Poster #PP-034-18

Isolation of neutrophils from mouse bone marrow and blood using a rapid, column-free negative enrichment method that does not require density centrifugation

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Introduction

Neutrophils are regarded as "professional phagocytes" and act as the immune system's first line of defense. They work alongside mononuclear phagocytes (macrophages, dendritic cells, and monocytes). Their main function is to eliminate deleterious agents while facilitating healing of damaged tissue, however they may also release proinflammatory mediators resulting in chronic inflammation diseases such as arthritis. In mouse they have been defined by their Gr-1(Ly6G/C) expression. Recently it has been shown that inflammatory mouse monocytes share the Gr-1 antigen through expression of Ly6C, but not Ly6G. Therefore it is important that neutrophils be distinguished from other cell types when assessing cell function, such as anti-inflammatory cytokine and chemokine production following innate stimulus.

We describe an immunomagnetic, column-free negative enrichment (EasySep®) of neutrophils from mouse bone marrow (BM) and peripheral blood that does not require a density gradient and yields high purity and recovery of viable cells. This protocol will facilitate studies of immune and inflammatory responses by providing easy access to unlabeled enriched mouse BM and peripheral blood neutrophils.

Conclusions

- No columns are required; the entire procedure takes 60 minutes
- No layering over a density medium is required to achieve high purity, viability (avg. 88%) and recovery of neutrophils
- Negative selection protocol – desired cells are not labeled with antibody
- Enrichment of mouse neutrophils can be automated using RoboSep®
- The rapidity and reproducibility of this method will facilitate the aquisition of neutrophils from mouse bone marrow or peripheral blood for further study

Methods

Bone Marrow:

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

Assessing Purity:

Neutrophils in the BM can express CD11b, Gr-1 and Ly6G. We have defined neutrophils as $CD11b^+Ly6G^+$ using clones M1/70 (CD11b-APC) and 1A8 (Ly6G-PE) when evaluating purity by flow cytometry.

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 (STEMCELL Catalog #07800 or #07850). After incubation on ice for 15 minutes, cells were centrifuged at 300xg for 6 minutes. Supernatant was discarded and cell pellet was washed 1x with PBS +2% FBS and 1mM EDTA. Cells were typically resuspended at 2-6 $x10^7$ cells/mL in PBS +2% FBS and 1mM EDTA with 5% normal rat serum added. On average 4.9 $x10^6$ leucocytes were obtained per mL of lysed blood.

Typically, 5 $x10^7$ BM cells or 1-2 $x10^7$ blood leucocytes were used for each protocol.

FIGURE 1: EasySep® procedure for column-free cell enrichment

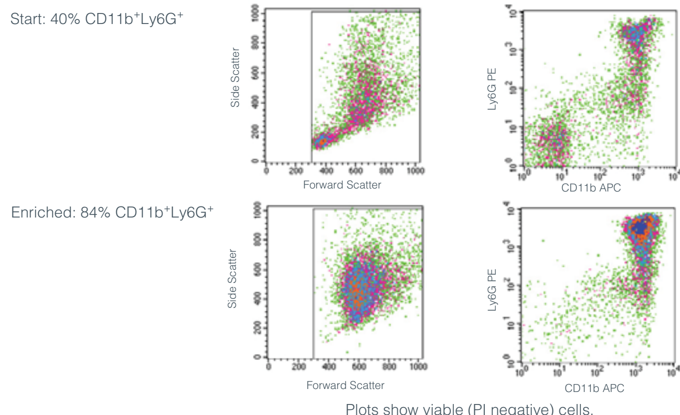
Results

TABLE 1: Purity and recovery of $CD11b^+Ly6G^+$ neutrophils enriched by negative selection from bone marrow and blood using EasySep® or RoboSep®

sample	n	% purity* start	% purity* enriched	yield of $CD11b^+Ly6G^+$ cells
Bone Marrow	15	34 ± 9	86 ± 4	1.0 x 10^7 per 1 x 10^8 total start BM cells
Blood	5	19 ± 3	88 ± 6	4.1 x 10^5 per 10^7 total start lysed blood cells**

*Purities determined by flow cytometry. All samples gated on viable (PI negative) cells and reported as $CD11b^+Ly6G^+$.
**Or approximately 2 $x10^5$ $CD11b^+Ly6G^+$ cells per mL of blood based on an average of 5 $x10^6$ leucocytes per mL of blood and 1 $x10^7$ cells used per experiment.
Values are expressed as means ±SD.

FIGURE 2: Phenotypic characterization of mouse bone marrow cells before and after neutrophil enrichment



Plots show viable (PI negative) cells.

FIGURE 3: Cytospin preparations stained with Wright-Geimsa before and after neutrophil enrichment