Magnetic enrichment of tumor-infiltrating T cells reduces experimental time and prevents biased functional characterization

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Introduction

Tumor-infiltrating T cells play an important role in anti-tumor immunity, highlighted by multiple immunotherapy strategies approved by the FDA. However, the benefit of current immunotherapies is still limited, such that additional research is needed to improve efficacy. Preclinical tumor models are essential to advance immunotherapy research. However, tumor composition is highly variable and T cell numbers can be very limited. Even flow cytometry can be quite time consuming. Importantly, tumor-infiltrating T cells are embedded in a cellular environment where antigen is abundant and surrounding cells express immunomodulatory molecules, potentially affecting the readout of functional assays. Pre-enrichment of tumor-infiltrating T cells is highly desirable to reduce hands-on time and generate high-quality and reliable data.

Results

1 Reliable and fast isolation of T cells from syngeneic mouse tumors

B16–F10, B16-OVA, CT26.WT, or 4T1 tumors were resected and dissociated using the gentleMACS™ Octo Dissociator and the Tumor Dissociation Kit, mouse (TDK). CD4⁺, CD8⁺, and Pan T cells were then magnetically isolated directly from dissociated mouse tumors with our newly developed reagents based on MACS® Technology (see fig. 1A). Cells were separated manually using MS or LS Columns. Percentages of tumor-infiltrating T cells among living cells (excluding erythrocytes) before or after isolation are shown for different tumor models, as assessed by flow cytometry. Frequencies of tumor-infiltrating T cells among unseparated bulk tumor cells ranged from 0.2% to 9%. Purities of the isolated cells were above 80% with yields ranging from 60% to 95% (fig. 1A), hence reducing time required for flow cytometry analysis (table 1). Pan T cell isolation maintained the original ratio between CD4⁺ and CD8⁺ cells (fig. 1B).

Cell type	Cells to analyze	Frequency	Events to collect	Flow cytometry time/sample*	Total flow cytometry time**
CD4⁺ T cells					
Bulk	5,000	0.18%	7.96×10^6	66.3 min	>22 h
Isolated***	5,000	92.4%	5.41×10^4	0.5 min	~20 min
CD8⁺ T cells					
Bulk	5,000	0.96%	2.80×10^6	23.3 min	>7.5 h
Isolated***	5,000	80.5%	4.37×10^4	0.4 min	~17 min
T cells					
Bulk	10,000	6.24%	8.13×10^5	6.8 min	>2.4 h
Isolated***	10,000	84.9%	3.24×10^4	0.3 min	~16 min

* Flow rate: 2,000 events/s
** Considering 20 samples. Includes automated mixing and rinsing between samples on the MACSQuant X
*** Isolation using CD8 (TIL), CD4 (TIL), or CD4/CD8 (TIL) MicroBeads, respectively

Table 1

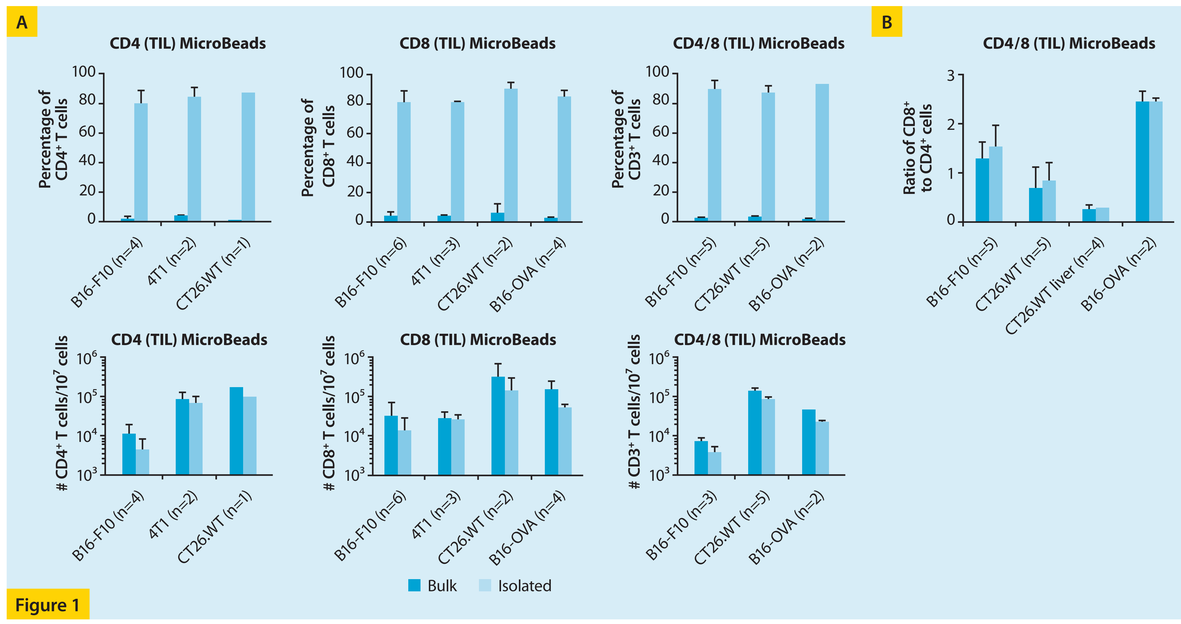

Figure 1

2 Magnetic isolation of tumor-infiltrating T cells maintained activation status

Figure 2 shows that isolation of CD4⁺ and CD8⁺ T cells did not lead to activation of the cells. B16-OVA tumors were collected, mixed with splenocytes from a naive C57BL/6 mouse, and dissociated. T cells were isolated (or not) using CD4/CD8 (TIL) MicroBeads. Unseparated bulk or isolated cells were cultured in RPMI supplemented with 10% FCS and L-glutamine at 37 °C. After 16 h, expression of the activation markers CD69 and CD25 on CD8⁺ or CD4⁺ T cells derived from the naive splenocytes was analyzed by flow cytometry.

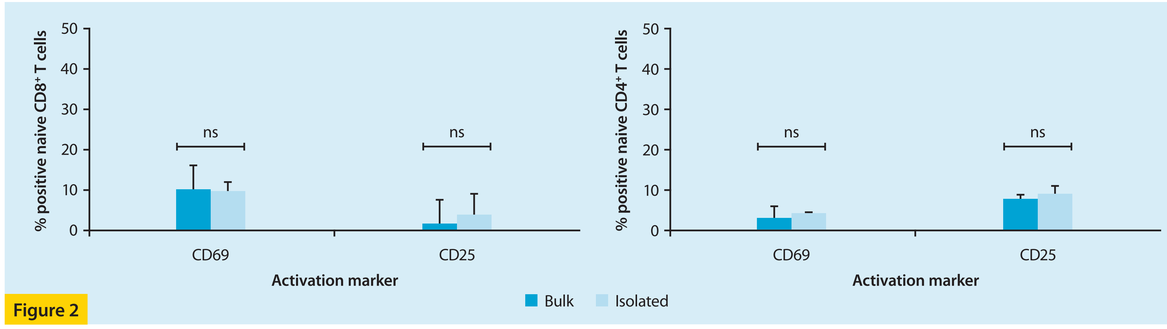

Figure 2

3 Isolation of tumor-infiltrating T cells revealed cell-intrinsic functional capabilities

4T1 tumors were collected and dissociated. T cells were isolated (or not) and cultured in the presence or absence of plate-bound CD3 antibody for 4 h (+Brefeldin A). Intracellular accumulation of IFN-γ was measured by flow cytometry. Results show that, compared to CD8⁺ cells in the unseparated bulk tumor cells, a higher percentage of isolated CD8⁺ T cells expressed IFN-γ (fig. 3A), suggesting that immunosuppression by cells present in the tumor microenvironment hindered assessment of cell-intrinsic functional characterization. As a control, T cells were isolated but then cocultured with the negative fraction, thereby reconstituting the bulk cell population (fig. 3B). Results show that the functional difference observed in the left panel was due to the presence of non-CD8⁺ T cells during the assay, not to the isolation process. ns: non-significant.

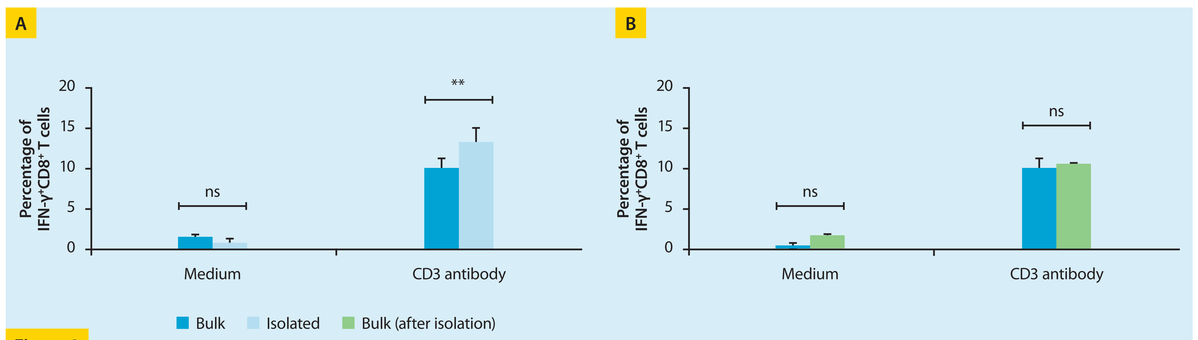

Figure 3

4 Semi- or fully automated separation for convenience, speed, and high-quality performance

(A) B16-OVA tumors from 20 mice were collected and dissociated as described above. Tumor-infiltrating T cells from all mice were isolated in parallel, using the MultiMACS™ Cell24 Separator Plus and CD4/CD8 (TIL) MicroBeads. Numbers indicate the frequency of cells that are either CD4⁺ or CD8⁺, in relation to live cells. (B) B16F10 tumors were collected and dissociated as described above. CD4⁺ or CD8⁺ T cells were isolated with CD4 (TIL) MicroBeads or CD8 (TIL) MicroBeads, either manually using an MS Column or by the autoMACS® Pro Separator for convenient fully automated walk-away separation.

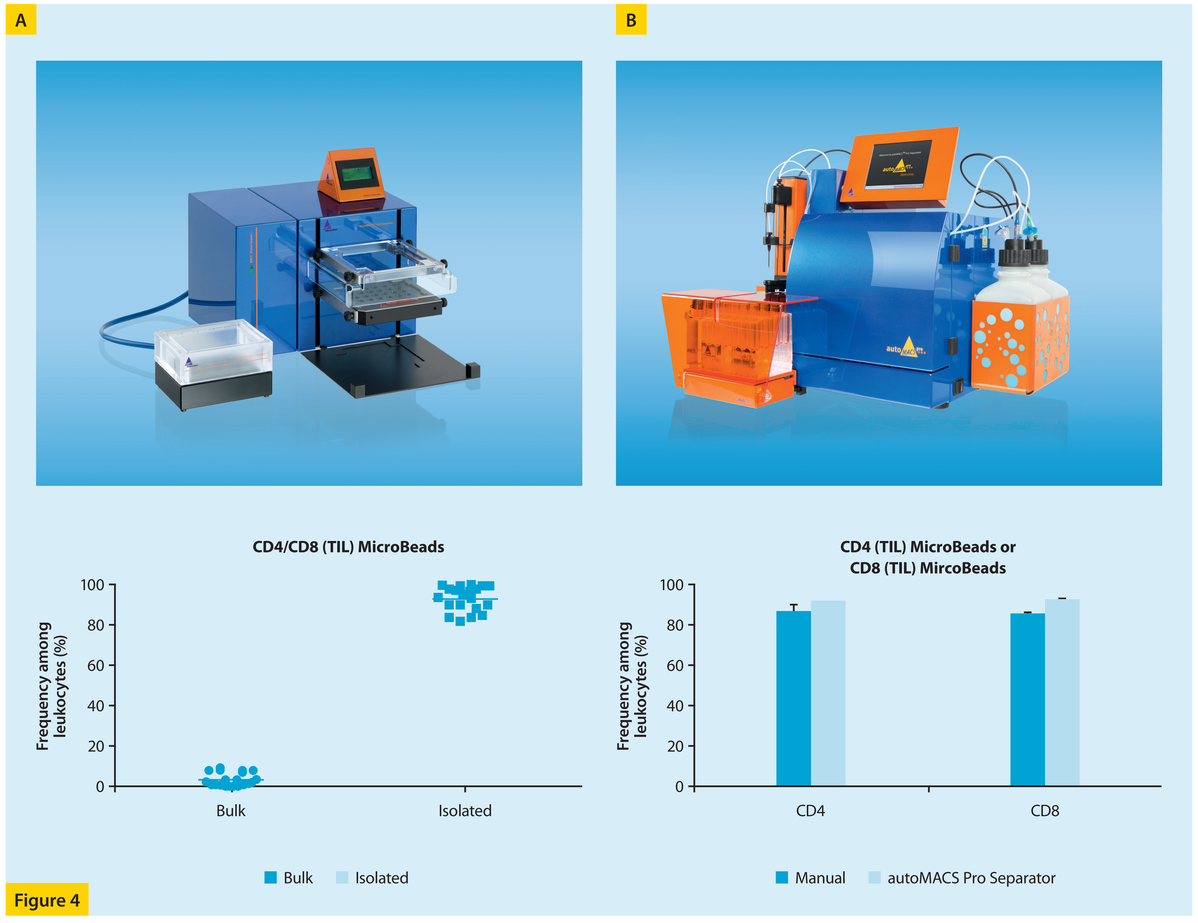

Figure 4

Conclusion

Our data, validated in four different mouse tumor models, clearly demonstrate that magnetic enrichment significantly reduces time of analysis, increases quality of analysis, and is essential for the functional characterization of tumor-infiltrating T cells.