Analysis of T cells made easy – Complete workflows improve the isolation and analysis of T cells

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Introduction

T cells play a central role in immunity to pathogens and tumors, but also in autoimmunity. T cell research has generated a vast body of knowledge that led to multiple clinical breakthroughs, such as the generation of T cell vaccination and tumor immunotherapy approaches. However, additional work is required to fully understand T cell biology, harness the cells' therapeutic potential, and control immunopathologies. Cutting-edge experimental protocols that help to explore and manipulate T cell biology often require prior purification of T cell populations. Strategies to simplify and accelerate T cell purification are highly desirable to save time, reduce bias in functional assays, and allow complex experiments to be performed.

Methods

1 Workflow for the isolation of CD90.1$^+$ and CD90.2$^+$ T cells from mouse spleen

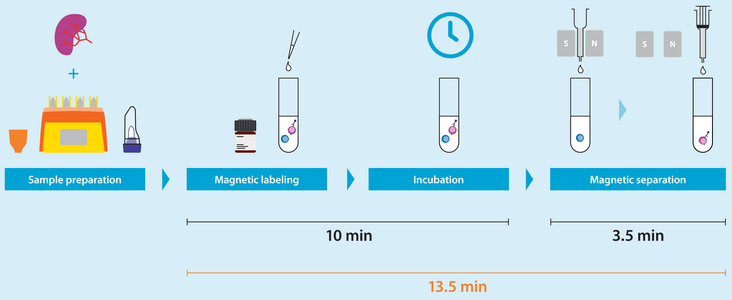


Figure 1

Results

1 Fast and reliable magnetic enrichment of CD90.1$^+$ or CD90.2$^+$ T cells from mouse spleen

Mouse spleens were automatically dissociated using the gentleMACS™ Octo Dissociator to obtain viable single-cell suspensions. To facilitate a fast isolation of T cells from these suspensions, we developed a new type of MACS® MicroBead reagents (CD90.1 MicroBeads and CD90.2 MicroBeads) and a short protocol that does not require a washing step after magnetic labeling. To evaluate purification, cells were labeled with fluorochrome-conjugated antibodies, CD3 (clones REA641, REA606), CD90.1 (REA838) or CD90.2 (30-H12), CD4 (REA604), CD8a (REA601), and CD45 (REA737) as well as 7-AAD before and after cell separation. Frequencies of CD90.1$^+$ or CD90.2$^+$ T cells in unseparated versus purified cells (among live, CD45$^+$ leukocytes) are shown (fig. 2A). Results for the purification of CD90.2$^+$ cells were similar for C57BL/6 and BALB/c mice. To isolate CD90.1$^+$ T cells, FVB/N mice were used. Low numbers of splenocytes collected from a CD90.1$^+$ mouse were added to a single-cell suspension obtained after dissociating a spleen from a CD90.2$^+$ mouse (fig. 2B, top) and vice versa (fig. 2B, bottom). Subsequently, the rare, added T cell populations were isolated by the new CD90.1 or CD90.2 MicroBeads. Frequencies of CD90.1$^+$ or CD90.2$^+$ T cells in unseparated versus purified cells are shown. The new type of MicroBeads enabled the isolation of target cells from low initial percentages to high purity.

A

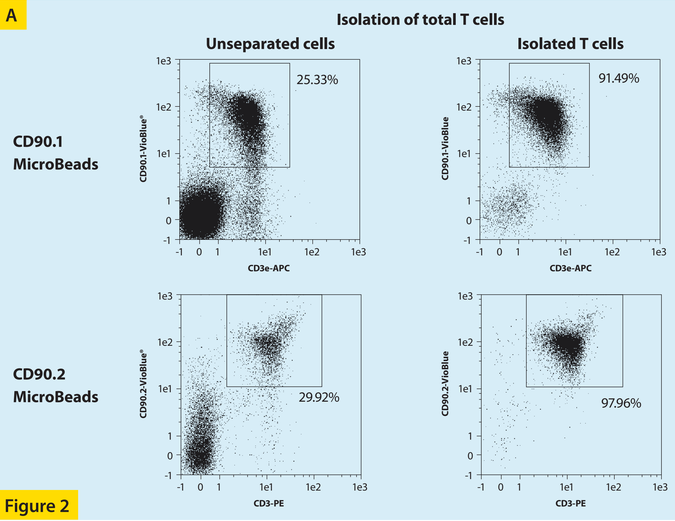


Figure 2

B

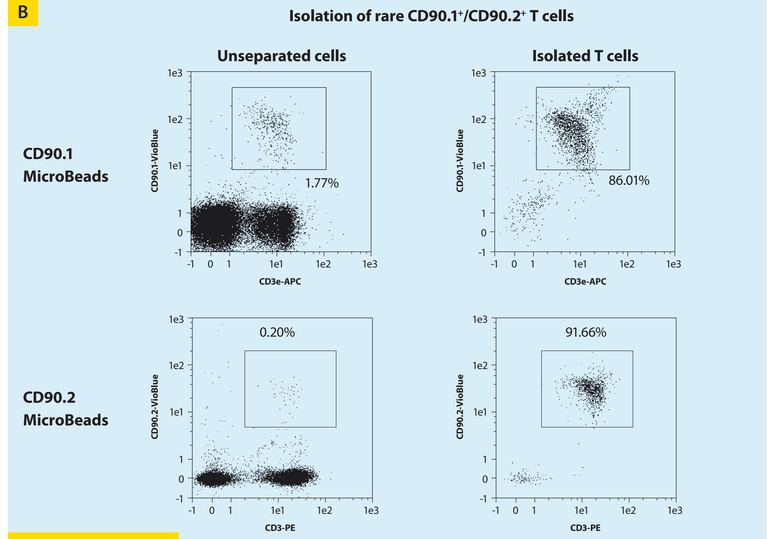


Figure 2 (continued)

2 Isolation of CD90.1$^+$ and CD90.2$^+$ T cells retained their naive state

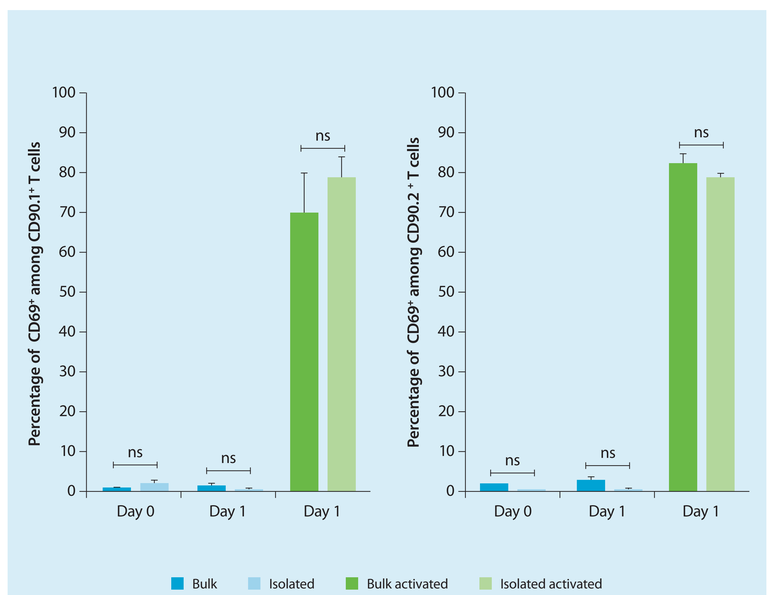


Figure 3

Bulk spleen cells or T cells isolated from naive mice using the new CD90.1 or CD90.2 MicroBeads were cultured unstimulated in complete medium overnight at 37 °C. As a control, bulk and isolated cells were activated with the T Cell Activation/Expansion Kit, mouse. The percentage of cells expressing the activation marker CD69, among CD90.1$^+$ or CD90.2$^+$ T cells, was analyzed prior to culture (day 0) and after overnight culture (day 1). Cells were gated on viable CD45$^+$ leukocytes and CD90.1$^+$ or CD90.2$^+$ cells. Statistical analysis was performed using 2-way ANOVA with Sidak's multiple comparisons test (means±SD; day 1: two independent samples for two experiments performed in triplicates; day 0: two independent samples; ns: non-significant).

3 Positively selected T cells retained their proliferation and cytokine expression capacity

T cells were isolated by positive selection using CD90.1 MicroBeads or by depletion of unwanted cells using the Pan T Cell Isolation Kit II, mouse, respectively. Cells were activated using the T Cell Activation/Expansion Kit, mouse and cultured for 7 days. On day 0 and day 7 the number of CD90.1$^+$ T cells and the total expansion on day 7 were determined (fig. 4A; means±SD; two independent experiments performed in triplicates).

CD90.2$^+$ T cells were isolated using CD90.2 MicroBeads and activated using the T Cell Activation/Expansion Kit, mouse and cultured for 2 days. Subsequently, cells were restimulated with PMA/ionomycin and brefeldin A for 4 hours and cytokine expression was assessed by intracellular staining and flow cytometry analysis (fig. 4B; means±SD; two independent experiments performed in triplicates).

A

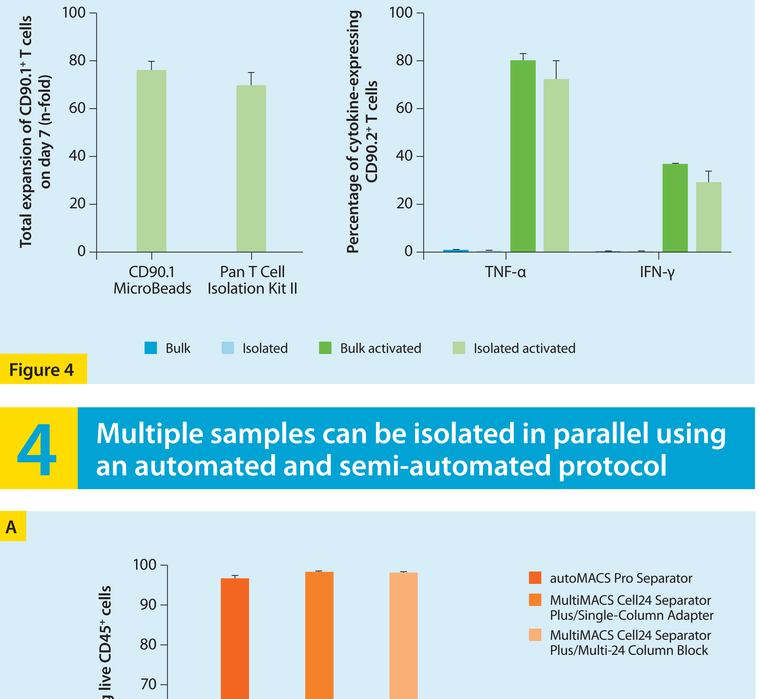
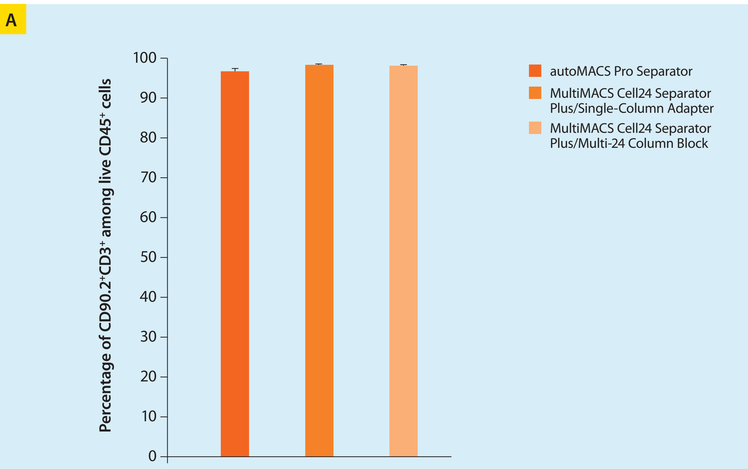


Figure 4

4 Multiple samples can be isolated in parallel using an automated and semi-automated protocol

A



B



Figure 5

T cells were isolated using CD90.2 MicroBeads (fig. 5A), automatically on an autoMACS® Pro Separator (fig. 5B, right) or semi-automatically on a MultiMACS™ Cell24 Separator Plus (fig. 5B, left) using a Single-Column Adapter or a Multi-24 Column Block.

Conclusion

- We established workflows combining automated tissue dissociation with T cell isolation.
- We developed new separation reagents specific for CD90.1$^+$ and CD90.2$^+$ T cells. The reagents enabled a significantly accelerated isolation of target cells to high purities, even when initial cell frequencies were very low, e.g., after adoptive transfer experiments.
- Isolated T cells retained their phenotype and were functional.
- T cell isolation could be automated, and multiple samples could be processed in parallel.

Our new workflow greatly reduces time required for downstream analysis while preserving cell phenotype and functional properties. We believe that these innovative tools significantly shorten otherwise time-consuming experiments and can be used to increase reproducibility and the quality of data obtained in T cell research.

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