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 dysfunction. 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. Aβ1-42 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 ddH2O)
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 Aβ_{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. Aβ_{1-42} is used immediately or stored at -20 °C.
2. Immunize C57BL/6 mice by subcutaneous injection into the rear footpad with Aβ_{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](http://www.bio-protocol.org/)

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 10⁶ cells per ml with Aβ₁₋₄₂ (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 Aβ₁₋₄₂ (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 cytokines in supernatants by ELISA.

10. Inject cells i.p. into recipient mice (typically 1.5 x 10⁷ 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

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References
