Workflow automation and parallelization improves the isolation and analysis of tumor-infiltrating immune cell subpopulations

Permanent Abstract Number: 1672

Janina Brauner, David Agorku, Anne Langhammer, Lukas Pohlig, Jan Drewes, Thomas Dino Rockel, Oliver Schilling, Stephan Mehlhose, Wael Al Rawashdeh, Andreas Bosio, and Olaf Hardt
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Immunotherapy against cancer has proven clinical efficacy and tremendous potential in multiple tumor entities. Syngeneic mouse tumor models represent the gold standard to analyze effects of immunotherapy, as they possess a fully competent immune repertoire. However, the amount and composition of tumor-infiltrating leukocytes (TILs) is highly variable, complicating the analysis of individual subpopulations. In particular, small subpopulations might escape analysis as they could get lost in the background noise. When working with large cohort sizes, even immunophenotyping of TILs by flow cytometry is time consuming and data processing highly work intensive. Therefore, pre-enrichment of TILs is highly desirable to increase the sensitivity of analysis and save time and effort during flow cytometry. To this end, we have established an automated workflow combining tissue dissociation with specific TIL isolation. Tumor dissociation was automated using the gentleMACS™ Octo Dissociator and optimized for epitope preservation to overcome bias in immunophenotyping caused by dissociation with aggressive or impure enzymes. Moreover, isolation of TILs was improved by developing a new CD45-specific enrichment reagent for magnetic cell sorting, based on MACS® Technology, directly from dissociated tumor tissue. The whole workflow takes only about 90 min to complete. To fulfill the need for parallelization and automation of sample processing in large cohort sizes used in *in vivo* studies, a fully automated version of the MultiMACS™ Cell24 Separator was developed by integrating the instrument into a robotic liquid handling platform. This new system, the MultiMACS X, can perform 24 cell separations in parallel with minimal hands-on time.

Results

1 Reliable and fast isolation of TILs from syngeneic mouse tumors

We have developed a new CD45-specific enrichment reagent for magnetic cell sorting (MACS Technology) directly from dissociated tumor tissue (fig. 1A). To validate this method for starting material showing variable frequencies of TIL infiltration, we used syngeneic mouse tumors induced by injection of three different murine tumor cell lines. Tumors derived from B16-F10 melanoma cells showed TIL frequencies of 2–4% among total viable cells after dissociation. CT26.WT cell–derived colon carcinoma contained 15–21%, and 4T1 cell–derived breast carcinoma 32–37% TILs. Using a manual separation system, TILs were enriched to purities above 90% at yields above 70% for CT26.WT and 4T1 tumors, and purities above 80% at high yields above 95% for B16-F10 tumors (fig. 1B and C).

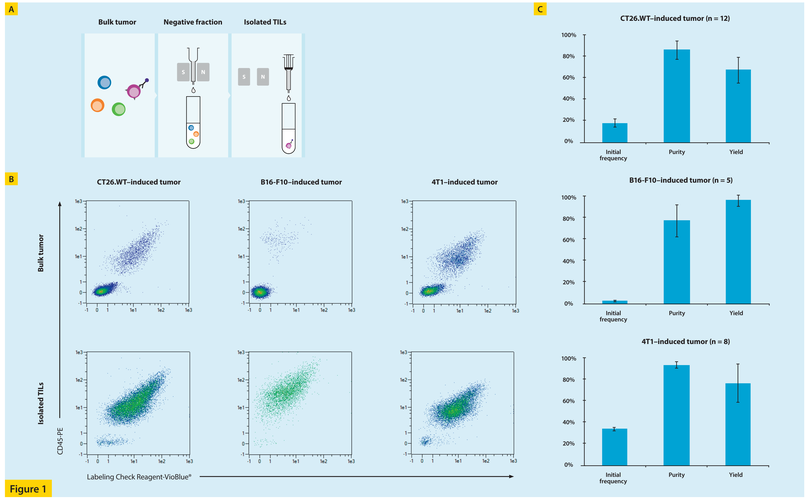


Figure 1

2 Immunophenotyping of TIL subpopulations

We used flow cytometry to detect and quantify TIL subpopulations in syngeneic mouse tumors induced by subcutaneous transplantation of B16-F10 melanoma cells. This tumor model showed TIL frequencies of only 2–4%, making it difficult to analyze smaller immune cell subpopulations in a reliable way. To directly compare the results from samples without (fig. 2A) or with (fig. 2B) prior CD45-specific TIL enrichment, we kept the number of acquired events, and therefore acquisition time, constant. In both cases, 100,000 events were acquired for subsequent analysis. In the sample without TIL enrichment, it was hard to detect and quantify immune cell subpopulations. This was particularly obvious for small subpopulations, such as plasmacytoid dendritic cells (pDCs), which could be detected reliably only in the pre-enriched sample. Importantly, the composition of infiltrating immune cell populations was not affected, excluding the risk of introducing a bias by this method (fig. 2C). An alternative approach to increase sensitivity would be to acquire more events during flow cytometric analysis. However, for this tumor, the overall TIL enrichment factor was 48-fold. This means that an acquisition of 4,800,000 events from the unseparated fraction would have been necessary to reach an equal level of detection as the acquisition of 100,000 events from the enriched fraction.

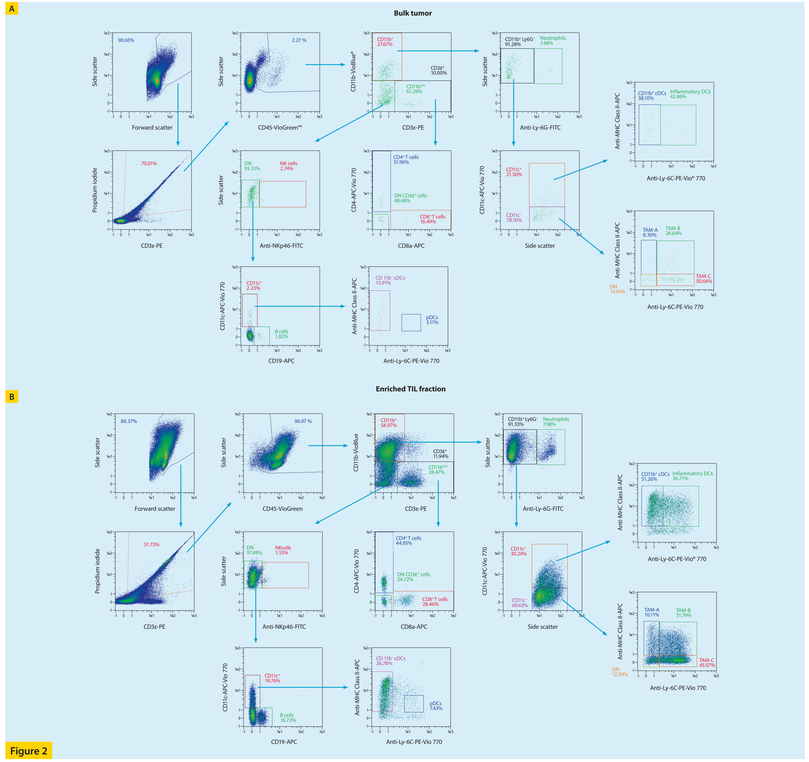


Figure 2

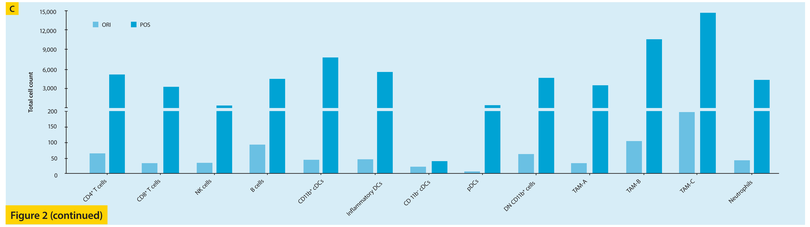
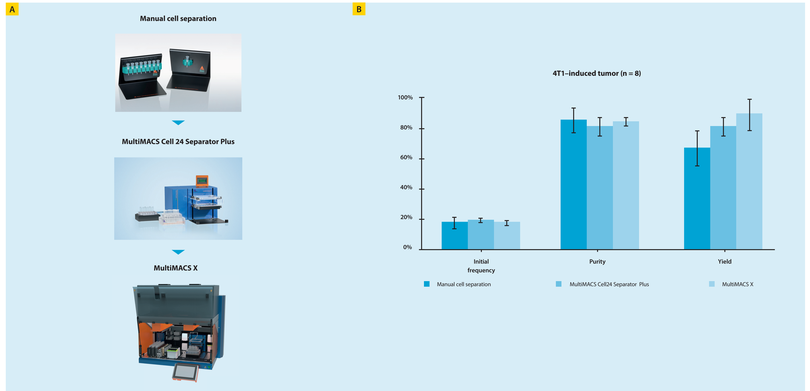


Figure 2 (continued)

3 Automation and parallelization of TIL isolation

Automation and parallelization of tumor dissociation has been achieved previously by developing the gentleMACS Octo Dissociator. As TIL subpopulation analysis is a common readout for pre-clinical *in vivo* studies in the immuno-oncology field, the method for TIL isolation had to be adapted to large cohort sizes as well. To address this need, a fully automated version of the MultiMACS Cell24 Separator was developed by integrating the instrument into a robotic liquid handling platform. This new system, the MultiMACS X (fig. 3A), can process 24 cell separations in parallel with minimal hands-on time. When compared to the manual system, equal purities were achieved, while the overall yield of target cells was increased from 70% to 90% as shown for TILs from CT26.WT tumors (fig. 3B).



Conclusion

- We have developed an automated workflow for the isolation of TILs from mouse tumors, streamlining downstream analysis while standardizing and enhancing the detection and quantification of immune cell subpopulations.
- Importantly, while the TIL enrichment significantly reduced time and reagent costs for immune cell subset analysis, the composition of infiltrating immune cell populations was not affected, excluding the risk of introducing a bias by this method.