Abstract 118 Table 1

		Stroke	Control	Difference (95% CI)	p Value
Number of participants		27	30		
Age (years)	Mean	68	58	10	0.001
	SD	11	11	4 to 16	
Sex	Male/female	17/27	15/15	0.13	0.420†
	Proportion male	0.63	0.50	−0.12 to 0.36	
Height (centimetres)	Mean	169.6	169.7	−0.1	0.997
	SD	7.9	12.2	−5.6 to 5.6	
O_2 saturations breathing air (%)	Median	97	97	0	0.660*
	IQR	92 to 98	95 to 98	−1 to 1	
Smoking	Number ever/ never smoked	13/14	12/18	0.1	0.599†
	Proportion ever smoked	0.30	0.40	−0.2 to 0.3	
Functional residual capacity (litres)	Median	2.500	2.780	−0.270	0.003*
	IQR	2.323 to 3.601	2.258 to 2.898	−0.710 to 0.115	
Functional residual capacity (% predicted)	Median	76.0	90.0	−14.0	<0.001*
	IQR	66.5 to 89.5	79.8 to 105.0	−22.0 to −5.0	
Peak cough flow rate (litres/min)	Mean	297	380	−83	0.019
	SD	133	121	−153 to −14	
Peak cough flow rate (% predicted PEF)	Mean	61.2	86.3	−25.1	<0.001
	SD	32.6	17.3	−38.8 to −11.4	
Volume inspired before cough (litres)	Mean	2.219	3.409	−1.190	<0.001
	SD	0.828	0.720	−1.715 to 0.665	
Volume Inspired before cough (% predicted VC)	Mean	64.3	94.6	−30.1	<0.001
	SD	19.5	15.6	−42.2 to 18.5	

p Values calculated using t tests except.
*p Value calculated using Mann–Whitney U test.
†p Value calculated using Fisher's exact test.
PEF, peak expiratory flow rate; O_2, oxygen.

REFERENCE

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S119 VARIATION IN PHARYNGEAL PH IN THE DIAGNOSIS OF AIRWAY REFLUX

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Introduction and objectives Reflux of gastric contents to the laryngopharynx has been implicated in the pathogenesis of chronic cough and may exacerbate other respiratory conditions. Direct measurement of pharyngeal pH is available but standard analysis relies on the pH crossing a lower threshold. Non-acid gaseous reflux may cause respiratory symptoms without producing a significant drop in pharyngeal pH. We theorised that variation in pharyngeal pH might be a useful marker of airway reflux.

Methods Measurements of pH in the pharynx over 24 h were made in patients with a variety of respiratory diagnoses suspected to have reflux contributing to their symptoms. Diagnoses included chronic cough, cystic fibrosis and asthma. Results were analysed using a predefined, threshold-based scoring system and our novel system based on variation in pH. Comparison was also made with oesophageal physiology where available.

Results 60 studies were performed on 58 patients; median age 48 years (range 17–81). 43 studies had an abnormal threshold score. 31 patients had an abnormal variation score (>30 events per hour). Both were positive in 21 patients and both negative in 7. Cough symptom scores were similarly high in patients with abnormal variation to those with abnormal threshold scores (mean 34.7 and 38.3 respectively) and higher than patients with both negative (23.0; n/s). Cough patients who had undergone fundoplication demonstrated less variation than those who had not (mean events 55 per hour vs 115; n/s). Asthma patients had similar overall variation to other groups but had higher numbers of events over fewer peak hours (468 vs 368 events per peak hour; p=0.08) with the opposite seen in cystic fibrosis patients (276; p=0.26). Of 25 patients with an abnormal pharyngeal study and an oesophageal study available, 15 had normal oesophageal studies.

Conclusions These results show that the interaction between pharyngeal pH and airway symptoms is complex, not easily assessed using a pH threshold alone and not well correlated with oesophageal physiology. Assessment of variation suggests different patterns of reflux may relate to disease phenotypes. The ideal analysis should include correlation of clinical symptoms with peaks in variation and pH threshold events.

Inflammation: an important regulator of the fibrotic response

S120 IL-1 IS A KEY EPITHELIAL ALARMIN WHICH PROMOTES FIBROBLAST ACTIVATION

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Background Alarmins are molecular 'danger signals' released by injured cells that can contribute to the innate immune response by activating immune cells via multiple receptors including Toll-like receptors (TLR), Nod-like receptors (NLR) and the receptor for advanced glycationend products (RAGE). Pulmonary fibrosis is associated with the upregulation of alarmins such as Interleukin 1 α (IL-1α) and High mobility group box 1 (HMGB1) in bronchoalveolar lavage fluid (BAL), however it remains unclear whether alarmins can contribute directly to the fibrogenic process by interacting with fibroblasts. We hypothesised that alarmins released from damaged epithelial cells act as damage associated molecular patterns (DAMPs) which are recognised by fibroblasts and lead to their activation.

Methods The 16HBE14o- human bronchial epithelial cell line was damaged by hydrogen peroxide (H_2O_2) induced oxidative stress and alarmin release (Heat Shock Protein 60 (HSP-60), HMGB1, IL-1α) measured via ELISA. MRC5 human lung fibroblasts were treated with media from damaged lung epithelia and cell proliferation (XTT proliferation assay), phosphorylation of downstream TLR signalling molecules (interleukin 1 receptor associated kinase 1 (IRAK1), TGFβ associated kinase 1 (TAK1)—western blotting) and gene expression of proinflammatory cytokines (interleukin 6 (IL-6) and Interferon β (IFNβ)—qRT-PCR) assessed.

Results Conditioned media from 16HBE14o- cells damaged with 200 μM H_2O_2 contained elevated concentrations of HSP-60 (16.7 vs 0.64 ng/ml; p<0.05, n=3), HMGB1 (71 vs 12 ng/ml; p<0.001, n=3) and IL-1α (434 vs 130 pg/ml; p<0.001, n=3) compared to untreated controls. Treatment of MRC5 cells with media from damaged lung epithelia enhanced cell proliferation by 29% (p<0.01, n=3), increased TAK1 and IRAK1 phosphorylation and increased IL-6 and IFNβ gene expression 2.7-fold (p<0.001, n=3) and threefold (p<0.001, n=3) respectively. Blocking IL-1R (500 ng/ml of IL-1R antagonist (IL-1Ra)) diminished IL-6 (85%; p<0.01, n=3) and IFNβ (34%; p<0.05, n=3) gene expression compared to treatment with conditioned media from damaged cells alone.

Conclusions The results suggest that alarmins such as the intrerleukin-1 family, released by damaged human lung epithelia may be

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