Isolation and Characterisation of Dendritic Cells from Peripheral Blood

Matthew Reeves* and John Sinclair

Institute of Immunity and Transplantation, University College London, London, UK
*For correspondence: matthew.reeves@ucl.ac.uk

[Abstract] Latency and reactivation of human cytomegalovirus (HCMV) is intimately associated with the myeloid lineage. Multiple studies have used *in vitro* protocols to generate dendritic cells (DCs) from myeloid precursors. Here we describe the direct isolation of DCs from peripheral blood to study HCMV latency directly in this cell type.

Materials and Reagents

1. 100 ml peripheral blood obtained via subcutaneous venepuncture
2. Heparin - to prevent clotting of blood
3. Histopaque-1077 (Sigma-Aldrich, catalog number: 10771) or any equivalent ficoll solution (density 1.077 g/ml)
4. Fetal calf serum (FCS)
5. PBS (chilled)
6. DC isolation kit (Miltenyi Biotec, catalog number: 130-094-487)
7. Antibodies (BD biosciences unless stated otherwise)
   a. Anti-TCRa/b-FITC (catalog number: 347773) - isotype with mouse IgG_1-FITC
   b. Anti-CD19-FITC (catalog number: 553785) - isotype with IgG_{2a}-FITC
   c. Anti-CD14-FITC (catalog number: 561712) - isotype with IgG_{2a}-FITC
   d. Anti-HLA-DR-PE (catalog number: 556644) - isotype with IgG_{2a}-PE
8. MACS buffer (see Recipes)

Equipment

1. Magnetic-activated cell sorting (MACS) magnet
2. LS columns (Miltenyi Biotec, catalog number: 130-042-401)
3. Centrifuge (50 ml tube capacity)
4. 50 ml polypropylene conical tubes (Falcon™)
5. 14 ml snap-cap polypropylene tubes

Procedure

A. Mononuclear cell isolation

1. Cool MACS magnet adaptor and LS column at 4 °C. LS columns have a total loading capacity of 2 x 10^9 unpurified cells (which would allow the isolation of 10^8 labelled cells).
2. Cool MACS Buffer and PBS on ice.
3. Dilute the blood 1:1 in PBS.
4. Add 15 ml histopaque-1077 (density 1.077 g/ml) to a 50 ml conical tube.
5. Carefully layer 30-35 ml of blood/PBS on top.
6. Spin at 800 *x g* for 20 min at RT. No brake on centrifuge.
7. Take WBC at interphase. To do this use a Pasteur pipette. Squeeze the teat prior to adding to Falcon and then suck up the white fluffy looking layer resting on histopaque. Avoid aspirating excessive histopaque-1077 as it can prevent pelleting of cells.
8. Pellet cells at 300 *x g* for 10 min (RT). If there is a problem pelleting cells possibly due to contaminating lymphoprep then dilute a further 1:4 in PBS and repeat centrifugation step.
9. Wash in cold PBS 300 *x g* for 10 min. Add 50 ml of PBS to pelleted cells.
10. Count cells. May need to dilute (1:100). A sample is taken as pre-sort control for FACs analysis to determine enrichment.
11. Centrifuge at 300 *x g* for 10 min (RT).
12. Re-suspend pellet in 300 µl of MACS buffer per 10^8 total cells. So for 3 x 10^9 the cells would be re-suspended in 9 ml. Add 100 µl of FcR Blocking reagent (in kit) per 10^8 and 100 µl of Microbead dendritic antibody cocktail.
13. Incubate for 10 min at 4 °C.
14. Transfer to a 50 ml Falcon and wash in PBS. Spin 300 *x g* for 10 min (RT).
15. As spin ends, charge the column.
16. To charge the column insert LS column into magnet. Then run 3 ml of MACS buffer through the column. Discard flow through.
17. Re-suspend the centrifuged cells in 500 µl of MACS buffer per 10^8 cells and add to column.
18. Collect flow through. Re-apply this flow through to the column. Collect again and then wash the column a further two times with 3 ml washes. The flow through represents the dendritic cell population.
19. Count cells.
20. Cells are then used for downstream application.

B. FACS staining

1. Using the cell count above. 10^5 cells are pelleted (400 *x g* for 5 min) in FACs tubes and re-suspended in residual PBS in tube (approximately 50 µl).
2. Add 1 µl of normal mouse serum to cells and incubate for 10 min (RT).
3. Proceed directly to adding directly conjugated antibody to cells (or isotype matched controls) as dictated by antibody datasheet. Stain in presence of normal serum. Stain for 20 min at RT in the dark (unless IgM which is then done at + 4 °C). Have one tube unstained for setting the parameters for collection.
4. Wash cells in 10x PBS and then spin (400 *x g*; 5 min, RT). Resuspend in 500 µl and proceed to FACs analysis.
5. Cells are characterized by the expression of a panel of cell surface markers consistent with a DC phenotype. The cells should be HLA-DR^{high} whilst TCR □□□, CD14 and CD negative. Cells will also express CD1a, CD86 and CD83^{low}.

Representative data

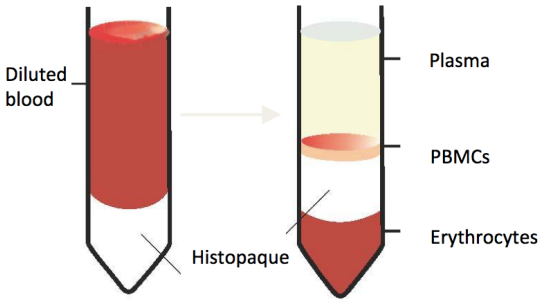

Figure 1. Isolation of PBMC on histopaque 1077

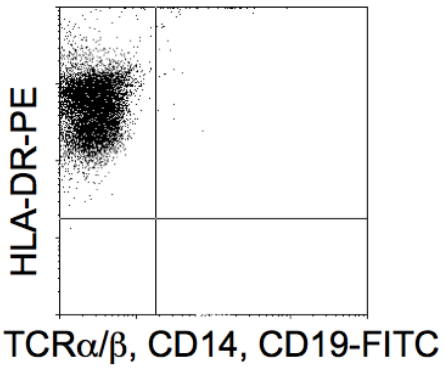

Figure 2. FACs analysis of putative DCs post isolation shown to be HLA-DR positive and CD14, 19 and TCRα/β negative

Recipes

1. MACS buffer
   PBS supplemented with 0.5% fetal calf serum and 2 mM EDTA

References

1. Reeves, M. B. and Sinclair, J. H. (2013). Circulating dendritic cells isolated from healthy seropositive donors are sites of human cytomegalovirus reactivation *in vivo*. *J Virol* 87(19): 10660-10667.

