OPTIMIZATION OF AUTOMATED CD8+ REGIONAL MEMORY T CELL ISOLATION

Nikolett Lupsa[1], Barbara Molnár-Érsek[1], Tünde Simon[1], Anett Tóth[1], Bence Bagita[1], András Falus[2], Edit Buzás[2], Zoltán Pós[1]

[1]"Lendület" Experimental and Translational Research Group, Hungarian Academy of Sciences - Semmelweis University, Budapest
[2]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

Tissue resident cytotoxic memory T cells (CD8+ Trm) represent a unique group of the CD8+ T cell memory protecting peripheral tissues and providing early alarm signals upon recall antigen challenge. Depending on tissue localization Trm cells express different homing markers; on the other hand, increased integrin αE (CD103) and CD69 expression is a common feature of all known CD8+ Trm subsets, regardless of their organ of residence. This project aims at a better understanding of the organ-specific biomarkers, functional features, establishment, maintenance and recall response of the CD8+ Trm cells.

We believe that the most adequate way to analyze this complex and largely unexplored field is, to begin with a hypothesis-free, comprehensive, genomic scale gene expression analysis of regional memory T cells of various organs. Pursuing this goal, the first and probably most challenging issue to be solved is the isolation of these rare and rather vulnerable cells, that requires a methodology ensuring highly pure, effective, reproducible, and gentle cell retrieval that does not decrease viability or compromises functionality of these cells. To this end we set up several novel automated isolation protocols, individually tailored to the needs of distinct murine CD8+ organ-resident Trm cells, acting by means of mechanical and enzymatic tissue processing. The process is conducted with help of a highly sensitive cell separator platform supported by appropriate RNA isolation and amplification systems to allow subsequent microarray analysis.

We successfully isolated tissue resident CD8+ Trm cells from select organ samples (lung, small intestine, liver,) of mice on the C57Bl/6 background by using automated tissue processing with help of a GentleMACS Octo Dissociator. Subsequent cell sorting depending on Trm markers (CD8b, CD103) was carried out on an autoMACS Pro Separator system. Pure fractions (93-98%) of Trm cells were processed by an RNeasy Micro Kit for ultra-low input RNA amplification with an Arcturus RiboAmp HS PLUS Amplification Kit and subjected to microarray gene expression analysis.

This isolation strategy successfully retrieved thousands of CD8+ Trm cells from various organs, is highly effective, and ensured both high purity, and reproducibility. The isolated cells were used for microarray analysis and their gene expressing profile also confirmed their identity as CD8+ Trm cells. To our best knowledge, this is the first attempt to conduct a comparative, hypothesis-free, in-depth genomic scale analysis on CD8+ Trm cells to gain further insight into this unique branch of CD8+ T cell memory.