B regulatory cells are numerically but not functionally impaired in AAV

AAV are characterised by myeloperoxidase or proteinase 3 autoantibodies, produced by autoreactive B cells. In addition to promoting disease, a regulatory subset of B cells has been defined, which plays a role in maintaining tolerance to self-antigens (Bregs). We assessed frequency of B cell subsets and ability to inhibit T cell cytokine production *in vitro*, in AAV and healthy controls.

The AAV cohort comprised 46 patients, 25 PR3-ANCA and 16 MPO-ANCA; 20 with active disease, 21 in remission and 5 tolerant. PBMC were stained with CD19, CD24, CD38 and CD5, enabling definition of Breg, memory (Bmem) and naïve (Bnaive) populations by flow cytometry. For functional assays, B cell subsets and naive T helper cells were isolated as previously described [PA Blair 2010]. T and B cells were combined at a ratio of 4:1 and cultured for 5 days, in presence of CD3 and anti-CD28mAbs. At the end of the assay IFN-γ and TNF-α were assessed by intracellular staining. The percentage change in cytokine positive cells and mean fluorescence intensity (MFI) was assessed for co-cultures, relative to T cells alone.

Active and tolerant but not remission AAV patients had a lower frequency of Bmem cells relative to controls. Breg ($CD24^{hi}$ $CD38^{hi}$) % and absolute numbers were statistically lower during remission as were $CD19+CD5^{hi}$ cells. Breg:Bmem ratios were statistically greater than control only during disease remission.

Bregs statistically suppressed T cell TNF α and INFγ production (% and MFI) more than Bmem or Bnaive cells (Table 1), but there was no statistical difference in suppressive capacity of AAV Bregs compared to controls.

In AAV disease remission there is a relative deficiency in Bregs, which is numerical but not functional, and preservation of Bmem. No differences were found between PR3-and MPO-ANCA patients. By contrast tolerant patients (ANCA negative off treatment) have preserved Breg and low Bmem populations. This imbalance in B cell homeostasis may contribute to the high rate of relapse observed in AAV.

Table 1 Overall change in T cell cytokine production in B cell co-cultures

Difference relative to T cells alone (%)		MEM	NAIVE	REG	ANOVA
MFI	TNF-α	37.3	28.56	-15.7	P<0.0001
	IFN-γ	36.7	34.11	-20.67	0.0013
Positive cells	TNF-α	25.3	18.22	-9	0.0050
	IFN-γ	5.4	6	-12.78	0.0179
	Double positive	25.6	19.22	-5.889	0.0145