Protocol 204_CFSE proliferation assay using T cells derived from SMARTA mice

A. Red blood cell lysis

1. Prepare single cell suspension from spleen from C57BL/6 and SMARTA mice.
2. For enhanced yield of DC, isolate DC from peripheral lymph node (protocol 208).
3. Lyse erythrocytes using NH_4Cl treatment.
4. Centrifuge splenocyte suspension in a 50ml Falcon tube for 5min at 1500rpm and re-suspend in 3ml RPMI.
5. Add 3ml of 1.66% NH_4Cl and wait exactly 3min.
6. Continuously shake the tube. After 3min fill up with RPMI and centrifuge for 5min at 1500rpm.

B. Sorting of T cells and DC

1. Resuspend pelleted cells in 300 μl MACS buffer
2. Add 40 μl MACS antibody of anti CD4 microbeads (for CD4 T cells) or CD11c microbeads (for DC).
3. Incubate for 30 mins on ice and flick tube from time to time
4. Wash with MACS buffer [2% FCS (20 ml), 2 mM EDTA (4 ml of 0.5 M EDTA stock), 976 ml 1×PBS] and centrifuge 1300 rpm for 5 mins.
5. Resuspend cells in 500 μl MACS buffer.
6. Prewet MC columns mounted on OctoMACS™ Separation with 500 μl MACS buffer
7. Apply cell suspension into the MS column
8. Wash column with 3×500 μl MACS buffer, adding buffer each time the column reservoir is empty.
9. Pipette 1 ml MACS buffer onto the column.
10. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
11. Collect positive fraction containing $CD4^+$ T cells.
12. Centrifuge 1300 rpm for 5 mins.

C. Labelling of T cells with CSFE

1. Label T cells by diluting 0.5 mM CFSE stock 1000-fold into the cell suspension to a final concentration of 0.5 uM.
2. Incubate for 10 mins at 37°C.
3. After labelling, add fetal calf serum to a final concentration of 2%.
4. Spin at 1,500rpm for 8 minutes and wash in PBS.
5. Resuspend the labelled cells at $1x10^6$ cells/ml.

D. DC and T cell co-culture

1. If stimulating with proteins, for example, ompC protein containing Gp61 sequences, stimulate DCs first with 1 ug/ml ompC proteins overnight. Wash DCs the following day and add CFSE labeled T cells.
2. For peptides, no pre stimulation is required.
3. Culture $5x10^5$ labelled T cells from SMARTA with $1x10^5$ DC from C57BL/6 mice in the presence of 20 ng/ml GP61 peptide for 4 days at 37°C.
4. Alternatively, culture $1x10^5$ labelled T cells from SMARTA with $1x10^5$ APC (from negative fraction when sorting CD4 T cells) from C57BL/6 mice in the presence of 20 ng/ml GP61 peptide for 4 days at 37°C.
5. For the negative control, incubate T and DC at 37°C for 4 days without the GP61 peptide.

D. Acquisition of day 2,3 and 4

1. Wash cell suspension with 1xFACS buffer.
2. Stain T cells using the following antibodies for 15 mins
3. Flow panel:
   (CFSE fluoresces in the FITC channel).
   CD4 Pacblue
   Va2 Biotin Strep APC
   CD3 PercpCy5.5
   CD90.2 Alexa Fluor 700
4. Wash with 1xFACS buffer and acquire on the LSRII SORP.