TRANSDUCTION OF ANTIGEN-SPECIFIC CD8$^+$ T CELLS WITH RETROVIRAL VECTORS

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An easy and flexible protocol to transduce CD8$^+$ T cells and differentiate them into central or effector memory cells.

MATERIALS

ACK buffer to lyse red blood cells (NH_4Cl 150mM, $KHCO_3$ 10mM Na_2EDTA 0.1mM)
T cell medium: RPMI 10% FCS HEPES (1:66) Sodium Pyruvate (1:100) GlutaMax (1:100) β-mercaptoethanol (1:1000)
Cognate peptide (e.g. OT-I or CL4 peptide)
Polybrene solution 4 mg/ml
If cells are from Balb/c, you need IL-12 (R&D 419-ML)
To generate effector memory cells, you need IL-2 (R&D 1150-ML)
To generate central memory cells, you need IL-15 (R&D 447-ML)

METHOD

Day 1:

1. Culture and stimulate CD8$^+$ T cells:
    a. Sacrifice mice, take spleen and LN (axillary, brachial, inguinal).
    b. Smash organs in sterile conditions. Put them in a petri dish and keep wet with PBS. LNs can be ground using 2 frosted and ethanol-wiped glass slides (be careful not to break them). Filter the suspension (40μm filter) and thoroughly wash the Petri dish. Centrifuge the cells.
    c. Lyse red blood cells by resuspension in 1ml ACK for 5' at room temperature. Block lysis by adding 9ml PBS.
    d. Resuspend cells in 10ml T cell medium + 10 μM of cognate peptide.
    e. **PRO TIP:** If cells come from B6 mice, APC produce enough IL-12. If they come from Balb/c, this is not the case. Supplement IL-12 20ng/ml.
    f. Put in a 10-cm Petri dish and culture overnight.

Day 2:

1. First transduction:
    a. Thaw retroviral supernatant (10ml per sacrificed mouse) and add polybrene 4 μg/ml. If cells are of Balb/c origin, also add 20ng/ml IL12.
    b. Harvest the T cells stimulated yesterday and spin them down at 500g for 5'.
    c. Resuspend the cells in the viral supernatant prepared in step a, and aliquot them 1ml/well of a 24-well plate.
    d. Spinfect 1000g 90' at 32C.
    e. After spinfection, add to each well 1ml of T cell medium (+ 20ng/ml IL-12 if cells are from Balb/c mice).
    f. Incubate overnight.

Day 3:

1. Second transduction:
    a. Repeat Day 2 steps 1a to 1d.

b. After spinfection, accurately harvest cells, which are stuck to the bottom of the wells, and transfer them in a 15ml Falcon tube. Spin 500g 5'.
c. Resuspend cells in T cell medium with 20ng/mlIL-2, to generate effector memory cells.
d. Plate cells in Petri dishes at the concentration of $1x10^6$/ml.
PRO TIP: Substituting IL-2 with 20ng/ml IL-15 will generate central memory cells. In this case maintaining cell concentration to at least $1x10^6$/ml is critical, as IL-15 is trans-presented by APC still present in the culture.

Day 5:

1. Select transduced cells. This can occur in three ways, depending on the selection gene encoded by the retroviral vectors:
    i. Retro encodes puromycin resistance: apply puromycin 8 µg/ml;
    ii. Retro encodes ΔLNGFR: do immunomagnetic selection for $\Delta LNGFR^+$ cells, or
    iii. Retro encodes fluorescent proteins: FACS sort cells.
2. Culture cells in T cell medium with 20ng/ml IL-2 or 20ng/ml IL15.

Day 6 or 7:

1. Use cells in experiments.