**Introduction:** Spleen tyrosine kinase (Syk) is a cytoplasmic protein tyrosine kinase which has been shown to play a role in signal transduction in a variety of cell types. Syk phosphorylation has been implicated in activation of neutrophils by ANCA. We have previously demonstrated the presence of Syk in renal biopsy tissue from patients with ANCA associated vasculitis (AAV) and shown that a Syk inhibitor is an effective treatment in experimental autoimmune vasculitis, a rat model of AAV. Here, we have investigated the activity of Syk in neutrophils, its role in cytokine production and expression of Syk in extra-renal sites of vasculitis.
**Methods:** Neutrophils were isolated from healthy controls and patients with acute AAV using dextran sedimentation and percoll gradient. Intracellular staining for total (T-Syk) and phosphorylated Syk (P-Syk) at two distinct phosphotyrosine residues (319 and 348) was analysed using flow cytometry. 22 AAV samples and 10 healthy controls were included. For functional studies neutrophils were primed with TNFα 2ng/ml for 30 minutes and stimulated with 100 µg/ml of IgG containing MPO-ANCA for 4 hours. Cells were incubated with R406, a small molecule Syk inhibitor, or with vehicle for 20 minutes prior to the addition of MPO-ANCA. Control experiments utilised healthy donor IgG in replacement of MPO-ANCA IgG. Immunostaining for T-Syk was carried out on formalin fixed paraffin embedded (FFPE) sections of tissue from patients with extra-renal AAV including ENT, lung and skin.
**Results:** There were similar levels of T-Syk present in neutrophils from patients and healthy controls. Levels of P-Syk at both the 319 and 348 residues were increased in AAV (Figure 1a). The levels of Syk phosphorylation were similar between patients who were MPO-ANCA positive and those who were PR3-ANCA positive. When TNFα primed neutrophils were stimulated with MPO-ANCA there was significant IL-8 release and this was inhibited by R406 in a dose-dependent manner (Figure 1b). There was also evidence of T-Syk in infiltrating inflammatory cells at non-renal sites of AAV.
**Conclusions**: Our results show that Syk is phosphorylated at the 319 and 348 residues to a greater degree in patients with AAV than in healthy controls. Phosphorylation of these sites occurs after Syk activation indicating greater neutrophil Syk activity in patients with AAV. Syk plays a role in cytokine production from neutrophils which may be pathogenic and Syk can be identified at sites of tissue inflammation. These results suggest there may be a role for Syk inhibition in decreasing neutrophil mediated damage in patients with AAV.

