Table 1.

BMT Group	Pathology Index	BALF Analysis: Total Cells ($\times 10^6$)	macs ($\times 10^6$)	$CD8^+$ T Cells ($\times 10^3$)	$GR1^+$ Cells ($\times 10^3$)	Protein (μg/ml)	TNFα (pg/ml)
Syngeneic	0.7 ± 0.3	0.72 ± 0.15	0.69 ± 0.14	7.2 ± 1.5	7.2 ± 1.5	247 ± 38	0 ± 0
Allo $CCR1^{+/+}$	5.2 ± 0.8	2.6 ± 0.6	1.9 ± 0.5	473.2 ± 69.3	87.3 ± 14.3	517 ± 58	24.6 ± 1.4
Allo $CCR1^{-/-}$	2.7 ± 0.6*	1.1 ± 0.1*	0.82 ± 0.1+	68.8 ± 7.0**	15.3 ± 1.6**	320 ± 51*	1.0 ± 0.6*

*$p < 0.05$; **$p < 0.01$; +$p = 0.06$.

137

REVERSAL OF SEVERE GRAFT-VERSUS-HOST DISEASE AFTER NON-MYELOABLATIVE MATCHED UNRELATED DONOR STEM CELL TRANSPLANT BY INFUSION OF BACKUP AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS

Khan, S.A.; Moreb, J.S. University of Florida College of Medicine, Gainesville, FL.

Acute graft vs. host disease (GVHD) is major concern in stem cell transplantation and is associated with significant morbidity and mortality. The incidence of grade II-IV acute GVHD is 78% and grade III-IV is 36% after HLA matched unrelated donor (MUD) transplant. We report a successful use of infusion of autologous peripheral blood stem cells to treat severe, refractory grade 4 GVHD after a non-myeloablative MUD transplant. Patient was a 55 years old white female with non-secretory MM presenting with pathological fracture of her arm. She was initially treated with VAD and then hyper-CVAD chemotherapy and achieved partial remission (PR). Subsequently, she underwent autologous peripheral blood stem cell transplant (PBSCT). She progressed 2 months after autologous PBSCT and became refractory to Thalidomide as well as chemotherapy combination of CCNU, VP-16, Cytoxan and prednisone. Therefore, she underwent a non-myeloablative MUD PBSCT after conditioning with Fludarabine, ATG and Busulfan. Post transplant evaluation revealed persistent disease with 60% plasma cells and mixed chimerism with 91% male donor cells, so immunosuppressive therapy was tapered. Two weeks later she developed severe grade 3 GVHD involving skin and gut which progressed to grade 4 GVHD and was refractory to therapy with FK506, Steroids, Cellcept, Daclizumab, Remicade and Thalidomide. She developed severe malabsorption with albumin as low as 1.6. We gave her cyclophosphamide followed by backup autologous stem cells in order to stop the GVHD by creating "host-versus-graft effect". On day +90 she received 10×10^6/kg $CD34^+$ cells and 1.4×10^7/KG $CD3^+$ cells. Four weeks later she still had 99% donor chimerism with no significant change in her GVHD symptoms and therefore she was given 2nd infusion of 42×10^6/kg $CD34^+$ cells and 5.7×10^7/kg $CD3^+$ cells. Subsequently, she recovered slowly from her GVHD and was discharged after 3 months of hospitalization. Currently she is 2.5 years post transplant without any immunosuppressive therapy and most recent BM test showed 100% donor chimerism and stable MM with 10% plasma cells. This case demonstrates feasibility of infusion of autologous stem cells to control refractory GVHD. A similar case was previously reported (Ricordi et al, Cell Transplantation 1994;3:187). The mechanism for such effect is not clear but may involve development of tolerance of donor T lymphocytes vs. host without inhibiting GVL effect or causing mixed chimerism.

138

CARDIAC MANIFESTATIONS OF GRAFT-VERSUS-HOST DISEASE

Gilman, A.L.[1]; Rackley, C.[2]; Goldman, F.[3]; Serrano, A.[4]; Chan, K.W.[5]; Schultz, K.R.[6] 1. University of North Carolina School of Medicine, Chapel Hill, NC; 2. Children's Memorial Hospital, Chicago, IL; 3. University of Iowa, Iowa City, IA; 4. Children's Mercy Hospital, Kansas City, MO; 5. M.D. Anderson Cancer Center, Houston, TX; 6. British Columbia Children's Hospital, Vancouver, BC, Canada.

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after bone marrow transplantation. Well-documented manifestations of GVHD include dermatologic, gastrointestinal, hepatic, pulmonary, musculoskeletal, and hematologic manifestations and sicca syndrome. To date, the heart has only rarely been reported to be a target of GVHD. We report 11 patients who developed bradycardia, coronary artery disease, or cardiomyolysis in association with acute or chronic GVHD. Eight patients were male and 3 were female. Patients were 4 months-19 years old (median 12 yrs). Three patients received post-transplant immunotherapy. The patient with Fanconi anemia/AML received IL-2 for recurrent leukemia and two recipients of T cell-depleted grafts received DLI, one for relapse and one for graft rejection. Regarding possible risk factors, six patients had received total body irradiation and five patients had received anthracyclines prior to transplant. Other possible causes for heart block and bradycardia including electrolyte abnormalities and medications were excluded. Of eight patients with bradycardia, seven had resolution of bradycardia (including heart block and sinus node failure) and one had improvement with increased immunosuppression. Three patients had a recurrence of bradycardia and GVHD following weaning of immunosuppression. The severity of these manifestations ranged from asymptomatic to fatal. The incidence of cardiac manifestations was estimated at 9.3%. TBI and prior anthracycline therapy did not seem to be significant risk factors because approximately half of patients had not received either. The majority of patients had alternative donors and 3 had post-transplant immunotherapy. Although uncommon, it is important to recognize these cardiac manifestations as they may reflect GVHD activity and may be reversible by increasing immunosuppression.

Cardiac Manifestations of GVHD

Age	Disease	Stem Cell Source	GVHD	Cardiac Manifestation	HR during Bradycardia	Normal HR for Age	Cardiac Outcome
4 mo	SCID	Haplo father BM	Hyper-acute & chronic	Complete heart block	30–40's	90–140's	Resolved w/ MP/ CSA
2 yr	Fanconi, AML	MSD BM	Atypical, after IL-2 for GVL	Sinus node failure	30–40's	90–140's	Resolved w/ MP/ Tac
18 mo	Wiskott-Aldrich	5/6 MUD cord	Acute & chronic	Bradycardia	50–90's	90–140's	Resolved w/ MP/ HCQ
21 mo	CID	4/6 MUD cord	Acute & chronic	Bradycardia	50–80's	90–140's	Improved w/ MP/ Pento-ostatin
13 yr	Refractory LCL	MSD PBSC	Acute & chronic	Bradycardia	50's	60–110	Resolved w/ MP
17 yr	ALL, CR4	6/6 MUD PBSC	Acute	Bradycardia	50's	60–110	Resolved w/ MP/ Zena-pax
12 yr	ALL, CR3	Haplo mother PBSC	Acute & chronic	Bradycardia	50–60's, low 40	60–110	Resolved w/ MP
13 yr	MDS, AML	Haplo mother PBSC	Acute & chronic	Bradycardia	50–60's	60–110	Resolved w/ MP
18 yr	ALL, CR3	MSD PBSC	Acute & chronic	Cardiomy-olysis	—	—	Sudden death
19 yr	AML, relapse	MSD BM	Acute & chronic	Coronary arterioscler-osis	—	—	Sudden death
25 mo	Wiskott-Aldrich	6/6 MUD BM	Acute & chronic	Coronary arterioscler-osis	—	—	Sudden death

139

RELATIONSHIP BETWEEN RACE AND RISK OF ACUTE GRAFT-VERSUS-HOST DISEASE

Klumpp, T.R.; Jillella, A.P.; Ulicny, J.A.; Emmons, R.V.B.; Mangan, K.F. Fox Chase-Temple BMT Program, Philadelphia, PA.

Several groups have reported an increased incidence of acute GVHD among African-American transplant recipients. However, informal communications with other BMT teams suggest that

these reports may be the result of publication bias, i.e., it appears that multiple groups have looked at this issue, but only those groups that have found a significant relationship, potentially as a result of random chance, have reported their findings. To further investigate this question we developed a multivariate logistic regression model for factors predictive of acute GVHD based on a series 167 patients, including 18 African-Americans, who underwent allogeneic BMT at our institution between January 1, 1992 and May 30, 2004. Factors examined in the multivariate model included age, CD34 dose, CD3 dose, sex mismatch pattern (female-to-male vs other), category of conditioning regimen (full-dose vs reduced-dose), presence or absence of an antigen-level HLA mismatch, type of donor (related vs unrelated), stem cell source (marrow vs PBSC), and race (African-American vs Caucasian). It was not possible to separate out the potential effect of the GVHD prophylactic regimen because our data set is characterized by a very strong correlation between type of GVHD prophylactic regimen and category of conditioning regimen. We observed a univariate trend toward an increased incidence of Grade 3 or higher acute GVHD among African Americans (39% vs 21%, p = 0.13). Race was not independently predictive of acute GVHD in the multivariate model, although the statistical power of the model to detect this relationship was limited due to the fact that only 18 African Americans were included in the series. Interestingly, among patients transplanted since 1996, there was a statistically significant (univariate) increase in the incidence of grade 3 or higher acute GVHD among African Americans (64% vs 25%, p = 0.01) but also a parallel disparity in the incidence of HLA-mismatched transplantation (27% vs 6%, p = 0.036) such that race remained non-contributory in the multivariate model. We conclude that the purported relationship between race and acute GVHD may be less strong that has been previously reported due to publication bias and/or to an increased incidence of HLA-mismatched transplant in this population. Further examination of this issue using larger data sets appears warranted.

140

EXTRACORPOREAL PHOTOCHEMOTHERAPY FOR (ECP) THE TREATMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE

Couriel, D.R.; Hosing, C.; Anderlini, P.; Ippoliti, C.; Hsu, Y.; Neumann, J.; De Lima, M.J.; Ghosh, S.; Rhodes, B.; Saliba, R.; Champlin, R.; Donato, M. University of Texas M.D. Anderson Cancer Center, Houston, TX.

Introduction: Chronic graft-versus-host disease (cGVHD) is a major cause of long term morbidity and mortality following allogeneic hematopoietic stem cell transplant (HSCT). Beyond the combination of steroids and calcineurin inhibitors, a variety of immunosuppressive methods have been used to manage this condition, most of them of limited efficacy and steroid-sparing capacity. ECP is becoming increasingly used in the treatment of acute and chronic GVHD, with promising preliminary data. **Methods:** Retrospective evaluation of all ECP procedures performed between 1/30/98 and 10/2/92 in patients with GVHD occurring after day 100. Patients were considered evaluable for response when they were treated with ECP for at least 1 month, without the addition of any further immunosuppression. Complete (CR) and partial responses (PR) had a minimal duration of 2 weeks. **Results:** A total of 82 patients with chronic GVHD were analyzed. The median age was 40 (5–70), and the donor origin was matched sibling (48, 59%), matched unrelated (24, 29%) or mismatched (10) HSCT. Most of them received tacrolimus-based prophylaxis (74, 90%) and had a history of acute GVHD (64, 78%). Most patients were on steroids (64) and had received a median of 2 (1–6) treatments prior to ECP. Out of 82 patients, only 51 were evaluable for response, and the remainder received further immunosuppression within a month from the start of ECP (30), or lacked documentation (1). The CR/PR rate for evaluable patients was 82% (n= 42). Thirty-four patients (67%) had skin involvement and the majority (32, 94%) responded to ECP. All types of skin involvement (lichenoid, scleroderma, mixed, acute-appearing) responded to therapy. ECP also demonstrated activity in visceral GVHD, with response rates of (15, 88%) and (6, 67%) respectively. The CR/PR rate for oral GVHD was 100% (n= 6), and for ocular GVHD 75% (n= 3). Of 51 patients, 32 required further immunosuppression for control of their GVHD. Since initiation of ECP, the overall survival for the whole group was 37%, with a median survival of 10 months. **Conclusions:** In this heavily pretreated group of patients with chronic GVHD, the response to ECP seems promising in all organs, including GVHD of the lung and the liver. The prospective and systematic evaluation of the role of ECP in the management of GVHD is warranted.

141

SUBEROYLANILIDE HYDROXAMIC ACID MODULATES THE INNATE AND ALLOSTIMULATORY RESPONSES OF DENDRITIC CELLS AND REGULATES EXPERIMENTAL ACUTE GRAFT-VERSUS-HOST DISEASE

Reddy, P.[1]; Maeda, Y.[1]; Lowler, K.[1]; Liu, C.[2]; Dinarello, C.[3]; Ferrara, J.L.M.[1] 1. University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; 2. University of Florida College of Medicine, Gainesville, FL; 3. University of Colorado Health Sciences Center, Denver, CO.

Dendritic cells modulate the innate (via Toll like receptors-TLRs) and the allostimulatory immune responses. Suberoylanilide hydroxamic acid (SAHA) is an anti-tumor agent that also exhibits potent anti-inflammatory properties. We investigated the effect of SAHA on modulating DC functions. Pre-incubation of BM derived DCs with 100 nM-500 nM concentrations of SAHA (s-DCs) significantly reduced the secretion of TNF-α and IL-6 upon TLR stimulation with lipopolysaccharide (TLR-4), peptidoglycan (TLR-2), poly IC (TLR-3) and CpG (TLR-9). Pre-treatment of DCs with SAHA significantly reduced the proliferation of allogeneic T cells in MLR cultures. Mixing experiments at 1:1 ratio significantly suppressed the allostimulatory capacity of control DCs. Incubation of DCs with SAHA significantly decreased the expression of CD40, CD80, CD86 and OX40L and but caused no significant changes in MHC class II expression. We determined the in vivo relevance of SAHA mediated effects on DCs by utilizing Class I disparate $CD8^+$ mediated [bm1 $\rightarrow$ $\beta2MG^{-/-}$], and Class II disparate $CD4^+$ mediated [bm12 $\rightarrow$ MHC-$II^{-/-}$] murine models of alloreaction. Recipient $\beta2MG^{-/-}$ and Class-$II^{-/-}$ B6 animals received 11 Gy, 2–4 $\times$ 10^6 T cells and 5 $\times$ 10^6 TCDBM cells from syngeneic B6 or allogeneic bm-1 and bm-12 donors respectively. The recipients were also injected on days −1 and 0 with 4–5 $\times$ 10^6 wild type B6 BM derived c-DC and s-DCs. Injection of s-DCs significantly reduced in vivo allogeneic donor T cell expansion of both $CD4^+$ and $CD8^+$ T cell subsets. We next utilized the well characterized mouse [BALB/c $\rightarrow$ B6] model of acute GVHD to determine the effect of s-DCs on an ongoing GVHD reaction. On day −1, the recipient B6 animals received 11 Gy and on day 0 were injected with 2 $\times$ 10^6 T cells and 5 $\times$ 10^6 BM cells from syngeneic B6 or allogeneic BALB/c donors. Injection of 5 $\times$ 10^6 host type s-DCs on days −1, 0, and +2 resulted in significantly better survival, reduced serum levels of TNF-α, donor T cell expansion and histopathology of GVHD ($P < 0.01$) on day +7 after BMT. Lastly, we determined the effect of SAHA on the DCs derived from the peripheral blood of normal healthy human volunteers and found that SAHA significantly reduced allogeneic T cell proliferation. Together our results demonstrate that SAHA modulates innate and allostimulatory functions of DCs in vitro and in vivo, and suggest that SAHA treatment of DCs might represent a novel cellular therapy that can reduce clinical GVHD.

142

MATURE T CELLS ALONE SEPARATE GVL FROM GVHD: THE NEED FOR BETTER CONTROLS

Chen, B.J.; Son, J.; Cui, X.; Chao, N.J. Duke University Medical Center, Durham, NC.

Separation of graft-vs.-leukemia effect (GVL) from graft-vs.-host disease (GVHD) is the phenomenon that patients with leukemia/lymphoma achieve complete remission without GVHD after allogeneic stem cell transplantation. We demonstrate in this study that separation of GVL from GVHD could be achieved using only unmanipulated mature T cells in a mouse model. Graded numbers (10^3-10^6) of purified T cells from C57BL/6 mice were transplanted