Generation of Aβ-specific T cell lines and *in vivo* Transfer

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[Abstract] Amyloid-β (Aβ)-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 Aβ plaques and associated cognitive dysfuncation. Active immunization with Aβ peptides and adjuvants has been evaluated as a therapy for AD, based on the premise that it induces Aβ-specific antibodies that may help to clear the Aβ plaques. However, immunization with Aβ peptides and adjuvants also promotes the development of Aβ-specific T cells (McQuillan *et al*., 2010) and there is evidence that Aβ-specific T cell may influence the development of Aβ plaques and disease progression in AD patients. In the mouse model, Aβ-specific T cells that secrete IFN-γ (Th1 cells) have been shown to enhance the plaque burden (Browne *et al*., 2013). Adoptive transfer of Aβ-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. $A\beta_{1-42}$ 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–IFNγ (BD, catalog number: 554430)
11. Dexamethasone (Sigma-Aldrich, catalog number: D4902)
12. High-performance liquid chromatography (HPLC)-grade water (sterile ddH_2O)
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 $A\beta_{1-42}$ 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. $A\beta_{1-42}$ is used immediately or stored at -20 °C.
2. Immunize C57BL/6 mice by subcutaneous injection into the rear footpad with $A\beta_{1-42}$ (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.

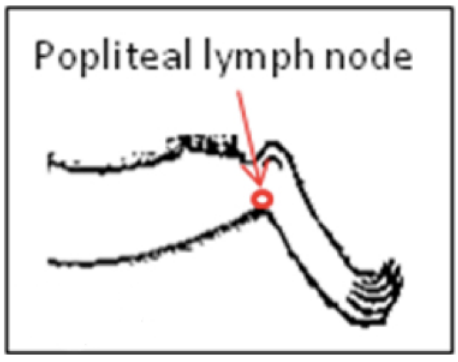

Figure 1. A schematic of popliteal lymph node

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 x 10^6 cells per ml with $A\beta_{1-42}$ (25 µg/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 µg/ml) and IL-12 (10 ng/ml). Th2 cells are amplified using dexamethasone (1 x 10^{-8} M), IL-4 (10 ng/ml), and anti-IFNγ (5 mg/ml), and Th17 cells are generated with IL-1 (10 ng/ml), IL-23 (10 ng/ml) and anti-IFNγ (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 *x 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-γ, IL-5 or IL-17 and FACS analysis or by assessing the quantity of these cyokines in supernatants by ELISA.
10. Inject cells i.p. into recipient mice (typically 1.5 x 10^7 cells/mouse) in 100 µl 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

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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.

