Supplementary data

Supplementary Table S1. Acute patient clinical details and B cell subset results

	Days since presentation	BVAS score	ANCA titre	Creatinine mg/dL	Lymphocyte x10^9/L	Current treatment	M:R(n)	Bmem%	Breg%
MPO_1	3	12	58	633	0.86	CYC, PRED	2.0	17.4	8.6
MPO_2	2	14	>100	383	0.74	MP, single dose	3.6	15.2	4.2
MPO_3	1	12	>100	665	0.37	CYC, PRED	0.9	9.1	10.6
MPO_4	0	14	37	142	1.63	CYC, PRED	3.3	19.6	5.9
MPO_5	4	14	>100	279	1.44	MP, single dose	2.7	11.0	4.1
PR3_1	15	13	44	150	0.61	CYC, PRED	7.9	21.6	2.7
PR3_2	17	13	176	67	1.49	NONE	3.7	21.0	5.7
PR3_3	6	13	23	74	0.86	MTX, PRED	4.1	11.8	2.9
PR3_4	0	17	96	151	2.62	NONE	9.0	16.0	1.8
PR3_5	17	12	>177	153	0.78	CYC, PRED	9.0	24.3	2.7
PR3_6	0	12	92	531	1.23	MP, single dose	7.6	15.1	2.0
PR3_7	14	14	35	494	0.37	CYC, PRED	6.8	18.1	2.7

All acute patients were tested within 17 days of initial presentation (median 3.5 days). At the time of sampling, median BVAS was 13 and patients were strongly positive for ANCA. There was no significant difference in creatinine or lymphocyte count between MPO-ANCA and PR3-ANCA, assessed by Mann Whitney U (P=0.2020 and P=0.9747, respectively). Breg frequency was significantly lower in PR3-ANCA acute patients, compared to MPO-ANCA (Figure 1, P=0.0101); although treatment varied, there was no trend apparent relative to B cell subset frequency or lymphocyte count (abbreviations used: CYC, cyclophosphamide; PRED, oral prednisolone; MP, intravenous methylprednisolone; MTX, methotextrate)

Supplementary Table S2. Patients tested in IL-10 and co-culture assays

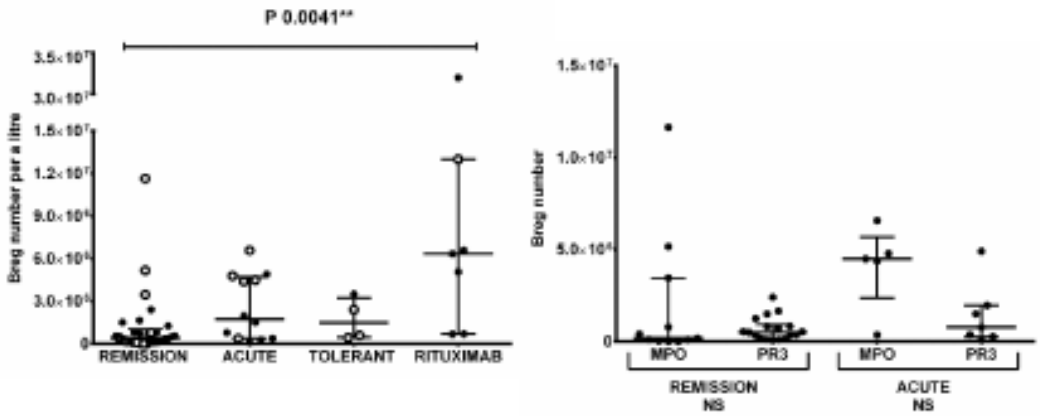
Assay	Sex	Age	ANCA	Creatinine mg/dL	Lymphocyte x10^9/L	Duration (years)	Treatment PRED	Treatment Other
IL10	F	69	MPO	54	3.38	10	-	MMF
IL10	F	64	MPO	183	0.76	4	-	MMF
IL10	F	64	MPO	93	1.03	0.9	2mg	AZA
IL10	F	72	PR3	112	2.29	>13	-	AZA
IL10	M	60	PR3	128	1.48	0.9	20mg	AZA
IL10	F	82	PR3	227	0.72	0.6	7.5mg	MMF
IL10	F	27	PR3	61	2.18	0.3	5mg	MTX
IL10	M	81	PR3	92	2.21	9	5mg	AZA
IL10	M	67	PR3	243	1.64	0.3	5mg	-
IL10	M	72	PR3	130	1.14	2	10mg	-
IL10	F	83	PR3	76	1.9	8	2mg	MMF
IL10	M	65	PR3	95	0.84	2	10mg	AZA
IL10	F	52	PR3	70	1.19	7	-	MTX RTX
IL10	M	47	PR3	99	1.36	>13	2.5mg	RTX
IL10	F	48	PR3	85	0.71	0.3	5mg	RTX
IL10	M	65	PR3	161	0.4	6	75mg	RTX MMF
Co-Culture	F	46	MPO	98	0.91	4	-	AZA
Co-Culture	F	54	PR3	61	1.76	20	2.5mg	AZA
Co-Culture	F	16	MPO	48	2.68	7	-	-
Co-Culture	F	62	MPO	101	0.84	0.5	10mg	AZA
Co-Culture	M	71	PR3	102	1.34	1	7.5mg	-

The patients tested in the functional assays were all in clinical remission; these were consecutive samples from the main cohort. Statistical analysis was conducted to show that they were representative of the main remission cohort (Table 1), with no bias in selection. Mann Whitney U was conducted for continuous variables: age (IQR 50-72, P=0.6796); disease duration (IQR 0.75-8.50, P=0.5212); lymphopcyte count (IQR 0.84-2.11, P=0.8838) and prednisolone dose (IQR 0.00-8.75, P=0.2866). Fisher's exact test was performed for discrete variables: sex (13:8, P=0.5628); ANCA specificity (6:15, P=0.5532) and treatment (oral prednisolone (PRED) P=0.7635; methotextrate (MTX) P=1.0000; azathioprine (AZA) P=0.3930; mycophenolate (MMF) P=0.7442).

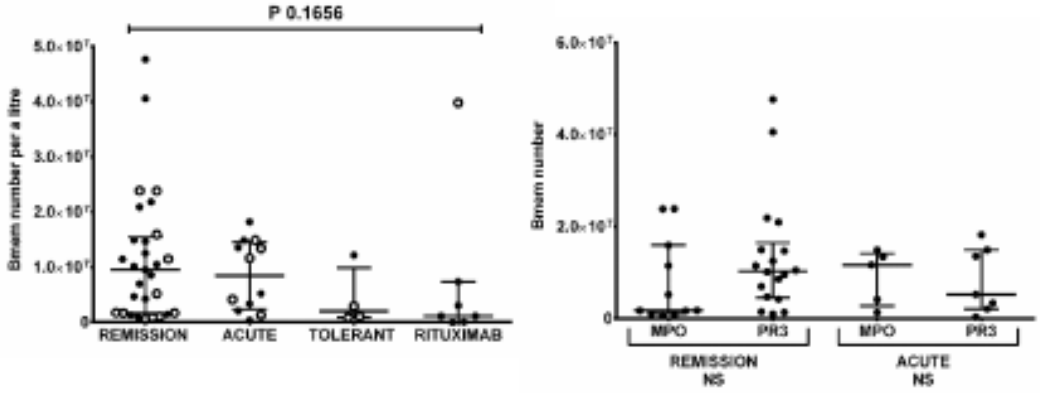
Supplementary Figure S1. Relative B cell number per litre

Patients segregated according to disease activity on the left; white data points depict MPO-ANCA and black, PR3-ANCA (1-way ANOVA and Dunn's multiple comparison conducted). Panels on the right show remission and acute patients, separated by ANCA (Mann Whitney U conducted). [A] Breg lower in remission, compared to rituximab treated patients (P=0.0020). Breg also reduced in remission, relative to acute disease (P=0.0360, Mann Whitney U, *P<0.05). [B] No significant difference in Bmem, although median values were lower in acute and tolerant patients. [C] CD19 number did not differ according to disease activity, despite significant differences in treatment and disease duration.

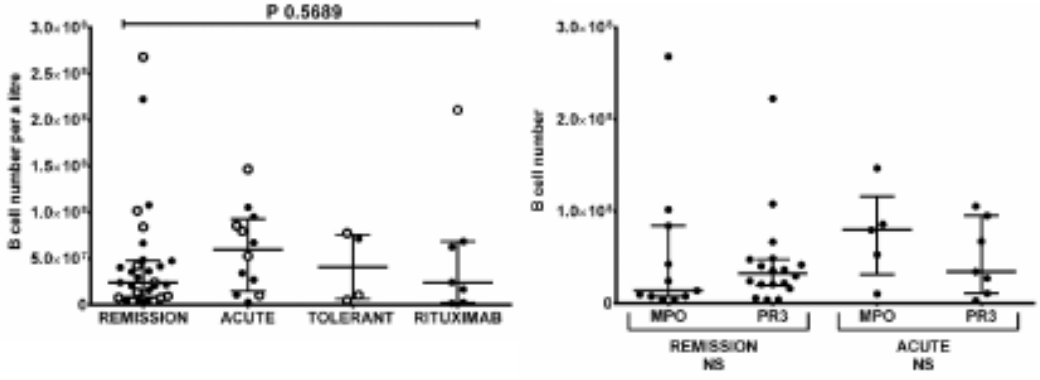
A Breg (CD24^{hi} CD38^{hi}) relative number per a litre of blood



B Bmem (CD24^{hi} CD38^{lo}) relative number per a litre of blood

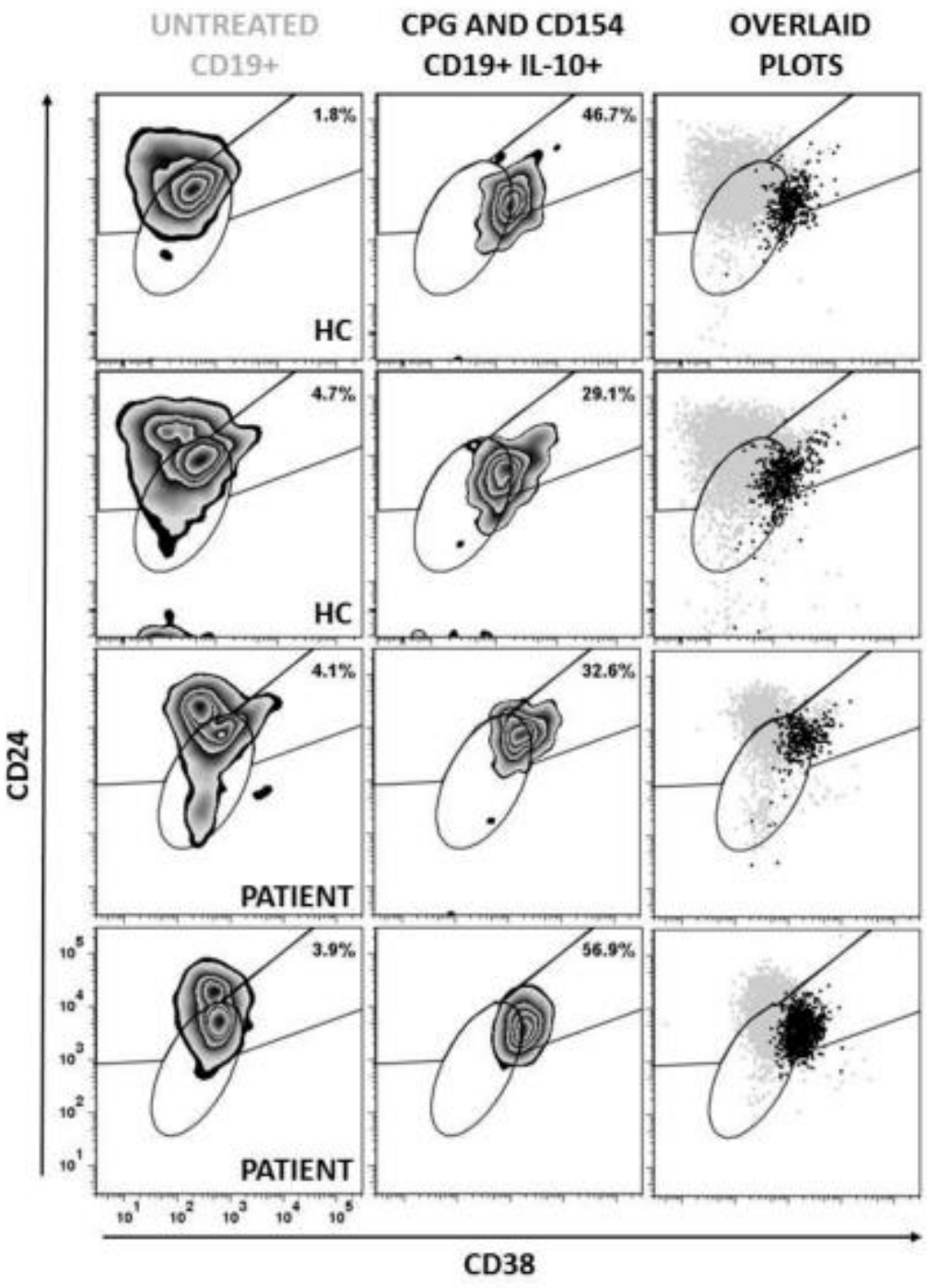


C B cell (CD19+) relative number per a litre of blood



Supplementary Figure S2. IL-10 B cells induced by CPG and CD154 treatment are predominately CD24^{hi} CD38^{hi}

Representative plots for two patients and two controls shown; CD19, CD24, CD38 and IL-10 staining conducted on PBMC after 48 hours culture. The plots on the left show untreated CD19+ cells (Brefeldin A alone); plots in the middle show IL-10 positive CD19+ cells, induced by CPG and CD154 treatment. Overlaid plots on the right side show untreated CD19+ cells in grey and IL-10 positive CD19+ cells in black. When the same gates were applied, IL-10 positive cells were enriched within Breg region (CD24^{hi} CD38^{hi}), percentage frequencies shown.


UNTREATED
CD19+
CPG AND CD154
CD19+ IL-10+
OVERLAID
PLOTS
1.8%
46.7%
HC
4.7%
29.1%
HC
4.1%
32.6%
PATIENT
3.9%
56.9%
PATIENT
CD24
CD38
10^1
10^2
10^3
10^4
10^5