PROTOCOL EXCHANGE | *COMMUNITY CONTRIBUTED*

Generation of mouse bone marrow-derived dendritic cells (BM-DCs)

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Introduction

Protocol for generating mouse dendritic cells from bone-marrow progenitor cells used in our Nature paper.

Subject terms: Cell culture Tissue culture Immunological techniques Cell biology Developmental biology

Keywords: dendritic cell differentiation GM-CSF

Reagents

GM-CSF-transduced B16 cell line.

BMDCs culture medium recipe (conditioned medium):
HI FBS (EuroClone) – 10%
L-Gln (EuroClone) – 2mM
Penicillin/Streptomycin (EuroClone) – 50 U/ml
Beta-mercaptoethanol (EuroClone) – 50 microM
B16-GMCSF growth supernatant – 10%
IMDM (EuroClone) – to volume

Procedure

1.Flush mouse tibiae and femurs with ice-cold PBS through a 70 µm-wide cut-off cell strainer.
2.Centrifuge 5' at 1400 rpm. Resuspend pelleted cells in conditioned medium (supplemented with 10% of growth supernatant of GM-CSF-transduced B16 cells).
3.Seed 7×10^6 cells in 100×20 mm non-treated cell culture plates in 10 ml of conditioned medium.
4.Incubate at 37 °C – 5% CO2.
5.On day 4 and 7 add 5 ml of pre-warmed conditioned medium.
6.At day 8/9 the percentage of CD11c+ cells should be higher than 90% as measured by FACS analysis. BMDCs are then ready for experimental use.
7.Harvest the supernatant and gently wash the plate once with 5 ml of pre-warmed PBS.

8.Incubate 2’ with 5 ml of 2mM EDTA at 37 °C – 5% CO_2.

9.Collect cells, wash once with PBS.

10.Centrifuge 5’ at 1200 rpm and resuspend pelleted cells in conditioned medium.

Associated Publications

This protocol is related to the following articles:

- CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation
  Ivan Zanoni, Renato Ostuni, Giusy Capuano, Maddalena Collini, Michele Caccia, Antonella Ellena Ronchi, Marcella Rocchetti, Francesca Mingozzi, Maria Foti, Giuseppe Chirico, Barbara Costa, Antonio Zaza, Paola Ricciardi-Castagnoli, and Francesca Granucci

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Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

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