TO52 REQUIREMENT OF VASCULAR ENDOTHELIAL GROWTH FACTOR FOR INDUCTION OF PRO-ANGIOGENIC CHANGES BY ASTHMATIC HUMAN AIRWAY SMOOTH MUSCLE

Stuart Hirst, Varsha Kanabar, Charlotta Karner, Tak Lee & David Simcock
King's College London, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London, SE1 7EH, United Kingdom

Neovascularisation is a feature of airway remodelling in asthma leading to airflow obstruction and hyperresponsiveness. Human airway smooth muscle (hASM) cells express multiple pro-angiogenic growth factors. Previously, we have demonstrated that factors secreted by asthmatic hASM induce vascular tubule formation in vitro. Here, we investigate the importance of vascular endothelial growth factor (VEGF) in hASM-induced angiogenesis.

Methods: hASM cells were cultured from healthy, non-atopic volunteers and those with atopic, mild or moderate asthma (n=3/group). Cell-conditioned medium (CM) from unstimulated hASM cells was collected and incubated with co-cultures of human endothelial cells and dermal fibroblasts before and after selective VEGF immunodepletion. Pro-angiogenic changes were visualised after 11 days following anti-CD31 labelling; numbers of vascular junctions, tubules and vascular length were quantified by image analysis. VEGF in CM was quantified by ELISA. VEGF mRNA alternative splice variants in hASM were detected by real time qRT-PCR.

Results: Vascular junctions and tubule length were increased by 2-fold above basal by CM from unstimulated healthy hASM and by 3.6-fold by CM from unstimulated mild or moderate asthmatic hASM ($p<0.05$). Selective VEGF immunodepletion abolished the induction of all vascular indices by CM from healthy or asthmatic hASM ($p<0.01$). Depletion of VEGF from CM samples was confirmed by specific ELISA and was undetectable ($p<0.001$). VEGF splice variant mRNAs and VEGF levels in undepleted CM were not different between mild or moderate asthma and healthy controls ($p>0.05$).

Conclusions: VEGF is required but not sufficient for induction of vascular tubules by CM from asthmatic ASM cells.

Supported by Asthma UK, MRC UK & King's College London
Conflict of Interest: NO