PROPAGATION OF HUMAN T-CELL CLONES

Dr. Robert F. Siliciano, Johns Hopkins University School of Medicine

Continuous growth of human T-cell clones normally requires periodic restimulation of clones with antigen and irradiated, histocompatible antigen presenting cells. Under some conditions, clones can also be grown using non-specific mitogens such as PHA and allogeneic feeder cells. In any event, it is necessary to restiumlate the cells approximately every week to maintain growth and viability. The following protocol works well for most CD4+ human T-cell clones.

Culture Medium: RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 4 mM L-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 50 U/ml recombinant human IL-2

Wash Medium: PBS (1X) containing 2% fetal bovine serum, 0.5% glucose, 12 mM HEPES, pH 7.2

Preparation of Feeder Cells:

Obtain irradiated (5000R) peripheral blood mononuclear cells (PBMC) from any donor. At least twice and preferably four times as many PBMC as cloned T cells are required. Wash twice with wash medium after irradiation.

Re-stimulation Procedure:

Culture T-cell clones at $2.5x10^5$ cells/ml with feeder cells at $0.5\text{-}1.0x10^6$ cells/ml in culture medium containing 0.25 µg/ml PHA (Burroughs Wellcome Catalog #HA16) in a 24-well plate, 2.0 ml/well.

Feeding:

After 2 days, aspirate most of the medium from the wells and replace it with fresh culture medium without PHA. For all subsequent feedings, use medium without PHA. Five days after re-stimulation, harvest the cells, centrifuge, and count. Resuspend the cells in fresh culture medium at $2.5\text{-}5.0x10^5$ cells/ml and culture in 24-well plates. Feed in this manner every 2-3 days.

Notes:

1. An aliquot of cells should be stimulated every week.

2. Cells generally live for 2-3 weeks after re-stimulation and should be frozen before they begin to die.

3. We use highly purified PHA. If less pure PHA is used, higher concentrations may be required.

4. Cells generally grow best in 24-well plates rather than flasks.