Mouse embryonic stem cells to macrophage-like cells

A robust 20 day differentiation protocol to direct mouse embryonic stem cells (mESCs) towards the macrophage lineage.

Macrophages display plasticity in both phenotype and function; therefore standardized culture and differentiation protocols are required to ensure consistency between different cell populations. Throughout this protocol we refer to these cells as embryonic stem cell-derived macrophages (ESDMs).

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Preparation of media for differentiation of mESCs to ESDMs

All media preparation should be carried out in a tissue culture hood under aseptic conditions.

Preparation of L929 conditioned medium*

1. For L929 medium, supplement DMEM/F12 GlutaMAX™ with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin.
2. Swirl to mix components and store at 4°C.
3. Harvest conditioned medium (CM) containing colony stimulating factor (CSF-1) from adherent L929 cells 3 days following confluency. Filter through a 0.22 µm membrane to remove cell debris, and store at -20°C.

Preparation of GMEM$_{SR}$ medium for mESC maintenance

1. For GMEM$_{SR}$ medium, supplement GMEM with 10% (v/v) FBA, 1% (v/v) non-essential amino acids (of a 100X solution), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol and 100 U/mL leukemia inhibitory factor (LIF).
2. Swirl to mix components and store at 4°C.

Preparation of ESDM$_{Diff}$ medium for macrophage differentiation

1. For ESDM$_{Diff}$ medium, supplement GMEM with 10% (v/v) FBS, 1% (v/v) non-essential amino acids (of a 100X solution), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol and 100 U/mL leukemia inhibitory factor (LIF).
2. Swirl to mix components and store at 4°C.

For EDSM$_{Cult}$ medium for macrophage maturation and growth

1. For EDSM$_{Cult}$ medium, supplement GMEM with 10% (v/v) FBA (pre-screened for optimal hematopoietic differentiation**), 1% (v/v) non-essential amino acids (of a 100X solution), 2mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol, 15% (v/v) L929 cM (contains CSF-1), 100 U/mL penicillin and 100 µg/mL streptomycin.
2. Swirl to mix components and store at 4°C.

**** L929 conditioned medium is an alternative source of macrophage colony stimulating factor (CSF-1). L929 cells (ATCC) are derived from mouse subcutaneous tissue.***

***** FBS was pre-screened for optimal hematopoietic differentiation using methylcellulose assay for the detection of hematopoietic progenitors.***

Generation of ESDMs

1. Culture mESCs (E14) on 0.1% gelatin-coated tissue culture flasks in $GMEM_{SR}$ medium.
2. Initiate macrophage differentiation by seeding $6x10^5$ trypsin-dissociated mESCs in suspension culture in 20 mL $ESDM_{Diff}$ per 95 mm bacteriological-grade Petri dish (Day 0). During the first 4 days the mESCs should start to differentiate and form embryoid bodies (EBs).
3. On Day 4, to avoid adherence the EBs should be gently dislodged and transferred to new Petri dishes in fresh $ESDM_{Diff}$ medium using a wide-bore pipette.
4. Repeat step 3 again on Day 6, to ensure that the EBs do not attach to the Petri dishes.
5. On Day 8 transfer the EBs to 95 mm gelatin-coated tissue culture dishes, in fresh $ESDM_{Diff}$ medium. The EBs will become adherent after 2 days.
6. At Day 10 collect the medium that contains the non-adherent macrophage precursor cells, centrifuge, re-suspend in 20 mL $ESDM_{Cult}$ and plate on 95 mm diameter bacteriological Petri dish. At the same time add 20 mL of fresh $ESDM_{Diff}$ medium to the original plate of adherent EBs, do that non-adherent cells can then be harvested again 2 days later. Harvesting in this way can be repeated every 2 days for up to a total of 20 days.
7. Between days 12 and 17 of this differentiation, ESDMs will adhere to the plastic Petri dishes and proliferate to form a confluent monolayer. For functional experiments that are performed in suspension, cells can be cultured for 1 day prior to experiments in Teflon® pots. Between days 15 and 20, ESDMs can be harvested for testing. To do this, wash twice in PBS, then add 5 mL TVP (1 mM EDTA, 1% chicken serum,, 0.025% trypsin) solution and incubate for 20 min at 37°C. Add 10 mL serum-containing medium to stop the trypsin, centrifuge, and wash once with PBS before use in further experiments.

Since each dish of mESCs can yield 2–4x10^6 ESDMs and supernatant can be collected 6 times (i.e. every 2 days from Day 10–20), a total of 12–24x10^6 ESDMs can be harvested from one starting dish of 6x10^5 mESCs.

Taken from:

Zhuang L, Pound JD, Willems JJ, Taylor AH, Forrester LM and Gregory CD (2012). Pure populations of murine macrophages from cultured embryonic stem cells. Application to studies of chemotaxis and apoptotic cell clearance. *J Immunol Methods,* 385, 1-2.