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New strategies for allogeneic hematopoietic stem cell transplantation with umbilical cord

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ABSTRACT

Umbilical cord blood is enriched in hematopoietic stem cells. For this reason, cord blood units may be utilized for allogenic hematopoietic stem cell transplantations when no adult human leukocyte antigen (HLA)-matched donor is found. Cord blood units are rapidly available from international cord blood banks and the naivety of cord blood cells allows the transplantation of HLA-mismatched units without an increase in graft-versus-host disease. But cord blood is also beset with some drawbacks compared to other stem cell sources, the most apparent being a slow immune reconstitution after transplantation leading to increased infection related mortality. The overall aim of this thesis work has been to develop new strategies and tools for handling patients transplanted with umbilical cord blood.

Donor lymphocyte infusions (DLI), i.e. an additional boost of donor lymphocytes, can be used to treat threatening rejections or malignant relapses in the adult donor setting. However, due to the limited cell dose, this treatment option is currently not available for cord blood transplanted patients. For this reason, we aimed to expand cord blood-derived T cells for possible use as DLI after transplantation. Starting with an aliquot from the original cord blood graft, we successfully expanded T cells in eight days to adequate numbers for DLI preparation. By studying the cells with multicolor flow cytometry for surface and intracellular markers, functional assays and spectratyping techniques we concluded that the T cells had polyclonal T cell receptor repertoire, were of central and effector memory phenotype and responded in a similar manner towards mitogenic and allogeneic stimulation compared to peripheral blood T cells.

The cytokine IL-7 has previously been shown to protect T cells from apoptosis induced by, e.g. cytokine withdrawal. This feature should be especially important for cord blood T cells due to their sensitivity to activation induced cell death as well as their high expression levels of the IL-7 receptor. Hence, we aimed to optimize our expansion protocol by adding IL-7 to a range of IL-2 concentrations. When IL-7 was added to low-dose IL-2, the resulting T cells presented with a higher degree of polyfunctionality and superior proliferation potential compared with cells expanded without IL-7. The T cells also had a higher CD4/CD8 ratio and a higher frequency of effector memory cells, which may have positive implications for their use as DLI.

The overall one-year 55% survival after cord blood transplantations at our center highlights the need for predictive risk markers for earlier interventions. We hypothesized that the T cell expansions could be utilized as indirect indicators of graft quality and, thus, as a tool for risk prediction. We correlated phenotypical and functional data from expanded cord blood T cells with clinical features after transplantation. The results indicated that higher frequencies of CD69+ T cells in the expansions were predictive of prolonged patient survival. Since many of the deaths were due to infections, this marker may thus be used as an indicator for e.g. the administration of prophylactic antiviral drugs.

To overcome the problem of low cell dose, the strategy of double cord blood transplantations (DCBT) in which two cord blood units are transplanted simultaneously, has been effectively employed. This provides the patient with an increased total nucleated cell dose during the initial critical weeks after transplantation but, in the vast majority of cases, one of the units eventually prevails. However, three out of seven evaluable patients undergoing DCBT at our center presented with a mixed donor chimerism more than two years after transplantation. Since these patients are extremely rare we characterized the phenotype and functionality of their immune systems to gain insight into the significance of mixed donor chimerism. Results indicate that patients with long-term mixed donor chimerism after double cord blood transplantation have a less functional immune system compared to control patients with one donor immune system. This could be because one of the two immune systems had a more naive T cell profile with poor cytokine production. Moreover, we speculate that the mixed donor chimerism in part may be explained by a graft-versus-graft tolerance induced by our use of high-dose anti-thymocyte globulin and an inter-unit match of HLA-C.

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