Isolation of ILC2 from Mouse Liver

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[Abstract] Group 2 innate lymphoid cells (ILC2) are a recently characterized cell population which lacks specific antigen receptors and contributes to immune responses at mucosal surfaces of lung and gut. Recently, we demonstrated that ILC2 expand in the context of chronic liver diseases in mice and contribute to pathology in an IL-33 dependent manner. Here, we describe a protocol to isolate highly purified ILC2 from mouse livers. This procedure provides effective digestion of liver tissue and limits proteolytic degradation of cell surface receptors leading to increased yields of biologically functional cells. The number of liver resident ILC2 in the steady state is low, however their number dramatically increase upon systemic or local treatment of mice with cytokines such as IL-25 and IL-33. Using minicircle-based expression constructs for these cytokines high numbers of functional ILC2 can be isolated with the protocol provided here.

Materials and Reagents

1. Liver from a IL-33 or IL-25 treated mouse (*e.g.* C57BL/6 or Balb/c)
2. Fetal calf serum (FCS)
3. RPMI-1640 medium
4. Easycoll separation solution (density 1.124) (Biochrom, catalog number: L6145)
5. Anti-Mouse CD16/CD32 (Fc-Block) (eBioscience, catalog number: 14-0161)
6. 0.5 M $CaCl_2$ solution
7. 0.2 M $MgCl_2$ solution
8. AutoMACS Rinsing Solution (Miltenyi Biotec)
9. Brilliant Violet 421 Streptavidin (BioLegend, catalog number: 405226)
10. Antibodies
    a. FACS antibodies
        i. Anti-Sca-1 (clone D7, FITC conjugated) (eBioscience, catalog number: 11-5981)
        ii. Anti-KLRG1 (clone 2F1, APC conjugated) (eBioscience, catalog number: 17-5893)
        iii. Anti-ICOS (clone 7E.17G9, PE conjugated) (eBioscience, catalog number: 12-9942)
    b. Anti-mouse lineage-antibodies biotin labelled

i. Anti-CD3 (clone 145-2C11) (eBioscience, catalog number: 13-0031)
ii. Anti-CD45R (clone RA3-6B2) (eBioscience, catalog number: 13-0452)
iii. Anti-Ly-6G (clone RB6-8C5) (eBioscience, catalog number: 13-5931)
iv. Anti-CD11b (clone M1/70) (eBioscience, catalog number: 13-0112)
v. Anti-NK1.1 (clone PK136) (eBioscience, catalog number: 13-5941)
vi. Anti-Ter-119 (clone Ter-119) (eBioscience, catalog number: 13-5921)
vii. Anti-SiglecF (clone ES22-10D8) (Miltenyi Biotec, catalog number: 130-101-861)
viii. Anti-CD5 (clone 53-7.3) (Miltenyi Biotec, catalog number: 130-101-960)

11. Krebs-Ringer-Buffer (KRB) (see Recipes)
12. Collagenase IV solution (Sigma-Aldrich, catalog number: C5138) (see Recipes)
13. DNase I solution (Roche Diagnostics, catalog number: 10104159001) (see Recipes)
14. 30% Biocoll (see Recipes)
15. 80% Biocoll (see Recipes)
16. FACS buffer (see Recipes)
17. ACK buffer (see Recipes)
18. PEB buffer (see Recipes)

Equipment

1. GentleMACS C tube (Miltenyi Biotec)
2. 6 well plates
3. GentleMACS Dissociator (Miltenyi Biotec)
4. MACSmix Tube Rotator (Miltenyi Biotec)
5. Centrifuge (Thermo Fisher Scientific, model: Multifuge X1R)
6. Cell sorter (*e.g.* FACSaria, BD Biosciences)
7. 100 µm cell strainer (Corning, catalog numer: 08-771-19)
8. Hemocytometer
9. 15 ml Falcon tubes
10. 50 ml Falcon tubes
11. FACS tubes

Procedure

1. Freshly prepare liver digestion solution by pipetting 4.4 ml Krebs-Ringer-Buffer (KRB), 25 µl DNase I solution, 500 µl Collagenase IV solution, 20 µl 0.5 M $CaCl_2$ and 50 µl 0.2 M $MgCl_2$ into a gentleMACS C tube. Warm up by keeping at 37 °C for 30 min. This solution

ensures effective liver tissue digestion while proteolytic degradation of immune cell surface receptors is limited.

2. Sacrifice mouse by cervical dislocation and remove the liver from a mouse aseptically under the sterile bench and transfer the organ into a well of a sterile 6 well plate filled with 5 ml KRB buffer. If co-isolation of some ILC2 cells potentially present in the vasculature should be avoided include liver perfusion *e.g.* via the portal vein system using standard techniques described elsewhere.
3. Rinse the liver with KRB buffer and transfer it into the C tube containing pre-warmed liver digesting solution.
4. Dissociate the tissue by applying the C tube into the gentleMACS Dissociator and running the program m_liver-01.02.

   Note: One mouse liver can be proceeded per C tube, the program can be run 2-3 times till the tissue is fully homogenized.
5. Attach the C tube containing the homogenized tissue to the MACSmix Tube Rotator and incubate the sample at 37 °C for 30 min under continuous rotation.
6. Apply the tube to the gentleMACS Dissociator again and run the program m_liver-02.02.
7. Prepare a 50 ml tube and a 100 µm cell strainer by rinsing them with PEB buffer.
8. Apply the homogenized tissue from C tube on the strainer on the 50 ml tube and wash the strainer with additional 10 ml PEB buffer.
9. Discard the cell strainer and add further 20 ml PEB buffer to the tube. Centrifuge the sample at 20 *x g* at 4 °C for 4 min. This step removes unwanted hepatocytes from the liver cell suspension.
10. Carefully remove the supernatant from the sample and transfer it into a new 50 ml tube.
11. Wash the cells by adding 30 ml PEB buffer and centrifugation at 300 *x g* for 10 min at 4 °C.
12. Carefully discard the supernatant and resuspend the cell pellet in 1 ml of PEB buffer.
13. Apply 10 ml of ACK buffer on the suspension to remove red blood cells. Incubate the sample at room temperature for 5 min.
14. Wash the sample two times by adding 30 ml PEB buffer and centrifugation at 300 *x g* for 10 min at 4 °C.
15. Fill 15 ml tube with 5 ml of 80% Biocoll separation solution.
16. Resuspend isolated cells in 10 ml 30% Biocoll separation solution and overlay slowly to the 80% Biocoll solution. Centrifuge the cells at room temperature with 1,400 *x g* for 20 min without breaks.
17. Collect the target cells by carefully pipetting up the interface fraction between the gradients into a new 50 ml tube.
18. Wash with 30 ml PEB buffer and centrifuge at 300 *x g* for 15 min.

19. Count the cells by using a hemocytometer and distribute to FACS tube for staining.
20. Prior to staining block unspecific bindings by incubating cells with 1 µl/10^7 cells of Fc-Block at 4 °C for 5 min.
21. Proceed immediately to staining using the following antibodies using the manufacturer's recommended concentrations: anti-Sca-1, anti-ICOS, anti-CD25, biotinylated lineage antibodies. Add 0.015 µg/10^6 cells of Brilliant Violet 421 Streptavidin.
22. Incubate the tube at room temperature for 20 min.
23. Wash the cells by adding 2 ml FACS buffer and centrifuge at 300 *x g* for 10 min.
24. Proceed to FACS sorting. Gate and sort ILC2 as lineage negative, Sca-1$^+$, ICOS$^+$, KLRG1$^+$ cells (see gating strategy in Figure 1).

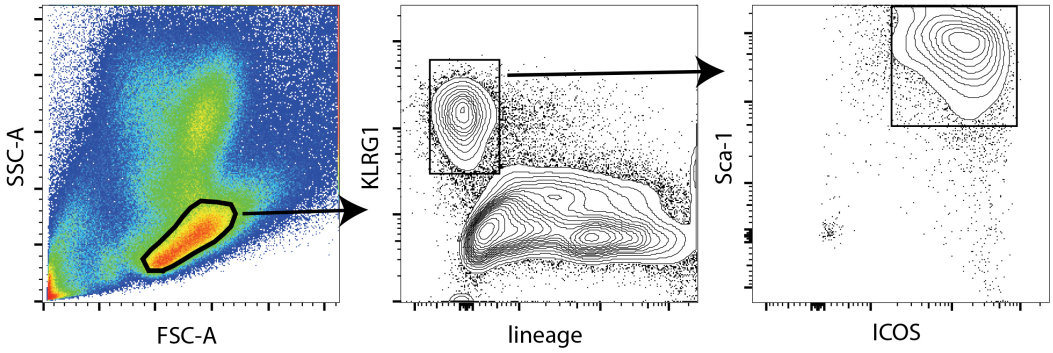

Figure 1. Gating strategy

Recipes

Note: All materials and reagents should be low endotoxin cell culture quality.

1. Krebs-Ringer-Buffer (KRB)
   154 mM NaCl
   5.6 mM KCl
   5.5 mM glucose
   20.1 mM $NaHCO_3$
   Adjust pH to 7.4 with NaOH
   Bring to final volume of 1,000 ml
   Filter sterilize and store at 4 °C
2. Collagenase IV solution
   5,000 Digestion Units/ml in KRB buffer
   Store stock solution at -20 °C
3. DNase I solution
   30,000 Units/ml in KRB buffer

Store stock solution at -20 °C

4. 30% Biocoll
   36.97 ml Biocoll 100%
   63.03 ml PBS
   Keep sterile at 4 °C
5. 80% Biocoll
   79.8 ml Biocoll 100%
   20.1 ml PBS
   Keep sterile at 4 °C
6. FACS buffer
   Add 5 ml Fetal Calf Serum to 495 ml of PBS
7. ACK buffer
   4.145 g NH_4Cl
   0.5 g $KHCO_3$
   18.6 g EDTA
   500 ml distilled water
   Filter sterilize and keep at 4 °C
8. PEB buffer
   Phosphate buffered saline (PBS) containing 0.5 % BSA and 2 mM EDTA

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References

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