

Murine *In vitro* Memory T Cell Differentiation

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[Abstract] Upon pathogen encounter, naïve CD8⁺ T cells are primed and undergo massive clonal expansion. A fraction of effector CD8⁺ T cells remains during the contraction phase and differentiate into memory T cells critical for mounting robust recall responses in response to secondary infection. Low frequency of memory T cells *in vivo* is a major obstacle to investigate their functional aspects including migration capacity and genetic regulation. Here, we describe detailed protocol for memory T cell differentiation developed by von Andrian's group to generate large number of CD44^{hi}CD62L^{hi} antigen-specific memory T cells *in vitro*.

Materials and Reagents

1. Recombinant mouse IL-15 (rmIL15) (BioLegend, catalog number: 566302)
2. RPMI-1640 medium (Life Technologies, Gibco[®], catalog number: 11875-119)
3. Fetal bovine serum (Atlanta Biologicals, catalog number: S11055H)
4. Penicillin/streptomycin (Gemini Bio-Products, catalog number: F52M00E)
5. L-Glutamine (Life Technologies, Gibco[®], catalog number: 25030-081)
6. 100x 1 M HEPES (Life Technologies, Gibco[®], catalog number: 15630-080)
7. 100x MEM Non-essential amino acids (Life Technologies, Gibco[®], catalog number: 11140-050)
8. 100x sodium pyruvate (100 mM) (Life Technologies, Gibco[®], catalog number: 11360-070)
9. 100x 2-mercaptoethanol (Life Technologies, Gibco[®], catalog number: 21985-023)
10. OVA₂₅₇₋₂₆₄ synthetic peptide (Sigma-Aldrich, catalog number: S7951)
11. Ficoll-Paque[™] Premium 1.084 (GE Healthcare, catalog number: 17-5446-02)
12. Antibodies:
 - a. Anti-CD44 PerCpCy5.5 (clone: IM7) (eBioscience, catalog number: 45-0441)
 - b. Anti-CD62L APC (clone: MEL-14) (eBioscience, catalog number: 17-0621)
13. RBC lysis buffer (eBioscience, catalog number: 00-4333-57)

14. Bovine serum albumin (Thermo Fisher Scientific, catalog number: BP1605-100)
15. NaN₃ (Sigma-Aldrich, catalog number: S8032)
16. T cell media (see Recipes)
17. Staining buffer (in PBS) (see Recipes)

Equipment

1. Centrifuge (Thermo Fischer Scientific, Sorvall™ Legend RT)
2. 70 µm cell strainer (BD Biosciences, Falcon®, catalog number: 352350)
3. 15 ml and 50ml Falcon tubes
4. 24 well plates (BD Biosciences, Falcon®, catalog number: 353226)
5. T75 culture flask (Corning, catalog number: 430641)
6. 37 °C 5% CO₂ Cell Culture incubator

Procedure

- A. CD44^{hi}CD62L^{lo} Memory T cell differentiation proceeds under sterile tissue culture conditions
 1. Euthanize a OT-1 CD8 TCR transgenic mouse and take spleen, and (optional) lymph nodes.
 2. Splenocytes are RBC lysed followed by washing with PBS twice.
- B. OT-1 TCR stimulation with cognate peptide antigen
 3. Resuspend cells in 1 ml of T cell media and add OVA₂₅₇₋₂₆₄ synthetic peptide to 1 µM.
 4. Incubate in the 5% CO₂ at 37 °C for 1 h.
 5. Spin down cells at 1,500 rpm for 3 min at 4 °C and wash once with T cell media.
 6. Resuspend cells in 12 ml of T cell media and plate 1ml/well of a 24 well plate.
 7. Incubate in the 5% CO₂ at 37 °C for 2 days.
 8. Harvest the cells by pipetting up and down, and pellet cells.
 9. Resuspend cells in 5 ml of T cell media, and load on to 2.5 ml of Ficoll.
 10. Spin down at 400 x g for 15 min at 4 °C.
 11. Transfer live cells on the interphase to a new 15 ml tube and fill up the tube with T cell media.
 12. Spin down cells at 1,500 rpm for 3 min at 4 °C.
- C. Memory T cell culture in the presence of IL-15
 13. Resuspend cells in 24 ml of T cell media containing rmlL15 (20 ng/ml). Culture cells in T75 flask for four days.

14. Harvest and pellet cells for Ficoll gradient (repeat steps 9-12).
15. Resuspend cells in 40 ml of T cell media containing rmlL15 (20 ng/ml). Culture in T75 flask for two days.
16. Staining cells with anti-CD44 and CD62L antibodies in staining buffer for 15 min on ice.
17. Wash with staining buffer twice, then proceeds flow cytometry analysis.

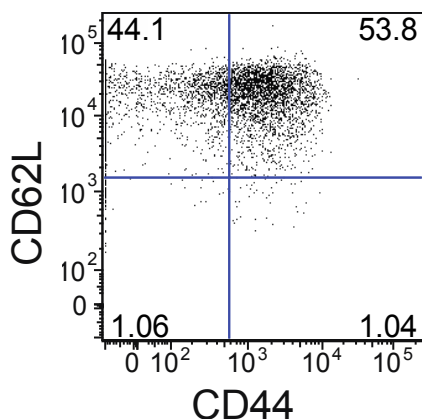


Figure 1. CD44 and CD62L expression of differentiated memory T cells

Recipes

1. T cell media
 - RPMI-1640
 - 10% fetal bovine serum
 - 1% penicillin/streptomycin
 - 1% L-Glutamine
 - 1x 1 M HEPES
 - 1x MEM non-essential amino acids
 - 1x sodium pyruvate 100 mM
 - 1x 2-mercaptoethanol
2. Staining buffer (in PBS)
 - 1% BSA
 - 0.02% NaN₃

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