MojoSort™, a versatile nanoparticle for magnetic isolation of CX3CR1^{+} microglia with high purity, yield and preserved functionality

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Abstract

Magnetic cell separation is becoming a cost-effective and ubiquitous method of isolating pure and functional cell populations from a variety of tissues. Of particular interest are microglia, colloquially known as the macrophages of the brain, as their role in neurodegenerative diseases is becoming more apparent. Utilizing our novel CX3CR1 antibody in our recently developed MojoSort™ magnetic cell separation system, microglia were isolated with higher purity than with the commonly used CD11b marker in a competitor's kit. Samples were prepared from neonate Balb/C mice brain, and isolated with a CX3CR1 biotinylated antibody and streptavidin nanobeads. Purity was assessed using CD11b and CX3CR1, to define the percentage of CD11b^{+} cells that are truly microglia. Microscope images of cultured positively selected cells indicate the viability and functionality of purified target cells, particularly through the presence/retraction of branches.

Introduction

CX3CR1, also known as fractalkine receptor or G –protein coupled receptor 13, is a chemokine receptor expressed on monocytes, macrophages, dendritic cells and natural killer cells. It is involved in cell recruitment during inflammation and participates in cell adhesion and extravasation from blood vessels. In the CNS, CX3CR1 expression is restricted to resident and activated microglia cells, which allows us to utilize it as a tool for highly specific magnetic isolation. Microglia respond to pathogens and injury by releasing cytokines/chemokines and phagocytosing unwanted cells. In doing so, they take on various forms and characteristics. Notably, ramification, a characteristic of microglia in their quiescent state, are a physical property we can image to confirm viability and adherence. Similarly, the ability of microglia to retract ramifications and enter an "activated form" upon stimulation can be used to identify a fully functional cell. The role of microglia is becoming clear in neurotoxicity, as absence of CX3CR1 leads to dysfunction in microglial response, as well as neuronal death. Isolation of these functional cells will be beneficial in facilitating discovery of mechanisms of communication between neurons and microglia and of factors pertaining to neuroinflammation.

Materials and Methods

Preparation of Brain Microglia

1. Sacrifice post-natal mice 14 days or younger (<P14), adult mice may be used but brain will be more myelinated.
2. Enzymatically digest with trypsin, while dissociating the tissue every minute.
3. Filter through a 70 µm membrane and homogenize the suspension.
4. Remove myelin debris though sucrose, commercial myelin removal kits, or percoll gradients (30/37/70%).
5. Viability achieved is 70-90%.
6. Incubate with biotin anti-mouse CX3CR1 antibody (Cat. No. 149018) followed by one wash.
7. Incubate with MojoSort™ Streptavidin Nanobeads (Cat. No. 480016) followed by one wash.
8. Proceed to magnetic separation using MojoSort™ Magnet (Cat. No. 480019).

Flow Cytometry (Fig. 1)

After isolation, cells were stained with PE anti-mouse/human CD11b antibody (Cat. No. 101207) and APC anti-mouse CX3CR1 antibody (Cat. No. 149007). The dead cells were excluded by 7AAD viability dye. Analysis was done on the BD LSRIIB.

Cell Culture

The isolated CX3CR1^{+} microglia cells were cultured in RPMI 1640 supplemented with 10% FBS and 1% Penicillin/Streptomycin. The cells were then analyzed by microscopy.

Microscopy (Fig. 2a/2b)

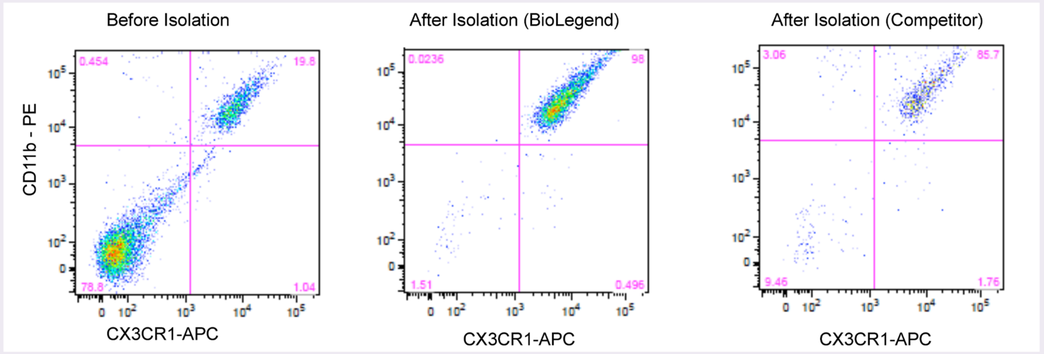
The 12 day cultured cells were fixed with 1% PFA and subsequently stained with Alexa Fluor® 594-anti CD11b and DAPI mounting solution for nuclear staining. Brightfield images were taken post LPS stimulation (100ng/mL) every minute for 90 minutes (Fig. 2b).

Conclusions

1. MojoSort™ CX3CR1 Nanobeads positively isolated mouse microglia can be enriched to 98% purity using MojoSort™ Nanobeads.
2. MojoSort™ CX3CR1 Nanobeads positively isolated mouse microglia yield a purer true microglia population than competitor CD11b Microglia.
3. MojoSort™ CX3CR1 Nanobeads positively isolated microglia are viable and functional.

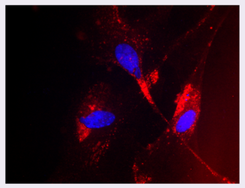
Results

Figure 1. MojoSort™ CX3CR1 Nanobead-isolated microglia shows higher purity than competitor's CD11b positive selection kit



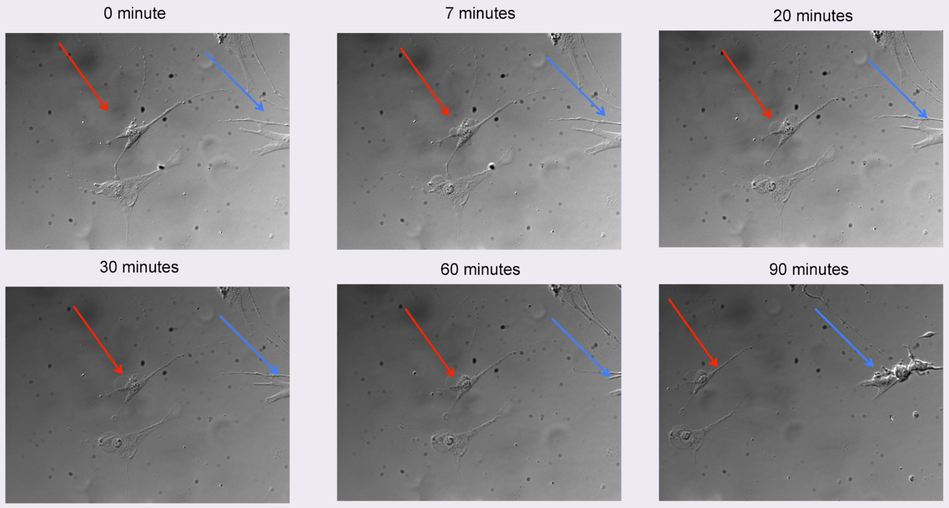
Mouse brain cells before isolation but after preliminary myelin removal (left), BioLegend post-CX3CR1 isolation (middle) and competitor's CD11b kit post-isolation (right). Cells were stained with PE-CD11b, APC-CX3CR1. Dead cells and cell debris were gated out based on scatter signals and 7AAD. Viability of BioLegend's positively selected cells = 95.2%, Yield of BioLegend's positively selected cells = 53.6%, Yield of Competitors positively selected cells = 20.5%.

Figure 2a. MojoSort™ CX3CR1 Nanobead-isolated microglia maintain functionality –Adhere to the glass



Positively isolated CX3CR1 cells were cultured for 12 days. Then the cells were stained with CD11b (red) and DAPI (blue). The image was captured by using 40X objective.

Figure 2b. MojoSort™ CX3CR1 Nanobead-isolated microglia maintain functionality -Retract ramifications upon LPS stimulation



Positively isolated CX3CR1 cells were cultured for 12 days. Then the cells were stimulated with 100 ng/mL of LPS. Brightfield images (40X objective) were captured every minute for 90 minutes, from left to right (0 min, 7 min, 20 min, 30 min, 60 min, 90 min). Red and blue arrows represent two separate cells.