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

granules containing perforin. Products treated with LLME demonstrated a median depletion of 94.9% CD56+ cells, 75.8 % CD8+ cells, but only 37.2% CD4+ cells. Patients initially received 10e4 (haploidentical (HAPLO)), 10e5 (HLA identical unrelated (URD)), or 10e6 (HLA identical sibling (SIB)) CD3+ cells/kg in cohorts of 3, and were eligible for additional infusions at a one log higher dose after 28 days if there was no evidence of GVHD or the CD4 count was less than the target of 100 cells/ml. The initial dose was escalated by one log in subsequent cohorts if no complications were observed in the previous cohort. Nine patients received grafts from SIBs (4 at 10e6, 5 at 10e7), six from URDs (3 at10e5, 3 at 10e6), and three from HAPLOs (3 at 10e4). All patients had pre-DLI CD4 counts of <5.0 cells/ml, and received 1 to 3 infusions post PCT. Since ATG was used during PCT cytoablation, the first DLI infusions were administered after ATG levels were <2 mcg/ml. After DLI #1, 6/8 evaluable matched SIB recipients and 2/6 evaluable URD recipients demonstrated early recovery of donor derived CD4+ cells prior to day 35 s/p DLI, with a median CD4 count of 139 cells/ul (range 57–471). Ten of these achieved a CD4+ count above 100 after 1–3 total infusions. In contrast, none of the haploidentical recipients (receiving the smallest doses) had an increase in CD4+ counts. Five patients developed GVHD (Grade I–II (n =4), Grade III (n = 1 following 4×10e7 LLME DLI). In recovering patients, spectratype analysis of CD4+ cells comparing DLI before LLME treatment with lymphocytes recovered from the patients post DLI demonstrated that 94% of the resolvable Vb families were equally complex. Perforin positive cells, as well as adenovirus (n = 2), EBV (n = 1) and CMV (n = 2) specific lymphocytes, also recovered post-PCT in these patients. These preliminary results suggest that LLME treated DLI can accelerate CD4+ reconstitution without causing severe GVHD.

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REGULATORY T CELL (Treg) RECONSTITUTION FOLLOWING T CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION—KINETICS OF RECOVERY AND RELATIONSHIP WITH GVHD

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Regulatory T cells (Treg) that specifically express the Foxp3 transcriptional repressor are instrumental in the maintenance of tolerance. They may suppress GVHD and regulate lymphocyte recovery after SCT. Animal transplantation models show that Tregs suppress GVHD. However, current data in humans on the impact of Tregs on GVHD following SCT is conflicting, possibly due to differences in surface phenotyping techniques used to characterize these cells. To characterize Treg recovery and the relationship with acute GVHD we studied CD4+ CD25+ Foxp3+ T cells reconstitution in the first 120 days following a T-depleted allogeneic SCT in 17 patients with leukemia. We used surface phenotyping for CD4 and CD25, intracellular staining for Foxp3 protein, Foxp3 gene expression and functional assays to define Treg cells. We found a strong correlation between Foxp3 gene expression and Foxp3+ CD4+ T cell number by intracellular staining. There was a direct correlation between Foxp3 gene expression and CD4+ CD25+ T cell numbers in healthy donors. Interestingly, this strict correlation was lost in patients with leukemia post-SCT due to a disparity between CD4+ CD25+ and CD4+ Foxp3+ T cell numbers in some patients, most likely related to expansion of the CD4+ CD25+ Foxp3− effector T cell population. Although, the proportion of CD4+ T cells that are CD25+ reached normal as early as 30 days post-SCT (median 5.0, range 2.0–8.9), absolute circulating Treg numbers were low in the first 30–45 days post-SCT reaching near-normal levels by day 120, indicating that Tregs generally recover at a similar rate to CD4+ CD25− T cells. However higher day 30 Foxp3 gene expression inversely correlated with the development of grade II–IV acute GVHD ($P = .02$) suggesting that suppressor activity rather than Treg numbers may be more relevant to GVHD suppression. Chimerism analysis was performed on sorted CD4+ CD25+ and CD4+ CD25− T cell fractions from leukapheresis samples collected 120 days post transplant on 7 patients. In most patients, the expanded Treg population was enriched in cells of donor origin. Our results suggest that early Treg reconstitution may affect the development of GVHD. These results suggest that Treg function (as measured by Foxp3 gene expression) is important in controlling alloreactions post-transplant. Our data support the use of ex vivo expanded activated Tregs given in the first 30 days post SCT to prevent acute GVHD.

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GANCICLOVIR SUPPRESSES HUMAN T LYMPHOCYTE PROLIFERATION IN VITRO

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Clinically significant cytomegalovirus (CMV) infection in allogeneic blood or marrow transplant recipients has dramatically declined in recent years by prophylactic or preemptive therapy with ganciclovir (GCV). However, patients at risk for CMV reactivation (seropositive donor and/or recipient) consistently have worse outcomes than patients who are not at risk for CMV reactivation. We have previously shown that even in the absence of overt CMV disease, persisting post-transplant antigenemia predicts for increased late relapse and treatment failure (Nakamura et al BBMT 2004). In other words, frequent CMV reactivation serves as a surrogate for impaired post-transplant immune reconstitution. To explain the observed association between CMV reactivation and relapse, we also raised the possibility that several weeks of GCV therapy could exert a deleterious effect on a fragile immune system. We present in vitro data that GCV inhibits normal human T-lymphocytes and identify the mechanism of immunosuppression. Human PBMCs were extracted from normal volunteers and subjected to mitogenic (PHA) or antigen-specific (CMV-antigen) stimulation in the absence or presence of varying concentrations of GCV. Thereapeutic concentrations of GCV greatly reduced both PHA-induced as well as antigen-specific T cell proliferation in vitro compared to foscarnet. The uptake of 3[H]-thymidine by PBMCs after 5 days incubation in RPMI-10% AB serum was reduced by 35% at peak therapeutic concentrations (10 mcg/ml) of GCV as opposed to 73% by Tacrolimus (10ng/ml). GCV did not induce apoptosis of the Raji (B cell, Burkitt's leukemia) cell line nor did it induce apoptosis of normal peripheral blood lymphocytes in the presence or absence of stimulation. Flow cytometry-based BrdU incorporation assays show that GCV exerts a time-dependent impairment of DNA synthesis in lymphocytes. Collectively, these results show that GCV is a potent suppressor of T-lymphocyte proliferation in vitro at therapeutic concentrations and the likely mechanism of action is inhibition of DNA synthesis. GCV therapy in BMT patients probably increases the level and/or duration of overall T cell immunosuppression and may account for the differences in clinical outcome.

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INSULIN-LIKE GROWTH FACTOR I IS A POSITIVE REGULATOR OF THYMIC FUNCTION

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Neuroendocrine growth factors have been implicated to play a role in regulating thymic function and serve as potential agents to accelerate thymic T cell production following hematopoietic stem cell transplantation (HSCT). We present evidence supporting insulin-like growth factor I (IGF-1) as a thymic regulator. Continuous 2-week infusions of IGF-1 (100 ug/day) into young adult mice resulted in significant increases in muliple thymocyte subpopulations, including early CD4-CD8- subpopulations and earliest thymocyte precursors (ETP). BrdU incorporation with continuous oral administration was also increased in these populations, suggesting a stimulatory effect of IGF-1 on thymocyte proliferation and developmental kinetics. Expansion of peripheral naive T