A simple and rapid method for the enrichment of mouse naïve CD4+ T cells from spleen

 Vesna Posarac, Armon Molavi, Karina McQueen, and Terry E. Thomas
 STEMCELL Technologies Inc., Vancouver, BC, Canada

Abstract

Naïve CD4+ T cells are a mature subset of CD4+ T cells with no previous antigen exposure. The CD62L<sup>hi</sup> CD4<sup>+</sup> naïve phenotype cells circulate throughout the secondary lymphoid organs where they become activated by foreign antigens presented on MHC class II molecules. Activation is marked by phenotypic changes (down- and up-regulation of CD62L and CD44, respectively), proliferation, and acquisition of effector functions. Current protocols for the isolation of naïve CD4+ T cells are time-consuming and require the use of columns. We have developed a one-step, column-free, immunomagnetic cell separation (EasySep<sup>™</sup>) method for the isolation of naïve CD4+ T cells from single-cell suspensions of splenocytes. Non-CD4+ T cells, regulatory cells, and memory CD4+ T cells are targeted for depletion using biotinylated antibodies cross-linked to streptavidin-coated magnetic particles. The labeled cells are separated using an EasySep<sup>™</sup> magnet and the desired naïve CD4+ T cell fraction is poured off. The entire protocol is performed in 15 minutes and can be fully automated using RoboSep<sup>™</sup>. The average purities and recoveries of CD4<sup>+</sup>-CD62L<sup>hi</sup> CD4<sup>+</sup> naïve cells are 93.1 ± 2.1% and 28.8 ± 7.6%, respectively (n=22). This new method will be invaluable to researchers studying T cell differentiation, signaling pathways, and immune response to infectious disease.

Methods

Preparation of Starting Cell Suspension

To prepare a single-cell suspension, spleens were disrupted in phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS). The cells were centrifuged at 300 x g for 10 minutes and resuspended at 1x10<sup>6</sup> cells per ml in PBS + 2% FBS with 5% normal rat serum.

EasySep™ Labeling of Mouse Cells

Unwanted cells, including CD4<sup>+</sup> T, regulatory cells, and CD44<sup>+</sup> CD4<sup>+</sup> T cells are specifically labeled with biotinylated antibodies cross-linked to streptavidin-coated magnetic particles. The magnetically labeled cells are then separated from unlabeled cells using an EasySep™ magnet (Figure 1), and the desired naïve CD4+ T cell fraction is poured off.

FIGURE 1: EasySep™ procedure for column-free enrichment of naïve CD4+ T cells from mouse splenocytes

Start: 14.3% CD4<sup>+</sup>-CD62L<sup>hi</sup>-CD44<sup>+</sup> viable cells

Enriched: 94.7% CD4<sup>+</sup>-CD62L<sup>hi</sup>-CD44<sup>+</sup> viable cells

This procedure can be fully automated using RoboSep™.

Purity Assessment

EasySep™-isolated naïve CD4+ T cells were assessed by flow cytometry after staining with CD44 FITC, CD62L PE, CD4 APC, and 7-AAD. Naïve CD4+ T cells are CD4<sup>+</sup>-CD62L<sup>hi</sup>-CD44<sup>+</sup> (Figure 2).

Results

TABLE 1: Purity and recovery of naïve CD4+ T cells enriched from mouse splenocytes by EasySep™ or RoboSep™

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>% Purity</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasySep™</td>
<td>16</td>
<td>93.8 ± 1.6</td>
<td>29.6 ± 7.8</td>
</tr>
<tr>
<td>RoboSep™</td>
<td>6</td>
<td>91.2 ± 2.4</td>
<td>26.8 ± 7.5</td>
</tr>
</tbody>
</table>

Purities were determined by flow cytometry. All samples gated on viable (7-AAD<sup>-</sup>) cells. Naïve CD4+ T cells are defined as CD4<sup>+</sup>-CD62L<sup>hi</sup>-CD44<sup>+</sup>. Values are expressed as means ± 1 SD.

FIGURE 2: Flow cytometric assessment of naïve CD4+ T cells before and after enrichment using EasySep™

TABLE 2: Comparison of naïve CD4+ T cell isolation protocols using EasySep™/RoboSep™ or the column-based competitor kit

<table>
<thead>
<tr>
<th></th>
<th>EasySep™</th>
<th>RoboSep™</th>
<th>Competitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total time</td>
<td>15 min</td>
<td>22 min</td>
<td>1 hr 50 min</td>
</tr>
<tr>
<td>Columns</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Centrifugations</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Isolation method</td>
<td>untouched</td>
<td>untouched</td>
<td>positive selection</td>
</tr>
</tbody>
</table>

Conclusions

- Isolate naïve CD4+ T cells from mouse splenocytes in 15 minutes.
- Naïve CD4+ T cell isolation can be fully automated with RoboSep™.
- Average purities and recoveries for naïve CD4+ T cell enrichments are 93.1% ± 2.1% and 28.8% ± 7.6%, respectively.