GENERATION OF MONOCYTES-DERIVED DENDRITIC CELLS FROM CHICKEN BLOOD FOR IN VITRO STUDIES

1. Material

1.1. REAGENTS AND DISPOSABLES

- Nunc™ Flat-bottomed 6-wells culture plates (Thermo Scientific, cat n°140675);
- SepMate 15 mL tubes (StemCell Technologies, cat n° 85415)
- Cell scraper (TPP, cat n° Y9902)
- 15-mL conical Falcon tubes
- Sterile tips
- Sterile Pasteur pipettes
- Sterile pipettes
- Heparin (SIGMA, Cat No. H-0878)
- Ficoll Histopaque 1083 density gradient (Sigma-Aldrich, cat n°1086-1)
- Recombinant ChIL-4 (KingFisher Biotech, cat n° RP0110C-025)
- Recombinant ChGM-CSF (KingFisher Biotech, cat n° RP0290C-025)
- Lipopolysaccharide from Escherichia coli O127:B8 (Sigma-Aldrich, cat n° L4516)
- Polyinosinic–polycytidylic acid (Poly(I:C), Sigma-Aldrich, cat n°P9582)

1.2. EQUIPMENT

- Biosafety cabinet
- Liquid waste container
- Centrifuge

1.3. SAMPLES

Heparinized chicken blood

1.4. BUFFERS AND SOLUTIONS

- Complete culture medium:
    - RPMI 1640 medium (Life Technologies, cat n° 52400025)
    - 10% heat-inactivated fetal bovine serum (Biowest, cat n° S1860)
    - 50 U/mL Penicillin-Streptomycin (Gibco, Cat n° 15140122)

- Heparin solution:
    - Resuspend the stock in 10 mL of RPMI (final concentration = 10 U/µL)
    - Stored at 4°C.

2. Method

2.1. PERIPHERAL BLOOD MONONUCLEAR CELLS ISOLATION

- The blood is collected in heparinized tubes or in a syringe containing heparin (100 µL/mL of blood) in 1 mL RPMI and then transferred to a 15-mL conical tube.
- In a laminar flow hood, dilute the blood with an equal volume of complete culture medium.
- Add Ficoll Histopaque 1083 density gradient medium to the SepMate™ tube by carefully pipetting it through the central hole of the SepMate™ insert.
- Keeping the SepMate™ tube vertical, add the diluted sample by pipetting it down the side of the tube.
- Centrifuge at 1200 x *g* for 10 minutes at room temperature, with the brake on.
- Pour off the top layer, which contains the enriched PBMCs, into a 15-mL Falcon tubes.
- Wash enriched PBMCs with 10 mL complete culture medium. Centrifuge at 300 x *g* for 8 minutes at room temperature, with the brake on. Repeat wash.
- Count the cells, and resuspend them in the complete culture medium at a concentration of 10^7 cells/mL.

2.2. MONOCYTES ENRICHMENT BY PLASTIC ADHESION

- Seed 2x10^7 PBMC into each well of 6-well plates.
- Culture PBMCs in a humidified incubator for 4h at 41°C in 5% CO2 atmosphere to allow monocyte adherence.
- After 4h, remove and discard the non-adherent cells.

2.3. GENERATION OF IMMATURE MODC

- Add 2 mL complete culture medium supplemented with 10 ng/mL IL-4 and 10 ng/mL GM-CSF into each well.
- Incubate for 5 days at 41°C in 5% CO2 atmosphere to allow differentiation.
- On day 3, refresh the culture medium by adding 0.5 mL of fresh complete culture medium supplemented with 10 ng/mL IL-4 and 10 ng/mL GM-CSF.

2.4. TREATMENT OF IMMATURE MODC

- On day 5, culture the cells for 6 to 24h in complete culture medium supplemented with 10 ng/mL IL-4, 10 ng/mL GM-CSF and 500 ng/ml LPS or 50 µg/mL poly(I:C). Non-stimulated cells are used as negative control.
- After 6 or 24h of stimulation, harvest cells by gentle scraping for phenotype and functional analysis of the induced maturation.