Video Article

Culture of myeloid dendritic cells from bone marrow precursors

Jeanette Boudreau[1,2], Sandeep Koshy[2,3], Derek Cummings[2], Yonghong Wan[2]

[1]Medical Sciences Program, McMaster University

[2]Centre for Gene Therapeutics, McMaster University

[3]Department of Chemical Engineering, University of Waterloo

Correspondence to: Jeanette Boudreau at boudreje@mcmaster.ca

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Abstract

Myeloid dendritic cells (DCs) are frequently used to study the interactions between innate and adaptive immune mechanisms and the early response to infection. Because these are the most potent antigen presenting cells, DCs are being increasingly used as a vaccine vector to study the induction of antigen-specific immune responses. In this video, we demonstrate the procedure for harvesting tibias and femurs from a donor mouse, processing the bone marrow and differentiating DCs in vitro. The properties of DCs change following stimulation: immature dendritic cells are potent phagocytes, whereas mature DCs are capable of antigen presentation and interaction with CD4+ and CD8+ T cells. This change in functional activity corresponds with the upregulation of cell surface markers and cytokine production. Many agents can be used to mature DCs, including cytokines and toll-like receptor ligands. In this video, we demonstrate flow cytometric comparisons of expression of two co-stimulatory molecules, CD86 and CD40, and the cytokine, IL-12, following overnight stimulation with CpG or mock treatment. After differentiation, DCs can be further manipulated for use as a vaccine vector or to generate antigen-specific immune responses by in vitro pulsing using peptides or proteins, or transduced using recombinant viral vectors.

Video Link

The video component of this article can be found at https://www.jove.com/video/769/

Protocol

This protocol has been adapted from Lutz et al.[1]

Harvest and processing of bone marrow

1. **Isolation of the tibia and femur:** Euthanize the donor mouse and spray the legs with 70% ethanol. Grasp the first ankle firmly with blunt forceps and begin to cut away the skin and underlying musculature to expose the tibia. To avoid damaging the bone, cut slowly and parallel to the tibia, leaving the knee joint intact.
   To clean the femur, immobilize the knee joint by grasping with blunt forceps. Clean away the musculature with a pair of sharp scissors and curved forceps. Continue upward until the hip joint is exposed and scissors can be placed between the head of the femur and the hip joint. Remove the bones by cutting between femur and hip joint and place into phosphate buffered saline (PBS) on ice.
2. **Removal of bone marrow:** Clean remaining musculature from the bones using scissors and curved forceps. Cut off the epiphyses of each bone and locate the center cavity. Using a 10mL syringe loaded with PBS and a 25G0.5" needle, flush the bone marrow into a non-tissue coated petri dish.
3. **Processing of bone marrow:** Use the rubber end of the plunger from a 1mL syringe to dissociate the bone marrow into a single-cell suspension (use an up and down, not scraping motion). Collect bone marrow in a conical falcon tube, and rinse residual bone marrow with PBS.

Culture of dendritic cells

1. Resuspend cells in DC media and count DC precursors on a hemocytometer (Figure 1). You will see cells of varying sizes; the DC precursors are the largest and brightest. Differentially count only these cells. From two femurs and two tibias of a wild-type C57Bl/6 mouse (6-8 weeks old), you should expect between 25-40 x 10^6 total DC precursors.

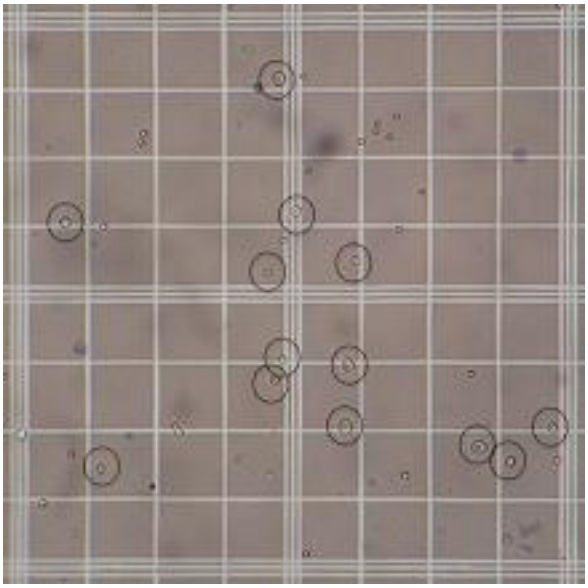

Figure 1

2. Culture cells at a density of 2 x 10^5 DC precursors/mL in DC media supplemented with 40 ng/mL recombinant murine GM-CSF. Plate cells in non-tissue-coated polystyrene petri plates.

Add media (day 3)

To refresh the media, add half of the total volume of fresh media supplemented with 40 ng/mL GM-CSF.

Replace one third of the media (day 6) and maturation

To refresh the media, carefully remove one third of the total volume of media and replace this volume with fresh DC media supplemented with 40 ng/mL GM-CSF on day 6 of culture.

If desired, dendritic cells can be stimulated for maturation using cytokines or toll-like receptor ligands. In the video, DCs were matured by overnight stimulation with 5 ng/mL CpG.

Harvest of dendritic cells

DC culture is complete (Figure 2). Cells will be both in suspension and loosely adhered to the plate. Adhered cells can be removed by scraping the dish with a tissue culture scraper and rinsing with PBS. The total number of cells will increase 5-8 fold during the week-long culture and differentiation period, therefore, expect to harvest 1-1.6 x 10^6 cells/mL.

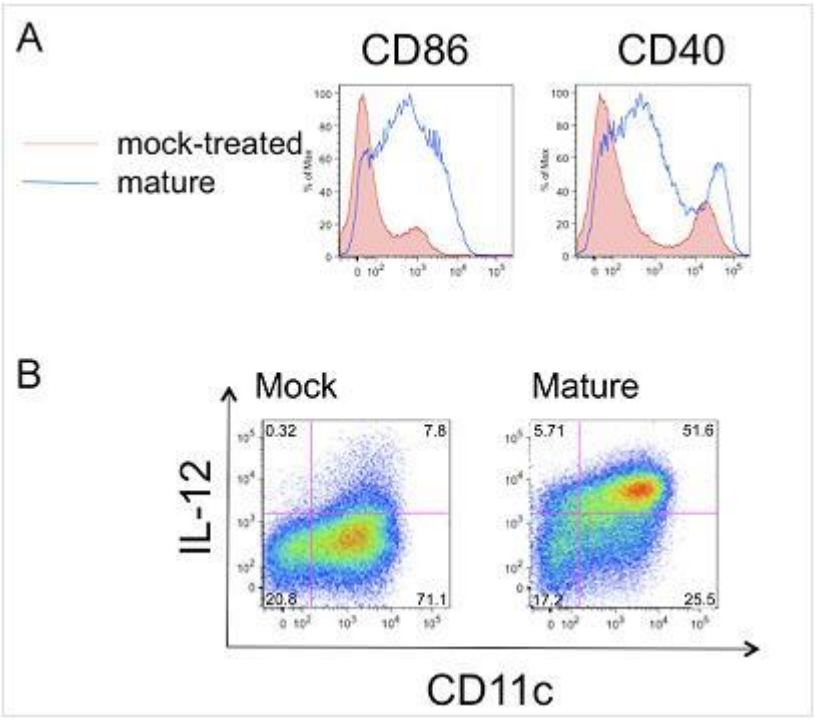

Figure 2

Reagents

1. **DC media**
    1. RPMI-1640 media, supplemented with:
    2. 10% Fetal bovine serum
    3. 100 IU/mL penicillin
    4. 100 µg/mL streptomycin
    5. 1%L-glutamine
    6. 0.1% 2-mercaptoethanol
    7. 1X non-essential amino acids
    8. 1X sodium pyruvate
2. **Recombinant murine GM-CSF**
    1. rmGM-CSF (Peprotech inc, New Jersey, catalogue no. 315-03)

Discussion

DCs are useful for studies of innate and adaptive immune interactions, and can be employed as a vaccine vector. In this video, we have demonstrated the steps to isolate bone marrow and differentiate myeloid dendritic cells in vitro. Following the culture period, these cells can be visualized microscopically both as adherent cells, which often possess dendrites, and non-adherent round cells. The DCs can be further manipulated for antigen presentation by pulsing with antigen or stimulated for cytokine production and costimulatory molecule upregulation using cytokines and/or toll-like receptor ligands (for review, see Gilboa, 2008(2)).

Acknowledgements

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References

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