PBMC or monocyte differentiation to macrophage and polarization

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PBMC isolation

The 8 mL BD VACUTAINER® Mononuclear Cell Preparation Tube (CPT™) with Sodium Citrate (BD, Franklin Lakes, NJ) for the separation of mononuclear cells from whole blood was used for blood collection using the standard technique for BD Vacutainer™. After collection, the tube was stored upright at room temperature (RT) and was processed within 2 h. Tubes were centrifuged at 1,800 RCF for 30 minutes at RT. After centrifugation, lymphocyte and monocyte band (PBMC layer) was collected using a serological pipette, and was washed with ample amount of Dulbecco's Phosphate Buffered Saline, e.g. transfer cell suspension collected from one CPT tube to one 50 ml conical tube, and add up to 40-45 ml total volume with DPBS (PBS, modified, without calcium chloride and magnesium chloride) followed by centrifuging at 330 RCF for 10 minutes at RT. PBMC cryopreserved in freezing media (90% FBS + 10% DMSO) at 2-3 x 10^6 cells/ml freezing media was stored in liquid nitrogen vapor phase for subsequent recovery if not seeded immediately.

Other methods: Order buffy coat from blood bank and isolate by CD14 beads selection.

Media:

RPMI-1640 and 20% FBS with 100 ng/ml Human M-CSF (Peprotech) (1 vial of M-CSF aliquot with 25 uL of 1 mg/mL stock prepared per manufacturer instruction)

For 250 ml Mac Media:

200 ml RPMI
50 ml FBS
2.5 ml Pen Strep
1 vial of M-CSF aliquot
Use within 2 weeks

Culture Vessel:

Primaria treated 6-well tissue culture plate: Fisher Cat# 353846
Primaria treated 24-well tissue culture plate: Fisher Cat# 353847
Primaria treated 100 mm tissue culture dish: Fisher Cat# 353803
Primaria treated 60 mm tissue culture dish: Fisher Cat# 353802

Differentiation:

D0:

1. Wash with PBS, spin down at 1,200 rpm for 5 min at RT
2. Resuspend in proper amount of Mac media, count cells.
3. General guidelines re seeding density
    a. 8-10 M fresh PBMC in 8 ml media/ 100 mm dish

b. 12-15 M fresh PBMC in 9 ml media / 6-well plate
c. 0.5M fresh PBMC in 12 ml media / 24-well plate
d. For fresh monocyte (isolated by apheresis or Ficoll/CD14+ beads selection), use 5M for 2 6-well plates
e. If using frozen cells, may need to use more than freshly isolated (e.g. at least 15M for 6-well plate, or 6M for 2 6-well plates), or culture for longer time. Below are what's already tried.

ND393 3M monocytes for 9 wells of 24-well plate;
ND422 3M monocytes for 18 wells of 24-well plate;
If frozen monocytes were stored for long time showing lower viability: 5-6 M for 12 wells of 24-well plate.

D1: Feed with 1.5 ml Mac media / well of 6-well plate
D4: Feed with 1.5 ml Mac media / well of 6-well plate
D6: Polarize if needed
D7: Collect cell pellets by trypsinization (scraping causes cell death); ideally, lyse in lysis buffer for DNA, RNA or protein collection directly.

(May use 2 ml media to feed if cell density is higher)

Notes: Also tried to polarize at D13 and collect on D14, for the few markers tested, there were not obvious difference in terms of gene expression and polarization response. There were also no obvious difference in polarization response for freshly isolated or frozen PBMC. But when using frozen PBMC/monocyte to differentiate, may need to do D8 or D9 so that cells are fully recovered.

Polarization:

18-20 h in 1:1 diluted Mac media with RPMI, 1:1000 dilution of stock solution

Cytokines	Company	Catalog	Size	Final Concentration	Location
Recombinant Human M-CSF	PeproTech (or GoldBio since 2015)	300-25	1 mg	100 ng/ml	-80, box "Human M-CSF"
Recombinant Human IFN-γ	PeproTech	300-02	100 ug	20 ng/ml	-80, box "Miscellaneous"
Recombinant Human IL-4	R&D	204-IL	10 ug	20 ng/ml	-80, box "Miscellaneous" and 10th floor
Lipopolysaccharides (LPS)	Sigma	L3129	10 mg	100 ng/ml	HZ 4C box "LPS", 1 mg/ml, dilute to 100 ug/ml with H2O first