally large alveolar airspaces detected. In contrast, mice with at least one floxed Gαq or null Gα11 allele showed no abnormalities at either 6 or 8 weeks. We also detected no abnormal lung phenotype in 6- and 8-week old mice with a homozygous or heterozygous deficiency in SpC-Gα12/13. These data suggest that Gα11/q signalling is required to prevent

pulmonary inflammation and our findings would be consistent with impaired epithelial TGFβ activation in the lungs of these mice. Further studies are required to determine the origin of the cells activating TGFβ in these lungs.

S124 MACROPHAGE DELETION OF VHL RESULTS IN ALTERNATIVE ACTIVATION AND ENHANCED LUNG FIBROSIS INDEPENDENT OF HIF-1

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Background Hypoxia-inducible factor (HIF-1) is a master regulator of the cellular hypoxic response and has been implicated in the pathogenesis of inflammatory and fibrotic disease including IPF.
Aims To study the role of hypoxia and HIF-1 activation in macrophages in the i.t. bleomycin-induced lung fibrosis model.
Methods The i.t. bleomycin model was used to study the effect of HIF-1 manipulation in mice. The primary end-point was lung collagen content at day 24 post i.t. bleomycin instillation. The HIF-1α inducer dimethyloxallyl glycine (DMOG) was administered i.p. on days 14, 17 and 21. The role of myeloid-HIF-1 activity in lung fibrosis was determined using mice in which either HIF-1α or vHL (the dominant negative-regulator of HIF-1α) was selectively knocked out of lysosyme M expressing cells (LysM-Cre-Hif-1 and Cre-LysM-vHL). Lung tissue hypoxia was determined using Hypoxyprobe-1TM administered on day 24. Alternative activation status of HIF-1 null and vHL null macrophages was studied in bone-marrow derived cells from LysM-Cre-Hif-1 and Cre-LysM-vHL mice.
Results Pharmacological induction of HIF-1 in the late period of the bleomycin model with i.p. dimethyloxallyl glycine (DMOG) resulted in significantly enhanced lung collagen (mean±s.e.mμg/lung) on day 24 compared to controls (193±15 vs 152±8, $p<0.05$, $n>7$ per gp). Hypoxyprobe-1 staining in the bleomycin-injured lung revealed hypoxic alveolar macrophages even in areas of lung distant to patches if severe fibrosis, implying a role for hypoxic/HIF-1 expressing alveolar macrophages in lung fibrosis. However, lung collagen content was identical in myeloid-cell Hif-1 null mice and wild-type litter-mate controls (276±23 vs 277±22, n=8 per gp). In contrast, myeloid-cell vHL-null mice exhibited significantly enhanced lung collagen deposition versus controls (373±36 vs 282±54, $p<0.05$, $n>9$ per gp). Isolated vHL-null macrophages exhibited enhanced expression of the alternative activation markers YM-1, mannose receptor, arginase-1 and FIZZ-1.
Conclusions vHL deletion in macrophages enhances alternative activation and promotes lung fibrosis independent of HIF-1.

S125 LY6CHI CIRCULATING MONOCYTES DIRECT ALTERNATIVELY ACTIVATED, PRO-FIBROTIC, LUNG MACROPHAGE REGULATION OF PULMONARY FIBROSIS

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Introduction and objectives Idiopathic pulmonary fibrosis (IPF) remains one of the few respiratory conditions for which there are *no* effective therapies. The role of monocytes and macrophages in IPF has been disputed as anti-inflammatory therapies produce questionable benefit. Corticosteroids, however, actually induce an alternatively activated, pro-fibrotic, macrophage phenotype. We sought to determine whether monocytes and macrophages play a role in disease pathogenesis in an attempt to explain why current hypotheses and anti-inflammatory therapies have produced limited clinical benefit despite years of research.
Methods Using multiple in vivo depletional strategies, backed up by an adoptive transfer technique, we extensively investigated the role of monocytes and macrophages during lung fibrogenesis. We performed studies on samples from patients with IPF in an attempt to determine the translational importance of our findings.
Results Depletion of lung macrophages during fibrogenesis *reduced* pulmonary fibrosis as measured by lung collagen (p=0.0079), fibrosis score (p=0.0051), and qPCR for surrogate markers of fibrosis *Col1* (p=0.0083) and *a-smooth muscle actin* (p=0.0349). There was an associated reduction in expression of markers of alternative macrophage activation, *Ym1* (p=0.0179), and *Arginase1*. This reduction was confirmed by immunohistochemistry (IHC) for Ym1 (p=0.0233). IHC on lung macrophages from patients with IPF demonstrated the novel finding of expression of the human alternative macrophage marker CD163. Depletion of $Ly6C^{hi}$ circulating monocytes reduced pulmonary fibrosis (p=0.0052). Adoptive transfer of $Ly6C^{hi}$ BMDMS during fibrogenesis exacerbated pulmonary fibrosis (p=0.0304). Furthermore, depletion of *circulating* $Ly6C^{hi}$ monocytes lead to a subsequent reduction in the number of Ym1-positive alternatively activated *lung* macrophages (p=0.0310) with a concomitant reduction in the expression of *Ym1* and *Arginase1*.
Conclusions We have demonstrated that monocytes and macrophages *do* modulate pulmonary fibrosis and suggest that $Ly6C^{hi}$ monocytes (possible fibrocyte precursors) are precursors of alternatively activated, pro-fibrotic, lung macrophages. These findings could link the 'inflammatory' and aberrant wound healing hypotheses and explain the lack of effectiveness of corticosteroids in treating IPF. By enhancing our understanding of the pathogenesis of this dreadful disease, our results may enable new therapeutic targets to be developed, facilitate targeted cell-based therapy, and bring hope to one of the longstanding enigmas of respiratory medicine.

Lung infection: a multi-faceted problem

S126 MEASURING QUALITY IN PNEUMONIA CARE. THE NORTH WEST ADVANCING QUALITY PROGRAMME 2008–2009

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As part of an initiative within the North West Strategic Health Authority to improve the quality of care, 'quality markers' (QMs) were measured in all adult admissions with pneumonia in all 24 Acute Trusts in the North West Region for 1 year (discharges from October 2008 to September 2009). Only adults who fulfilled a prescribed definition of 'pneumonia' were included. QMs were taken from a USA initiative and adapted for UK use. Patient identification was based on clinical coding. Data were recorded in each individual Trust and centrally collated.
Combined data from all trusts QMs were recorded with the following frequencies (no in parentheses is number of patients included): Oxygenation assessment within 24 h or prior to hospital arrival 96.9% (11 127), blood culture performed in the A & E prior to initial antibiotic received in hospital 58.5% (3323), smoking cessation advice/counselling given in 38.1% (2788), initial antibiotic consistent with local CAP guidelines 80.8% (6337) and initial antibiotic received within 6 h of hospital arrival 64.6% (7889). Over the four