T cells would ameliorate the development of graft-versus-host-disease (GVHD) in recipients of an allogeneic bone marrow transplantation (allo-BMT). To analyze the expression of ICOS on donor T cells, we transferred CFSE-labeled donor T cells into irradiated allogeneic recipients and observed an increased expression of ICOS on alloreactive T cells compared to non-alloreactive T cells. We then performed GVHD experiments in two models with full MHC class I and II disparity and observed significantly less GVHD morbidity and mortality in recipients of ICOS−/− donor T cells. Interestingly, in GVHD/graft-versus-tumor (GVT) experiments, ICOS−/− donor T cells displayed intact GVT activity, while their GVH activity was diminished. We are currently performing experiments to assess the alloreactivity of ICOS−/− T cells in vivo, the infiltration of ICOS−/− donor T cells into GVHD target organs, and the degree of GVHD damage in target organs by histopathological analysis. In conclusion, we have found that ICOS−/− donor T cells cause less GVHD morbidity and mortality in comparison to wild-type donor T cells while preserving GVT activity. This data suggests that strategies to inhibit ICOS could be useful for the prevention and/or treatment of GVHD in recipients of an allo-BMT.

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ADOPTIVE TRANSFER OF PRIMED T CELLS PRIOR TO ABLATIVE CONDITIONING AND BMT RESULTS IN SYNGENEIC ANTI-TUMOR IMMUNITY EARLY POST-TRANSPLANT

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We have shown that mice vaccinated against a B6 myeloid leukemia cell line (MMB3.19) prior to BMT followed by tumor challenge post-BMT exhibited tumor specific immunity. Vaccinated BMT recipients exhibited prolonged survival compared to unvaccinated BMT recipients indicating that anti-tumor reactive T cells survived the conditioning regimen and subsequent autologous BMT. To investigate T cell survival and function early after ablative conditioning and BMT, an adoptive transfer BMT model (AT-BMT) was developed. Since memory cells appear less susceptible to irradiation induced apoptosis vs. naive cells, B6-gfp$^+$ mice were immunized with irradiated MMB3.19 cells 5-6 weeks before the BMT as a source of anti-tumor primed T cells for adoptive transfer (AT). Two days prior to BMT, 2.5x10^7 CD3$^+$ cells from vaccinated donor B6-gfp$^+$ mice were injected (i.p.) into naive C57BL/6 (B6-gfp$^-$) recipients. One day later (D-1), recipient mice were conditioned with 8.5Gy TBI and at D 0 were injected with TCD syngeneic B6-gfp$^-$ marrow. On D+1 post-BMT, mice received an ip challenge of 2.5x10^4 viable MMB3.19 cells. AT-BMT recipients exhibited significantly increased MST vs. non-ATBMT controls (control: MST = 13.8 days vs. AT-BMT: MST > 40 days). To examine if so-called "low quality" CD8$^+$ T cells could protect in this model, B6-wt and B6-CD4$^{-/-}$ mice were primed 6 weeks prior to AT. Recipients received an AT of 2.5x10^7 CD4$^+$ and CD8$^+$ B6-wt (CD8$^+$ = 1.2x10^7) or CD4$^-$CD8$^+$(1.2x10^7 cells) on D-2 and were irradiated, transplanted and challenged with tumor as above. In contrast to B6-wt T cells, recipients of CD4$^-$ CD8$^+$ T cells were not protected from tumor challenge. Following transplant, spleen and peritoneal exudate cells (PEC) were analyzed for the presence of surviving AT cells. Transferred B6-gfp$^+$ cells were readily detected at Days +4 and +11 in the spleen (0.5% and 1.5%, respectively) of AT-BMT recipients injected with tumor post-BMT. Notably, a large percentage of gfp$^+$ cells were identified in the PEC of these AT-BMT mice at these time points (10.7% and 50.9%, respectively). In total, these results demonstrate that memory T cells can survive ablative BMT conditioning and contribute to an effective immune response (i.e., anti-tumor) in the early post-transplant period during lymphohematopoietic reconstitution. These observations suggest that approaches to increase selective host T cell survival during BMT may be useful to diminish immune deficiency in the immediate post-transplant period.

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UNRELATED DONOR STEM CELL TRANSPLANTATION DOES NOT RESULT IN LONG TERM REMISSION IN PATIENTS WITH MULTIPLE MYELOMA DESPITE THE PRESENCE OF CHRONIC GVHD

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Autologous SCT increases survival for patients with multiple myeloma compared to conventional chemotherapy but is not considered curative as most patients eventually relapse. Allogeneic SCT improves the CR rate and can result in a graft versus myeloma (GVM) effect, but not all patients have a related donor. We performed unrelated SCT using either a full or reduced intensity preparative regimen. Patients with stage II or III multiple myeloma who had relapsed following at least one prior therapy were eligible. From January 1996 to May 2001 thirteen patients were enrolled. Prior to SCT, patients had received a median number of 3 prior regimens (range, 1-5). Six patients had previously received radiation, and three had previously undergone an autologous transplant. The median age at transplant was 50 years (range 38-62). Six patients received a full intensity preparative regimen of total body irradiation (TBI), busulfan, and cyclophosphamide, and seven patients received a reduced intensity regimen of total lymphoid irradiation, fludarabine, and busulfan. All patients received HLA-matched, unrelated donor BM (n = 7) or peripheral stem cell (n = 6) transplants. The median mononuclear cell dose was 3.4 x 10^8 per kg (range, 1.2–7.0). GVHD prophylaxis consisted of tacrolimus and methotrexate. Three patients developed grade 3 aGVHD and six developed cGVHD (1 limited, 5 extensive). The median followup for all patients was 26.6 months (range 0.5–77.8). At the time of SCT, nine patients had chemosensitive disease and two patients were in CR. After transplant, six patients were in CR, five had a PR and one had a stable monoclonal restriction. Seven patients developed progressive disease at a median of 20.8 months (range 3.2–39.8) post-transplant, four in the setting of extensive cGVHD. Ten patients died (5 from relapse, 3 GVHD, 1 VOD, and 1 pneumonia/ARDS). Three patients are alive with stable, asymptomatic monoclonal restrictions at 26.6, 36.8, and 77.8 months after SCT. The Kaplan-Meier estimates at 1, 3 and 5 years for overall survival were 54%, 26%, and 13 %, and for progression-free survival were 54%, 15% and 7%, respectively. In this population of heavily pretreated patients, unrelated donor transplantation did not result in long-term disease control despite the presence of cGVHD and suggests that the GVM effect was not sufficient to prevent relapse.

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UNRESTRICTED CTL RECOGNITION OF HFE, AN EMPTY-PEPTIDE–GROOVE MHC CLASS I MOLECULE CONTROLLING IRON METABOLISM HIGHLIGHTS ITS POTENTIAL ROLE AS A MAJOR HISTOCOMPATIBILITY ANTIGEN IN UNRELATED BONE MARROW TRANPLANTATION

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Unrestricted cytolytic T lymphocytes (CTL) responses against human HFE, a non classical MHC class I molecule with an empty peptide groove which controls iron metabolism, were induced in HLA-A*0201 transgenic/H-2 class I knock-out mice. HFE recognition was mediated by the alpha-beta T cell receptor. Similar unrestricted CTL responses against mouse HFE molecule were induced in DBA/2 HFE knock-out mice. Predominant usage of AV α6 segments was documented, contrasting with diversified Vβ segment usage. Thus absence of bound ligand to a histocompatibility class I molecule does not preclude αβ TCR interaction. These results support the hypothesis that the immune system could participate in the control of iron metabolism and indicate a novel unrestricted allogenic recognition mechanism that could explain the high proportion of allogenic cytotoxic precursors encountered in any individual.

Ten to 15% of the individuals carry a mutated H41D HFE molecule. Since HFE is encoded on the chromosome 6 and thus cosegregates with the HLA haplotypes in siblings, a HFE mis-

match in related transplant is unlikely. In contrast, investigating the frequency of a donor-recipient HFE mismatch in a series of 60 unrelated bone marrow transplants otherwise perfectly matched on the HLA-A,B,C,DRB1,DQB1 loci, we found a mismatch in 10 cases. These data suggest that HFE could be a potent histocompatibility antigen in humans in view of its structural polymorphism.

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MULTIDISCIPLINARY APPROACH TO DEVELOPMENT OF A GVHD OUTPATIENT CLINIC

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Managing GVHD is a particularly challenging aspect of the care of blood and marrow transplant recipients. In the Blood and Marrow Transplant program at Baylor University Medical Center, the GVHD patients were identified as a population that could benefit from an interdisciplinary approach in a specialized clinic setting. A questionnaire was sent to the transplant physician group to gauge interest in and the need for this clinic. It was determined that enough interest was present and a patient population would support the organization of this program. The interdisciplinary team consisted of an oncologist as team leader, dermatologist, clinic manager, contract administrator, nurse manager, pharmacist, biostatistician, physical therapist, occupational therapist, dietitian, enterostomal therapist, social worker, and chaplain. The team gathered for regular meetings to decide on structure, services, and supplies that would be needed to support such an endeavor. The clinic manager and contract administrator investigated financial reimbursement for each discipline in the outpatient setting. Organization of the GVHD clinic recommended that each patient see the medical team leader followed by other specialty areas that would benefit the patient. A key component for a successful clinic is data collection for patients before their appointments to assist clinicians with a thorough needs assessment. Each discipline offered a data collection tool and these were compiled by a team member. This tool includes sections on medication, medical symptoms, skin assessment, nutrition, physical and occupational therapy, and psychosocial needs. Prior to patient's appointments, questionnaires are mailed with the instructions to complete and bring to their appointment. Research protocols for disciplines in this clinic are being developed. Taking a multidisciplinary approach to a GVHD clinic is a key factor in developing a successful program.

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IL-7 IS NECESSARY FOR THE DEVELOPMENT OF EXPERIMENTAL GRAFT-VERSUS-HOST DISEASE (GVHD)

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Interleukin 7 (IL-7) promotes both thymopoiesis and the survival and proliferation of mature T lymphocytes. Although IL-7 enhances immune reconstitution following hematopoietic stem cell transplantation (HSCT), its effect on mature T lymphocytes in allogeneic bone marrow transplantation (BMT) could lead to exacerbation of GVHD. However, recent experiments to determine whether IL-7 treatment worsens GVHD have produced conflicting results. Therefore, this study was designed to examine whether IL-7 is necessary for the induction of GVHD by maintaining donor mature T cells following allogeneic BMT. In order to induce GVHD, B6 or B6/IL-$7^{-/-}$ ($CD45.2^+$ $H2K^b$) recipient mice were lethally irradiated (1300 cGy) and co-transplanted with $4x10^6$ lymph node (LN) and $1x10^6$ T cell depleted (TCD) BM cells from either congenic B6.SJL ($CD45.1^+$, $H2K^b$) or major MHC mismatched allogeneic Balb/C ($CD45.2^+$, $H2K^d$) donor mice. Following transplantation, the recipient mice received either human recombinant IL-7 (rhIL-7) 500ng BID or PBS from day 1 to 60. The survival rate was similar in all groups of mice for the first 25 days after transplantation and no evidence of GVHD was detected from allogeneically transplanted B6/IL-$7^{-/-}$ recipients treated with PBS. In contrast, GVHD-related mortality and morbidity in allogeneic recipients treated with IL-7 were increased compared to the PBS treated groups. IL-7 treatment significantly reduced survival in the B6/IL-$7^{-/-}$ mice (15%, n = 20) compared to the PBS treated B6/IL-$7^{-/-}$ recipients (59%, n = 17 [$p < 0.005$]). Furthermore, the overall GVHD clinical index of IL-7 treated B6/IL-$7^{-/-}$ was significantly lower than the PBS treated B6/IL-$7^{-/-}$ recipients ($p < 0.05$). The recovery of donor $CD4^+$ or $CD8^+$ T cells in the periphery of the PBS treated B6/IL-$7^{-/-}$ recipients was significantly lower than the IL-7 treated B6/IL-$7^{-/-}$ mice by day 30 post-transplantation. In addition, IL-7 treatment increased the number of activated (CD69 positive) donor-derived CFSE labeled CD4 and CD8 LN T lymphocytes in the lymph nodes of B6/IL-$7^{-/-}$ mice compared to PBS treated B6/IL-$7^{-/-}$ recipients indicating that IL-7 enhances maintenance of dividing activated allogneic mature T cells. Therefore, it is likely that IL-7 is necessary for the development of GVHD presumably by maintaining the adequate number of activated donor T cells in the periphery of the recipient animals post-allogeneic BMT.

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THE ABSENCE OF PEYER'S PATCHES OR HOST TNF-A DOES NOT AFFECT GVHD PROGRESSION IN AN ALLOGENEIC BMT MODEL USING EXTENSIVE CONDITIONING REGIMENS

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Peyer's patches (PP) have been previously shown to play a critical role in the initiation of acute lethal GVHD in a non-irradiated murine model (Murai et al., 2003). This report utilized IL-7 receptor antibody treatment of pregnant mice to inhibit the development of PPs in the fetus. We wanted to assess GVHD progression in mice congenitally deficient in PP presence and function. The absence of various members of the TNF and TNF-R families also affects secondary lymphoid structure and organization. The presence of PP has varied between different independently generated TNF knockout strains which may be related to the close linkages of *tnf* with *lta* and *ltb* genes. A new TNF deficient strain has been developed that lacks PPs, displays the defects characteristic of TNF ablation but not the LT associated defects characterized by lack of lymph nodes and defects in splenic microarchitecture. To examine the role of host-derived TNF family members in acute lethal GVHD, we also had to assess the role of secondary lymphoid structures. We utilized a full MHC mismatched mode of BALB/c ($H2^d$) cells into myeloablated C57BL/6 ($H2^b$) recipients. We lethally irradiated wild type (WT) mice, TNF-α deficient mice with Peyer's patches (PP+) and TNF-α deficient mice without Peyer's patches (PP-) followed by infusion of allogeneic bone marrow and spleen cells. We observed no difference in the survival of the three groups of mice (median day of death ranged from 26 to 27). In addition, no significant differences were observed in GVHD associated histopathological lesions in the small intestine. Hisopathological lesions observed in the colon were similar in both the PP+ and PP- TNF-α deficient recipients. Based on our observations, we conclude that (1) Peyer's patches and (2) host TNF-α are not required for the development of acute lethal GVHD in mice that received extensive conditioning and allogeneic bone marrow transplantation.

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DEVELOPMENT OF NUTRITION ALGORITHMS FOR CHRONIC GVHD

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One of the challenges for patients with chronic GVHD is to maximize quality of life by maintaining optimal nutritional intake and minimizing loss of lean body mass. Registered Dietitians (RD's) play an integral role in the nutritional care of patients in the Blood and Marrow Transplant program at Baylor University Medical Center and are part of the team establishing a new GVHD clinic. Patients referred to the GVHD clinic are seen by the RD