Anneli Jaeger, Kuchroo laboratory

Differentiation of T effector cells

→ Take spleen and lymph nodes of 6-10 week old 2D2 mice, prepare a single cell suspension by mashing spleens and lymph nodes and passing through a 70 μm mesh. After erythrocyte lysis with ACK-buffer (BioWhittaker) purify CD4+ T cells using magnetic beads coated with anti-CD4 antibody (L3T4) according to the manufacturer's instructions (Miltenyi Biotech).

→ Stain CD4+ cells with anti-CD4 and anti-CD62L antibodies and sort into naïve CD4+CD62L^{hi} T cells with a BD FACS Aria.

→ Culture naïve cells at a concentration of $2x10^6$ ml^{-1} in RPMI medium with 10% FCS in the presence of 10^7 ml^{-1} irradiated splenocytes and 2.5 μg/ml soluble anti-CD3 antibody.

→ Th1 cells: IL-12 at 10 ng/ml and anti-IL-4 antibody (11B11) at 20 μg/ml
→ Th17 cells: IL-6 at 30 ng/ml, TGF-β at 3 ng/ml, anti-IFN-γ (XMG1.2) and anti IL-4 antibody at 20 μg/ml

→ After 48h split Th1 cells with RPMI medium containing 20 U/ml of IL-2. Split Th17 cells using RPMI medium containing 10 ng/ml of IL-23. (All cytokines from R&D) Keep splitting/feeding cells with these media as necessary.

→ Monitor differentiation process daily under the microscope. After initial phase of proliferation and activation/blasting cells reach the resting stage after 5-7 days of culture (i.e. they become small and round again, do not form new clusters and do not use up a lot of medium any more – no color change).

→ When rested but before crashing re-stimulate polarized T cells at $2x10^6$ ml^{-1} for 48h in the presence of plate-bound anti-CD3 and anti-CD28 antibodies both at 2 μg/ml in fresh medium without any cytokines.

Induction of EAE by transfer of T effector cells

Collect re-stimulated cells and wash 3x with 40 ml PBS. Inject $3\text{-}5x10^6$ cytokine producing cells resuspended in PBS into the tail vein of C57Bl/6 recipients. (Calculate with the percentage of cytokine production observed 4 days after initial stimulation by intracellular cytokine staining – usually between 30 and 60%)