Whole Blood Staining of Human Monocyte Subsets for Flow Cytometry

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[Abstract] This is a general protocol to stain whole human blood for flow analysis with minimal spontaneous activation of monocytes. This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research.

Materials and Reagents

A. Antibodies

1. Mouse anti-human CD56 FITC (BD Biosciences, catalog number: 340410)
2. Mouse anti-human CD2 FITC (BD Biosciences, catalog number: 555326)
3. Mouse anti-human CD19 FITC (BD Biosciences, catalog number: 555412)
4. Mouse anti-human CD14 PerCP (Life Technologies, Invitrogen™, catalog number: MHCD1431)
5. Mouse anti-human CD16 Pac Blue (BD Biosciences, catalog number: 558122)
6. Mouse anti-human HLA-DR APC-Cy7 (BioLegend, catalog number: 307617)

B. Other materials

7. Human blood
8. 10x BD FACS Lysing Solution (BD Biosciences, catalog number: 349202)
9. 20% formaldehyde (Tousimis, catalog number: 1008A)
10. Phosphate buffered saline (PBS) (Life Technologies, Gibco®, catalog number:

20012-027)

Equipment

1. Standard Bench-top Centrifuge
2. 5 ml polypropylene tubes (BD Biosciences, Falcon®, catalog number: 352063)
3. BD LSR II flow cytometer
4. Glass Whole Blood Tube with K3EDTA (BD Vacutainer®, catalog number: 366450)

Software

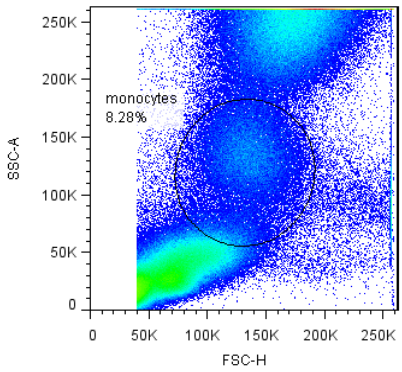
1. FlowJo (Tree Star)

Procedure

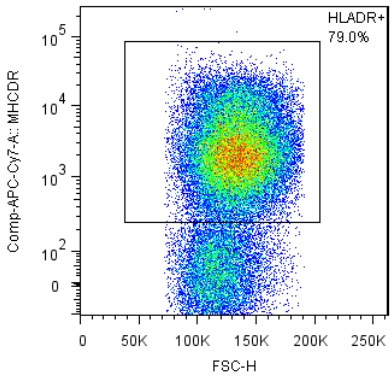
1. Harvest human blood into a 13 ml Glass Whole Blood Tube with K3EDTA and mix the blood by gently inverting the tube several times.
2. Transfer 100 µl whole blood into 5 ml polypropylene tubes and label the samples accordingly.
3. Add 5 µl of each antibody into the blood sample and mix by gently tapping the tubes.
4. Incubate the samples for 15 min in dark at room temperature.
5. Add 2 ml 1x BD FACS Lysing Solution (10x solution diluted in ddH_2O) in each sample.
6. Vortex sample briefly three times, check for clarity and take to centrifuged within 2-3 min.
7. Centrifuge 1,200 rpm for 7 min at room temperature.
8. Remove supernatant and resuspend pellet in 200 µl 2% formaldehyde.
9. Acquire the samples on BD LSR II flow cytometer.
10. Analyze data using FlowJo.

Gating strategy

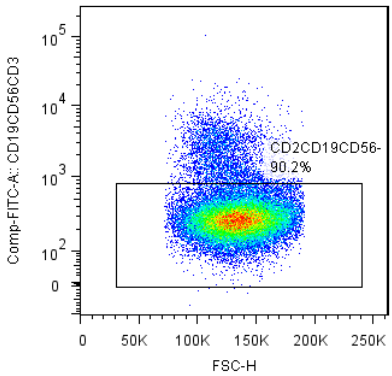
1. Gate on lymphocytes



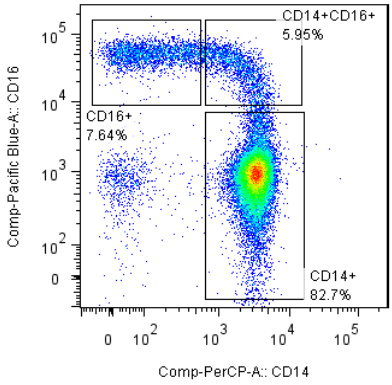
2. Gate on HLA-DR$^+$ cells



3. Exclude CD2$^+$, CD19$^+$, and CD56$^+$ cells



4. Gate CD16^{hi}, CD14^{hi}, and CD14^{hi}CD16^{hi} monocytes



Acknowledgments

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References

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2. Ramanujam, M., Wang, X., Huang, W., Liu, Z., Schiffer, L., Tao, H., Frank, D., Rice, J., Diamond, B., Yu, K. O., Porcelli, S. and Davidson, A. (2006). Similarities and differences between selective and nonselective BAFF blockade in murine SLE. *J Clin Invest* 116(3): 724-734.

