ONCODAILY MEDICAL JOURNAL

abstract

Features of the analysis of pathogen-specific T-lymphocytes in CD45RA-depleted cell products during manufacturing and storage

Irina Kazmina, Elena Osipova, Ekaterina Kovalenkova, Anastasia Melkova, Yakov Muzalevsky, Maria Efimenko, Ruslan Nikolaev, Pavel Trahtman, Michael Maschan

DOI: 10.69690/ODMJ-018-0425-592

SIOP Asia, 2025, Saudi Arabia

abstract

Features of the analysis of pathogen-specific T-lymphocytes in CD45RA-depleted cell products during manufacturing and storage

Authors: Irina Kazmina, Elena Osipova, Ekaterina Kovalenkova, Anastasia Melkova, Yakov Muzalevsky, Maria Efimenko, Ruslan Nikolaev, Pavel Trahtman, Michael Maschan

Affiliation: Dmitry Rogachev National Medical Research Center Of Pediatric Hematology, Oncology and Immunology

DOI: 10.69690/ODMJ-018-0425-592

Introduction: CMV, EBV, and ADV infections pose a significant risk to patients after hematopoietic stem cell transplantation (HSCT). Accurate quantitative and functional assessment of virus-specific T-lymphocytes (VSTs) in donor cell products is critical to improving the effectiveness of cellular therapies.

Methodology: The study included: donor's peripheral blood mononuclear cells (PBMC) (n=199); CD45RA-depleted cell products (n=122), processed with single-step procedure on CliniMACS Plus or Prodigy instrument, aliquoted and cryopreserved for further use. Three groups of CD45RA-depleted products were analyzed: fresh fractions (n=122), cryopreserved and stored for 2 weeks (n=30) and stored for 5 years (n=15). FN-gamma ELISpot assay was performed to determine the frequencies of CMV-, EBV-, and ADV-VSTs. The excess monocyte amounts were deleted by adhesion on plastic for 16-18 hours at 37°C. Overnight resting for cryopreserved cells was performed in serum-free media. The Monocytes/CD3 ratio was monitored by flow cytometry.

Results: The median quantity of VSTs in donors peripheral blood was: CMV-203, EBV-69, ADV-34 per 300,000 PBMC. High monocyte content (median Mon/CD3=0.5) in CD45RA-depleted products dramatically decreased the efficiency of VSTs detection (CMV-4, EBV-3, ADV-0 per 300,000 cells). Reduction in monocytes numbers significantly enhanced VSTs detection (CMV-101, EBV-78, ADV-26). The efficiency of VSTs detection in cryopreserved and twained after 2 weeks CD45RA-depleted products was markedly reduced (CMV-9, EBV-3, ADV-2 per 300,000 cells), however, overnight resting restored detection (CMV-102, EBV-64, ADV-15). 5-years storage significantly reduces VSTs activity. In these CD45RA-depleted cell products after overnight resting 28 CMV-specific, 5 EBV-specific, and 2 ADV-specific cells were identified per 300,000 cells.

Conclusion: Monocyte depletion and overnight resting are essential for the reliable evaluation of VSTs in CD45RA- depleted products. Long-term storage significantly reduces the antiviral activity of T-lymphocytes. For clinical application of thawed aliquots after cryopreservation, it is recommended to perform ELISpot assay to assess the functional activity of VSTs.