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®), 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^{neg}). The isolated cells retained the expression of several markers associated with an NK phenotype, including NKG2D and LY49C/I/F/H. The purified cells could be readily expanded when cultured in the presence of IL-2 (> 8-fold expansion after 5 days). ^{51}Cr release assays demonstrated the cytotoxic 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

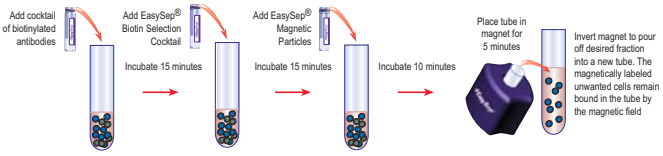

Figure 1. EasySep® procedure for negative selection of mouse cells

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® magnetic particles. The tube containing the labeled cell suspension is placed directly in the specially designed EasySep® 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.

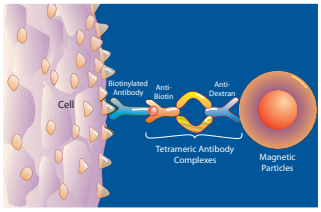

Figure 2. Schematic drawing of EasySep® magnetic labelling of mouse cells

Tetrameric Antibody Complexes are comprised of two mouse IgG_1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG_1 antibody molecules. One mouse antibody recognizes biotin while the other recognizes dextran on the EasySep® magnetic particles.

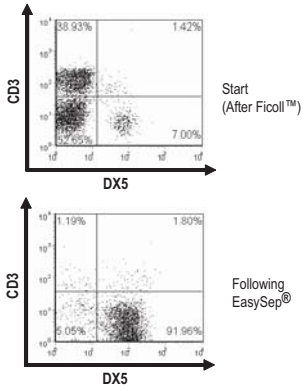

Figure 3. Isolation of NK cells using the EasySep® Mouse NK Cell Enrichment Kit

NK cells were isolated from splenocytes of either C57BL/6 or BALB/c mice. Red blood cells were initially separated from splenocytes by Ficoll-Paque™. NK cells were then purified using the EasySep® 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.

Results

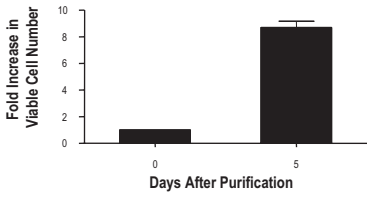

Figure 4. Isolated NK cells proliferate and expand in number when cultured in IL-2 supplemented medium

Isolated NK cells were cultured in complete media (RPMI 1640 supplemented with 10% fetal bovine serum containing penicillin, streptomycin, glutamine and β-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.

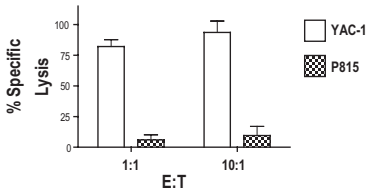

Figure 5. Culture-expanded NK cells display NK cell-mediated cytotoxicity and target specificity

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 ^{51}Cr release assay using either the NK-sensitive YAC-1 line or NK-resistant P815 line.

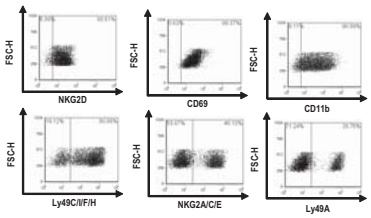

Figure 6. Isolated NK cells retain their polyclonal nature following expansion

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/I/F/H, NKG2A/C/E, and Ly49A, and detection using flow cytometry analysis. Similar results were obtained in three separate experiments.

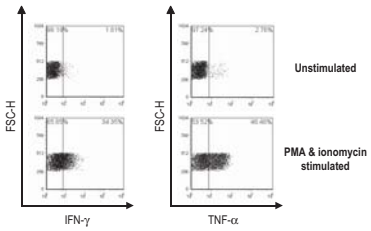

Figure 7. Expanded NK cells produce IFN-γ and TNF-α when stimulated with PMA and ionomycin

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-γ and TNF-α production. The cells were intracellularly stained with IFN-γ-AlexaFluor and TNF-α-PE and analyzed by flow cytometry.

Conclusions

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

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