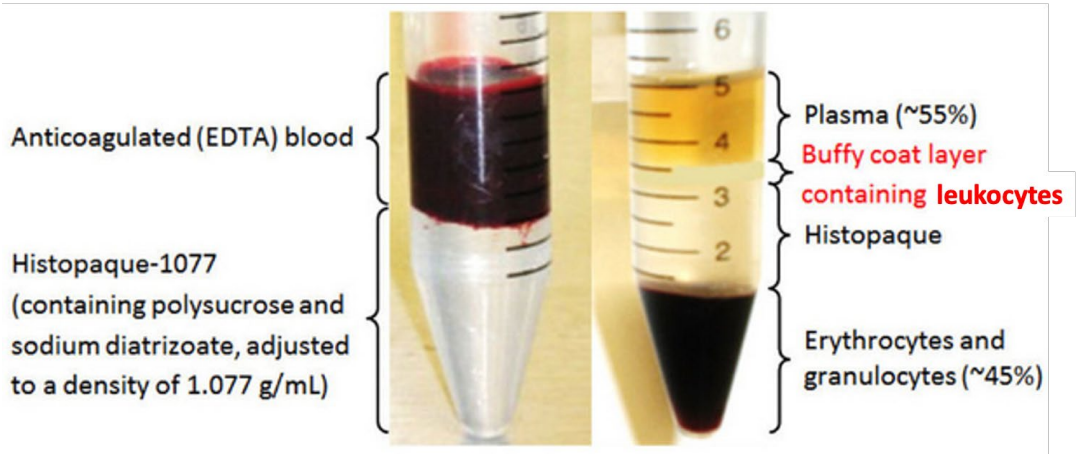
Human macrophage isolation

Peripheral human blood monocytes are isolated from buffy coats of anonymous, de-identified healthy adult volunteers, with informed consent (New York Blood Center), according to the protocol below.

- Fill 15 mL tubes with 7 mL of preheated (37 °C) Histopaque solution-1077 (Sigma-Aldrich), and carefully layer 7 mL of donor blood on top
    - Write the last three numbers of the donor code down, as identification
    - One donor gives ~35 mL of blood in total, so five 15 mL tubes per donor
- Centrifuge the 15 mL tubes at 3000 rpm for 30 min at room temperature without break (acceleration=9, break=0)
- Remove the leukocytes from the buffy coat layer (as indicated in the picture below), ~1 mL in total
- Wash the isolated leukocytes with RPMI-1640 (Gibco, stored in cold-room Tall lab), containing 10% FBS and 1% penicillin/streptomycin, and then centrifuge at 400 g for 5 min (live cells should not be centrifuged at speeds higher than 500 g)
- Repeat the washing step once more
- Resuspend the pellet in RPMI-1640 medium and plate the cells into 70 mm tissue culture treated plates (not petri dishes, these are only used for murine BMDMs which are more adherent)
    - The cells of one donor can go into ~6 culture treated plates
    - Optionally, plate the cells in only 2 plates, and freeze the rest down in the -80 °C freezer, in 90% FBS and 10% DMSO (for 2 plates in one vial)
- If there is time, refresh the medium after ~3-4 hours with new RPMI-1640
- Add 20 ng/mL of human GM-CSF or M-CSF (PeproTech) to the plates, by diluting the 20 µg/mL stock 1:1000 (so add 10 µL to 10 mL medium)
- Every 2-3 days, replace the medium with fresh GM-CSF or M-CSF containing medium
- After 7-14 days, the cells can be used for experiments, when they are more than 80% confluent



Human macrophage polarization

M0 (GM-CSF) – can be differentiated into **M1** by incubating with 100 ng/mL LPS and 20 ng/mL human IFNγ for 24 hours

M0 (M-CSF) – can be differentiated into **M2** by incubating with 20 ng/mL human IL-4 for 24 hours*

** For evaluating gene expression in IL-4 treated macrophages, 8-hour stimulation is sufficient, while 24 hours is required for protein expression*

Compound dilutions

Compound	Stock concentration	Final concentration	Dilution
Human IL-10	4 μg/mL	20 ng/mL	1:250
Human IL-4	20 μg/mL	20 ng/mL	1:1000
Human IFNg	20 μg/mL	20 ng/mL	1:1000
LPS	1 mg/mL	100 ng/mL	1:10,000
Oxidized LDL	1 mg/mL	25 μg/mL	1:40

** For evaluating gene expression in IL-4 treated macrophages, 8-hour stimulation is sufficient, while 24 hours is required for protein expression*