FACS Staining for Follicular Helper T Cells

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[Abstract] Germinal center is the primary site for B cells to undergo somatic hypermutation and class switching. A recently discovered subset of T cells known as T follicular helper (TFH) cells are essential for germinal center reaction and thus have been the subjects of intensive study in the recent years. This protocol describes a reliable way to stain this population for flow analysis. This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research.

Materials and Reagents

A. Antibodies

1. Rat anti-mouse CXCR5-biotin (BD Biosciences, Pharmingen™, catalog number: 551960)
2. Goat anti-rat IgG-biotin (BD Biosciences, Pharmingen™, catalog number: 554014)
3. Straptavidin-PE Cy7 (BD Biosciences, Pharmingen™, catalog number: 557598)
4. Rat anti-mouse B220-pacific blue 450 (eBioscience, catalog number: 48-0452-82)
5. Rat anti-mouse CD11b-pacific blue 450 (eBioscience, catalog number: 48-0112-82)
6. Rat anti-mouse CD4-APC eFluor 780 (eBioscience, catalog number: 47-0042-82)
7. Hamster anti-mouse PD-1-PE (eBioscience, catalog number: 12-9985-82)
8. Mouse Fc block (BD Biosciences, Pharmingen™, catalog number: 553141)

B. Other materials

9. Single cell suspensions derived from murine spleen samples
10. Fetal bovine serum (FBS) (Hyclone)
11. Dulbecco's modification eagle medium (DMEM) (Life Technologies, Invitrogen™)
12. Ammonium chloride (0.17 M, filtered, autoclaved) (pH7.4)
13. DAPI nucleic acid stain (Life Technologies, Invitrogen™)
14. FACS buffer (see Recipes)

Equipment

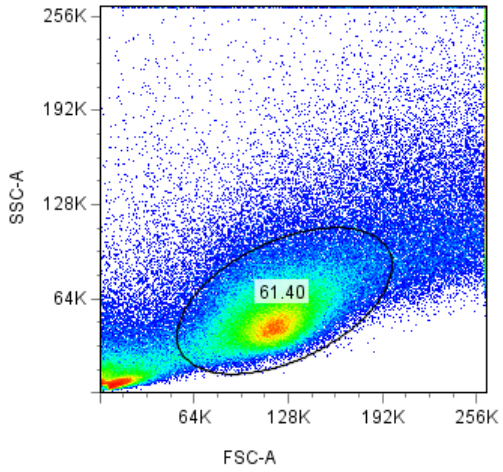
1. BD LSR II flow cytometer
2. Conical tubes (Corning)

Procedure

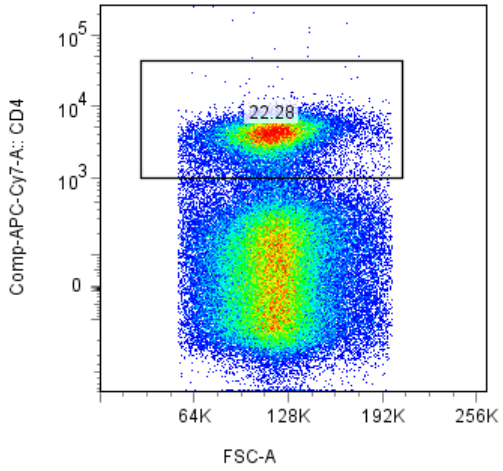
1. Harvest the spleen and create single cell suspension by gently smashing spleen pieces with the frosted surface of a pair of microscope slides in 5 ml of DMEM.
2. Transfer the cells into 50 ml conical tubes and spin down the cells at 300 RCF for 5 min at 4 °C.
3. Discard the supernatant with aspiration without disturbing the pellet.
4. Resuspend the cells with 5 ml of 0.17 M ammonium chloride and keep the cells on ice for 5 min.
5. Add 15 ml DMEM to the cells and spin at 300 RCF for 5 min at 4 °C.
6. Discard the supernatant and resuspend the cells with 20 ml of DMEM and count the cells.
7. Resuspend 2 millions spleen cells in 50 µl of 1:200 Fc block in FACS buffer and incubate for 30 min on ice.
8. Wash the cells with 200 µl of PBS and spin down the cells at 300 RCF for 5 min at 4 °C.
9. Discard the supernatant and resuspend the cells with 30 µl of anti-mouse CXCR5-biotin (1:50 in FACS buffer) and incubate for 30 min on ice.
10. Wash the cells with 200 µl of PBS and spin down the cells at 300 RCF for 5 min at 4 °C.
11. Discard the supernatant and resuspend the cells with 30 ul of anti-Rat IgG-biotin (1:50 in FACS buffer) and incubate for 30 min on ice.
12. Wash the cells with 200 µl of PBS and spin down the cells at 300 RCF for 5 min at 4 °C.
13. Discard the supernatant and resuspend the cells with 30 µl of the mixture of antibody 3-7 (1:50 in FACS buffer) and incubate for 30 min on ice.
14. Wash the cells with 200 µl of PBS and spin down the cells at 300 RCF for 5 min at 4 °C.
15. Resuspend the cells in 400 µl DAPI (3 µM in PBS) and analyze the cells using BD LSR II flow cytometer.

Gating strategy

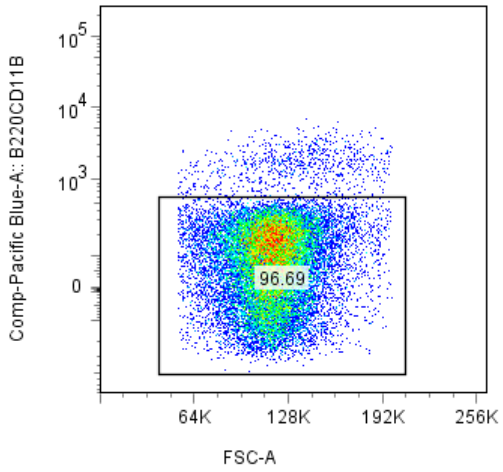
1. Gate on lymphocyte gate



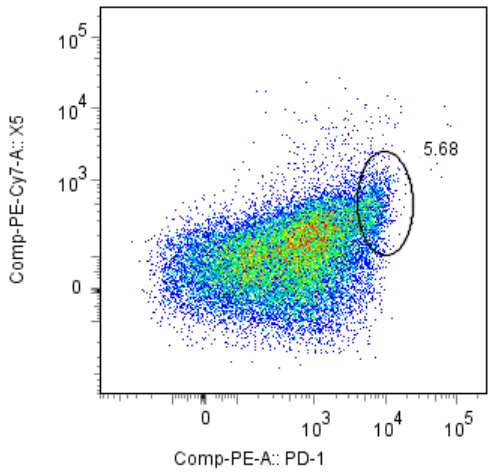
2. Gate on CD4 positive population within the lymphocyte gate



3. Exclude B220 and CD11b positive cells from CD4 positive gate



4. Gate on Tfh population based on CXCR5 and PD-1 expression



Recipes

1. FACS buffer
   3% fetal bovine serum in PBS

Acknowledgments

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References

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