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The mononuclear phagocyte system is comprised of tissue macrophages, dendritic cells, blood monocytes and their bone marrow (BM) progenitors. Transgenic mouse models have recently provided insight into the biology of monocytes in vivo. However a general method for isolating monocytes from mice is needed to better study their functions.

We describe a rapid and simple method for the enrichment of monocytes from mouse BM and peripheral blood that does not require a density gradient and yields high purity and recovery. BM was harvested from femurs and tibia by crushing the bones. Blood was collected with heparin and red blood cells were removed by ammonium chloride lysis. The monocytes were then enriched using immunomagnetic, column-free negative selection (EasySep®). Briefly, unwanted cells were specifically labeled with dextran-coated magnetic particles using a cocktail of bispecific tetrameric antibody complexes. The sample was placed in a magnet and the supernatant containing unlabeled monocytes was collected. The separation procedure can be automated with a pipetting robot (RoboSep®). Purity of $CD11b^+Ly\text{-}6G^-$ cells as assessed by flow cytometry ranged from 80-93% for BM and 92-98% for blood with recovery of 46 ±11 % (n=38) and 25 ±10 % (n=20) respectively. This protocol will provide easy access to monocytes, enriched from peripheral blood and BM, for further studies of immune and inflammatory responses.

A simple new method for negative enrichment of monocytes from mouse blood and bone marrow

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Introduction

Monocytes are derived from a common myeloid progenitor in the bone marrow (BM). Once released into the peripheral blood they circulate for several days before entering tissues and differentiating to macrophages. Taken together, BM progenitors, blood monocytes and tissue macrophages comprise the mononuclear phagocyte system.

Mouse monocytes are morphologically and phenotypically heterogeneous. Although their study has been facilitated by a recently developed transgenic mouse, no antigenic marker has yet been shown to be uniquely expressed by mouse monocytes. In the blood, they are known to express CD11b, F4/80 and CD115 (M-CSF receptor) but lack expression of other lineage markers (T, B, NK, dendritic cells)[1,2]. Furthermore, monocyte subsets have been defined by their Ly-6C expression. Monocytes newly released from the bone marrow are $Ly\text{-}6C^{hi}$ and Ly-6C expression is down-regulated in the circulation[2].

We describe a rapid and simple method for the enrichment of monocytes from mouse BM and peripheral blood that does not require layering over density gradient. Briefly, monocytes were enriched using immunomagnetic, column-free negative selection (EasySep®). Using this method, unwanted cells were specifically depleted by cross-linking them to magnetic particles using biotinylated antibodies. The sample was placed in a magnet and the supernatant containing unlabeled monocytes was collected. The separation procedure can be automated with a pipetting robot (RoboSep®). Monocytes were defined as $CD11b^+Ly\text{-}6G^-$ and purity was assessed by flow cytometry. This protocol will provide easy access to monocytes, enriched from BM or peripheral blood, for further studies of immune and inflammatory responses.

Methods

Preparation of a single cell suspensions

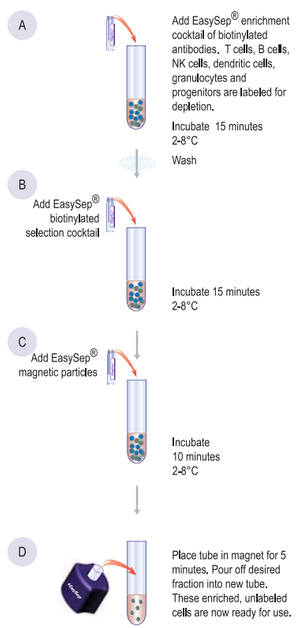
Bone marrow:
Bone marrow was harvested from 6-10 week old C57/Bl6 mice and femur and tibia bones were crushed in PBS+2% FBS and 1mM EDTA using a mortar and pestle. Clumps of cells and debris were removed by passing cell suspension through a 70 µm mesh nylon strainer. Strainer was rinsed with buffer and cells were centrifuged at 300x*g* for 6 minutes. Supernatant was discarded and cells resuspended at $1x10^8$ cells/mL in PBS + 2% FBS and 1mM EDTA with 5% normal rat serum added.

Blood:
Blood was collected into sodium heparin anticoagulant and lysed prior to use. Blood from 6-10 week old C57/Bl6 mice was mixed at a ratio of 1 part blood to 9 parts Ammonium Chloride (Catalog # 07800 or 07850). After incubation on ice for 15 minutes, cells were centrifuged at 300x*g* for 6 minutes. Supernatant was discarded and cell pellet was washed 1x with PBS + 2% FBS and 1mM EDTA. Cells were finally resuspended at $1x10^8$ cells/mL in PBS + 2% FBS and 1mM EDTA with 5% normal rat serum added. On average 4.9 x 10^6 leucocytes were obtained per mL of lysed blood. Typically, $5x10^7$ BM cells or 1-2 x 10^7 blood leucocytes were used for each protocol. The starting cell number range per experiment was 1.0 – 60.0 x 10^7 leucocytes.

Assessing Purity:
Monocytes can express CD11b, F4/80, CD115 (M-CSFR), Gr-1 and Ly-6C. We have defined monocytes here as $CD11b^+Ly\text{-}6G^-$ using CD11b-APC and Ly-6G-PE when evaluating purity by flow cytometry.

Differentiation of enriched cells:
Enriched monocytes derived from BM were plated in MethoCult® 3234 (STEMCELL Technologies) + 10ng/mL M-CSF. Cells were plated at 2000 cells/ 35mm culture dish. At d13 colonies were plucked into 100µL PBS and cytospins made. Slides were stained with May-Grünwald Giemsa.

Figure 1. EasySep® Procedure for column-free cell enrichment



Results

Table 1. Purity and Recovery of $CD11b^+Ly\text{-}6G^-$ monocytes enriched by negative selection from bone marrow or blood using EasySep® or RoboSep®

sample	n	% purity* start	% purity enriched	Average yield of $CD11b^+Ly\text{-}6G^-$ cells
Bone marrow	38	13.2 ± 2.8	88.3 ± 4.1	2.66 x 10^6 per $5x10^7$ total start BM cells
Blood	20	14.9 ± 3.4	94.4 ± 3.0	4.08 x 10^5 per 10^7 total start lysed blood cells**

Values are expressed as means +/- SD
* Purities determined by flow cytometry. All samples gated on viable (PI negative) cells.
** Or approximately 2.0 x 10^5 $CD11b^+Ly\text{-}6G^-$ cells per mL of blood based on an average of 4.9 x 10^6 leucocytes per mL of blood and $1x10^7$ cells used per experiment.

Figure 2. Phenotypic characterization of mouse bone marrow or blood cells before and after enrichment (plots show viable (PI negative) cells)

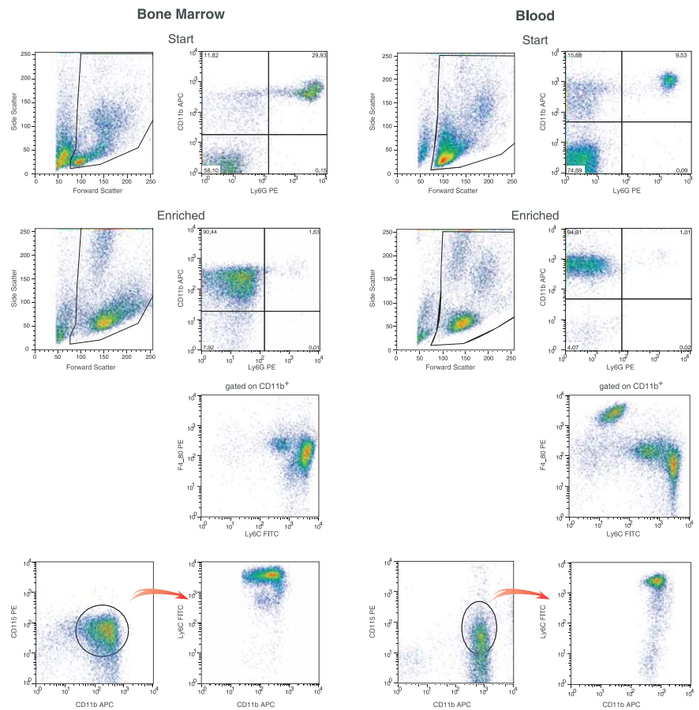
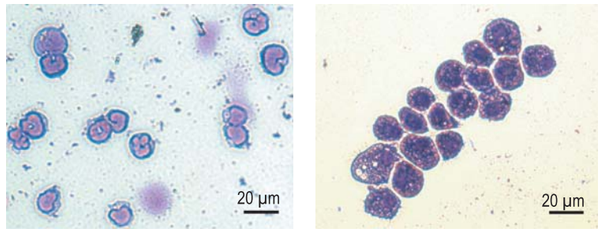


Figure 3. Cytospin preparations stained with May-Grünwald Giemsa showing bone marrow enriched monocytes before and after 13 days of culture in MethoCult® 3234 + M-CSF.



Day 0 Day 13

Conclusions

- No columns are required. Entire procedure takes 60 minutes
- No layering over a density medium is required to achieve high purity, viability (95%) and recovery of monocytes
- Negative selection protocol – target cells are not labeled with antibody
- Enrichment of mouse monocytes can be automated using RoboSep®
- Monocytes enriched from bone marrow and depleted of hematopoietic progenitors will differentiate to macrophages in the presence of M-CSF
- The rapidity and reproducibility of this method will facilitate the aquisition of monocytes from mouse bone marrow or blood for further study

References:
1. F. Geissmann, S. Jung, D. R. Littman, Immunity. 19, 71 (2003)
2. D. Strauss-Ayali, S. M. Conrad, D. M. Mosser, J. Leukoc. Biol. 82, 244 (2007)