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Fostamatinib treatment of a new model of MPO-ANCA vasculitis in WKY rats induced by administration of a sub-nephritogenic dose of nephrotoxic serum after immunisation with human myeloperoxidase

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Background:
Experimental autoimmune vasculitis (EAV) is a rat model of MPO-ANCA vasculitis induced by immunising WKY rats with human myeloperoxidase (hMPO) in complete Freunds adjuvant (CFA). Although animals develop pulmonary haemorrhage and proliferative glomerulonephritis (GN), crescent formation is infrequent and the model is milder than ANCA associated vasculitis (AAV) in humans. In order to augment disease severity in EAV, we immunised animals with an additional sub-nephritogenic dose of rabbit nephrotoxic serum (NTS).

Methods:
In order to define a sub-nephritogenic dose of NTS, male WKY rats were immunised IV with 100µl NTS diluted from 1:2 to 1:100, and assessed for disease severity. For combination experiments, animals were immunised IM with 800µg/kg hMPO in CFA (or human serum albumin (HSA) in CFA control) on day 0, and 100µl 1:100 NTS IV (or normal rabbit serum (NRS) control) at day 14. Where a small molecule Syk inhibitor (fostamatinib, Rigel Pharmaceuticals) was used, this (or vehicle) was administered by oral gavage (40mg/kg twice daily) from day 24-27. Blood and urine were collected weekly and animals were sacrificed at day 28 with collection of tissues for processing. Infiltrating glomerular leucocytes were isolated by serial sieving and collagenase digestion, stained with cell surface markers, and analysed by flow cytometry.

Results:
Immunising animals with a serial dilution of NTS from 1:2 to 1:100 identified 1:100 as a sub-nephritogenic dose, with no animals developing urinary abnormalities, deposited antibody as detected by direct immunofluorescence, or glomerular histological injury. Animals serially immunised with hMPO & 1:100 NTS had significantly more proteinuria, glomerular abnormalities and glomerular infiltrating cells at day 28 than those immunised with hMPO & NRS, HSA & NTS, or HSA & NRS (Figure 1A). There was no deposited glomerular rat or rabbit IgG as measured by direct immunofluorescence in any of the groups. When fostamatinib was administered to animals immunised with hMPO & NTS, there was a significant reduction in lung haemorrhage, urinary and glomerular abnormalities compared to vehicle treated animals (Figure 1B). There was no difference in anti-MPO antibodies between animals immunised with hMPO +/- NTS, NRS, or after fostamatinib treatment. In animals immunised with hMPO and 1:100 NTS there were significantly more infiltrating glomerular leukocytes with a greater increase in classical compared to non-classical monocytes. In animals treated with fostamatinib, cellular infiltrate was similar to control animals (Figure 1B).

Conclusions:
Immunisation with a sub-nephritogenic dose of NTS 14 days after hMPO significantly augments disease severity, without evidence of deposited antibody. Characterisation of glomerular infiltrating cells by flow cytometry shows significant infiltration of classical monocytes and we suggest it may be these cells which

are stimulating crescent formation. Administration of fostamatinib for 4 days was sufficient to significantly decrease disease severity in this model.