

## Generation of A $\beta$ -specific T cell lines and *in vivo* Transfer

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**[Abstract]** Amyloid- $\beta$  (A $\beta$ )-containing plaques accumulate in the brains of patients with Alzheimer's disease (AD). Studies in transgenic mice which over-express amyloid precursor protein and presenilin 1 (APP/PS1 mice) have suggested that T cells that infiltrate the brain may influence the development of A $\beta$  plaques and associated cognitive dysfunction. Active immunization with A $\beta$  peptides and adjuvants has been evaluated as a therapy for AD, based on the premise that it induces A $\beta$ -specific antibodies that may help to clear the A $\beta$  plaques. However, immunization with A $\beta$  peptides and adjuvants also promotes the development of A $\beta$ -specific T cells (McQuillan *et al.*, 2010) and there is evidence that A $\beta$ -specific T cell may influence the development of A $\beta$  plaques and disease progression in AD patients. In the mouse model, A $\beta$ -specific T cells that secrete IFN- $\gamma$  (Th1 cells) have been shown to enhance the plaque burden (Browne *et al.*, 2013). Adoptive transfer of A $\beta$ -specific T cells that have been polarized *in vitro* to Th1, Th2, Th17 or Treg cells can be used to examine the function of these cells *in vivo*.

### Materials and Reagents

1. C57BL/6 mice (adult, >6 weeks old; typically 4 per experiment)
2. A $\beta$ <sub>1-42</sub> peptide (Life Technologies, Invitrogen™, catalog number: 03112)
3. CpG (CpG-oligodeoxynucleotide 1668; 5'-tccatgacgttccgatgct-3'; Sigma-Genosys)
4. PBS (Sigma-Aldrich, catalog number: D8537)
5. IL-1 (Immunotools, catalog number: 12340013)
6. IL-2 (Immunotools, catalog number: 12340024)
7. IL-4 (Immunotools, catalog number: 12340043)
8. IL-12 (Miltenyi, catalog number: 130-096-707)
9. IL-23 (Miltenyi, catalog number: 130-096-676)
10. Anti-IFN $\gamma$  (BD, catalog number: 554430)
11. Dexamethasone (Sigma-Aldrich, catalog number: D4902)
12. High-performance liquid chromatography (HPLC)-grade water (sterile ddH<sub>2</sub>O)
13. RPMI medium (Sigma-Aldrich, catalog number: R0883)
14. Penicillin-streptomycin (Sigma-Aldrich, catalog number: P4333)
15. L-glutamine (Sigma-Aldrich, catalog number: G7513)

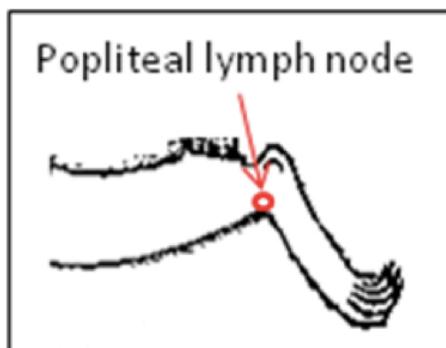
16. FBS (Sigma-Aldrich, catalog number: F9665)
17. Complete RPMI medium (see Recipes)

### **Equipment**

1. Shaker capable of 200 rpm at 37 °C
2. 24-well cell culture plates (Greiner Bio-one, catalog number: 662160)
3. 1 ml tuberculin syringes (BD, catalog number: 300013)
4. 70 µm nylon mesh filter (Corning, catalog number: 352350)
5. Tissue culture facilities including class II laminar flow hood
6. Centrifuge

### **Procedure**

1. Dissolve A $\beta$ <sub>1-42</sub> peptide in HPLC-grade water to provide a 12 mg/ml stock solution, which is diluted to 2 mg/ml using sterile PBS and allowed to aggregate for 48 h at 37 °C and with agitation of 200 rpm. A $\beta$ <sub>1-42</sub> is used immediately or stored at -20 °C.
2. Immunize C57BL/6 mice by subcutaneous injection into the rear footpad with A $\beta$ <sub>1-42</sub> (75 µg/mouse) and CpG (25 µg/mouse) in a total volume of 50 µl *i.e.* 25 µl per foot.
3. Administer a sec (booster) immunization with the same doses of antigen and adjuvant after 21 days.
4. After a further 7 days, sacrifice the mice and remove the popliteal lymph nodes (the draining lymph node; see Figure 1) and spleens.



**Figure 1. A schematic of popliteal lymph node**

5. Dissociate lymph node and spleen tissue through a sterile 70 µm nylon mesh filter, wash with complete RPMI and centrifuged at 280 x g for 5 min and perform a cell count.

6. Stimulate the cells at  $2 \times 10^6$  cells per ml with  $A\beta_{1-42}$  (25  $\mu\text{g/ml}$ ) in the presence of cytokines and antibodies depending on the type of cell line required. To generate Th1 cells, the cells from the lymph nodes and spleens are stimulated with  $A\beta_{1-42}$  (25  $\mu\text{g/ml}$ ) and IL-12 (10 ng/ml). Th2 cells are amplified using dexamethasone ( $1 \times 10^{-8}$  M), IL-4 (10 ng/ml), and anti-IFN $\gamma$  (5 mg/ml), and Th17 cells are generated with IL-1 (10 ng/ml), IL-23 (10 ng/ml) and anti-IFN $\gamma$  (5 mg/ml).
7. After 4 days, add IL-2 (5 ng/ml) to the Th1 and Th2 cell cultures, or medium only to the Th17 cells and incubation continued for a further 7 days.
8. Wash cells with complete RPMI, centrifuge at  $280 \times g$  for 5 min and count.
9. Confirm that cells are polarized to Th1, Th2 or Th17 either by performing intracellular cytokine staining for IFN- $\gamma$ , IL-5 or IL-17 and FACS analysis or by assessing the quantity of these cytokines in supernatants by ELISA.
10. Inject cells i.p. into recipient mice (typically  $1.5 \times 10^7$  cells/mouse) in 100  $\mu\text{l}$  serum-free medium or PBS.

### **Recipes**

1. Complete RPMI medium  
RPMI medium supplemented with 1% penicillin-streptomycin, 1% L-glutamine and 10% FBS

### **Acknowledgments**

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### **References**

1. Browne, T. C., McQuillan, K., McManus, R. M., O'Reilly, J. A., Mills, K. H. and Lynch, M. A. (2013). [IFN-gamma Production by amyloid beta-specific Th1 cells promotes microglial activation and increases plaque burden in a mouse model of Alzheimer's disease.](#) *J Immunol* 190(5): 2241-2251.
2. McQuillan, K., Lynch, M. A. and Mills, K. H. (2010). [Activation of mixed glia by Abeta-specific Th1 and Th17 cells and its regulation by Th2 cells.](#) *Brain Behav Immun* 24(4): 598-607.