scaffold protein involved in the proteasomal processing of p105 into the p50 subunit. Inhibition of this process impairs the nuclear translocation of NF-κB and the consequent p65/p50 regulated gene transcription. Hence, TRUSS may be a novel target for modulating the inflammatory functions of TNFα-TNF-R1 signalling.

S31 CHARACTERISATION OF CELL ADHESION MOLECULE-1 IN LUNG MAST CELLS

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Introduction and Objectives Cell adhesion molecule 1 (CADM1) is implicated in several diseases and a prognostic factor for lung cancer. In human lung mast cells (HLMC) it contributes to cell-cell adhesion and proliferation. Functional CADM1 isoforms arise from alternative splicing between exons 7 and 11. Exon 9 may be functionally important because it encodes a specific cleavage site for TNF-alpha-cutting enzyme, TACE, in the longest SP1 and SP6 isoforms. Our aim was to characterise the isoforms of CADM1 expressed in HLMC.
Methods CADM1 expression in isolated HLMC and human mast cell lines (HMC-1 and LAD2), was investigated using RT-PCR, cloning, and transfection.
Results Multiple highly glycosylated CADM1 isoforms were found in HLMCs and cell lines. The SP4 isoform containing exon 8 represented ~80% of clones in both HLMCs and 'differentiated' LAD2 cells, and ~96% in non-differentiated HMC-1 cells. The SP1 isoform with exons 8+9 represented ≤20% of clones in HLMCs and LAD2 cells. A novel SP6 isoform with exons 8+9+10 was found only in HLMCs (<5% of clones). More sensitive PCR analysis detected all these isoforms and an additional isoform SP3, lacking exons 8–10, in HLMCs and in cell lines. In contrast to these functional isoforms, non-functional isoforms were also found with two cryptic exons between exons 1 and 2, which cause a translational frame shift and premature termination of the protein. The cryptic exon B is located within the hot spot for SNPs. When the isoforms SP4, SP1 and SP6 were expressed in the HMC-1 cell line and the epithelial cell line HEK293 as fusion CADM1-GFP proteins, they were found at the cell periphery as well as in the cytoplasm. They were concentrated in cell boundaries in clumps of HMC1 cells.
Conclusions Multiple and novel CADM1 isoforms are found in HLMCs. The longest SP1 and SP6 isoforms might be involved in the negative regulation of HLMC adhesion following secretion of TACE after activation. Some cryptic exons are likely to be a result of specific SNPs, creating new branching points for splicing. Non-functional cryptic CADM1 isoforms may reduce mature protein expression and affect the function of CADM1 expressing cells.

S32 CYCLICAL MECHANICAL STRETCH ENHANCES THE PRO-FIBROTIC RESPONSES OF PRIMARY EMBRYONIC FOETAL FIBROBLASTS, BUT NOT ADAM33 EXPRESSION

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Rationale *A Disintegrin And Metalloprotease (ADAM)33* is a susceptibility gene associated with asthma, bronchial hyperresponsiveness (BHR) and reduced lung function in young children. It is selectively expressed in mesenchymal cells, including bronchial smooth muscle and fibroblasts. We have previously shown that *ADAM33* expression increases during lung development when spontaneous peristaltic contractions of the airways commence. Therefore we hypothesised that mechanical strain induces *ADAM33* expression and affects smooth muscle/myofibroblast differentiation.
Methods Primary human embryonic fibroblasts from the pseudoglandular stage of lung development were cultured on flexible collagen-coated membranes and exposed to cyclical mechanical stretch (30% amplitude, 12 cycles per minute) for 48, 96 and 168 h. Control cells were cultured on the same membranes without mechanical stretch. Quantitative RT-PCR was performed for *ADAM33* and a-smooth muscle actin (a-SMA), a marker of smooth muscle and myofibroblast differentiation. We also measured collagen III and IL-8 mRNA. Soluble collagen protein levels in culture supernatants were measured using soluble collagen assay.
Results *ADAM33* and a-SMA mRNA expression were not significantly affected by mechanical strain. In contrast, collagen III mRNA expression was increased fourfold by cyclical mechanical strain and there was a threefold increase in soluble collagen proteins in culture supernatants of stretched cells. Unexpectedly, IL-8 expression was also increased by cyclical mechanical strain.
Conclusion Mechanical strain did not appear to influence markers of smooth muscle differentiation. However, the increase in ECM production may indicate a requirement for stiffening of the airways as the tubular structures develop. We postulate that IL-8 is not proinflammatory in the context of airway development, and may be a paracrine growth factor for developing epithelial cells.

S33 SUPPRESSION OF CONSTITUTIVE AND STIMULATED SECRETION OF HISTAMINE FROM HUMAN LUNG MAST CELLS BY A SECRETED FACTOR FROM LUNG EPITHELIAL CELLS

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Introduction Constitutive and IgE-dependent secretion of histamine from human lung mast cells (HLMC) is suppressed by direct contact with the BEAS-2B bronchial epithelial cell line, but not conditioned epithelial cell media. This suggests that direct contact or a low concentration/labile secreted factor is involved. We explored this relationship further using a Transwell co-culture system of HLMC with BEAS-2B monolayers or air liquid interface primary bronchial epithelial cultures (ALI) derived from asthmatic and healthy subjects.
Methods ALI and BEAS-2B were grown on Transwell membranes. IgE-sensitised HLMC were then cultured i) on the luminal surface of a BEAS-2B monolayer, ii) separated from the BEAS-2B basal surface by a Transwell membrane, or iii) on the well bottom with BEAS-2B and ALI sitting on the Transwell insert. Cells were co-cultured for 16 h, media removed, and HLMC stimulated with anti-IgE for 30 min. Parallel controls without epithelial cells were performed. Histamine concentrations were determined by radio-enzymic assay.
Results *BEAS-2B (n=3)*: Compared to no epithelium control, constitutive histamine secretion from HLMC in direct contact with BEAS-2B was suppressed by a mean (±SEM) 57±15% (p=0.04), and by 55±9% when separated by Transwells (p=0.02). IgE-dependent secretion was suppressed by 79±8% (p=0.04) from HLMC in direct contact with BEAS-2B and by 88±7% (p=0.03) when separated by Transwells, compared to control. *ALI-culture*

(n=6 healthy, 6 asthmatic): Compared to control, healthy ALI suppressed constitutive HLMC histamine release by 39±5% (p=0.01), but asthmatic ALI did not (mean 19±11% suppression, p=0.07). There was a significant difference between healthy compared to asthmatic ALI (p=0.01). Healthy and asthmatic ALI suppressed IgE-dependent histamine release by 55±4%, p=0.001 and 48±1%, p=0.001, respectively.

Conclusions BEAS-2B and healthy airway epithelial cells suppress constitutive and IgE-dependent HLMC histamine secretion when separated by Transwell membranes. Asthmatic ALI cultures do not suppress constitutive HLMC histamine secretion, but do suppress IgE-dependent secretion. These results suggest that the normal regulation of this process is by a secreted, probably labile factor(s), which may be partially deficient in asthma. Isolation and manipulation of this factor may have interesting therapeutic potential.

S34 EFFECTS OF THE CYCLIN-DEPENDENT KINASE INHIBITOR R-ROSCOVITINE ON EOSINOPHIL SURVIVAL AND CLEARANCE

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Background Eosinophils are pro-inflammatory cells implicated in the pathogenesis of asthma and atopy. Apoptosis has been proposed as a potential mechanism underlying the resolution of eosinophilic inflammation and studies have indicated the ability of interventions that induce human eosinophil apoptosis to promote the resolution of eosinophilic inflammation. Recently, the cyclin-dependent kinase (CDK) inhibitor R-roscovitine was shown to enhance neutrophil apoptosis and promote the resolution of neutrophilic inflammation.

Aim The purpose of this study was to examine the expression of CDKs in human blood eosinophils, the effects of R-roscovitine on eosinophil survival and phagocytosis *in vitro* and determine whether R-roscovitine could influence eosinophilic lung inflammation *in vivo*.

Methods Eosinophils were isolated from human peripheral blood and the effects of R-roscovitine on apoptosis, degranulation and phagocytic uptake examined *in vitro*. The effects of R-roscovitine on eosinophilic lung inflammation *in vivo* were also assessed using an ovalbumin mouse model.

Results Our data demonstrate that human eosinophils express five targets for R-roscovitine: CDK1, −2, −5, −7 and −9. R-roscovitine induced eosinophil apoptosis in a time- and concentration-dependent manner but also accelerated transition to secondary necrosis as assessed by light and electron microscopy, flow cytometry and caspase activation. In addition, we report that the pro-apoptotic effect of R-roscovitine is associated with suppression of Mcl-1L expression and that the apoptotic eosinophils are phagocytosed by human monocyte derived macrophages. R-roscovitine also induced apoptosis in mouse eosinophils purified from the bone-marrow, spleen and peripheral blood. Despite this, R-roscovitine did not modulate the tissue and lumen eosinophilia characteristic of the ovalbumin mouse model of airway eosinophilia.

Conclusions These data demonstrate that R-roscovitine is capable of inducing rapid apoptosis and secondary necrosis in human eosinophils but does not affect the onset or resolution of eosinophilic airway inflammation *in vivo*.

How should we be investigating suspected lung cancer?

S35 A RANDOMISED CONTROLLED TRIAL COMPARING COMBINED EBUS/EUS FOLLOWED BY SURGICAL STAGING VERSUS SURGICAL STAGING ALONE IN NON-SMALL CELL LUNG CANCER: THE ASTER STUDY

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Background For many years the standard approach to staging of the mediastinum in non-small cell lung cancer (NSCLC) has been surgical using cervical mediastinoscopy, left anterior mediastinotomy or video assisted thoracoscopic surgery (VATS). More recently endobronchial ultrasound (EBUS) and endoscopic ultrasound (EUS) have been reported. We conducted a randomised phase III trial to compare surgical staging versus endoscopic staging. The primary endpoint was detection of mediastinal nodal metastasis (N2/3); secondary endpoints were complication and futile thoracotomy rates.

Methods Consecutive patients with potentially resectable (suspected) NSCLC in whom invasive mediastinal staging was indicated based on CT or PET-CT findings were randomly assigned to either Arm A, surgical staging or Arm B, combined EBUS/EUS (followed by surgical staging if endoscopic findings were negative for malignancy). Surgical staging involved mediastinoscopy and/or mediastinotomy and/or VATS. Subsequently, in the absence of mediastinal disease, thoracotomy with systematic lymph node sampling was performed. 240 pts were required to show a 20% increase in sensitivity (power 80% and α=0.05) to detect mediastinal nodal disease with a prevalence of 50%.

Results 118 patients were randomised to Arm A and 123 to Arm B. The sensitivity for detection of mediastinal metastases by surgical staging in Arm A was 80% (95% CI, 68 to 89) vs 94% (95% CI, 85 to 98) for endoscopic (± surgical) staging in Arm B (p=0.04). Nodal metastases were found in 41 (35%) of surgically staged patients in Arm A and 62 patients (50%) (56 by EBUS/EUS + 6 by subsequent surgical staging) in Arm B (p=0.019). Overall, the prevalence of mediastinal disease in each arm was similar (p=0.24). Thoracotomy was considered futile in 21 (18%) in those staged in Arm A vs 8 patients (7%) in Arm B (p=0.009). Complication rate was similar in both arms (6 vs 7 patients, p=0.8); however, 12 of 13 complications were due to surgical staging procedures.

Conclusions Mediastinal staging for NSCLC should commence with combined EBUS/EUS (followed by surgical staging if endoscopic findings are negative for malignancy) as this improves the detection of nodal metastases and reduces futile thoracotomies compared to surgical staging alone.

S36 CHECK NOVEL *IN VIVO* REAL TIME IMAGING OF THE BRONCHIAL MUCOSA USING AN ENDO-CYTOSCOPY

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Objectives We investigated the capabilities of an Endo-Cytoscopy system (ECS) that enables microscopic imaging of the