Video Article

Preparing T Cell Growth Factor from Rat Splenocytes

Christine Beeton[1], K. George Chandy[1]

[1]Department of Physiology and Biophysics, University of California, Irvine (UCI)

Correspondence to: K. George Chandy at gchandy@uci.edu

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Abstract

Maintenance of antigen-specific T cell lines or clones in culture requires rounds of antigen-induced activation separated by phases of cell expansion [1,2]. Addition of interleukin 2 to the culture media during the expansion phase is necessary to prevent cell death and sufficient to maintain short-term T cell lines but has been shown to increase Th1 polarization [3]. Replacement of interleukin 2 by T cell growth factor (TCGF) which contains a mix of cytokines is more effective than interleukin 2 in maintaining long-term T cell lines in vitro [3]. Moreover, TCGF can easily be prepared in large amounts in the laboratory and is much cheaper than recombinant interleukin 2.

Here, we show how to prepare TCGF from rat splenocyte culture supernatants. For this procedure, we harvest spleens from naive Lewis rats euthanized for thymus and blood collection. We prepare single-cell suspensions from the spleens, lyze the red blood cells by osmotic shock, and seed the splenocytes in culture medium. The cells are stimulated with concanavalin A, a mitogen that non-selectively activates all rat T lymphocytes, inducing the production of cytokines. The culture supernantant is collected 48 hours later andexcess concanavalin A is bound to alpha methyl mannoside to prevent it from activating T cell lines to which TCGF will be added. The TCGF is then sterile-filtered, aliquoted, and stored at -20°C.

Video Link

The video component of this article can be found at http://www.jove.com/video/402/

Protocol

1. Take Lewis rat spleens (rats between 160-200g are best). Dilacerate on ice in a petri dish containing PBS + antibiotics (PBS-PS) using a cell strainer. Put in a 50 ml tube. Fill with PBS-PS.
2. Spin for 10 min at 4°C to pellet the cells.
3. Wash the cells twice.
4. Resuspend the pellet in NH_4Cl 0.15 M (5 ml per spleen). Mix gently and continuously with a pipet for **3 min** on ice to lyse the erythrocytes. Fill the tube with medium.
5. Spin for 10 min at 4°C to pellet the cells.
6. Wash the cells twice.
7. Count the cells. A rat spleen gives 200-250 million cells.
8. Seed the cells at 2 million per ml in complete medium: 50 ml per 75 cm^2 flask.
9. Let grow for 48 hours in the incubator.
10. Spin 15 min at 4°C to pellet the cells.
11. Collect the supernatant; discard the cells.
12. Add 15 mg/ml a methyl mannoside to the supernatant. Mix thoroughly.
13. Filter (0.2 mm)
14. Aliquot and store at -20°C. Can be kept at 4°C for 10 days if necessary.

Discussion

We prepare TCGF from Lewis rat splenocytes since we regularly euthanize naive rats from this strain to harvest serum and thymi to stimulate Lewis rat T cell lines in vitro. This TCGF can be used to promote the growth and survival of T cell lines from other rat strains. TCGF can also be prepared from other strains of rats.

Disclosures

The authors have nothing to disclose.

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