sensitivity (0.94), specificity (0.96), PPV (0.978) and Youden's index (0.9). The surface protein expression of KIR3DL1, measured by flow cytometry (DX9) correlated well with the relative expression by Q-PCR ($r = .418$; $P < .027$). We then applied the Q-PCR assay to 3 discrete subpopulations of blood NK cells sorted using a cocktail of 3 antibodies (GL183, EB6 and DX9) that recognize 6 KIR genes ($CD56^{+bright}$, $CD56^{+dim}KIRAb^{+}$, and $CD56^{+dim}KIRAb^{-}$). Compared to the combined $CD56^{+dim}$cell populations, $CD56^{+bright}$NK cells (thought to be developmentally immature) had lower levels of gene expression for all KIR except 2DL4. Interestingly, the $CD56^{+dim}KIRAb^{+}$ cells expressed higher amounts of the 7 KIR genes not included in the sorting cocktail than did the $CD56^{+dim}KIR^{-}$ cells (median ratio of 5.05; $P < .0001$). The $CD56^{+dim}KIRAb^{-}$ cells had low gene expression for all KIR. KIR2DL4, which is regulated differently than the other KIR, was the most highly expressed gene in the $CD56^{+dim}KIRAb^{-}$cells. Additionally, $CD56^{+dim}KIRAb^{-}$ cells express a compensatory rise in NKG2A (which precedes KIR expression during NK development) when compared to a $CD56^{+dim}KIRAb^{+}$ population using flow cytometry ($75.3 \pm 12.5\%$ vs $32.0 \pm 6.6\%$; $P = .038$). This demonstrates that the mature, circulating population of $CD56^{+dim}$ NK cells is comprised of KIR-expressing and KIR-negative populations. Together, these results suggest that KIR expression may be a marker of maturation with KIR acquisition under a common locus control mechanism. It also identifies a large population of mature NK cells lacking inhibitory KIR that have the potential to become alloreactive if other inhibitory interactions can be evaded.

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THE CLINICAL USE OF DONOR-DERIVED VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES REACTIVE AGAINST CYTOMEGALOVIRUS (CMV), ADENOVIRUS, AND EPSTEIN BARR VIRUS (EBV)

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CMV, Adenovirus (Ad), and EBV are major viral pathogens post allogeneic SCT. Adoptive immunotherapy with donor-derived Cytotoxic T Lymphocytes (CTL) can prevent the clinical manifestations of EBV and CMV. We have extended these studies by generating CTL from donor PBMC that can restore cellular immunity to CMV, EBV, and Ad simultaneously. Our protocol utilizes stimulation with mononuclear cells transduced with a recombinant Ad type 5 vector pseudotyped with a type 35 fiber carrying a transgene for the CMV antigen pp65, followed by 2 stimulations with EBV-lymphoblastoid cell lines transduced with the same vector. After 3 stimulations, 10 CTL cultures contained a mean of 83% (range 8.4–98.99%) CD8+ve and a mean of 19.6% (range 2.2–91.6%) CD4+ve cells. In Cr release +/or IFNγELISPOT assays, all CTL lines showed specific activity against CMV and EBV targets; 9/10 lines also showed specificity against Ad targets. Further, using MHC-peptide multimers we have demonstrated the simultaneous presence of CD8+ve cells recognizing peptide epitopes from CMV pp65 (range 2.32–21%) and Ad hexon (1.07–8.08%) in the CTL cultures. We have treated 6 patients in this phase I CMV prophylaxis study, 3 on DL1($1 \times 10^7/m^2$) and 3 on DL2($5 \times 10^7/m^2$). Patients received one infusion of virus-specific CTL from 54–120 days post SCT. We observed up to a 28-fold increase in CMV pentamer +ve CD8 T cells post CTL. At last follow-up (12–40 wks post CTL infusion) all patients are CMV and EBV neg. Two patients were transiently +ve for CMV by PCR 4–9 weeks post CTL but both were neg 7 days later without anti-viral therapy, with a corresponding rise in CMV-specific CTL detected in the peripheral blood. Two patients were culture +ve for Ad in stool +/− blood pre-CTL therapy. One of these patients was infected with Ad species from subgroups A, C, and D and the other with group C alone. In both patients, we observed a 2-log reduction of adenoviral load within 2–3 weeks post CTL infusion at which time their symptoms (fever, loose stools) resolved. In summary, we can efficiently generate multi-virus specific CTL. Infusion of these cells included virus-specific CD8+ve T cells in the peripheral blood post CTL infusion. Further, reduction in Ad load in stool/blood suggests efficacy of Ad-specific CTL in vivo. However, expansion of virus-specific CTL in vivo may require presence of antigen. We will complete this prophylaxis study and then proceed to using virus-specific CTL for the treatment of CMV and Ad disease post SCT.

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ENHANCED IMMUNE RECONSTITUTION AND FUNCTION BY $CD4^{+}CD25^{+}$ REGULATORY T CELLS FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Regulatory T cells (Treg) reduce the incidence and severity of acute graft-versus-host disease (GvHD) in murine models of major-MHC mismatched hematopoietic cell transplantation (HCT). It is unclear whether the effect of Treg on effector T cells is a selective or nonselective process or if Treg regulate the process of intrathymic and peripheral T cell maturation and selection following HCT. The current study assessed the impact of Treg on the quantitative and functional lymphoid reconstitution after HCT. Treg from FVB/N (H2q) mice were co-transplanted into lethally irradiated Balb/c (H2d) host along with FVB/N T cell depleted bone marrow cells and CD4+/CD8+ cells, the latter to induce GvHD. At days 14 and 40 post-transplantation, total lymphoid reconstitution was delayed in the GvHD group and enhanced in recipients transplanted with Treg ($P = .0005$ on day 14, $P < .0001$ on day 40). T cell reconstitution, particularly CD4+ cells, was enhanced in the Treg group at both time points and reached statistical significance on day 40 ($P = .003$). The number of donor natural killer cells was particularly increased on day 14 in the Treg recipients ($P = .0003$). B-cell reconstitution was enhanced at day 40 in recipient animals that received Treg ($P = .006$). T cell repertoire assessed by V-beta TCR screening with FACS analysis showed a polyclonal distribution. To determine if the improved and diverse lymphoid reconstitution is associated with increased immune function, mice were challenged with murine CMV intraperitoneally at day 14. Two weeks after infection, 66% of animals that received Treg in addition to Tcon, and 11% of animals that received Tcon alone were alive ($P = .05$). Uninfected mice in the respective groups served as controls to separate the effect of CMV infection and GvHD on survival. Compared to infected animals, no deaths were observed in the respective uninfected groups at this time point (Tcon alone, $P = .0004$; Treg + Tcon, $P = .21$). In both infected and control uninfected animals, Treg treated animals had no evidence of significant clinical GvHD compared to animals that received Tcon alone. Decreased viral load and increased CMV-specific T cell response in the Treg group confirmed survival outcomes ($P < .05$). These findings indicate that Treg enhance both the quantitative and functional recovery of the lymphoid cell populations while providing protection against GvHD.

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ACCELERATED IMMUNE RECOVERY FOLLOWING LLME TREATED DONOR LYMPHOCYTE INFUSION

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Delayed immune reconstitution is a major cause of morbidity and mortality after T cell depleted allogeneic progenitor cell transplant (PCT). To accelerate immune reconstitution without GVHD, we have administered escalating doses of L-leucyl-L-leucine methyl ester (LLME) treated lymphocytes (DLI) to 18 patients post CD34+ cell-enriched PCT in an ongoing phase I trial. LLME's cellular toxicity occurs after polymerization by dipeptidyl peptidase, leading to selective depletion of cells with cytotoxic effector