Abstract

Introduction: Viral infections and reactivations still remain a cause of morbidity and mortality after hematopoietic stem cell transplantation due to immunodeficiency and immunosuppression. Transfer of unmanipulated donor-derived lymphocytes (DLI) represents a promising strategy for improving cellular immunity but carries the risk of graft versus host disease (GvHD). Depleting alloreactive naïve T cells (T_N) from DLIs was implemented to reduce the risk of GvHD induction while preserving antiviral memory T-cell activity. Here, we compared two T_N depletion strategies via CD45RA and CD62L expression and investigated the presence of antiviral memory T cells against human adenovirus (AdV) and Epstein-Barr virus (EBV) in the depleted fractions in relation to their functional and immunophenotypic characteristics. ***Methods:*** T-cell responses against ppEBV_EBNA1, ppEBV_Consensus and ppAdV_Hexon within T_N-depleted ($CD45RA^-/CD62L^-$) and T_N-enriched ($CD45RA^+/CD62L^+$) fractions were quantified by interferon-gamma (IFN-γ) ELISpot assay after short- and long-term *in vitro* stimulation. T-cell frequencies and immunophenotypic composition were assessed in all fractions by flow cytometry. Moreover, alloimmune T-cell responses were evaluated by mixed lymphocyte reaction. ***Results:*** According to differences in the phenotype composition, antigen-specific T-cell responses in $CD45RA^-$ fraction were up to 2 times higher than those in the $CD62L^-$ fraction, with the highest increase (up to 4-fold) observed after 7 days for ppEBV_EBNA1-specific T cells. The $CD4^+$ effector memory T cells (T_{EM}) were mainly responsible for EBV_EBNA1- and AdV_Hexon-specific T-cell responses, whereas the main functionally active T cells against ppEBV_Consensus were $CD8^+$ central memory T cells (T_{CM}) and T_{EM}. Moreover, comparison of both depletion strategies indicated that alloreactivity in $CD45RA^-$ was lower than that in $CD62L^-$ fraction. ***Conclusion:*** Taken together, our results indicate that CD45RA depletion is a more suitable strategy for generating T_N-depleted products consisting of memory T cells against ppEBV_EBNA1 and ppAdV_Hexon than CD62L in terms of depletion effectiveness, T-cell functionality and alloreactivity. To maximally exploit the beneficial effects mediated by antiviral memory T cells in T_N-depleted products, depletion methods should be selected individually according to phenotype composition and CD4/CD8 antigen restriction. T_N-depleted DLIs may

improve the clinical outcome in terms of infections, GvHD, and disease relapse if selection of pathogen-specific donor T cells is not available.