

P402

## P402 -The role of the calprotectin/ RAGE/ TLR4 axis in ANCA-Associated vasculitis

Dr Theresa Page<sup>1</sup>, Dr Francesca Brunini<sup>1</sup>, Josep Garnica Caparros<sup>1</sup>, Azin Kherkhah<sup>1</sup>, Fayez Dudhiya<sup>1</sup>, Dr Maria Prendecki<sup>1</sup>, Dr Stephen McAdoo<sup>1</sup>, Professor Charles Pusey<sup>1</sup>

<sup>1</sup>Imperial College, London, United Kingdom

**Objectives:** ANCA-associated vasculitis (AAV) patients exhibit raised levels of circulating calprotectin and HMGB1 (1,2), molecules recognised by the TLR4:CD14 receptor and the receptor for advanced glycation end products (RAGE). We have previously demonstrated raised monocyte CD14 levels in AAV (3) and others have reported raised TLR4 expression, however levels of RAGE and the functional consequence of receptor/ligand ligation have not been fully investigated in AAV. We have now investigated (a) the expression of TLR4, RAGE and its naturally occurring soluble decoy, sRAGE, in AAV serum, peripheral blood mononuclear cells (PBMC) and biopsy samples; and (b) the effect of RAGE/ TLR4 and ANCA stimulation on neutrophil responses.

**Methods:** Serum and plasma levels of sRAGE, calprotectin and HMGB1 were analysed using commercially available ELISAs. Tissue biopsy sections were stained for RAGE and TLR4 expression by standard immunohistochemical techniques. PBMC and neutrophils were from healthy volunteers. cDNA preparation and qPCR analysis were by standard techniques.

**Results:** Levels of calprotectin and HMGB1 were raised in active AAV, whilst sRAGE was significantly reduced ( $p=0.0001$ ) compared to those in remission and healthy controls. Within the active patient group, sRAGE levels were lower in untreated patients ( $p=0.04$ ) and showed an inverse correlation with serum calprotectin ( $p=0.02$ ). Reductions in sRAGE protein were mirrored by a reduction in levels of mRNA in AAV PBMC suggesting changes in the transcriptional profile in these patients. Sequential staining of AAV renal and lung biopsies revealed glomerular and interstitial RAGE+ and TLR4+ cells co-localising with areas of MPO staining, T cell (CD3), B cell (CD20) and macrophage (CD68) infiltrates. In vitro stimulation of neutrophils with combinations of ANCA and TLR4/ RAGE ligands produced enhanced cytokine production compared with ANCA alone.

**Conclusion:** AAV tissue infiltrating cells of a variety of lineages express both RAGE and TLR4, and will encounter raised levels of endogenous ligands (calprotectin / HMGB1), the activity of which may be unchecked by insufficient sRAGE in active disease. Consequently, exposure to a combination of calprotectin/ HMGB1/ANCA may produce an exaggerated TLR4/RAGE-mediated response.

(1) R. J. Pepper et al. *Kidney International* (2013) 83, p1150

(2) C. Wang, et al. *Autoimmunity Reviews* (2015) 14:11, p1057

(3) R.M.Tarzi, et al. *Clin. Exp. Immunol.* (2015) 181(1): p65

1 This research was supported by Vasculitis UK and the UK National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London

2 supported by the EDTA young fellowship programme