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## Murine *In vitro* Memory T Cell Differentiation

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### Abstract

Upon pathogen encounter, naïve CD8<sup>+</sup> T cells are primed and undergo massive clonal expansion. A fraction of effector CD8<sup>+</sup> 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<sup>hi</sup>CD62L<sup>hi</sup> 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<sup>®</sup>, 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<sup>®</sup>, catalog number: 25030-081)
6. 100x 1 M Heps (Life Technologies, Gibco<sup>®</sup>, catalog number: 15630-080)
7. 100x MEM Non-essential amino acids (Life Technologies, Gibco<sup>®</sup>, catalog number: 11140-050)
8. 100x sodium pyruvate (100 mM) (Life Technologies, Gibco<sup>®</sup>, catalog number: 11360-070)
9. 100x 2-mercaptoethanol (Life Technologies, Gibco<sup>®</sup>, catalog number: 21985-023)
10. OVA<sub>257-264</sub> synthetic peptide (Sigma-Aldrich, catalog number: S7951)

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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. NaN<sub>3</sub> (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% CO<sub>2</sub> Cell Culture incubator

## Procedure

### A. CD44<sup>hi</sup>CD62L<sup>lo</sup> 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 OVA<sub>257-264</sub> synthetic peptide to 1 µM.
4. Incubate in the 5% CO<sub>2</sub> 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% CO<sub>2</sub> 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  $400 \times g$  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 rmIL15 (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 rmIL15 (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.

### Recipes

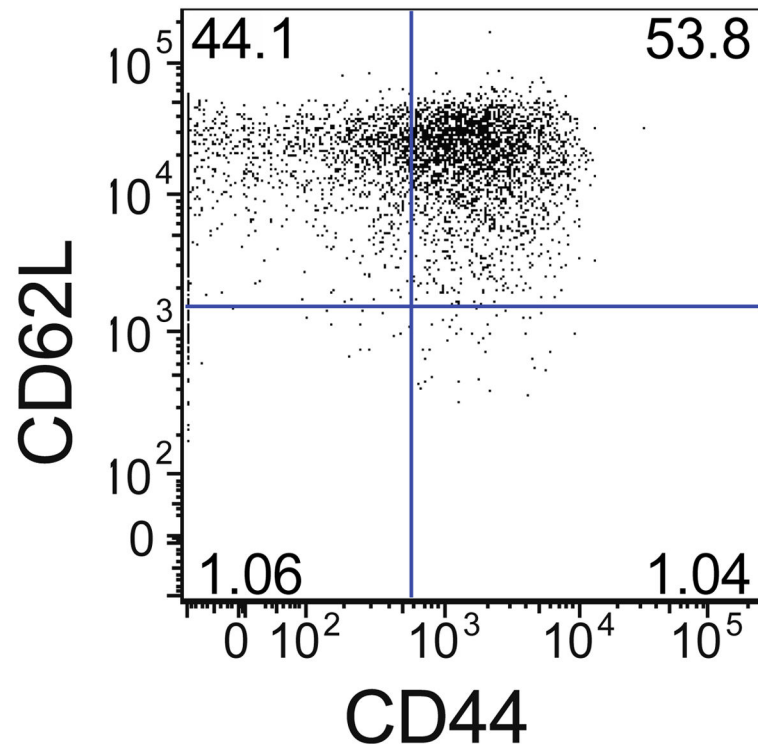
1. T cell media
  - RPMI-1640
  - 10% fetal bovine serum
  - 1% penicillin/streptomycin
  - 1% L-Glutamine
  - 1x 1 M HEPES
  - 1x MEM non-essential amino acids
  - 1x sodium pyruvate 100 mM
  - 1x 2-mercaptoethanol
2. Staining buffer (in PBS)
  - 1% BSA
  - 0.02% NaN<sub>3</sub>

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## References

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