Analysis of T cells made easy – complete workflow improves the isolation and analysis of T cells

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Introduction

T cells play a central role in immunity against 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 development of T cell vaccination and tumor immunotherapy approaches. However, additional work is required to fully understand T cell biology, harness their 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 CD4⁺ and CD8a⁺ T cells from mouse spleen

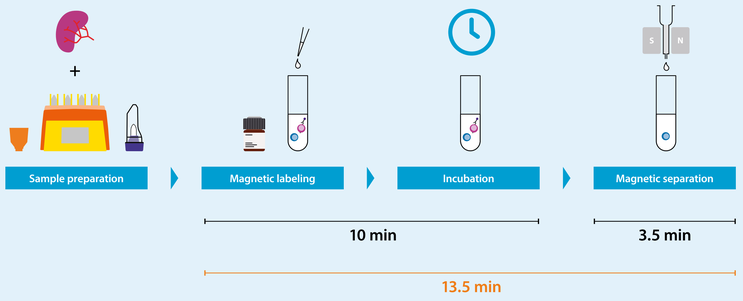

Figure 1

Results

1 Fast and reliable magnetic enrichment of CD4⁺ and CD8a⁺ T cells from mouse spleen

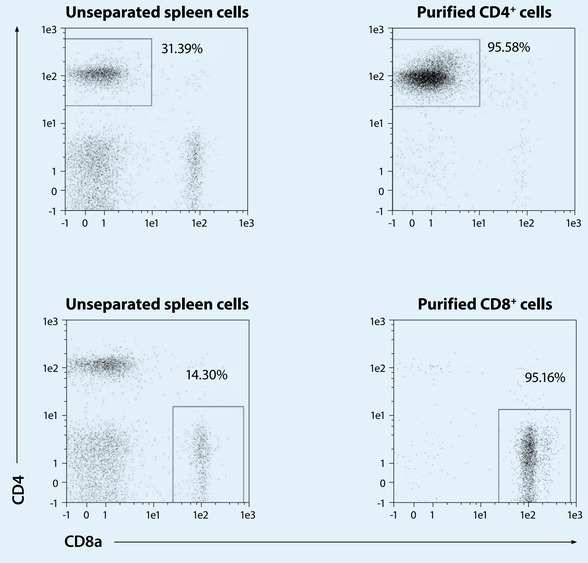

Figure 2

BALB/c mouse spleens were automatically dissociated using the gentleMACS™ Octo Dissociator to obtain viable single-cell suspensions. To facilitate a fast isolation of CD4⁺ and CD8⁺ T cells from these suspensions, we developed a new type of MACS® MicroBead reagents (CD4 (L3T4) MicroBeads and CD8a (Ly-2) MicroBeads) and a short protocol that does not require a washing step after magnetic labeling. To evaluate purification, cells were labeled with CD4-VioBlue®, CD8a-PE-Vio® 770, CD45-VioGreen™, and 7-AAD before and after cell separation. Frequencies of CD4⁺ and CD8⁺ T cells in unseparated versus purified cells (among live, CD45⁺ leukocytes) are shown. Results were similar for C57BL/6 and BALB/c mouse strains.

2 Isolated CD4⁺ and CD8a⁺ T cells retained their non-activated state

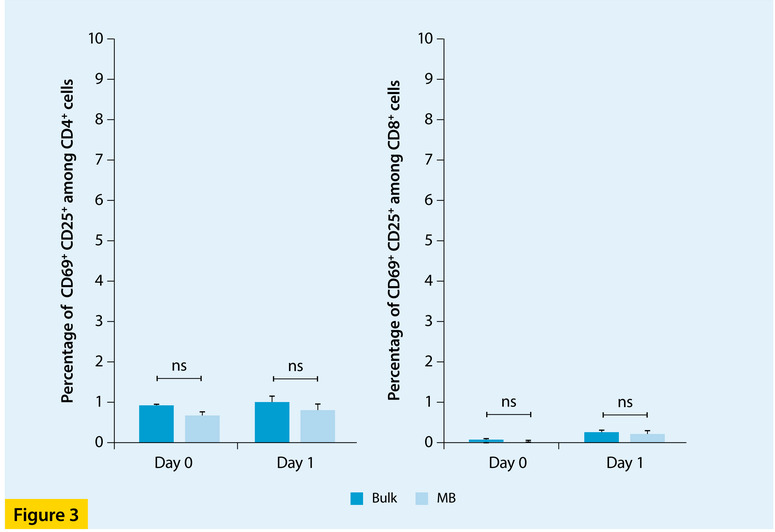

Figure 3

We tested whether T cells isolated by the new type of CD4 or CD8a MicroBeads and the short protocol were activated by the process. To this end, unseparated spleen cells (bulk) or T cells purified by the new type of MicroBeads (MB) from naive mice were cultured in RPMI supplemented with FCS and L-glutamine overnight at 37 °C. The percentage of cells expressing the activation markers CD25 and CD69 among CD4⁺ or CD8⁺ T cells was analyzed prior to culture (day 0) and after overnight culture (day 1). Cells were gated on i) viable cells, ii) leukocytes, iii) single cells, iv) CD45⁺ cells, and v) CD4⁺ or CD8⁺ cells. Statistical analysis was performed using 2-way ANOVA with Sidak's multiple comparisons test (n = 3).

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

CD4⁺ or CD8⁺ T cells were isolated by positive selection using the CD4 (L3T4) MicroBeads and CD8a (Ly-2) MicroBeads based on the new short protocol, or by depletion of unwanted cells using CD4⁺ and CD8a⁺ T Cell Isolation Kits, mouse, respectively. Depletion of unwanted cells leaves the CD4⁺ and CD8⁺ cells completely untouched.
(A) To investigate cell proliferation, cells were labeled with a fluorescent cell-tracking dye and activated using the T Cell Activation/Expansion Kit. Upon activation, cells were cultured for 3 d. Subsequently, the fluorescence of the dye was measured by flow cytometry. Numbers in the histograms specify the percentages of cells that underwent proliferation. Cells isolated by positive selection (MB) and untouched cells (TCIK) showed similar proliferation capacities, which indicates that labeling with the new type of MicroBeads had no effect on the cells' proliferative capability.
(B) Cells were activated using the T Cell Activation/Expansion Kit and cultured for 6 d. Subsequently, cells were restimulated with PMA/Ionomycin and Brefeldin A for 4 h and intracellular cytokine expression was assessed by flow cytometry (n = 3). Cells were gated on i) viable cells, ii) leukocytes, iii) single cells, iv) CD45⁺ cells, and v) CD4⁺ or CD8⁺ cells. The capacity to produce cytokines was comparable between cells isolated by positive selection (MB) and untouched cells (TCIK), which indicates that labeling with the new type of MicroBeads had no effect on the cells' capacity to produce cytokines.

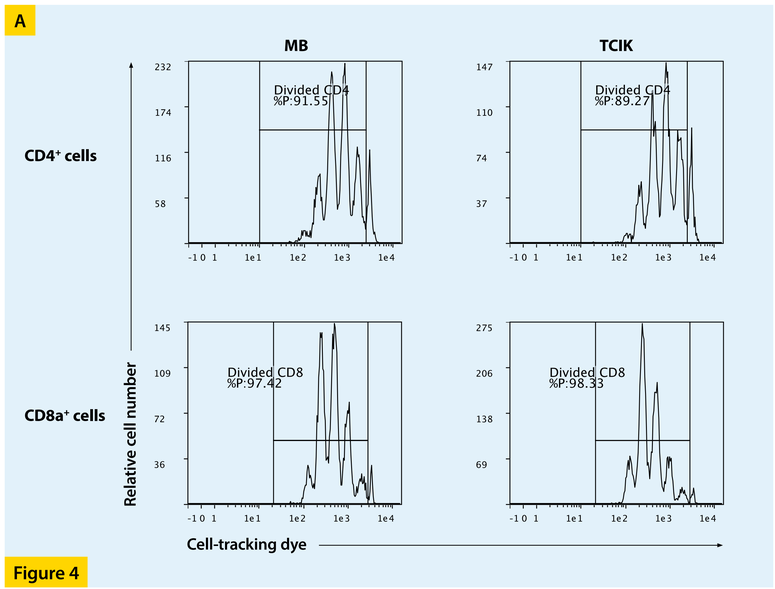

Figure 4

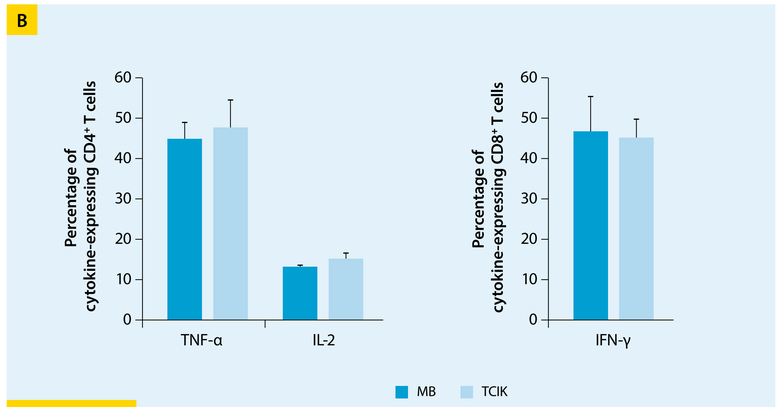

Figure 4 cont.

4 Multiple samples can be isolated in parallel by a semi-automated protocol

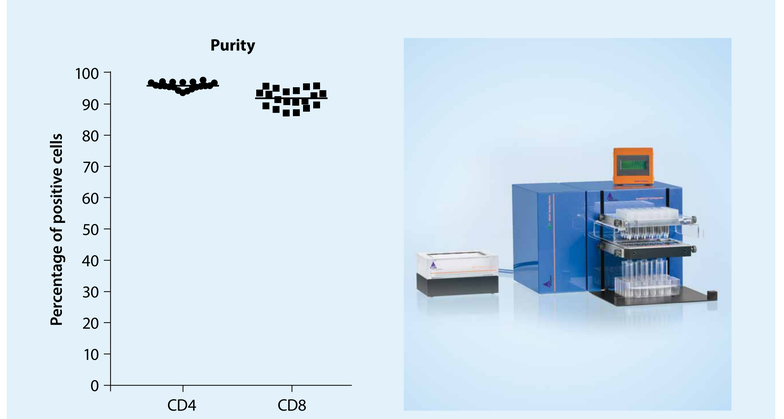

Figure 5

Cells were labeled with CD4 or CD8a MicroBeads and subsequently isolated in parallel on the MultiMACS™ Cell24 Separator Plus using the POSSEL_2 program. For flow cytometry analysis, cells were gated on i) viable cells, ii) leukocytes, iii) single cells, iv) CD45⁺ cells, and v) CD4⁺ or CD8⁺ cells. The percentages of CD4⁺ or CD8⁺ cells within the enriched fraction are shown.

Conclusion

- We established workflows combining automated tissue dissociation with T cell isolation.
- We developed new separation reagents specific for CD4⁺ and CD8⁺ T cells, which enable a significantly accelerated isolation protocol.
- T cells were isolated to purities above 90%. Pre-enrichment reduces the time required for downstream analysis.
- Importantly, culture of isolated T cells did not induce activation, based on analysis of the activation markers CD69 and CD25.
- Furthermore, magnetic isolation did not affect cell proliferation or the capacity to express cytokines upon *in vitro* stimulation.
- Finally, T cell isolation could be automated and multiple samples could be processed in parallel.

Our new workflow greatly reduces the 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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