achieved in 81.2% (13/16) of responders, and occurred a median 10 (2-34.7) months after ECP. Nine cases (56.2%) had recurrent cGvHD at 8.4 (3-19.3) months after achieving response and 1 (6.25%) had late primary disease relapse (>6 months after ECP initiation). Another non-responder had a late relapse. Factors associated with OR were younger age at cGvHD diagnosis and at ECP initiation (<50 years, P=0.046), while that with CR was early initiation of ECP within 18 months of cGvHD diagnosis (P=0.018). The 1-year, 2-year and 3-year post ECP initiation survival rates were 66.7%, 38.1% and 23.8%. Types of cGvHD onset and donor were associated with survival post ECP initiation (P=0.028 and P=0.05). The patients with non-progressive onset cGvHD and with HLA-MRD survived longer (1-year post ECP initiation survival rates of 88.9% and 78.6%) than those with progressive onset cGvHD and with HLA-MUD (50% and 33.3%). However, OR and CR to ECP were not associated with prolonged post ECP initiation survival. In summary, our results suggest that ECP is an effective second-line treatment option for cGvHD especially with early initiation. Progressive onset cGvHD and HLA-MUD were associated with poor survival rates in patients receiving ECP for cGvHD. Whether response to ECP would be translated to higher survival requires a larger study.

Table

Characteristics	Details
Sex: male/female (n/n)	**9/12**
Median age (median, range)	
-- At day 0	**42.6 (26.5-60.1) years**
-- At cGvHD diagnosis	**42.8 (26.8-60.4) years**
-- At ECP initiation	**43.4 (28-60.4) years**
Type: HLA-MRD/-MUD/-mismatched unrelated CBD (n)	**14/6/1**
GvHD Prophylaxis (n)	
-- Calcineurin inhibitor and methotrexate	**18**
-- Calcineurin inhibitor and methylprednisolone	**2**
-- Triple regimen	**1**
Acute GvHD (n)	**19**
-- Clinical Grade: I/II/III/IV	**5/12/1/1**
-- ECP for aGvHD	**1**
Chronic GvHD (n)	**21**
-- Onset: *De novo*/Progressive/Recurrent (n)	**1/13/7**
-- CIBMTR Overall Severity at ECP initiation (n)	
.....Mild	**2**
.....Moderate	**11**
.....Severe	**8**
-- Maximum Grade (n)	
.....Limited	**3**
.....Extensive	**18**
-- Median duration of ECP (median, range)	**10.8 (1.3-58.8) months**
-- Median follow-up time after ECP initiation (median,range)	**15.5 (1.3-62.4) months**
-- Overall and complete response to ECP by CIBMTR Severity	
.....Mild (n (%))	**1 (50%), 1 (50%)**
.....Moderate (n (%))	**5 (45.4%), 4 (36.4%)**
.....Severe (n (%))	**5 (62.5%), 3 (37.5%)**

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INTERACTION BETWEEN HOST NATURAL KILLER T CELLS AND DONOR $CD4^+CD25^+$ T_{REG} CELLS PROTECTS AGAINST GVHD AFTER TLI/ATS HOST CONDITIONING AND ALLOGENEIC BONE MARROW TRANSPLANTATION

Pillai, A.B.[1], Dutt, S.[1], George, T.I.[1], Strober, S.A.[1] [1]Stanford University School of Medicine, Stanford, CA.

The murine non-myeloablative regimen of total lymphoid irradiation (TLI) and anti-thymocyte serum (ATS) prevents acute graft-versus host disease (aGVHD) and was recently successfully applied for human hematolymphoid malignancies (Lowsky et al, NEJM, 2005). To investigate GVHD protection, we transplanted (BMT) 50×10^6 bone marrow cells and 60×10^6 splenocytes from wild-type (WT), $CD4^{-/-}$, IL-$4^{-/-}$, or IL-$10^{-/-}$ C57BL/6 (H-2^b) donor mice into wild-type (WT) or natural killer (NK) T cell deficient J$\alpha18^{-/-}$ BALB/c (H-2^d) hosts following TLI/ATS. Controls were WT BALB/c mice given 800cGy total body irradiation (TBI) and ATS with BMT from WT C57BL/6 donors. TBI/ATS-conditioned WT hosts given BMT from WT donors and TLI/ATS conditioned WT hosts given BMT from $CD4^{-/-}$, IL-$4^{-/-}$, or IL-$10^{-/-}$ donors developed aGVHD, with marked donor $CD8^+$ T cell accumulation in liver, mesenteric lymph nodes (MLN), and colon at day 6. Since GVHD protection depends on donor $CD4^+$ cells, IL-4, and IL-10, we investigated the role of donor $CD4^+$ T_{regs} in GVHD protection. TLI/ATS-conditioned WT hosts given BMT from WT donors survived without aGVHD and demonstrated a dramatic ($p< 0.01$) ten-fold increase in the absolute number of donor $CD4^+CD25^+Foxp3^+$ T_{regs} in the spleen at day 6 after BMT relative to TBI/ATS-conditioned WT hosts or TLI/ATS-conditioned J$\alpha18^{-/-}$ hosts that succumbed to aGVHD. These $CD4^+CD25^+Foxp3^+$ T_{regs} secreted IL-4 and IL-10 and had the GVHD suppression capacity of conventional $CD4^+CD25^+$ T_{regs}. Pre-transplant adoptive transfer of sorted WT host invariant NK T cells protected TLI/ATS-conditioned J$\alpha18^{-/-}$ hosts from day 6 donor $CD8^+$ T cell accumulation and caused a significant ($p < 0.01$) increase in the absolute number of donor $CD4^+CD25^+Foxp3^+$ T_{regs}. Using congenic markers (CD45.1/CD45.2), we found that these $CD4^+$ T_{regs} arise from donor splenic (peripheral) T cells after BMT. CD25 depletion of WT donor cells before BMT into TLI/ATS-conditioned WT hosts or into TLI/ATS-conditioned J$\alpha18^{-/-}$ hosts given adoptive transfer of WT host NK T cells resulted in loss of the donor $CD4^+CD25^+Foxp3^+$ T_{regs} subsets in the spleen, and significantly increased day 6 donor $CD8^+$ T cell accumulation in the host liver, MLN, and colon, accompanied by lethal aGVHD. These studies show that host invariant NK T cells are critical for GVHD protection and that they can facilitate the expansion of donor $CD4^+CD25^+Foxp3^+$ T_{regs}, which protect TLI/ATS treated hosts from lethal GVHD after allogeneic BMT.

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IN VIVO ACTIVATION OF APCS WITH TLR LIGANDS AND TISSUE DAMAGE RATHER THAN AMOUNT OF HOST APCS ARE CRITICAL FACTORS THAT DETERMINE DLI-MEDIATED GVL REACTIVITY AND GVHD IN MHC-MATCHED MINOR HISTOCOMPATIBILITY ANTIGEN (MHAG)-MISMATCHED CHIMERAS

Radojcic, V.[1], Skarica, M.[1], Murphy, G.F.[2], Luznik, L.[1] [1]Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; [2]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

We recently found that residual host $CD11c^+$ DCs persist in the skin of MHC-matched chimeras after myeloablative conditioning and transplantation of T cell-repleted BM, and that those residual host APCs are insufficient for the induction of optimal DLI-mediated alloreactivity (J Immunol.,2006). Since the latter observation in established C3H.SW→B6 chimeras differs from the current paradigm in freshly irradiated MHC-matched chimeras, we hypothesized that in established chimeras, beside host APCs and donor T cells, the induction of DLI-mediated GVL reactivity and GVHD requires two additional factors: (a) TLR-mediated activation of APCs and (b) tissue damage. *In vivo* coadministration

of TLR7 (imiquimod) and/or 9 (CpG1826) ligands with DLI resulted in powerful DLI-mediated GVL reactivity ($P<0.02$), without clinical signs of GVHD in established C3H.SW→B6 chimeras. The effect was mediated by DLI-derived anti-mHAg-specific T cells since adoptive transfer of B6 DLI to the syngeneic B6→B6 chimeras in the presence of TLR ligands did not result in antitumor immunity. To examine the effects of tissue damage, established C3H.SW→B6.SJL chimeras were lethally re-irradiated and received C3H.SW BM together with purified donor $CD8^+$ T cells. Consistent with previous observation, these chimeras did not develop severe GVHD despite the fact that skin DCs were predominantly host-derived. However, if TLR7 ligand was administered prior to re-irradiation and administration of donor $CD8^+$ T cells, all chimeras developed severe skin and systemic GVHD ($P<0.01$). To determine whether the donor $CD8^+$ T cells directly recognize host mHAgs and induce tissue damage, we constructed chimeras using as a host $B6\beta2m^{-/-}$ mice in which host DCs and parenchyma are unable to present MHC class I-restricted peptides. We found no clinical signs of skin or systemic GVHD in the re-irradiated C3H.SW→$B6\beta2m^{-/-}$ chimeras that received purified donor $CD8^+$ T cells after pre-treatment with TLR7 ligand. Our data suggest that in MHC-matched setting (1) despite the presence of host DCs the TLR-mediated signaling is critical for induction of DLI-mediated GVL reactivity in established chimeras; (2) induction of GVHD in established chimeras requires not only donor T cells, host APCs and TLR ligands, but also tissue damage. These requirements for GVL reactivity and/or GVHD in established MHC-matched chimeras clearly differ from those in freshly irradiated MHC-matched or in established MHC-mismatched chimeras.

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SUCCESSFUL THERAPY OF STEROID-REFRACTORY ACUTE GRAFT-VERSUS HOST DISEASE WITH SEQUENTIAL ALEMTUZUMAB

Repp, R.[1], Claviez, A.[2], Schrauder, A.[2], Schub, N.[1], Gahn, B.[1], Gramatzki, M.[1] [1]Division for Stem Cell Transplantation and Immunotherapy, 2nd Medical Department, University of Kiel, Kiel, Germany; [2]Department of Pediatrics, University of Kiel, Kiel, Germany.

After allogeneic stem cell transplantation severe acute graft-versus-host disease (GvHD) is the major limitation of this treatment modality. While in acute GvHD addition of steroids to immunosuppressive prophylaxis lead to a response rate of 30% to 70%, treatment options of steroid resistant acute GvHD are limited and no accepted standard for salvage therapy exists. Alemtuzumab (Campath-1H) is a commercially available humanized antibody targeting the CD52 antigen, which is expressed on T-cells, B-cells and on some monocyte derived dendritic cells. For years, Alemtuzumab has been shown to be effective in GvHD prevention when used in conditioning regimes, however infectious complications did limit this approach when routinely applied. In order to evaluate efficacy and safety of sequential alemtuzumab for severe acute GvHD, ten patients with steroid-resistant acute GvHD of grade III or IV involving the gut or/and liver were treated in our Division. The initial three patients, all with advanced grade IV GvHD of the liver and already receiving cyclosporine, mycophenolate mofetil and high-dose steroids, were treated with up to 80 mg of alemtuzumab followed by additional 40 mg CD52 antibody within the next four weeks. While dramatic clinical responses were seen, such as a bilirubin level of 48 mg/dl returning to normal within 6 weeks, virus reactivation and bacterial infections were limiting this approach. Although pronounced lymphocyte depletion seems inevitable for efficacy, additional patients were treated with lower doses of 20 to 30 mg Alemtuzumab, repeated approximately every two weeks, while concomitant immunosuppressive drugs could be tapered within 3 to 4 weeks. Acute GvHD improved in all patients treated so far. A complete response of liver and gut GvHD was seen in two patients, an additional patient developed a vanishing bile duct syndrome but has no evidence of ongoing GvHD. Another two patients are still on treatment with major improvement of gut and liver-GVHD, respectively. Thus, alemtuzumab given sequentially in moderate doses shows promising activity in severe, steroid refractory acute GvHD. However, monitoring for infectious complications, particularly by reactivated viruses (CMV, adenovirus) is mandatory for months after treatment.

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IN VIVO BIOLUMINESCENCE AND [^{18}F]-FHBG MICROPET IMAGING STUDIES OF HUMAN T CELL TRAFFICKING, EXPANSION, AND XENOGENEIC GRAFT-VERSUS-HOST-DISEASE FOLLOWING DIFFERENT ROUTES OF HUMAN T CELL ADMINISTRATION

Rettig, M.P.[1], Nervi, B.[1], Ritchey, J.K.[1], Prior, J.L.[1], Piwnica-Worms, D.[1], Wang, H.L.[1], Bauer, G.[1], Walker, J.[1], Bonyhadi, M.L.[2], Berenson, R.J.[2], Nolta, J.[1], DiPersio, J.F.[1] [1]Washington University School of Medicine, St. Louis, MO; [2]Xcyte Therapies, Inc., Seattle, WA.

GVHD is the major cause of morbidity and mortality following allogeneic hematopoietic stem cell transplantation. We previously demonstrated that 60% of sublethally irradiated NOD/SCID-$\beta2m^{null}$ mice develop lethal xenogeneic GVHD (X-GVHD) following retroorbital (r.o.) injection of 10^7 human T cells (huT). However, we observed no X-GVHD when the huT were administered through the lateral tail vein (i.v.). In the current studies, we used in vivo bioluminescence imaging (BLI) and [^{18}F]FHBG microPET to evaluate why the route of huT cell administration affected huT cell engraftment and the development of lethal XGVHD in NOD/SCID-$\beta2m^{null}$ mice. We generated huT that co-expressed chimeric CD34-thymidine kinase (CD34-TK; for [^{18}F]FHBG microPET) and click beetle red luciferase-egfp (CBRluc-egfp; for BLI) genes by transducing the cells with two retroviral vectors, selecting for CD34, and sorting for EGFP ($huT^{TK/CBR}$). As before, sub-lethally irradiated NOD/SCID-$\beta2m^{null}$ mice injected i.v. with 10^7 $huT^{TK/CBR}$ failed to develop lethal XGVHD, whereas 60% of mice injected r.o. developed lethal XGVHD. Serial whole body BLI and [^{18}F]FHBG microPET revealed very different trafficking patterns and expansion profiles between the i.v. and r.o. routes of huT cell administration. $HuT^{TK/CBR}$ cells injected i.v. immediately trafficked to the lungs and failed to expand during the first three weeks after infusion. In contrast, a significant portion of the r.o.-injected cells remained in the retroorbital cavity and trafficked to secondary lymphoid organs during the first 7 days after $huT^{TK/CBR}$ cell infusion. This altered trafficking of the r.o.-injected cells was associated with a 10-fold and 5-fold increase in the $huT^{TK/CBR}$ BLI signal and [^{18}F]FHBG standardized uptake value, respectively, during the first 2 weeks after huT cell infusion, with the $huT^{TK/CBR}$ cells accumulating in the skin, lymph nodes, lungs and gut. This homing pattern and strong BLI and [^{18}F]FHBG signals of the r.o.-injected $huT^{TK/CBR}$ cells remained until death from GVHD. Importantly, histological examination of the GVHD target tissues revealed changes consistent with human GVHD. Therefore, this NOD/SCID-$\beta2m^{null}$ mouse model provides a system to study the pathophysiology of acute GVHD induced by human cells and aids in the development of more effective therapies for human GVHD.

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DAY 30 POST-TRANSPLANT ABSOLUTE LYMPHOCYTE AND NATURAL KILLER CELL COUNT STRONGLY PREDICT OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCY

Savani, B.N.[1], Mielke, S.[1], Rezvani, K.[1], Yong, A.[1], Shenoy, A.[1], Read, E.J.[1], Hensel, N.[1], Childs, R.[1], Barrett, A.J.[1] [1]Hematology Branch, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD.

One hundred and fifty-seven patients with leukemia (80 CML, 48 AML, 29 ALL) received a T cell depleted myeloablative allogeneic stem cell transplant (SCT) (28 BM and 129 PB) from an HLA-matched sibling between 09/1993-09/2005. Conditioning consisted of TBI (12-13.6 Gy) + cyclophosphamide or combined with fludarabine. T cell dose ranged from 0.2 - 2 × 10^5CD3+ cells/kg. GVHD prophylaxis was low dose cyclosporine (level 100-200 ng/ml) in 103 and standard dose in 54. Patients without ≥ grade II acute GVHD received 1-2 donor lymphocyte infusions