A method for rapid isolation of highly purified monocytes using fully automated negative cell selection

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Summary

Monocytes participate in both innate and acquired immune responses. They are responsible for phagocytosis of foreign substance in the body and are capable of killing infected host cells through antibody dependent cytotoxicity. Moreover, monocytes are able to differentiate to macrophages and dendritic cells (DC), serving as scavenger and antigen presenting cells. The preparation of highly purified monocytes for experimentation has traditionally been difficult, requiring multiple steps and many hours of work. In addition, purity and recovery may be low, and purified monocytes are often activated by cell labeling and cross-linking to magnetic particles. We have developed a rapid negative selection method that enables the preparation of highly purified monocytes from human peripheral blood using EasySep® column-free immunomagnetic cell separation technology. We also optimized the method for use with the RoboSep® fully automated cell separator. Both the manual and fully automated separation procedures took less than 30 minutes and provided average CD14⁺CD16⁻ monocyte purity above 90% combined with average recoveries above 60%. Culture of purified monocytes in the presence of GM-CSF, IL-4 and LPS led to efficient differentiation into dendritic cells (DC) as identified by the expression of the DC markers CD1a and CD83, and the loss of the monocyte marker CD14. Incubation of the differentiated DC with allogeneic CD4⁺ T cells induced robust CD4⁺ T cell proliferation, which further confirmed that the isolated monocytes were fully competent to differentiate into functional DCs. We report here the successful development of a new one-step method for isolating highly purified and functional monocytes.

Methods

Figure 1. Schematic drawing of EasySep® magnetic labeling of human cells

TAC are comprised of two mouse IgG1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG1 monoclonal antibody molecules. One mouse antibody recognizes the specific cell surface antigen while the other recognizes dextran on the EasySep® magnetic particle.

Figure 2. EasySep® procedure for column-free enrichment of human monocytes by negative selection

1. Cell suspension
2. Incubate 10 minutes
3. Place tube in magnet for 2.5 minutes
4. Invert magnet to pour off desired fraction into a new tube. The magnetically labeled unwanted cells remain bound in the tube by the magnetic field.
5. Obtain purified cells in less than 30 minutes.

Figure 3. Fully automated enrichment of human monocytes using RoboSep®

Robotic arm: Uses 1 mL tips to add reagents and 5 mL tips to add buffer and transfer or mix the cell sample
Carousel: Each quadrant holds a magnet, sample, tip rack and reagents. This enables up to 4 samples to be separated at once.
Negative fraction tube: Supernatant is transferred to negative fraction tube after magnetic separation step.
Sample tube: Standard 14 mL tube loaded directly onto the carousel
Magnet and separation tube: Sample transferred to separation tube after magnetic labeling is complete
Reagent vials: Instrument calculates reagent addition volume based on sample volume.
Tip racks: Each disposable rack contains sterile tips to process a single sample. Tip rack segregates tips to avoid cross contamination.
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Results

Table 1. Results obtained using EasySep® and RoboSep® technology for human CD14+ CD16- monocyte enrichment from previously frozen mononuclear cells

| %CD14+CD16- Cells | Recovery from start Start EasySep® Enriched EasySep® RoboSep® EasySep® RoboSep® |
|--------------------|-------------------------------|-----------------|-----------------|-----------------|
| AVERAGE (n=7)      | 19.8                         | 90.1            | 90.1            | 63              | 71              |
| SD                 | 8.0                          | 3.4             | 4.6             | 19              | 21              |

*Mononuclear cells obtained from peripheral blood Leuko Paks collected from normal donors using an apheresis machine were further processed using density centrifugation and cryopreserved in FBS containing 7.5% DMSO prior to use.

Conclusions

- Functional monocytes can be isolated in single step using column-free immunomagnetic EasySep® technology.
- Highly purified cells can be obtained in less than 30 minutes.
- The method can be fully automated using the RoboSep® cell separator.