Monocyte-MSC Co-cultures

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[Abstract] To assess the effect of multipotent stromal cells (MSC) on monocytes, 3-day cultures were performed of freshly isolated monocytes in MSC-conditioned medium (CM). As a control condition, monocytes were stimulated with low dose macrophage colony-stimulating factor (M-CSF). Monocytes were isolated from peripheral blood mononuclear cell (PBMC) populations by magnetic activated cell sorting (MACS) using CD14 microbeads.

Materials and Reagents

1. Peripheral blood mononuclear cells (PBMC) [isolated from a buffy coat from a healthy donor using Ficoll-Paque (own pharmacy) density gradient (1.077 g/cm^3)]
2. Multipotent stromal cells (MSC) from healthy donors
3. Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies, catalog number: 31870-082)
4. Penicillin/streptomycin (5,000 U/ml) (Life Technologies, catalog number: 15070-063)
5. L-glutamin (200 mM) (Life Technologies, catalog number: 25030-024)
6. Fetal calf serum (FCS) (Greiner Bio-One GmbH, catalog number: 758072)
7. Phosphate buffered saline (PBS) (produced by in-house pharmacy)
8. M-CSF (Pepro Tech, catalog number: 300-25)
9. CD14 MicroBeads (human) (Miltenyi Biotec, catalog number 130-050-201)
10. Antibodies
    a. Anti-CD14 PE (BD Biosciences)
    b. Anti-CD206 APC (BD Biosciences)
    c. Anti-CD163 PerCP-Cy5.5 (BioLegend)
    d. Anti-CD80 PE-Cy7 (BioLegend)
11. Trypsin/EDTA (0.05%, phenol red) (Life Technologies, Invitrogen^{TM}, catalog number: 25300-054)
12. Culture medium (see Recipes)
13. Proliferation medium (see Recipes)

Equipment

1. T75 culture flasks
2. 6-well plates (Sigma-Aldrich, catalog number: CLS3506)
3. 37 °C, 5% CO_2 cell culture incubator
4. Microscope
5. Centrifuge
6. Hemocytometer (counting chamber)
7. MS Columns (Miltenyi Biotec, catalog number: 130-042-201)
8. MiniMACS separator (Miltenyi Biotec, catalog number: 130-042-102)
9. MACS MultiStand (Miltenyi Biotec, catalog number: 130-042-303)
10. 24-well plates (Sigma-Aldrich, catalog number: CLS3526)

Procedure

1. MSC cultures are generated from aspirated bone marrow: bone marrow-derived mononuclear cells are isolated using Ficoll-Paque density gradient (1.077 g/cm^3) and plated at 1.3 x 10^5 cells/cm^2 in proliferation medium.
2. Cultures were incubated at 37 °C and 5% CO_2. After 3-4 days non-adherent cells were removed, and medium was refreshed every 3-4 days until confluence was reached. The MSC monolayer was detached using trypsin/EDTA, and cells were reseeded at 4,000 cells per cm^2 for further expansion.
3. MSC (passage 2-5) are cultured at confluency in a T75 culture flasks in 10 ml culture medium for at least 3 days without refreshing the medium.
4. The medium is aspirated from the cultures (= MSC-CM). Spin down MSC-CM at 350 *x g* for 10 min to obtain cell-free MSC-CM.
5. Day 0: Isolate monocytes from freshly obtained PBMC from a buffy coat by MACS using CD14 microbeads and MS Columns according to the manufacturer's instructions.
6. Plate 2.5 x 10^6 monocytes in a 24-wells plate in 400 µl culture medium.
7. Add 600 µl of cell-free MSC-CM to the monocyte cultures.
8. As control condition, add M-CSF to the monocyte cultures at a concentration of 5 ng/ml.
9. Place the cultures for 3 days at 37 °C in a 5% CO_2 cell culture incubator.
10. Day 3: Collect monocytes.
    Note: When the monocytes are attached, place the plates for 15 min on ice.
11. Spin down monocytes at 350 *x g* for 10 min.
12. Add 120 µl of PBS and count dead/alive cells using a hemocytometer.
13. Analyze monocytes with flowcytometry/ isolate mRNA for follow-up analysis.

a. Antibodies for flowcytometry to analyze the monocytes:
   i. Anti-CD14 PE
   ii. Anti-CD206 APC
   iii. Anti-CD163 PerCP-Cy5.5
   iv. Anti-CD80 PE-Cy7

Recipes

1. Culture medium
   RPMI medium
   10% FCS
   P/S (100 U/ml)
   L-glutamin (100 U/ml)
2. Proliferation medium
   Dulbecco's modified Eagle's medium-low glucose (DMEM-LG)
   10% FCS
   P/S (100 U/ml)
   L-glutamin (100 U/ml)

References

1. Melief, S. M., Schrama, E., Brugman, M. H., Tiemessen, M. M., Hoogduijn, M. J., Fibbe, W. E. and Roelofs, H. (2013). Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells* 31(9): 1980-1991.

