Ex vivo Co-culture of Lymphoid Tissue Stromal Cells and T Cells

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[Abstract] Stromal cells within lymphoid tissues produce IL-7, which is critical for the survival and function of T cells. This protocol is to be used to isolate primary human lymphoid tissue stromal cells to study their impact on the survival of T cells in an *ex vivo* co-culture system.

Materials and Reagents

1. RPMI-1640 medium (Life Technologies, catalog number: 11875-093)
2. Fetal bovine serum (FBS) (GEMBIO, catalog number: 900-108)
3. Antibiotic-Antimycotic (Life Technologies, catalog number: 15240-062)
4. Anti-CD45RA (Dako, catalog number: M0754)
5. Anti-activated caspase-3 (Cell Signaling Technology, catalog number: 9665)
6. Anti-CD3 (AbD Serotec, catalog number: MCA1477)
7. Anti-IL-7 (R&D Systems, catalog number: MAB207)
8. Streck's tissue fixative (Streck Laboratories, catalog number: 265138)
9. TritonX-100 (Sigma-Aldrich, catalog number: X-100)
10. CellMicroSieves (BioDesign Inc. of New York, catalog number:N100S)
11. Alexa Fluor 488 donkey anti-rabbit IgG (Life Technologies, Invitrogen™, catalog number: A-21206)
12. Alexa Fluor 568 Donkey Anti-Mouse IgG (Life Technologies, Invitrogen™, catalog number: A10037)
13. Alexa Fluor 647 Chicken Anti-Rat IgG (Life Technologies, Invitrogen™, catalog number: A-21472)
14. Dimethylsulfoxide (DMSO) (Sigma-Aldrich, catalog number: D8418)
15. Phosphate-Buffered Saline (PBS) (Life Technologies, Invitrogen™, catalog number: 10010-023)
16. Complete RPMI-1640 culture medium (see Recipes)

    Note: The experimental protocols used here for human tissue samples had full IRB approval (Institutional Review Board: Human Subjects Committee, Research Subjects' Protection Program, University of Minnesota) and informed written consent was obtained

from individual patients, or the legal guardians of minors, for the use of tissue in research applications prior to the initiation of surgery.

Equipment

1. Biological Safety Cabinet
2. Chamber slides (Thermo Fisher Scientific, catalog number: 154526)
3. Centrifuges
4. Water bath

Procedure

1. Fresh human palatine tonsil tissues were obtained from routine tonsillectomies and processed within 1–2 h of completion of surgery. Viable tonsil lymphocyte suspensions were prepared by forcing cut tissue pieces through a metal sieve (*e.g.* CellMicroSieves), and collecting the released single cell suspension in complete RPMI-1640 culture medium.
2. The lymphocytes were washed twice (centrifuge and resuspend the cells in PBS) and immediately cryopreserved by resuspending cells in freezing medium (FBS with 10% DMSO). The cells were immediately transferred to -20 °C for one hour, followed by -80 °C overnight before permanent storage in liquid nitrogen.
3. By culturing the stroma left on the metal sieve (*e.g.* CellMicroSieves), in complete RPMI-1640 culture medium at 37 °C with 5% CO_2, adherent proliferating fibroblast-like stromal cells were first visible after 2-5 days in the culture, and confluent monolayers developed after 10-25 days.
4. For co-culture of lymphocytes and stromal cells, 2 x 10^5 lymphocytes isolated from human tonsil were cultured in chamber slides without stromal cells, with autologous stromal cells (2 x 10^4 cells/well), or with autologous stromal cells (2 x 10^4 cells/well) and IL-7 blocking antibody (50 µg/ml) for 2 to 3 days.
5. After co-culture, the slides were fixed in 0.5 ml Streck's tissue fixative for 20 min, permeabilized with 1% TritonX-100 for for 5 min.
6. The slides were washed with 500 µl/well PBS + 3% FBS for 3 times, blocked with 500 µl/well DPBS + 3% FBS + 0.5% Tween-20 for 2 h at room temperature.
7. After removing the blocking reagent, add 250 µl/well primary antibodies [activated caspase3 (1:100 dilution), CD45RA (1:100 dilution) and CD3 (1:100 dilution)] and incubate at room temperature for 1 h.

8. After removing the primary antibodies, the slides were washed with 500 µl/well PBS + 3% FBS for 3 times.
9. Add 250 µl/well fluorescent secondary antibodies and incubate at room temperature for 1 h.
10. Remove the secondary antibodies and wash the slides with washed with 500 µl/well PBS + 3% FBS for 3 times.
11. Use fluorescence microscope or confocal microscope to quantify the number of apoptotic naive T cells.

Recipes

1. Complete RPMI-1640 culture medium
   Supplemented with 10% heat inactivated fetal calf serum FBS and 1x Antibiotic-Antimycotic

Acknowledgments

This protocol is adapted from Link *et al.* (2007) and Zeng *et al.* (2012).

References

1. Link, A., Vogt, T. K., Favre, S., Britschgi, M. R., Acha-Orbea, H., Hinz, B., Cyster, J. G. and Luther, S. A. (2007). Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol* 8(11): 1255-1265.
2. Zeng, M., Southern, P. J., Reilly, C. S., Beilman, G. J., Chipman, J. G., Schacker, T. W. and Haase, A. T. (2012). Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. *PLoS Pathog* 8(1): e1002437.