Generation of Mouse Bone Marrow-Derived Macrophages (BM-MFs)

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[Abstract] Generating mouse macrophages from bone-marrow progenitor cells is a useful tool to study biological functions of mouse macrophages. Macrophages are one of the major populations of phagocytes and play many different roles during inflammatory process initiation and termination.

Materials and Reagents

1. M-CSF-transduced L929 cells
2. HI FBS (EuroClone, catalog number: EC S0180L)
3. L-Glutamine (EuroClone, catalog number: EC B3000D)
4. Penicillin/streptomycin (EuroClone, catalog number: EC B3001D)
5. IMDM (EuroClone, catalog number: EC B2072L)
6. Beta-mercaptoethanol (Sigma-Aldrich, catalog number: M6250)
7. M-CSF-transduced L929 growth supernatant
8. Phosphate buffered saline (PBS) (EuroClone, catalog number: ECM9605AX)
9. BMMFs culture medium/ conditioned medium (see Recipes)

Equipment

1. Centrifuges
2. 70 µm-wide cut-off cell strainer
3. Non-treated cell culture plates
4. Incubator (37 °C and 5% CO_2)
5. Fluorescence activated cell sortor (FACS)

Procedure

1. Flush mouse tibiae and femurs with ice-cold PBS through a 70 µm-wide cut-off cell strainer.

2. Centrifuge 5 min at 450 *x g*. Resuspend pelleted cells in conditioned medium (supplemented with 30% of growth supernatant of M-CSF-transduced L929 cells).
3. Seed 7 x 10^6 cells in 100 x 20 mm non-treated cell culture plates in 10 ml of conditioned medium.
4. Incubate at 37 °C and 5% CO_2.
5. Upon reaching confluence (approximately 6 days) use the cells or split adhered cells and seed 5 x 10^6 cells in 100 x 20 mm in non-treated cell culture plates in 10 ml of conditioned medium.
6. BMMFs are ready for experimental use when the percentage of $CD11b^+$ cells is higher than 90% as measured by FACS analysis.

Recipes

1. BMMFs culture medium (conditioned medium)
   HI FBS - 10%
   L-Gln - 2 mM
   Penicillin/streptomycin - 50 U/ml
   Beta-mercaptoethanol - 50 µM
   M-CSF-transduced L929 growth supernatant - 30%
   IMDM - to volume

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References

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