Methods Primary bronchial epithelial cells (PBEC) and macrophage-like cells (THP-1) were incubated with varying concentrations of cigarette smoke extract (CSE) before stimulation with whole cell lysates of HI prepared from clinical isolates. Cell viability was assessed by flow cytometry and PBEC proliferation was analysed using an XTT assay. Pro-inflammatory cytokine release (IL-8, TNFα, IL-1β) was assessed in PBEC and PMA-differentiated THP-1 cells by ELISA.
Results CSE concentrations ≤10% had no significant effect on PBEC or THP-1 cell viability, however CSE concentrations >5% inhibited PBEC proliferation ($p<0.05$, n=9). PBEC demonstrated increased release of IL-8 in response to treatment with 5% CSE and increasing doses of HI (n=3, $p<0.05$). Co-treatment with HI + CSE accentuated IL-8 release from PBEC even at CSE concentrations of 1–2.5% which had no effect alone ($p<0.05$, n=6). TNFα and IL-1β release from PBEC were below the detection limit of the ELISA. THP-1 cells increased the release of IL-8, TNF-α and IL-1β in response to treatment with HI (n=3, $p<0.05$) however CSE alone had no effect on cytokine production (n=3, $p>0.05$). Co-treatment of THP-1 cells with HI + CSE increased IL-8 release (61%), but reduced TNF-α (40%) and IL-1β (24%) release compared to treatment with HI alone (n=3, $p<0.05$).
Conclusions CSE alters epithelial cell and macrophage responses to bacterial pathogens by promoting release of the neutrophil chemokine IL-8 whilst at the same time suppressing TNFα and IL-1 β release. This dysregulation may promote continued neutrophil inflammation in the airway whilst insufficiently clearing pathogens and could be an important mechanism in COPD.

S145 THE IMPACT OF CIGARETTE SMOKE EXTRACT ON INFLAMMATORY RESPONSES AND TOLL-LIKE RECEPTOR-4 EXPRESSION IN HEALTHY NASAL EPITHELIAL CELLS

doi:10.1136/thx.2010.150946.46

[1]D Comer, [1]J S Elborn, [1]V Brown, [2]C McDowell, [1]M Ennis. *[1]Respiratory Medicine Group, Centre for Infection and Immunity, Queens University Belfast, Belfast, Northern Ireland; [2]Clinical Research Support Centre, Belfast, Northern Ireland*

Although cigarette smoke extract (CSE) is widely used in respiratory research, the methods used in its preparation are diverse and there is no consensus as to whether cigarette smoke extract (CSE) induces a pro-inflammatory or an immunosuppressive response in epithelial cells.[1] Although the adverse effect that cigarette smoke has on the lower airway has been extensively studied, the responses of the nasal epithelium are not well described. Our aims were to study the effects of a non-cytotoxic CSE exposure on LPS-induced innate immune responses from primary nasal epithelial cells (PNECs), to assess the constitutive gene expression and the localisation of TLR-4 in PNECs by RT-PCR and flow cytometry, and finally to analyse the modulation of TLR-4 expression after stimulation PNECs by LPS±CSE pre-treatment. CSE was prepared by combusting 1 or 2 12 mg tar Marlboro cigarettes through 25 ml of media. Submerged PNEC cultures were treated with CSE (or vehicle) followed by stimulation with LPS. Supernatants were assessed for IL-8 and IL-6 and the expression and localisation of TLR-4 was established. Cell viability was not affected except after 24 h incubation with 5% CSE made from two cigarettes, or using single cigarette along with a 24 h stimulation with 20–25 μg/ml LPS. A brief incubation with CSE (4 h) significantly inhibited LPS-induced cytokine release. In contrast, a more prolonged incubation with CSE (24 h) heightened LPS-induced cytokine release. Incubation with CSE (4 h and 24 h) had no significant impact on expression of TLR4 mRNA. A brief incubation with CSE (4 h) resulted in a lower percentage of surface and intracellular TLR4. A prolonged incubation with CSE (24 h) did not affect surface or intracellular TLR4. CSE exposure for 4 h suppresses the inflammatory response in PNECs to stimulation with LPS whereas 24 h CSE exposure was pro-inflammatory. A reduced surface TLR-4 may account for the immunosuppressive effects, but unaltered surface and intracellular TLR-4 after 24 h CSE treatment suggests that alternative pathways may be responsible for the pro-inflammatory effects.

REFERENCE

1. **Smith LA,** *et al*. Inflammatory response of lung macrophages and epithelial cells to tobacco smoke: a literature review of ex vivo investigations. *Immunol Res* 2010;**46**:94–126.

S146 MICROVASCULAR ENDOTHELIAL CELL APOPTOSIS AND DYSREGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (VEGF-R2) IN RESPONSE TO CIGARETTE SMOKE. NEW INSIGHTS INTO THE PATHOGENESIS OF EMPHYSEMA

doi:10.1136/thx.2010.150946.47

[1]L S Mackay, [2]S Dodd, [2]W Tomlinson, [2]I Dougall, [1]H Walden, [2]B Isherwood, [1]A Gardner, [1]L Borthwick, [2]K Pinnion Brown, [2]M Foster, [1]A J Fisher, [1]P A Corris. *[1]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK; [2]Astrazeneca R+D Charnwood, Loughborough, UK*

Microvascular injury may be a primary mechanism in the pathogenesis of emphysema. Rats treated with a VEGF-R2 antagonist develop emphysema that is caspase-3 dependent. Additionally, patients with emphysema have reduced VEGF. We hypothesised that cigarette smoke injury may disrupt homeostatic vascular repair mechanisms and investigated VEGF-R2 expression and endothelial apoptosis in the pulmonary microvasculature of patients with emphysema.
Methods Microvascular endothelial cells were isolated from explanted peripheral lung tissue from four patients with emphysema undergoing transplantation. Cells were characterised via flow cytometry and confocal microscopy. Concurrent immunolocalisation of CD31, VEGF, VEGF-R2 and caspase-3 was performed on peripheral lung tissue from each patient and expression compared with that in excess normal lung tissue obtained from lobectomies. Isolated endothelial cells were treated with freshly prepared cigarette smoke extract (CSE) (3%) for 0, 24, 48, 72 h and expression of VEGF-R2 investigated via q-PCR. A cell permeable caspase-3 substrate, NucView 488 (Biotium), which is cleaved by the enzyme to form a high-affinity fluorescent DNA binding dye, was used to detect apoptosis via live cell imaging in response to CSE (0–12%) over 64 h.
Results Isolated cells at low passage (P3-P6) expressed high levels of CD31, negligible CD90 and inducible CD62 confirming them as microvascular endothelial cells. VEGF-R2 expression was significantly reduced (68%; n=4, $p<0.01$) following treatment with 3% CSE at 48 h, compared with unstimulated cells. Live cell imaging demonstrated that cells underwent apoptosis in response to low dose CSE (3%, 1 h treatment) at 24 h ($p=0.05$) compared with unstimulated cells. Immunohistochemical analysis revealed reduced CD31 expression in the alveolar bed of emphysema tissue, indicating capillary loss. Regional alveolar expression of VEGF-R2 was also reduced compared with non-emphysematous tissue. Caspase-3 staining revealed positive cells in the alveolar bed, a sub-population of these indicating the presence of apoptotic endothelial cells in severe emphysema.
Conclusions Microvascular endothelial cells isolated from individuals with emphysema undergo accelerated apoptosis and down-regulate VEGF-R2 in response to CSE. These findings were consistent with the immunohistochemical analysis of emphysema tissue. This may represent a maladaptive response to CSE injury in susceptible individuals and be important in the pathogenesis of emphysema.

S147 DIESEL EXHAUST PARTICLES ALTER MONOCYTE DIFFERENTIATION IN VITRO BUT HAVE LITTLE IMPACT ON NEUTROPHIL FUNCTION

doi:10.1136/thx.2010.150946.48

[1]H Jary, [2]K Piddock, [3]K Donaldson, [2]L R Prince, [2]L C Parker, [2]I Sabroe, [2]N Chaudhuri. *[1]Academic Unit of Respiratory Medicine, University of Newcastle, Newcastle, UK;*