A single-step enrichment method for generating highly purified mouse NK cell populations by negative selection

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Summary

Natural Killer (NK) cells play a critical role in innate immunity and tumor immunology. They are particularly difficult to isolate because they are rare and express several markers found on other cell types. Using column-free immunomagnetic cell separation technology (EasySep\(^TM\)), we sought to develop a single-step negative selection method for NK cell isolation, where negative selection enables the isolation of cells with minimal impact on cell function. Unwanted cells were cross-linked to magnetic particles using a highly optimized cocktail of 10 biotinylated antibodies. This enabled us to achieve NK cell purities of 92.3 ± 2.6% (n=6; defined as %CD49b⁺CD3\(^-\))]. The isolated cells retained the expression of several markers associated with an NK phenotype, including NKG2D and Ly49Chi/Fi/F. The purified cells could be readily expanded when cultured in the presence of IL-2 (> 8-fold expansion after 5 days). \(^5\)Cr release assays demonstrated the cytotoxicity capacity of the isolated cells, and NK-specific killing was confirmed using both NK-sensitive and NK-resistant cell lines. In conclusion, we present here a novel and unique enrichment method for the isolation of pure NK cells in a single step.

Methods

Mouse cells are targeted for immunomagnetic depletion by first labeling the cells with a cocktail of anti-cell biotinylated antibodies, while cells of interest are not targeted for depletion. Labeled (unwanted) cells are then crosslinked to EasySep\(^TM\) magnetic particles. The tube containing the labeled cell suspension is placed directly in the specially designed EasySep\(^TM\) Magnet. This light, handheld magnet is gently inverted to remove the cells that are not bound to the magnetic particles and the desired cells are poured off into a new tube.

Figure 1. EasySep\(^TM\) procedure for negative selection of mouse cells

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Figure 2. Schematic drawing of EasySep\(^TM\) magnetic labelling of mouse cells

Tetrameric Antibody Complexes are comprised of two mouse IgG1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG antibody molecules. One mouse antibody recognizes b2m while the other recognizes dextran on the EasySep\(^TM\) magnetic particles.

Figure 3. Isolation of NK cells using the EasySep\(^TM\) Mouse NK Cell Enrichment Kit

NK cells were isolated from splenocytes of either C3H/HeJ or BALB/c mice. Red blood cells were initially separated from splenocytes by Ficoll-Paque\(^TM\). NK cells were then purified using the EasySep\(^TM\) Mouse NK Cell Enrichment Kit (STEMCELL Technologies). NK purity was assessed by surface staining with DX5-PE and CD3-PeCy5 and detection using flow cytometry.

Figure 4. Isolated NK cells proliferate and expand in number when cultured in IL-2 supplemented medium

Isolated NK cells were cultured in complete media (RPMI 1640 supplemented with 10% fetal bovine serum containing penicillin, streptomycin, glutamine and [l-mercaptoethanol]) supplemented with 1000 IU/mL IL-2 for 5 days. NK cell number was determined by viability counting with Trypan Blue. Values are expressed as a ratio of the number of cells obtained after culture relative to the number plated cells on day 0, and are averages of 3 experiments with error bars indicating standard deviation from the mean.

Figure 5. Culture-expanded NK cells display NK cell-mediated cytotoxicity and target specificity

Isolated NK cells were cultured in complete media supplemented with 1000 IU/mL IL-2 for 5 days. Activated NK cells were then used as effectors in a 4-hour \(^5\)Cr release assay using either the NK-sensitive YAC-1 line or NK-resistant P815 line.

Figure 6. Isolated NK cells retain their polyclonal nature following expansion

Isolated NK cells were cultured in media supplemented with 1000 IU/mL IL-2 for 5 days. NK cell surface receptor expression profiling was performed by staining with fluorochrome conjugated anti-NKG2D, CD69, CD11b, Ly49C/Fi/F, NKG2A/C/E, and Ly49A, and detection using flow cytometry analysis. Similar results were obtained in three separate experiments.

Figure 7. Expanded NK cells produce IFN-\(\gamma\) and TNF-\(\alpha\) when stimulated with PMA and ionomycin

Purified NK cells were cultured in media supplemented with 1000 IU/mL IL-2 for 5 days. Activated NK cells were stimulated in ionomycin and phorbol 12-myristate 13-acetate (PMA) overnight to induce IFN-\(\gamma\) and TNF-\(\alpha\) production. The cells were intracellularly stained with IFN-\(\gamma\)-AlexaFluor and TNF-\(\alpha\)-PE and analyzed by flow cytometry.

Conclusions

- Mouse NK cells can be highly purified in a single step using the EasySep\(^TM\) Mouse NK Cell Enrichment Kit.
- The purified cells can be expanded in culture while retaining the phenotypic and functional properties of mouse NK cells.