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HIGH RATE OF DE NOVO CHRONIC GRAFT-VERSUS-HOST (cGVHD) FOLLOWING BUSTULAN-FLUDARABINE CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION FROM A MATCHED-SIBLING DONOR (MSD) FOR AML/MDS  
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Bustulan-fludarabine is increasingly used as a conditioning regimen for allogeneic stem cell transplantation (ASCT). Fludarabine has complex immunologic effects which may impact the development of GVHD. Risk factors and outcomes of cGVHD in this setting have not been determined. We retrospectively evaluated all consecutive patients (pts) who received conditioning with IV Busulfan (130 mg/m² for 4 days) and Fludarabine (40 mg/m² for 4 days) and ASCT from a MSD at MD Anderson Cancer Center between 2001 and 2005. In a landmark analysis starting at day +100, we estimated the cumulative incidence (CI) of cGVHD (defined according to the recent NIH consensus diagnostic criteria); evaluated risk factors for overall and de novo cGVHD using Cox’s regression analysis; and evaluated the effect of cGVHD on outcomes. 104 pts were included in this analysis. Median age was 46 years (12–65). 46% were females and 49% were in complete remission at the time of ASCT. Peripheral blood (PB) was the stem cell source in 85% of pts. GVHD prophylaxis consisted of tacrolimus and methotrexate at standard doses. The incidence of grade II-IV aGVHD was 18%. 89 pts were alive day +100 without progression of their malignancy including 51 pts who had not developed aGVHD. With a median follow-up of 46 months in survivors, 57/89 pts developed cGVHD; half (n = 29) of these cases occurred de novo. The 3 years CI of de novo cGVHD was 57% in the 51 pts without antecedent aGVHD. Time of onset was later for de novo cGVHD compared with relapsing/progressive cGVHD; median 195 days (115–693) vs. 143 days (100–639). The use of PB was the only significant risk factor for development of cGVHD (HRoverall = 3.3, P<0.01; HRde novo = 5.0, P<0.03). Recipient age, recipient/donor sex and CMV serostatus, and disease status prior to ASCT had no significant impact on the rate of cGVHD. On univariate analysis considering cGVHD as time-dependent variable, there was a trend toward an association of de novo cGVHD, but not relapsing/progressive cGVHD, with a lower rate of disease progression (HR = 0.6, P=0.4) and mortality (HR = 0.4, P=0.1). There was also a trend for more favorable overall survival (HR = 0.4, P=0.08) and non-relapse mortality (HR = 0.3, P=0.2) in pts who developed de novo compared with relapsing/progressive cGVHD. There is a high frequency of aGVHD after busulfan-fludarabine conditioning and ASCT for AML/MDS. This suggests a shift in the epidemiology of GVHD that merits further consideration.

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EXPLORE T-CELL RECRUITMENT DURING THE EALIER PHASE OF GRAFT VS. HOST DISEASE IN VITRO  
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Graft-versus-host disease (GVHD) is the major complication following allogeneic blood and marrow transplantation (BMT). Although major advances have been achieved in understanding the immunopathology of GVHD, the rate of success in the clinic has been hampered by the inability to improve overall survival. T cell depletion of the hematopoietic stem cell inoculum has been proved efficient in preventing GVHD, however, this treatment abolished the graft-versus-leukemia (GVL) effect, also mediated by donor T cells, resulting in significant increases in the rates of relapse. Therefore, other means for separating GVHD and GVL need to be considered. Activation and recruitment of T cells to targeted organs during GVHD is preceded by a well orchestrated, but yet unknown in vivo, step. The epithelial cells of the skin and the gastrointestinal tract are the first targets of GVHD. In this study, we take advantage of our recently developed in vitro model of allogeneic T cell/epithelial interactions to investigate this less explored aspect of GVHD. Using a high throughput multiprotein assay, we examined the secretion of different chemokines in cocultures of skin primary epithelial cells, with alloreactive T cells, derived from minor histocompatibility antigen allogeneic C57Bl/6 (B6) mice, with alloreactive T cells, derived from minor histocompatibility antigen allogeneic C57Bl/6 (B6) mice. In addition to IFN-γ and IL-12, we detected the presence of MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5 and GM-CSF by 7 days after the addition of the alloreactive T cells. Although we do not know yet in detail whether T cells, epithelial cells, or both are responsible for the secretion of the chemokines in our system, their level was dependent on the amount of T cells added to the cocultures (p<0.03). However, we found that in the absence of T cells, conditioning of the pEC by exposure to 11 Gy of γ-irradiation induced the secretion of 109.6 pg/mL MCP-1 and 21.7 pg/mL RANTES within 24 hours. Thus, our preliminary data suggests that the conditioning of epithelial cells, alone, is enough to induce secretion of chemokines, which in turn could induce T cell recruitment to the site before any damage to the epithelial cells can be visualized, leading subsequently to an amplification cascade governed by chemokine secretion from both target and effector cells.

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WHAT IS THE ROLE FOR REGULATORY T-CELLS AFTER NONMYELOABLATIVE CONDITIONING?  
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Purpose: We investigated the association between regulatory T cell (Treg) levels and chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HCT) following nonmyeloablative conditioning.

Methods: Data from 74 patients given nonmyeloablative conditioning as treatment for hematologic malignancies or renal cell carcinomas were analyzed. Conditioning regimens consisted of...
low-dose TBI with (n = 45) or without (n = 17) fludarabine, or cyclophosphamide plus fludarabine (n = 12). T-reg (CD4+Foxp3+) levels on days 100, 180, and 365 were determined by flow cytometry. Chimerism levels among total white blood cells, CD3+ T-cells and CD4+CD8+CD127low/bright regulatory T-cells were determined by multiple STR PCR or X-Y FISH. Thyminic function was determined by assessing sTREC levels.

Results: Mean Treg (± SD) levels in controls were 31 ± 18 cells/μL. Mean (± SD) Treg levels on day 100 were 20 ± 24 cells/μL (p < 0.01 in comparison to controls) in patients without grade II-IV acute GVHD before day 100, and 27 ± 21 cells/μL (NS) in patients with an antecedent of grade II-IV acute GVHD. Mean ± donor CD3+ T-cells and Treg chimerism levels on day 100 were 75 ± 29% and 81 ± 18%, respectively (NS). Day 100 chimerism levels among CD3+ T-cells and Tregs were highly correlated (r = 0.78, p < 0.01). The 1-year probability of grade II-III NIH chronic GVHD in patients with day 100 Treg levels below or above median was 53% and 36%, respectively (P = 0.13). sTREC levels significantly increased from day 100 to day 365 after HCT (p < 0.01), demonstrating thymic recovery. Finally, Treg and sTREC levels correlated on days 100 (r = 0.48, p < 0.01) and 365 (r = 0.47, p < 0.01) after HCT.

Conclusions: Our data did not show thus far a significant correlation between Treg levels and occurrence of chronic GVHD. The association between Treg and TREC levels on days 100 and 365 might suggest a role for the thymus in regulating Tregs levels after HCT, or that similar factors affect thymic function and Treg levels after nonmyeloablative conditioning. Data including higher number of patients will be presented.

343 GENERATION OF CLL-SPECIFIC CTL EFFECTORS FROM PARTIALLY HLA-MATCHED UMBILICAL CORD BLOOD GRAFTS USING CD154-TRANS DUCED CLL CELL S AS APCs

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Introduction: Long-term remissions have been observed following allogeneic hematopoietic stem cell transplantation (allo-HSCT) for the treatment of chronic lymphocytic leukemia (CLL). Unfortunately, many CLL patients are ineligible for transplant due to the lack of an HLA-compatible donor. Umbilical cord blood (UCB) transplantation permits transplantation of many individuals who are ineligible for allo-HSCT due to its reduced requirement for stringent HLA matching; however, disease relapse remains a significant complication. Further, unlike allo-HSCT, donor lymphocyte infusion (DLI) cannot be employed as a post-transplant therapy. To address this issue we are developing a strategy to generate CLL-specific T-lymphocytes to be employed as a post-UCB-HSCT therapeutic intervention.

Methods: CLL-specific antigen presenting cells (APCs) were generated by the transplantation of CLL cells with an adenosine vector encoding CD154 (CD40L). Successful transduction and subsequent CD40 ligation in CLL cells were verified by monitoring CD95 expression. CD154-APCs were then used to prime partially HLA-matched (typically 4/6) UCB lymphocytes in the presence of IL-2, IL-7, and IL-15. Expanded UCB lymphocytes were phenotyped by flow cytometry, and CLL specificity was determined by ELISPOT and 31Cr lysis using non-CLL (CD19/CD3+) patient lymphocytes as allo-antigen controls.

Results: Effector cell phenotype was predominantly (>80%) CD4+; however, skewing toward CD8+ expansion could be achieved by incubation in IL-12 during priming. Significant populations of effector memory (CD62L+CD127+) and central memory (CD62L–CD127+) cells were observed following repeated stimulations with CLL-APCs. Significant expansion (>4 fold) of UCB lymphocyte populations was typically observed. In ELISPOT assays, CLL-APC primed UCB lymphocyte responders exhibited a significant increase (p < 0.05) in IFN-γ ELISPots when incubated with CLL stimulators in comparison to non-CLL control stimulators. Cytolytic activity was demonstrated against unmodified CLL cells by 31Cr release assays in which percent lysis of CLL targets was 30–50% at E:T ratios of 10:1 and 20:1. Lysis of autologous non-CLL control targets was negligible.

Conclusions: Our results demonstrate that in vitro generation of CLL-specific effectors from partially HLA-matched UCB lymphocytes is both feasible and practical. These preclinical results support further exploration of this technique as a promising treatment modality in conjunction with UCB-HSCT.

344 THE T CELL CYTOLYTIC MOLECULES FAS LIGAND AND TRAIL, THE TRAFFICKING MOLECULES CCR9, 7 INTEGRIN AND PSGL1, AND THE IMMUNE MODULATING MOLECULES OX40 AND CEACAM1 ARE REQUIRED FOR THYMIC GRAFT-VERSUS-HOST DISEASE


Thymic graft-versus-host-disease (tGVHD) contributes to impaired T cell reconstitution after allogeneic bone marrow transplantation, but its pathophysiology has not been well defined. We assessed thymic output (RAG2+ splenic recent thymic emigrants) as well as the thymic cellularity (esp. CD4+CD8+ cells) and found that these were inversely related to numbers of mature donor T cells in the allograft. Bc1-2 expression in donor BM-derived thymocytes was decreased in recipients with GVHD vs. those without GVHD, which suggests that survival of thymocytes is decreased during tGVHD. Consequently, tGVHD severity may be associated with thymic function. We studied the migration of alloreactive donor T cells in vivo and found that donor T cells infiltrated the thymus within the first week post-transplant. Upon adoptive transfer of CFSE-labeled donor T cells we noted that thymus-infiltrating alloreactive donor T cells were fast-proliferating (CFSElo) and highly activated (CD25+CD44+). We then analyzed T cell trafficking in tGVHD with mice deficient for certain trafficking molecules, and assessed tGVHD by loss of BM-derived CD4+CD8+ thymocytes. We found that CCR9, β7 integrin, and PSGL1 were all partially required for GVHD, while L-selectin and zE integrin may be dispensable. Similarly, we examined the role of T cell cytolytic pathways for tGVHD, and found that FasL and TRAIL were required for GVHD, but that perforin and TNF were dispensable. Finally, we assessed the role of various T cell regulatory molecules for tGVHD, and found that Ceacam1 and OX40 were required, while GITR was partially required and ICOS was dispensable. Host non-hematopoietic thymic stroma may be an important target for donor alloreactive T cells in GVH reaction models. We assessed the expression of the death receptors Fas and DR5 in thymic stroma from normal and irradiated (85 Gy) BALB/c mice, and observed that in particular, MHC class II-negative stroma (endothelial cells and fibroblasts), as well as a population of MHC class II-positive stroma (epithelial cells) upregulated the expression of both Fas and DR5 after irradiation. Thymic epithelial cell numbers were relatively preserved after irradiation of mice deficient for TRAIL and FasL. In conclusion, we found that TRAIL, TNF, CCR9, integrin β7, PSGL1, Ceacam1 and OX40 on donor T cells as important for tGVHD pathophysiology. These data suggest selective therapeutic targets to attenuate tGVHD and improve post-transplant T-cell reconstitution.

345 ORAL CHRONIC GRAFT-VERSUS-HOST DISEASE SCORING USING THE NIH CONSENSUS CRITERIA

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Background: The NIH Oral GVHD Activity Assessment Instrument is intended to be simple to use and to provide a reproducible objective measure of disease activity over time. The objective of this study was to assess inter- and intra-observer variability in the component and composite scores in patients evaluated with oral cGVHD.