Supplementary Material

Supplementary Figure 1. Summary of the B cell lineage differentiation and associated cell-surface phenotypes. Bone marrow emigrant naïve antigen-inexperienced B cells encounter antigen and T cells in a germinal centre. Germinal centres are most commonly located in lymph nodes and spleen. The T cells express CD40L and secrete IL-2, IL-21 and TNFα, amongst other factors which help naïve B cells differentiate into CD27+ unswitched (IgD+) and switched (IgG+) memory B cells. These then differentiate into antibody-secreting cells (below the dashed line: plasmablasts, short- and long-lived plasma cells) whose survival is supported by IL-6, BAFF and APRIL. Short-lived plasma cells may reside in tissues including bone marrow. Long-lived plasma cells typically niche in the bone marrow, but can reside in the CNS in states of inflammation. Antibodies in blue=IgG, red=IgD; yellow=IgM.

Supplementary Figure 2. Effects of immunotherapies and antibody-secreting cell phenotype. A. Resting B cell subsets from patients with and without mycophenolate mofetil (MMF) administration. B. No effects of freeze-thaw on IgG production from PBMC cultures under varying conditions for cells with membrane-bound CD40L co-cultures. Very similar results obtained in conditions with soluble CD40L and without CD40L (not shown). CD138 (C, blue) and CD20 (D, black) cells are shown within the CD19+CD27++CD38++ antibody-secreting cells generated in vitro. A mean of 60% of the CD19+CD27++CD38++ antibody secreting cells expressed CD20 (mean 60%, range 18–93) and a mean of 15% expressed CD138 (range 1-41). E. Total IgG production across all tested conditions in patients stratified by MMF, corticosteroids and their doses (F), and the percentage of B cell subsets in blood (G).

Supplementary Figure 3. Relationships between in vitro generation of total IgG (ng/ml) and AQP4-specific IgG (ΔMFI) across 21 culture conditions. Absolute values in cells are accompanied by corresponding heat maps. Black = no CD40L; blue = soluble CD40L (sCD40L); red = membrane-bound CD40L (mCD40L).

Supplementary Figure 4. In vitro culture observations. From wells with R848 and IL-2, supernatant IgG levels did not vary with addition of CD40L (A). From wells with IL-21, without IL-2, addition of CD40L (membrane or soluble) generated more IgG in vitro (p=0.0002, Mann Whitney U test; B). Total IgG per well correlated strongly with the
percentage of ASCs per well (black, Spearman’s r = 0.71, p<0.0001), and more modestly with tetanus-IgG (IU/ml; blue, Spearman’s r = 0.46, p=0.0084), but not with AQP4-IgG generated per well (C; red, ∆MFI). AQP4-IgG levels in culture supernatants did not vary with days from illness onset, days since last clinical relapse and duration of immunotherapy (time parameters, left y-axis) or with corticosteroid dose, dose of mycophenolate mofetil (MMF) or number of immunotherapies (D; immunotherapy parameters, right y-axis).
Supplementary methods

Peripheral Blood Mononuclear Cells were isolated from whole blood using a Ficoll gradient (Ficoll-Paque, GE Healthcare). Phases were separated by centrifugation at 400 g for 30 min at room temperature and slow deceleration. A 3mL sterile pastette was used to isolate the buffy coat layer in a 50mL conical tube. The cells were washed with PBS/1%BSA twice (200g, 10 minutes, RT, medium acceleration and deceleration). The cells were counted using 0.04% Trypan Blue exclusion and frozen at 10–20 × 10^6 per mL per cryovial in B cell medium (RPMI 1640 without phenol red, 5% FBS, 1% Penicillin/Streptomycin, 1% Glutamax, 0.1% beta-mercaptoethanol, 0.1% IgG-depleted transferrin (20 µg/ml)) with 40% Fetal Bovine Serum (FBS) and 10% DMSO chilled on ice. Cryovials containing cells were placed in a CoolCell® Cell Freezing Container (BioCision) and maintained at −80 °C for 24-72 hours before being transferred to liquid nitrogen tanks.

To thaw cells, the cryovials were removed from liquid nitrogen tanks into dry ice containers. Vials were thawed immediately with shaking in a lukewarm water bath. No more than two cryovials were thawed at the same time. The cell suspension was transferred dropwise to a 50mL conical tube containing 9 ml cold B cell medium per 1 ml of thawed cell suspension. The cells were washed twice with B cell medium (200g, 10 minutes) and counted. Viability was assessed using 0.04% Trypan Blue exclusion and ranged from 70–85% of the fresh cells. Cells were re-suspended in B cell medium at appropriate cell concentrations.
Supplementary Table 1. B cell subsets from 12 NMOSD patients and age- (± 5 years) and sex-matched healthy control (HC) subjects. HCs listed in order of matching to the patients 1-12. Percentages represent gating strategies from Figure 1.

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<th>Naïve</th>
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**Bone marrow**

- **Immature B**
  - CD19^+/
  - CD20^-
  - CD27^-
  - IgM^+

**Periphery**

- **Naïve B**
  - CD19^+
  - CD20^+
  - CD27^-
  - IgM^+

- **Activated B cell**
  - CD40
  - CD40L, IL-2, IL-21, TNFα

**Germinal centre**

- **Unswitched memory B**
  - CD19^+
  - CD20^+
  - CD27^-
  - IgD^+

- **Switched memory B**
  - CD19^+
  - CD20^+
  - CD27^+
  - IgD-IgG^+ Ag encounter

- **Activated B cell**
  - CD40
  - CD40L, IL-2, IL-21, TNFα

**Plasma cell niche**

- **Long-lived plasma cell**
  - CD19^+-
  - CD20^-
  - CD27^{++}/CD38^{++}
  - CD138^+

- **Short-lived plasma cell**
  - CD19^+
  - CD20^-
  - CD27^{++}/CD38^{++}
  - CD138^{++/-}

- **Plasmablast**
  - CD19^+
  - CD20^{low/-}
  - CD27^{++}/CD38^{++}
  - CD138^-

**Supplementary Figure 1**
A

All R848 plus IL-2 wells

No CD40L + CD40L

p=0.64

B

All IL-21 without IL-2 wells

No CD40L + CD40L

p=0.0002

C

ASCs per well (CD19-CD27++CD38++/CD3-CD14-DAPI-) or Tetanus-IgG (IU/ml)

Total IgG per well (ng/ml)

AQP4-IgG (r=-0.23, p=0.22)

% ASCs (r=0.71, p<0.0001)

Tetanus-IgG (r=0.46, p=0.0084)

D

Days from illness onset (r = -0.09, p = 0.78)

Days since last clinical relapse (r = -0.03, p = 0.93)

Days of immunotherapy (r = -0.11, p = 0.72)

Corticosteroid dose (divided by 10; mg daily; r = -0.50, p = 0.17)

Number of immunotherapies (r = 0.07, p = 0.85)

Dose of MMF (g daily; r = 0.01, p = 0.99)

Supplementary Figure 4