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Phenotypic analysis of circulating leukocytes

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1 Works for me dx.doi.org/10.17504/protocols.io.6zxhf7n

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ABSTRACT

Antibody panels for flow cytometry were prepared with specific antibodies for identification of lymphocyte populations, and antibodies specific to IL-23 receptor andIL-12 receptorβeta-2 subunit.

Single stains were used for compensation controls and fluorescence minus one (FMO) controls were used to help set gates for flow cytometry.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0224276

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Coakley JD, Breen EP, Moreno-Olivera A, Al-Harbi AI, Melo AM, O'Connell B, McManus R, Doherty DG, Ryan T (2019) Dysregulated T helper type 1 (Th1) and Th17 responses in elderly hospitalised patients with infection and sepsis. PLoS ONE 14(10): e0224276. doi: 10.1371/journal.pone.0224276

MATERIALS TEXT

PBA = phosphate buffered saline or PBS containing 1.5% bovine serum albumin and 0.02% sodium azide

LIVE/DEAD Fixable Aqua dead cell stain purchased from Molecular Probes,*Leiden, The Netherlands*.

Fluorochrome-conjugated monoclonal antibodies:
CD3 (clone REA613, BW264/56), CD4 (REA623), CD8 (BW135/80, REA734), CD14 (REA599, TÜK4), CD16 (REA423), CD25 (4E3), CD45RA (REA562), CD127 (REA614), CD197 (CCR7; REA546), HLA-DR (REA805), IL-12Rβ2 (REA333) and IL-23R (218213) (purchased from Miltenyi Biotec, Gladbach Bergische, Germany and R&D Systems, Abingdon, UK).

Optimal quantities to give full staining with minimal amount of each antibody were determnined for each vial to ensure optimal results prior to calculation of volume needed.

Cocktails of up to 8 mAbs were made up in PBA buffer.

BD FACS CANTO II flow cytometer

1 Acquire blood sample from patient - 3mls EDTA

2 Transfer 100mcl whole blood to each FACS tube (7 tubes per patient)

3 Add Dead Cell Stain into each tube except tube 7 (unstained), vortex, and incubate for 15 minutes in the dark

4 Wash with PBA, vortex, and spin down at 1500RPM for 7 minutes

5 Resuspend with appropriate antibody cocktail (50mcl per tube) into each tube except 7 (unstained), vortex, and incubate for 15 minutes

6 Add 1ml BD FACS lysing solution, vortex, and incubate for 15 minutes in the dark

7 Add 1ml PBA and spin down at 1500RPM for 7 minutes. DIscard supernatants

8 Resuspend in 2mls PBA, vortex, and spin and discard as per step 6

9 Resuspend cells in few drops of PBA, vortex, keep cool, and acquire by flow cytometer immediately