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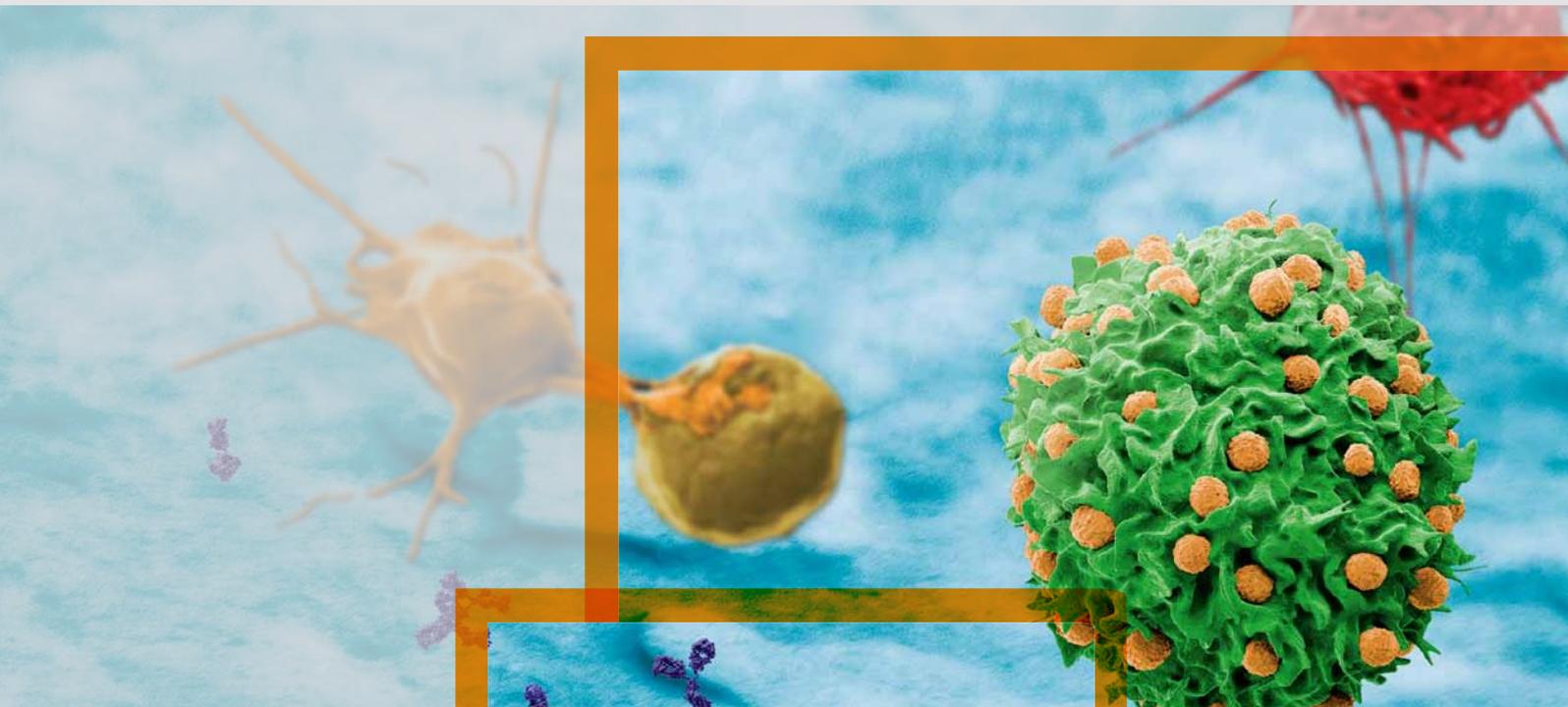
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Mouse NK cells isolated to high purity by MACS® Technology are fully functional



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Introduction

NK cells play a crucial role in both innate and adaptive immunity. Due to their capacity to lyse tumor cells without pre-activation, they hold great potential for use in cellular therapies¹. To investigate the function of NK cells in detail, it is crucial to work with cell populations of high purity. In this study, we compared two different strategies for NK cell isolation based on MACS® Technology: i) positive selection of NK cells according to the expression of CD49b (DX5) and ii) depletion of non-NK cells. Both approaches yielded NK cells with high purities from BALB/c mouse spleen. We further optimized the procedure for the depletion of non-NK cells, allowing for the isolation of highly pure NK cells also from C57BL/6 mouse spleen. Isolated NK cells were fully functional in terms of IFN- γ secretion and tumor lysis capacity.

Materials and methods

Sample preparation

Spleens from female, 7–9-week-old C57BL/6 and BALB/c mice were dissociated into single-cell suspensions as described².

Isolation of NK cells

CD49b (DX5)⁺ NK cells were isolated by positive selection using CD49b (DX5)

MicroBeads, mouse (Miltenyi Biotec) in combination with the autoMACS® Pro Separator (Miltenyi Biotec) and the program “Posseld” according to the standard protocol. Untouched NK cells were isolated using the NK Cell Isolation Kit, mouse (Miltenyi Biotec) and the autoMACS Pro Separator with program “DepleteS”. We further improved the depletion cocktail included in this kit to allow

for a more effective depletion of residual T cells, B cells, and macrophages. This optimized cocktail is now part of the protocol of the new NK Cell Isolation Kit II from Miltenyi Biotec. For NK cell isolation from C57BL/6 spleens, we recommend incubation periods of 10–15 minutes with antibodies and MicroBeads. Purified cell populations were subsequently analyzed by flow cytometry.

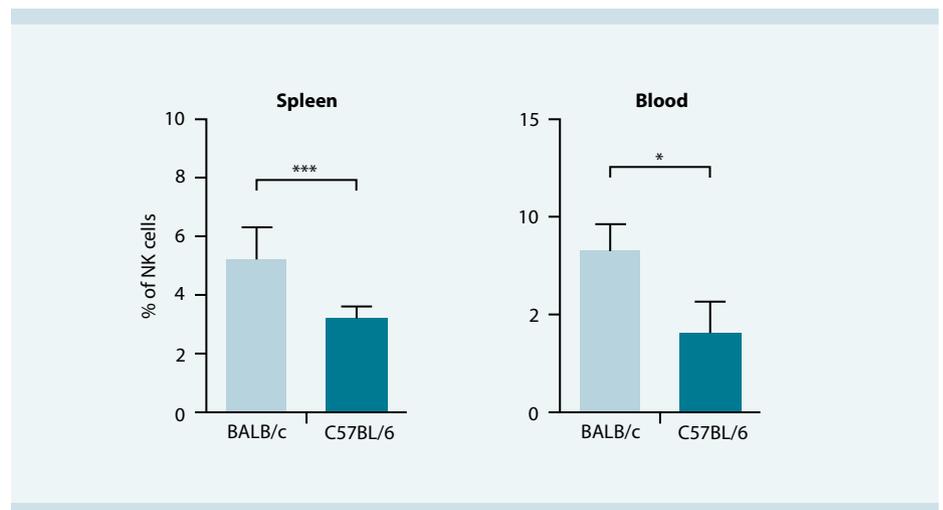


Figure 1 Frequencies of NK cells in spleen and peripheral blood from two different mouse strains. NK cells from spleens (n=7) and blood (n=4) from BALB/c and C57BL/6 mice were analyzed by flow cytometry with gating on viable CD49b⁺NKp46⁺ cells. *** $p=0.0006$, * $p=0.0286$. Data were adapted from reference 2.

Analysis of cytokine production by isolated NK cells

To assess the function of isolated NK cells, we determined their capacity to secrete IFN- γ . To this end, the cells were stimulated overnight with IL-2 (50,000 U/mL). The next day, cell culture supernatants were analyzed using the BD[®] OptEIA[™] Mouse IFN- γ ELISA Set (BD Biosciences).

Tumor lysis assay

The capacity of isolated NK cells to lyse tumor cells was analyzed by using the crystal violet assay as described previously¹. Briefly, 5,000 tumor cells were plated in 96-well plates and cocultured with isolated NK cells at an effector:target ratio of 10:1. To avoid MHC mismatch effects, we used B16F10 melanoma cells together with NK cells from C57BL/6 mice, and CT26 colon carcinoma cells with NK cells from BALB/c mice. The tumor cells were originally derived from the same background as the corresponding NK cells.

Results and discussion

NK cell frequencies in spleen and peripheral blood from different mouse strains

To gain insight into the overall NK cell content of mouse spleen and peripheral blood from the mouse strains C57BL/6 and BALB/c, we analyzed the frequency of CD3⁻CD49b⁺NKp46⁺ NK cells by flow cytometry. Figure 1 indicates that the percentage of NK cells in both spleen and blood from BALB/c mice was approximately twice as high as from C57BL/6 mice. There were no significant differences in the proportions of CD27⁺CD11b⁻, CD27⁺CD11b⁺, and CD27⁻CD11b⁺ NK cell subsets.²

Magnetic isolation of splenic NK cells

For detailed characterization of NK cells, in particular functional analysis, it is crucial to use cell separation methods that allow for high cell purities. Here we compared different strategies based on MACS Technology for the isolation of NK cells from BALB/c and C57BL/6 mice. The first strategy involved positive selection of NK cells using CD49b (DX5) MicroBeads, based on the expression of the CD49b antigen. The second approach utilized depletion of non-NK cells using the NK Cell Isolation Kit, resulting in the isolation of untouched NK cells. Figure 2 shows that both approaches allowed

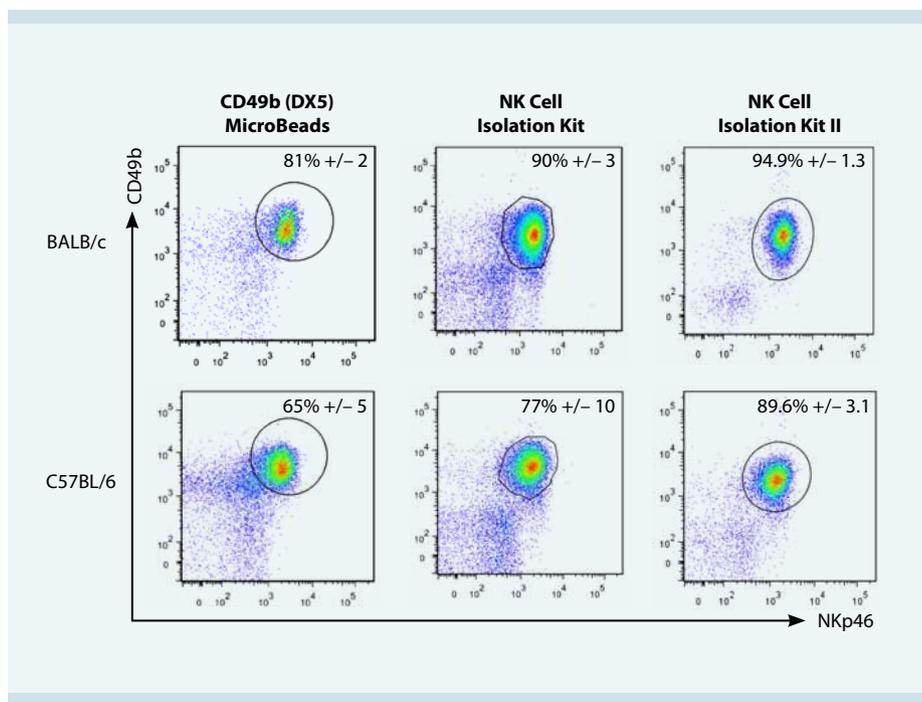


Figure 2 Isolation of splenic NK cells from BALB/c and C57BL/6 mice using different strategies based on MACS Technology. Cells were isolated as described in the materials and methods section. Subsequently, CD49b (DX5)⁺NKp46⁺ cells were analyzed by flow cytometry. Numbers indicate mean purities and standard deviations from at least five independent experiments. Data were adapted from reference 2.

for efficient isolation of NK cells from BALB/c mice with purities of up to 90%, whereas cells from C57BL/6 mice reached lower purities of up to 77%. This led us to modify the depletion

cocktail to achieve a more effective removal of residual T cells, B cells, and macrophages. Strikingly, the new depletion cocktail, i.e., NK Cell Isolation Kit II, resulted in high-purity NK

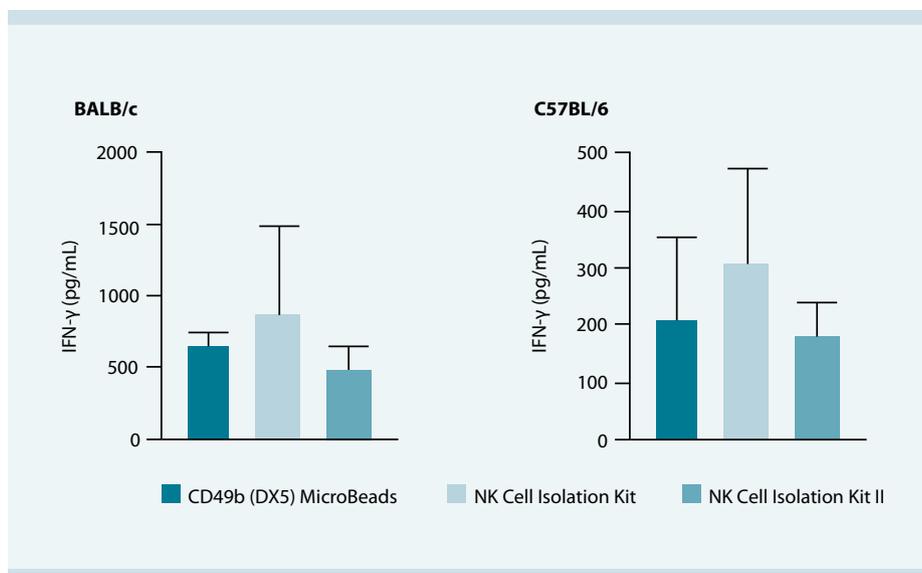


Figure 3 IFN- γ production by isolated NK cells. Cells were stimulated with IL-2 (50,000 Units/mL) overnight. Cell culture supernatants were analyzed by an IFN- γ -specific ELISA. One representative experiment of at least three independent experiments is shown. Experiments were performed in triplicates. Data were adapted from reference 2.

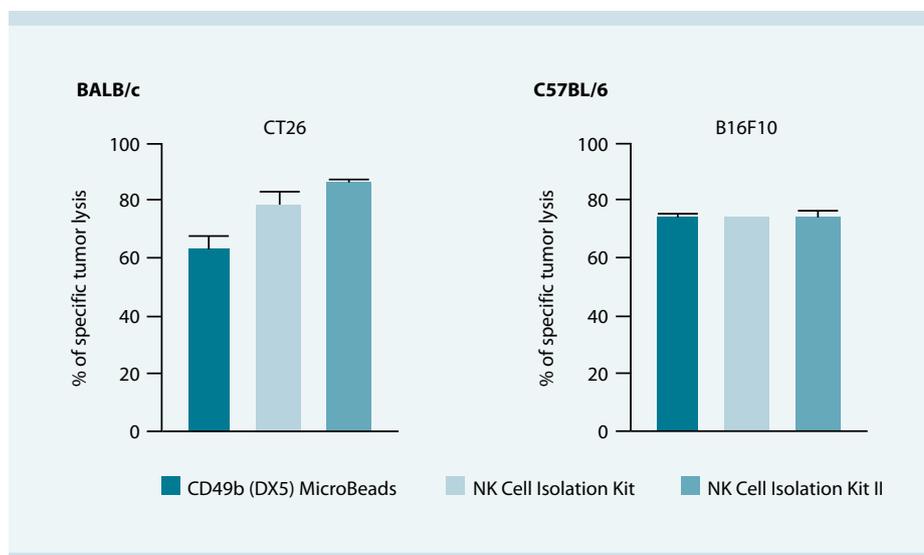


Figure 4 Tumor lysis assay. For the crystal violet assay, 5,000 tumor cells were plated in 96-well plates and cocultured with isolated NK cells at an effector: target ratio of 10:1. One representative experiment of at least three independent experiments is shown. Experiments were performed in triplicates. Data were adapted from reference 2.

cells from both C57BL/6 (approx. 90%) and BALB/c spleens (approx. 95%). Importantly, the subset distribution of CD27⁺CD11b⁻, CD27⁺CD11b⁺, and CD27⁻CD11b⁺ cells remained unaltered, regardless of whether the cells were isolated by positive selection or depletion (not shown).

Functional analysis of isolated NK cells

Next we tested whether the isolated cells retained their full functionality, i.e., the capacity to produce cytokines in response to stimulation with IL-2. Our results indicate that both BALB/c and C57BL/6 NK cells from all three isolation procedures secreted IFN- γ (fig. 3). NK cells prepared with the NK Cell Isolation Kit II showed slightly reduced IFN- γ production compared to the other approaches. This may be due to the higher NK cell purity in these preparations and thus a lower proportion of contaminating cells that might influence the IFN- γ production.

Moreover, we analyzed the isolated NK cells for their capacity to lyse tumor cells. Our results from the crystal-violet assay show that BALB/c and C57BL/6 NK cells effectively lysed CT26 colon carcinoma and B16F10 melanoma cells, respectively (fig. 4).

Conclusion

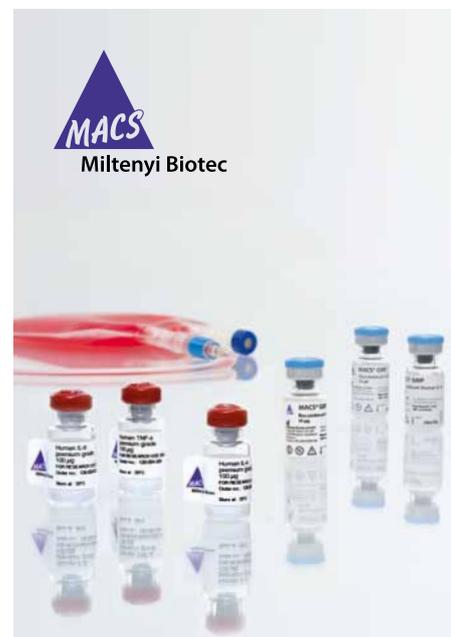
- Both CD49b (DX5) MicroBeads and the NK Cell Isolation Kit allow for the isolation of NK cells from BALB/c mouse spleens with high purity.
- The new NK Cell Isolation Kit II yields highly pure NK cells from BALB/c mice and represents the optimal solution for the isolation of C57BL/6 NK cells.
- Isolated NK cells are fully functional regardless of whether they were isolated by positive selection or depletion.

References

1. Terme, M. *et al.* (2008) *Nat. Immunol.* 9: 486–494.
2. Meinhardt, K. *et al.* (2012) *J. Immunol. Methods* 378: 1–10.

MACS Product*	Order no.
autoMACS Pro Separator – Starter Kit	130-092-545
CD49b (DX5) MicroBeads, mouse	130-052-501
NK Cell Isolation Kit II, mouse	130-096-892

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