IMPERIAL

Peptide EAV: A Novel Model of MPO-AAV

Lyndon Costa,
Maria Prendecki,
Stephen McAdoo

INTRODUCTION

AAV

- Anti-Neutrophil Cytoplasm Antibody (ANCA) Associated Vasculitis (AAV) is a rare and severe autoimmune.
- Multisystemic and can cause life-threatening lung haemorrhage and end-stage renal disease.
- Characterised by small blood vessel inflammation, tissue damage and organ impairment.
- Patients develop ANCA targeting neutrophil granules – Myeloperoxidase (MPO) and Proteinase 3 (PR3).
- Hallmark feature is pauci-immune necrotising and crescentic glomerulonephritis (GN).

Standard Experimental Autoimmune Vasculitis

- WKY rats immunised with hMPO with the addition of pertussis toxin and killed *M. Tuberculosis.*
- Crescentic glomerulonephritis and lung haemorrhage.
- Rats given an additional sub-nephritogenic dose of nephrotoxic serum (NTS) resulted in recapitulation of more important features in human disease.

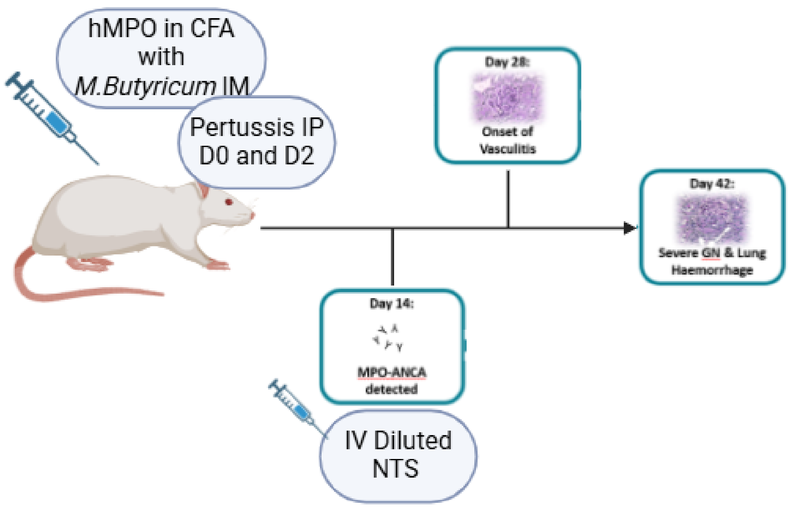

Figure 1: Boosted EAV Induction regimen in WKY Rat. Immunisation with human MPO in Complete Freund's Adjuvant along with supplementation with dead *M.Butyricum* and two administrations of pertussis toxin at day 0 and day 2 results in the development of anti-MPO disease

- Further refined the model by immunising with the immunodominant peptide of rat $MPO_{420-434}$.
- Rats develop more clinically relevant anti-MPO disease.

RESULTS

Boosted EAV

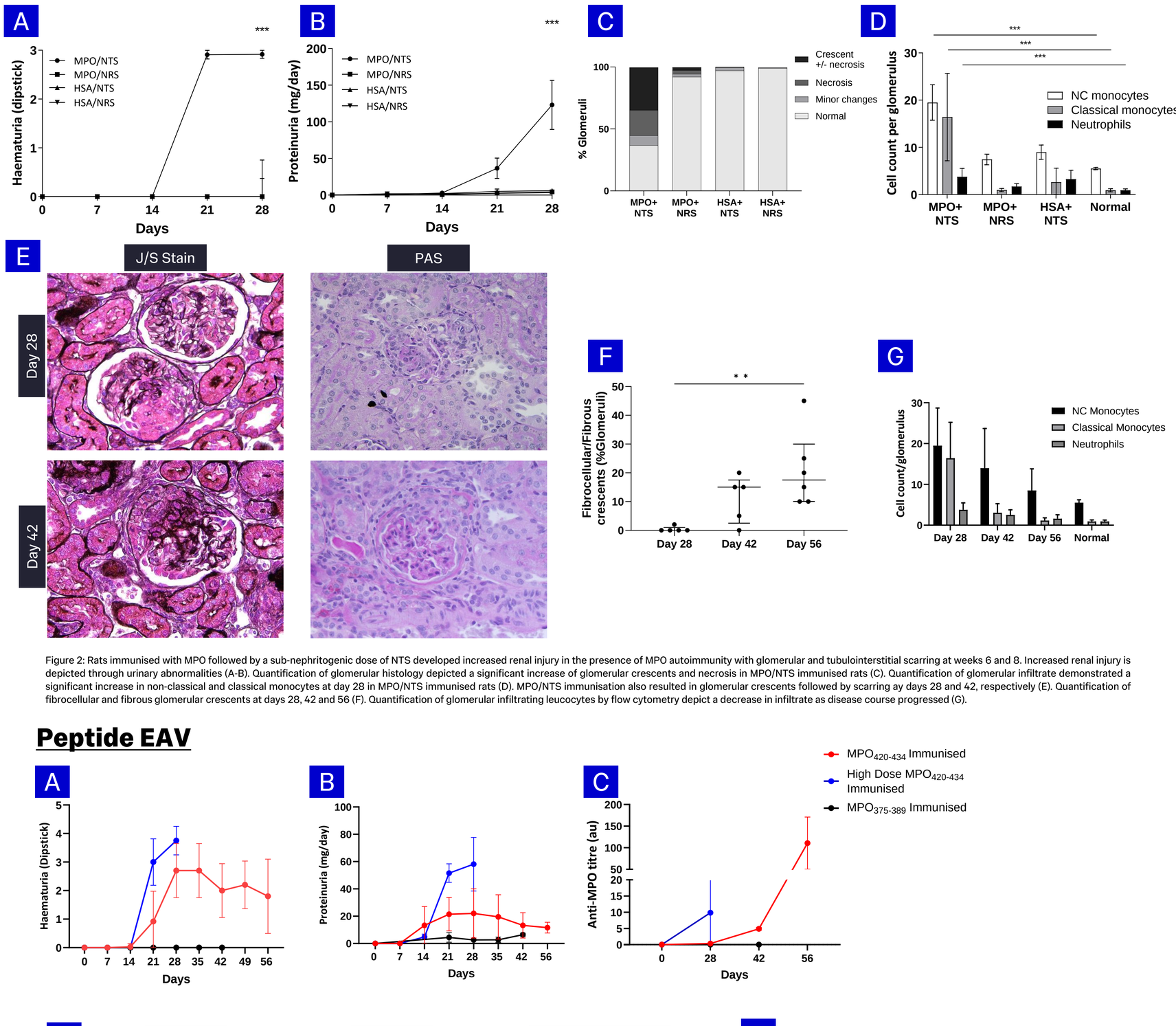

Figure 2: Rats immunised with MPO followed by a sub-nephritogenic dose of NTS developed increased renal injury in the presence of MPO autoimmunity with glomerular and tubulointerstitial scarring at weeks 6 and 8. Increased renal injury is depicted through urinary abnormalities (A-B). Quantification of glomerular histology depicted a significant increase of glomerular crescents and necrosis in MPO/NTS immunised rats (C). Quantification of glomerular infiltrate demonstrated a significant increase in non-classical and classical monocytes at day 28 in MPO/NTS immunised rats (D). MPO/NTS immunisation also resulted in glomerular crescents followed by scarring ay days 28 and 42, respectively (E). Quantification of fibrocellular and fibrous glomerular crescents at days 28, 42 and 56 (F). Quantification of glomerular infiltrating leucocytes by flow cytometry depict a decrease in infiltrate as disease course progressed (G).

Peptide EAV

Figure 3: Immunisation with $MPO_{420-434}$ induces glomerulonephritis with autoantibodies against the peptide that cross react with whole MPO. Renal injury is depicted via urinary abnormalities that peak by day 28 (A-B). $MPO_{420-434}$ immunised rats also develop an increasing titre of anti-MPO antibodies by day 28 (C). $MPO_{420-434}$ immunisation results in diffuse proliferative glomerulonephritis on day 28, which aggravates by day 56 (D). Quantification of glomerular infiltrate in $MPO_{420-434}$ immunised rats compared with conventional EAV, boosted EAV and NTN (E).

3Rs

- Boosted EAV shortened disease course from 42 to 28 days.
- Reduction in the number of rats needed to test any therapies as disease is more reproducible.
- Peptide EAV more accurately recapitulates AAV in humans.
- Peptide EAV does not use NTS so avoids IV injection.

Conclusion

- Boosted EAV is a better anti-MPO disease model than conventional EAV
- Peptide EAV induces a disease course that is different to conventional EAV
- Peptide EAV induces more severe glomerulonephritis with an early disease phase before the development of anti-MPO antibodies.
- The immunodominant peptide of MPO in rats is homologous with that in humans.

Imperial College London
imperial.ac.uk