NE as a primary efficacy endpoint in clinical trials or as a marker of inflammation within the clinic has been hampered by the lack of a robust and simple to use assay. ProteaseTag™ Active NE Immunoassay specifically measures only **active** NE in clinical samples, is quick and easy to use (<3 h) and has no dependency on a kinetic readout. ProteaseTag™ technology is currently being transferred to a lateral flow device for use at Point of Care.

P103 INHIBITION OF ASTHMA-RELATED IMMUNOLOGICAL RESPONSES BY CULTURED EPITHELIAL CELL LINES

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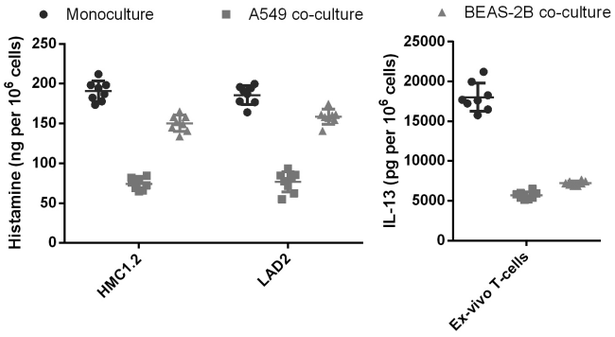
Background Previous studies have shown that constitutive and IgE-mediated histamine production by human lung mast cells is inhibited by a transferable factor produced by the airway epithelium. We have tested the hypothesis that a similar interaction exists between epithelial cell and mast cell lines. We have also investigated the effect of co-culture of epithelial cell lines and T_H2 cells on interleukin (IL)-13 production.
Methods A549 or BEAS-2B cells were grown to confluence overnight. Media was removed and LAD2, HMC1.2 or human-derived ex-vivo T-cells added for 16 h. For transwell experiments epithelial cells were added to a 24-well plate, replaced with fresh media after 16 h and mast cells media added to the insert, maintaining the mast cell/epithelium/volume ratio. Wells, and transwell insert media, were then centrifuged, supernatants harvested and mediator release quantified by histamine or IL-13 ELISA.
Results Neither mast cell line consistently produced histamine in response to IgE and anti-IgE. Flow cytometry suggested that this was due to absence of the high-affinity IgE receptor FcεR1. Constitutive histamine production by HMC1.2 was reduced from 191 ± 13 ng/10^6 cells by 60.9% (95% CI 54.1, 67.8; $p < 0.0001$) when co-cultured with A549 and 21% (95% CI 14.2, 28.1; $p < 0.0001$) with BEAS-2B cells. Similar findings were seen with the LAD2 mast cell line. Constitutive IL-13 production by T_H2 cells was reduced from 18000 ± 1800 pg/10^6 cells by 68.6% (95% CI 62.0, 75.1; $P < 0.0001$) by A549 and 59.9% (95% CI 53.3, 66.5; $p < 0.0001$) by BEAS-2B. Epithelial inhibition was similar when cells were separated by a transwell suggesting involvement of a transferable factor.



Abstract P103 Figure 1 -hour constitutive histamine (left) and IL-13 (right) release from mast or T-cells in the presence or absence of epithelial cell lines

Conclusion Epithelial cell lines inhibit a range of asthma-related immunological responses, probably by producing an inhibitory substance.

P104 STRUCTURAL AND CELLULAR RELATIONSHIPS IN THE PERIPHERAL LUNG: COMBINING MICRO-CT AND IMMUNOHISTOCHEMISTRY

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Introduction and objectives The peripheral lung contains a range of structural elements (small airways, blood vessels and lymphatics) together with infiltrating inflammatory cells. These components exist together in complicated spatial arrangements. Lung disease is frequently accompanied by changes in both lung architecture and the number and distribution of inflammatory cells. Light microscopy has been the conventional technique of choice in understanding these changes and relationships but provides only 2-D representations of a complex 3-D network.

We selected to use micro computed tomography (μ-CT) to image structural elements in the peripheral lung. We aimed to reconstruct the 3-D architecture by combining the μ-CT data with immunohistochemistry (IHC) to positively identify the principal structural elements and inflammatory cells.
Methods Human lung tissue was fixed in formalin, embedded in paraffin wax and subjected to μ-CT scanning. The tissue was then sectioned and immunostained for pancytokeratin (airways), collagen IV (blood vessels), D2–40 (lymphatic vessels) and CD68 (macrophages). The resulting images were used to guide the segmentation of the 3-D μ-CT image stack. IHC, using neurofilament antibodies, was also used on multiple lung samples to attempt to identify nerve fibres in the parenchymal tissue.
Results The main structural elements of the lung periphery could be identified, segmented out and their 3-D architecture examined. Macrophages were found throughout the tissue in large quantities and were most concentrated around the blood vessels and lymphatics. Lymphatic vessels were especially dense in the pleural region and elsewhere were intertwined with blood vessels. Despite being readily identifiable in bronchial samples, nerve fibres were not identified using IHC in the parenchyma.
Conclusions Combining μ-CT and IHC provides a robust method to positively identify important structural elements of the peripheral lung and to localise inflammatory cells in 3-D, thus allowing a detailed review of their spatial relationships. Alternative methodologies may however be advantageous regarding identifying parenchymal nerve fibres for reconstruction. μ-CT and IHC together create a highly accurate 3-D reconstruction but this method remains time consuming; advances in automation and improved tools are required to fully exploit the research potential.

P105 IDENTIFICATION OF 'LARGE' ALVEOLAR MACROPHAGES AND PULMONARY INTRA-VASCULAR MACROPHAGES IN COPD PATIENTS

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Background A population of small macrophages with increased pro-inflammatory activity has been reported in COPD sputum. We have investigated macrophage size in the alveoli of COPD