Clinical-scale isolation of 'minimally manipulated' cytomegalovirus-specific donor lymphocytes for the treatment of refractory cytomegalovirus disease.

Abstract:
Reactivation of cytomegalovirus (CMV) after hematopoietic stem cell transplantation remains a major cause of morbidity despite improved antiviral drug therapies. Selective restoration of CMV immunity by adoptive transfer of CMV-specific T cells is the only alternative approach that has been shown to be effective and non-toxic. We describe the results of clinical-scale isolations of CMV-specific donor lymphocytes with the use of a major histocompatibility (MHC) class I peptide streptamer-based isolation method that yields minimally manipulated cytotoxic T cells of high purity. Enrichment of CMV-specific cytotoxic T lymphocytes (CTLs) was performed by labeling $1 \times 10^{10}$ leukocytes from a non-mobilized mononuclear cell (MNC) apheresis with MHC class I streptamers and magnetic beads. Thereafter, positively labeled CMV-specific CTLs were isolated through the use of CliniMACS (magnetic-activated cell sorting), and MHC streptamers were released through the use of d-biotin. The purity of enriched CMV-specific CTLs was determined on the basis of MHC streptamer staining and fluorescence-activated cell sorting. A total of 22 processes were performed with the use of five different MHC
class I streptamers. The median frequency of CMV-specific CTLs in the starting apheresis product was 0.41% among CD3+ T cells. The isolation process yielded a total of $7.77 \times 10^6$ CMV-specific CTLs, with a median purity of 90.2%. Selection reagents were effectively removed from the final cell product; the CMV-specific CTLs displayed excellent viability and cytotoxicity and were stable for at least 72 h at 4°C after MNC collection. Clinical-scale isolation of "minimally manipulated" CMV-specific donor CTLs through the use of MHC class I streptamers is feasible and yields functional CTLs at clinically relevant dosages.