REGULATORY B CELLS IN ANCA ASSOCIATED VASCULITIS: THEIR ROLE IN RESTORING IMMUNE TOLERANCE

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Methods
Mononuclear cells were isolated from peripheral blood by density gradient centrifugation on Lymphoprep (Axis-Shield, Dundee, UK); viability was 95% or greater, as assessed by trypan blue exclusion (Invitrogen, Paisley, UK).

Flow cytometry was conducted with: CD19 PE-Cy7 (BDBiosciences), CD24 PE (eBioSN3) and CD38 FITC (HIT2) antibodies (eBioscience, Hatfield, UK). A healthy control was included in each analytical run and staining conducted, alongside isotype and single-colour compensation controls. Data was acquired on an LSRII Fortessa instrument (BD Biosciences, Oxford, UK) and analysis conducted with FlowJo software (TreeStar, Inc., San Carlos, CA).

Statistical analysis was performed with GraphPad Prism (GraphPad Software, San Diego, CA) using one-way ANOVA and student’s unpaired, two-tailed t-test.

Conclusions
Remission AAV patients have fewer B regulatory cells than active or tolerant patients. This is not likely to be solely due to immunosuppression as transplant recipients have a greater in-basal suppressive load and higher frequency of B regulatory cells.

The B regulatory cells characterized in this study by high expression of CD24 and CD24, are functionally suppressive, inhibiting T cell proliferation and cytokine production. Further investigation of B regulatory function in tolerant and remission patients is currently underway.

B regulatory cells are restored in those AAV patients who regain immunological tolerance. Harnessing B regulatory cells may be a novel therapeutic strategy for relapsing remitting AAV.