Redo experiment 4.1

7/24/15

In vivo 4.2

12 hour

Preparation of mouse lung single-cell suspension

1. Clean fat, lymph nodes, etc from ½ to 1 lung.
2. Place each lung in a separate Miltenyi Biotec gentlemacs tube containing
   a. 2.5 ml RPMI
   b. 35.7 ul of Roche Liberase TM (collagenase: 14 U/ml)
   c. 62.5 ul 1 mg/ml DNase I
3. Ensure gentlemacs tubes are tightly sealed
4. The gentlemacs is set up for several different tissues; choose program Lung_01
5. Place the tubes on the gentlemacs and start program Lung_01 (8 seconds). If the lung pieces look too big, run the program a second or even third time.
6. Incubate at 37 C for 40 minutes to one hour (I go for an hour, but others in my lab use only 40 minutes), gently mixing ½ way through.
7. After incubation, add 2.5 ml RPMI with 10% calf serum.
8. Choose program Lung_02 and start it (38 seconds).
9. Centrifuge to bring all the cells to the bottom of the tube (250 x g, 5 minutes).
10. Transfer contents to a 50 ml conical via a 70 um mesh. Wash the gentlemacs tube with 2.5 ml RPMI/calf serum and add the wash to the 50 ml conical (don't mix up the samples).
11. Centrifuge 8-10 minutes at 250 x g
12. Aspirate SN.
13. Lyse RBCs using ACK as per your favorite method.
14. Wash cells, resuspend in FCM buffer and stain.

6 samples
15 mL RPMI
214.2 mL
35 mL
2.6 mL

6 samples
15 mL RPMI
1.6

~1600 rpm

Don't aspirate

We use PE-conjugated anti-CD3, CD14, CD16/32, B220; PerCP cy5.5 conjugated anti-CD44 and APC-conjugated anti-CD25 (clone PC61): you can use the colors you have.

We use the gating strategy below.