



Abstract S40 Figure 1 EBUS-MP outcomes.

a role in investigation of peripheral pulmonary mass lesions. In a well selected cohort of patients it appears to be a safe alternative to CT-guided biopsy.

Immunity and fibrosis in chronic asthma

S41 EXPRESSION OF FUNCTIONAL RECEPTOR ACTIVITY MODIFYING PROTEIN (RAMP)-1 BY AIRWAY EPITHELIAL CELLS WITH DYSREGULATION IN ASTHMA

doi:10.1136/thx.2010.150912.41

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Introduction and Objectives Epithelial cell expression of calcitonin-gene-related peptide (CGRP) is a feature of provoked asthma. Receptor activity modifying protein 1 (RAMP1) and the calcitonin-receptor-like receptor (CRLR) combine to form the CGRP1 receptor. We determined whether functional RAMP1 is expressed by airway epithelial cells and if there are alterations in asthma at baseline and after allergen challenge.

Methods BEAS-2B and A549 cells lines were studied by RT-PCR, confocal microscopy, a quantitative immunofluorescence assay and

ELISA. Bronchial biopsies from normals and asthmatics were examined by immunohistochemistry and in situ hybridisation.

Results Inflammatory cytokines induced CGRP release and CGRP mRNA in BEAS-2B and A549 epithelial cell lines. RAMP1 was highly expressed by resting, unstimulated BEAS-2B and A549 cells. CGRP induced internalisation of RAMP1 and IL-6 production, both of which were inhibited by the CGRP antagonist, CGRP8-37. Activation of BEAS-2B and A549 cells by inflammatory cytokines induced CGRP secretion, binding of CGRP to RAMP1 and RAMP1 internalisation which was blocked by CGRP 8-37. RAMP1 immunoreactivity and RAMP1 mRNA expression in bronchial biopsies from asthmatics was significantly lower than in normal subjects ($p=0.002$ and $p=0.007$, respectively). Inhalational challenge of atopic asthmatics with allergen-derived peptides produced a significant decrease in the numbers of RAMP1-positive epithelial cells in responders ($p=0.027$) but not non-responders.

Conclusions RAMP1 was expressed both by airway epithelial cells in culture and in bronchial biopsies from normal subjects and internalised after epithelial cell activation through autocrine feedback of CGRP. There is an apparent dysregulation of RAMP1 in asthmatic epithelium suggesting continuous stimulation of pathways involving CGRP.

S42 AIRWAY EPITHELIAL TOLL RECEPTOR EXPRESSION IN ASTHMA AND ITS RELATIONSHIP TO DISEASE SEVERITY

doi:10.1136/thx.2010.150912.42

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Introduction Asthma is classically considered a Th2 mediated disease. However, severe and treatment-resistant disease is more heterogeneous and often associated with airway neutrophil recruitment. This may be related to an altered airway bacterial colonisation. Bacteria express pathogen associated molecular patterns (PAMP's) that are recognised as non-self by pattern recognition receptors (PRRs). These PRRs represent an essential component of the innate immunity and an important family of PRRs are the Toll-like receptors (TLR). These are expressed on a range of innate immune cells including epithelial cells. TLR-1, -2, -4, -5 and -6 are located on the cell surface membrane and respond to bacterial cell wall components. This study has investigated the expression of mRNA for TLR-2, -4 and -5 in airway epithelial cells in asthmatics and healthy volunteers.

Methods Epithelial brushings were obtained from the large central airways at fibre-optic bronchoscopy from 18 healthy non-asthmatic volunteers (8 female and mean age 26 years) and 34 asthmatic volunteers (25 female, mean age 43 years). The asthmatic group comprised 7 non-steroid treated and 27 steroid treated asthmatics. The brushings were placed in Trizol and the RNA subsequently extracted and converted to cDNA, prior to real time TaqMan RT-qPCR analysis for the Toll receptors, TLR2, TLR4 and TLR5, as well as IL-8.

Results Gene expression for TLR-2 ($p=0.008$) and TLR-4 ($p=0.012$) was significantly increased within the epithelial brushing sample from the asthmatics compared to the healthy control subjects whilst that for TLR-5 did not differ significantly. Interleukin 8 mRNA was also increased within the epithelial brushing sample in the asthmatics ($p=0.007$) compared to that in the healthy control subjects. These significant differences from the healthy population were also individually present in both the mild and severe asthmatic groups, with no significant difference being evident between mild and severe asthma.

Conclusions These findings reveal up regulation of epithelial gene expression for members of the Toll receptor family relevant to bacterial responses within the airways. Additionally there is enhanced IL-8 gene expression. These features are indicative of on-

going innate immune airway responses in asthma. The relevance of this to clinical disease expression requires understanding.

S43 THE EXPRESSION OF CRACM ION CHANNELS IN HUMAN LUNG MAST CELLS

doi:10.1136/thx.2010.150912.43

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Introduction and objectives The influx of extracellular Ca^{2+} into human lung mast cells (HLMC) is essential for the IgE-dependent release of preformed granule derived mediators and newly synthesised autacoids and cytokines. However the identity of the ion channels underlying the influx of Ca^{2+} remains unknown. Members of the recently discovered CRACM (also known as Orai) ion channel family are candidates for this function. The channels carry Ca^{2+} selective currents (I_{CRAC}) that are activated when endoplasmic reticulum Ca^{2+} stores are emptied. Here we investigate whether CRACM channels are expressed in HLMC and their possible contribution to Ca^{2+} influx in these cells.

Methods HLMC were purified from lung tissue by immunomagnetic affinity selection. Total RNA purified from cells was used in quantitative RT-PCR using TaqMan probes and primers (Applied Biosystems). For electrophysiology the whole cell method of patch clamp was used.

Results CRACM1, CRACM2 and CRACM3 mRNA transcripts were all readily detectable in HLMC. CRACM1 transcripts (4.50 ± 0.51 copies/ 10^3 beta-actin transcripts, $n=7$ HLMC donors) were more abundant than CRACM2 transcripts (1.79 ± 0.67 copies/ 10^3 beta-actin transcripts, $n=5$) and CRACM3 transcripts (3.33 ± 0.95 copies/ 10^3 beta-actin transcripts, $n=5$). Consistent with the presence of CRACM transcripts, an I_{CRAC} -like current was detected in HLMC following IgE-dependent activation and also in cells dialysed with 30 μM inositol triphosphate to empty endoplasmic reticulum Ca^{2+} stores. The Ca^{2+} -selective current obtained under both conditions was blocked by the addition of 10 μM La^{3+} , a known blocker of CRACM channels. Thus following addition of anti-IgE to 11 HLMC, current at -120 mV increased from -12.1 ± 1.1 pA at baseline to -34.2 ± 2.5 pA ($p < 0.0001$) and following addition of 10 mM La^{3+} this was reduced to -17.4 ± 2.1 pA. ($p = 0.0003$). When HLMC were dialysed with IP_3 ($n=12$), current increased from -15.3 ± 1.2 pA to -40.2 ± 3.2 pA ($p < 0.0001$), and decreased to -17.7 ± 1.5 pA ($p < 0.0001$) by La^{3+} .

Conclusions The presence of an I_{CRAC} -like current and CRACM1, -2 and -3 mRNA transcripts in HLMC supports a role for CRACM channels in the influx of extracellular Ca^{2+} into these cells following activation. Such a role would make CRACM channels a potential therapeutic target in the treatment of asthma.

S44 INCREASED EXPRESSION OF IMMUNOREACTIVE THYMIC STROMAL LYMPHOPOETIN IN SEVERE ASTHMA

doi:10.1136/thx.2010.150912.44

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Background Thymic stromal lymphopoietin (TSLP) is a cytokine implicated in the pathophysiology of asthma through two pathways: a TSLP-OX40L-T cell axis and a TSLP-mast cell axis. Whether these pathways operate in human asthma is unknown.

Aims To investigate whether mucosal TSLP protein expression relates to asthma severity, and distinct immunological pathways.

Methods GMA-embedded bronchial biopsies from healthy subjects ($n=12$) and patients with mild (BTS step 1, $n=8$), moderate (BTS steps 2 and 3, $n=12$) and severe (BTS steps 4 and 5, $n=16$) asthma were immunostained for TSLP, OX40, OX40L, CD83, IL-13, and inflammatory cell markers. Extent of immunostaining was correlated with clinical data.

Results Specific TSLP immunoreactivity was evident in both the airway epithelium and lamina propria of both healthy and asthmatic subjects. TSLP immunoreactivity was not present in airway smooth muscle. TSLP epithelial expression was significantly elevated in asthma as a whole compared to healthy controls ($p=0.0005$), particularly in mild and severe disease. The number of TSLP+ cells in the lamina propria was elevated in patients with severe asthma relative to other groups ($p=0.0058$). Co-localisation studies in 6 severe asthmatic subjects showed that 5% of TSLP+ cells in the lamina propria were CD68+ macrophages and 20% were tryptase+ mast cells. There were significant inverse correlations between TSLP counts in both the asthmatic bronchial lamina propria and epithelium with the FEV₁/FVC ratio ($r_s = -0.53$, $p=0.002$ and $r_s = -0.40$, $p=0.037$). Immunostaining for OX40, OX40L and CD83 in the airways was sparse, with no difference between asthmatic patients and normal control subjects. IL-13 staining was increased in non-epithelial cells within the airway epithelium in severe asthma ($p=0.033$).

Conclusions TSLP expression is elevated in severe asthma despite high dose corticosteroid therapy. Although we did not detect activity of the TSLP-OX40L-T cell pathway within asthmatic bronchial mucosa, it is possible that this pathway operates in secondary lymphoid organs such as draining lymph nodes. The close approximation of airway stroma and mast cells suggests that the TSLP-mast cell axis, in which TSLP and IL-13 operate in a positive regulatory loop, is active in asthmatic bronchial mucosa and may be important in maintaining chronic airway inflammation.

S45 FUNCTIONAL $\text{KCa}_{3.1}$ K^+ CHANNELS ARE REQUIRED FOR HUMAN FIBROCYTE MIGRATION

doi:10.1136/thx.2010.150912.45

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Background Fibrocytes are bone marrow-derived CD34⁺ collagen I⁺ cells present in peripheral blood that are implicated in the pathogenesis of tissue remodelling and fibrosis in both asthma and pulmonary fibrosis. Ion channels play key roles in cell function, but the ion channel repertoire of human fibrocytes is not known. The aim of this study was to assess whether human fibrocytes express the $\text{KCa}_{3.1}$ K^+ channel.

Methods Human fibrocytes were isolated from peripheral blood taken from both normal and asthmatic subjects and maintained in cell culture. Channel expression was examined using RT-PCR, Western blot, and patch clamp electrophysiology. Migration assays were performed using the Transwell system.

Results Fibrocytes expressed both $\text{KCa}_{3.1}$ mRNA and protein. At baseline unstimulated fibrocytes ($n=30$ healthy cells from 7 donors, $n=17$ asthmatic cells from 4 donors) demonstrated heterogeneity of the resting whole cell current, with a small outwardly rectifying current in 91% of cells, a steeply inwardly rectifying current in 30% of cells, and a linear current in 9% of cells. The $\text{KCa}_{3.1}$ opener 1-EBIO (100 mM) elicited typical $\text{KCa}_{3.1}$ currents in 46/47 cells tested. Whole cell current at +40 mV increased from 122 ± 25 pA pre-1-EBIO to 795 ± 104 pA post 1-EBIO ($p < 0.0001$). This was accompanied by a negative shift in reversal potential from -29.8 ± 3.0 mV to -61.8 ± 1.6 mV ($p < 0.0001$). 1-EBIO induced a greater current in