Xiao et al., http://www.jem.org/cgi/content/full/jem.20062498/DC1

SUPPLEMENTAL MATERIALS AND METHODS

Proliferation assays and cytokine intracellular staining

Female SJL mice were immunized with $PLP_{139-151}$/CFA. On day 10, spleen cells were isolated and plated in round-bottomed 96-well plates in culture medium with various concentrations of $PLP_{139-151}$ plus 10 μg/ml of control Ab, Fab' RMT1-10, or Fab' 3B3. Forty-eight hours later, plates were pulsed for 16 h with 1 μCi [^{3}H]-thymidine per well. Proliferation was measured as counts per minute (cpm) by using a Wallac liquid scintillation counter. Spleen cells were also cultured in 24-well plates with $PLP_{139-151}$ plus control mAb, 3B3, RMT1-10, or their Fab' fragments for 6 d. Then, cells were purified by Ficoll-Hypaque density gradient centrifugation and reactivated with PMA (20ng/ml) and ionomycin (300ng/ml) and 2mM monensin (GolgiStop, BD Biosciences) for 4 h at 37°C. After staining with APC-anti-CD4, and 7-AAD, the cells were fixed, permeabilized, and stained with PE-cytokine Ab as recommended by the manufacturer (BD Biosciences). The frequencies of cytokine-producing cells were then analyzed with the programs CELLQUEST and FlowJo by gating on $CD4^+7\text{-}AAD^-$ populations.

www.jem.org/cgi/doi/10.1084/jem.20062498