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INCREASED SENSITIVITY OF TWO-STEP CYTOMEGALOVIRUS PCR ANALYSIS ON PERIPHERAL BLOOD LEUKOCYTES PERMITS DETAILED ANALYSIS OF REACTIVATION AND IMMUNE RECONSTITUTION

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Although current methods to detect human cytomegalovirus (CMV) infection following hematopoietic cell transplantation (HCT) have adequate sensitivity to guide preemptive treatment, we chose to monitor CMV with a more sensitive PCR approach to try to better understand the degree and timing of reactivation. The purpose of this study was to compare the occurrence of CMV reactivation during the first 100 days post HCT in patients receiving a non-myeloablative (NMA) or myeloablative (MA) regimen prior to allogeneic HCT. We analyzed reactivation in 98 consecutive patients undergoing HCT (MA = 48 and NMA = 50) with the COBAS AMPLICOR™ quantitative PCR on plasma (n = 98) and a more sensitive two-step PCR (qualitative nested PCR and quantitative real-time PCR) on peripheral blood leukocytes (PBL, n = 77). The estimated cumulative incidence of reactivation was similar in both patient groups using either AMPLICOR™ (63% NMA, 68% MA) or nested PCR (80% NMA, 100% MA). However, reactivation was detected earlier in the NMA group compared to the MA group using either method (AMPLICOR™ P = .012, nested PCR P = .002). Of the 77 patients followed by both AMPLICOR™ and nested PCR, 9 patients in each of the NMA (21%) and MA (26%) groups had levels of CMV DNA detectable with nested PCR but not with AMPLICOR™. When levels of viral DNA were quantified using real-time PCR, we found that the CMV copy number was lower in the NMA group compared to the MA group at the first positive PCR result (P = .038) and at the peak viral load (P = .02), suggesting NMA patients are better able to control CMV early post-transplant. However, more NMA patients had late CMV disease. We also compared the timing of CMV reactivation in relation to acute graft vs. host disease (aGvHD) in the NMA and MA groups, as differences between these patient groups are unclear. When using nested PCR we found that all of the NMA patients (n = 5 of 5, 100%) reactivated before aGvHD, in contrast to the MA group where few patients (n = 2 of 10, 20%) reactivated before aGvHD (P = .037). There was a similar trend with AMPLICOR™, as almost half of the NMA patients (n = 3 of 7, 43%) and none of the MA patients (n = 0 of 10) reactivated before aGvHD. These data suggest that PCR on PBL may be an effective way to further understand the timing of CMV reactivation in relation to aGvHD in the NMA and MA groups. In addition, detection of CMV with PCR on PBL may be a sensitive method with which to monitor immune reconstitution after HCT.

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UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION USING TBI/FLAG CONDITIONING REGIMEN FOR ADVANCED HEMATOLOGICAL MALIGNANCIES

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A combined chemotherapy using fludarabine (Flu), cytosine arabinoside and granulocyte colony-stimulating factor (FLAG) has been reported to be effective in the treatment of relapsed or refractory leukemias. Rationale for FLAG therapy is (i) Flu infusion before Ara-C increases the accumulation of the active metabolite ara-C triphosphate (ara-CTP) (ii) G-CSF may sensitize leukemic blasts to S-phase specific Ara-C by recruiting quiescent cells into cell cycle and increasing Ara-C phosphorylation. Flu is also highly immunosuppressive agent and frequently used in preparative regimen for stem cell transplantation. We here report thirty-two patients with advanced hematological malignancies who underwent cord blood transplantation (CBT) with a conditioning regimen composed of 1200 cGy total body irradiation (TBI) and FLAG therapy (TBI/ FLAG). Graft-versus-host disease (GVHD) prophylaxis was FK506 or cyclosporin A and/or methotrexate. The cumulative incidence of sustained donor engraftment was 91%. The median number of days to an absolute neutrophil count of 500/ml was 22 (range, 19-31 days). The median time to an untransfused platelet count of 50000/ml was 46 (range, 21-208 days). The incidence of grades II-IV acute GVHD was 67%. Eleven patients were alive at a median follow-up of 22 months (range, 5-58 months). Three year overall survival and disease-free survival rates were 34 and 26%, respectively. The result suggests that this novel regimen is well tolerable and offers rapid donor cell engraftment. Thus, this study demonstrated the feasibility of FLAG/TBI as a conditioning regimen in CBT in advanced hematological malignancies.

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DEPLETION OF RECIPIENT NK CELLS SIGNIFICANTLY INCREASES ENGRAFTMENT OF HAPLO-MATCHED HIGHLY PURIFIED HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cell transplantation has been shown to be a potential curative treatment for malignant and nonmalignant hematopoietic disorders. However, many patients lack an HLA identical matched donor, and haplo-matched transplanted patients are at risk of developing acute and chronic graft versus host disease (GvHD). Preparations of purified hematopoietic stem cells (HSC) depleted of T cells greatly decrease the risk of GvHD. However, transplantation of allogeneic haplo or completely mismatched purified HSC results in greater resistance to engraftment compared to whole bone marrow. An earlier study demonstrated that barriers to engraftment of allogeneic HSC may be decreased by increasing the stem cell dose (Uchida et al., 1998); however, the mechanism of inhibition of engraftment of purified HSC was not shown. Our study evaluated hematopoietic recovery kinetics (WBC, RBC, and platelets) and long-term functional immune restoration of highly purified mouse KTLS HSC in syngeneic, MHC-matched unrelated (MUD) and haplo-identical transplantation models. Mice transplanted with haplo-identical HSC exhibited slower recovery kinetics, decreased levels of donor chimerism and survival at all HSC doses tested as compared to syngeneic or MUD HSC. Pretreatment of recipients with antibody to clear residual NK cells improved recovery kinetics, donor chimerism and survival of haplo-identical HSC transplants equal to levels observed with syngeneic HSC transplants. Analysis of transplanted mice revealed no quantitative differences of donor B and T lymphoid, myeloid, or NK cells in the bone marrow, spleen, thymus, lymph nodes and peripheral blood. Progenitor compartments and B and T lymphoid maturation pathways appeared normal. Immunization of haplo-identical HSC transplanted mice with ova, elicited a specific T-mediated B cell response in all three transplant models. These data illustrate that depletion of residual host NK cell activity in preparation for purified haplo-identical HSC transplantation results in rapid early immune recovery and a quantitative and qualitative restoration of the adaptive immune response equivalent to fully matched HSC. Our studies suggest the potential clinical use of purified haplo-identical HSC could be safely broadened by depletion of host NK cells in transplant patients without a matched related donor.

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GENOTYPING KILLER IMMUNOGLOBULIN-LIKE RECEPTORS USING MALDI-TOF MASS SPECTROMETRY FOR STEM CELL TRANSPLANT DONOR SELECTION

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The highly polymorphic receptors known as killer immunoglobulin-like receptors (KIR) are one of several families of receptors involved in regulating the activity of natural killer (NK) cells, a