

Poster Session I

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SEPARATION OF CD4⁺ T CELL-MEDIATED GRAFT-VERSUS-HOST RESPONSES FROM THE GRAFT-VERSUS-LEUKEMIA EFFECT

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Graft-versus-host disease (GVHD) can result from transplantation of blood and bone marrow cells into major histocompatibility complex (MHC)-matched recipient mice due to donor T cell recognition of minor histocompatibility antigens (miHA). Graft-versus-leukemia (GVL) responses can be directed to distinct or over expressed leukemia-restricted antigens presented by MHC class I or class II molecules. In addition, donor T cells which are responsible for the induction of GVHD may also mount a GVL effect directed to shared host miHA on tumor cells. We have previously described CD4⁺ T cell-mediated lethal GVHD in the B6 anti-BALB.B miHA model and characterized the B6 anti-host response over the course of disease development, in the absence of leukemia challenge. Using a BALB.B-derived myeloid leukemia cell line (MMBALB7), we subsequently investigated the GVL activity in this GVHD model. We first transferred B6 CD4⁺ T cells, presensitized to the BALB.B leukemia cell line, to bone marrow-reconstituted syngeneic recipients and observed effective GVL protection against the leukemia challenge. Then, in order to better characterize this B6 CD4⁺ T cell anti-leukemia response, T cell receptor V β CDR3-size spectratype analysis was performed. B6 mice were stimulated with the MMBALB7 leukemia cell line and the CD4⁺ T cell response from the draining lymph nodes was analyzed. The B6 anti-leukemia spectratype results were compared to the previously reported B6 anti-BALB.B GVHD responses. These results indicate anti-leukemia V β TCR usage that was either unique or overlapping in both the GVHD and GVL responses. Specifically, V β 1 and 5 families were uniquely involved in the anti-leukemia response, whereas V β 3, 8.2, 8.3, 11, 13, and 14 families were skewed in both the anti-leukemia and anti-BALB.B GVHD responses. The presence of V β families that are likely recognizing leukemia-restricted antigens in the GVL response suggests that it is possible to separate these responses from those responsible for GVHD, allowing for optimum immunotherapeutic potential.

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ANALYSIS OF GENE EXPRESSION PROFILE IN PATIENTS WITH ACUTE GRAFT VERSUS HOST DISEASE FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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To better understand the cellular events that precede clinical onset of GVHD we compared gene expression profiles in patients 3 weeks after transplantation from normal donor following myeloablative conditioning. Blood leucocytes were obtained at scheduled times prior to administration of steroids. RNA was biotin labeled and hybridized on Affymetrix HG U133A chips. In a first set of experiments we compared global gene expression profiles among 15 patients and 10 normal controls. A total of 1176 genes were differentially expressed between patients and controls. The expression profiles of these 1176 genes was further compared between 8 patients who developed GVHD within the next 1 to 5 days and 7 patients who remained GVHD free for at least 90 days. Nine genes were differentially expressed in these two groups (NFD=1): 3 were increased, 6 were decreased in GVHD patients. In addition to the global comparison, we examined expression for a candidate list of 189 genes and found 6 genes (NFD=1) associated with onset of GVHD: 4 were increased, 2 were decreased. In a second set of experiments we compared changes occurring within 7 patients between 3 and 4 weeks post-transplant. Using a pairwise comparison we found 55 increased genes and 88 decreased among 4 patients developing GVHD within 7 days. Among the increased genes 3 were associated with adaptive immune response and 5 with inflammation. Among the decreased genes 3 were associated with cell metabolism, 6 with DNA repair, replication and cell cycle, 5 with signal transduction, 10 with adaptive immune response in-

cluding 4 T cell associated genes. These results demonstrate extensive changes in gene expression during early post-transplant period the majority of which are not obviously associated with immune response. By candidate gene approach we detected a smaller number of genes associated with onset of GVHD. We found that the most informative approach was to compare longitudinal changes within the same patient. We observed a paradoxical decrease in the expression of genes associated with cell cycle and T cell function. This may be explained by previous studies demonstrating activation induced cell death during clinical GVHD, and the finding of profound lymphopenia in patients with GVHD. Further studies will be necessary to determine if gene expression profiling can be useful in identifying a molecular "signature" for GVHD, and if this approach can be of value in monitoring or predicting response to GVHD treatment.

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DENILEUKIN DIFTITOX (ONTAK) FOR THE TREATMENT OF ADVANCED STEROID REFRACTORY GRAFT-VERSUS-HOST DISEASE (SR-GVHD): A SINGLE INSTITUTION EXPERIENCE

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SR-GVHD is a leading cause of morbidity and mortality following allogeneic stem cell transplantation. Denileukin diftitox (DD), a recombinant fusion protein composed of the cytotoxic A chain of diphtheria toxin and binding portion of interleukin-2, has potent activity against activated CD25⁺T cells important in the etiology of GVHD. Recent reports suggest that DD may have therapeutic activity for the treatment of SR-GVHD. SR-GVHD was defined as lack of response or disease progression after at least 7 days of treatment with methylprednisolone at 2 mg/kg. Eleven patients received allogeneic transplant, 2 after MRD and 9 after URD (7 matched, 1 C antigen mismatch, 1 three allele mismatch). GVHD prophylaxis consisted of cyclosporine (CsA), methotrexate (MTX), plus steroids (10) or CsA and MTX (1). Seven patients developed acute SR-GVHD overall grade III (2) or IV (5) while 4 had chronic extensive SR-GVHD. Five patients had 2 organ (skin and GI) grade IV GVHD and 6 had 3 organ involvement (skin, GI, and liver). Six patients had prior admissions for treatment of GVHD and all patients had failed a median of 2.5 agents (range 1–5) prior to DD including dacluzimab (4), MMF (6), tacrolimus (7), sirolimus (1) and beclomethasone (1). Median day to DD therapy after stem cell transplant was day 76 (30–311). Planned treatment for DD was at a dose of 600 mcg (approximately 9 mcg/kg) on days 1, 3, 5, 15, 17 and 19 (Ho, Blood, 2004). Two patients with cGVHD developed hyperbilirubinemia without elevation of hepatic enzymes on DD. Five patients including 3 of 4 with cGVHD died prior to completing the full course of treatment (1–3 doses). These patients succumbed to GI bleed secondary to gut GVHD and CMV colitis (1), fungal pneumonia (2), and ARDS (2). Six patients received all six doses of DD. All 6 showed response to treatment with 1 patient with grade III aGVHD achieving a CR and 5 achieving a PR, defined as at least a one overall grade improvement in GVHD staging. Five of the 6 patients have died with patients dying of CMV pneumonia (1), multi-organ failure (1), IPS (1), aspergillus pneumonia (1), and respiratory arrest (1). Steroids could be tapered in 8 of 10 patients. Median survival for the entire group was 20 days from the onset of DD treatment (range 5–440 days) with median survival of 34 days for patients who received all 6 doses of DD. These results demonstrate that patients with advanced SR-GVHD can respond to DD and suggest that earlier treatment may improve outcomes.

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MURINE CIK CELLS SHOW TUMOR SPECIFIC CYTOLYSIS IN CD107A BASED DEGRANULATION ANALYSIS

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Immunotherapy with cytokine induced killers cells (CIK) is effective in the treatment of malignancies in several murine models.