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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 Vβ 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 Vβ TCR usage that was either unique or overlapping in both the GVHD and GVL responses. Specifically, Vβ 1 and 5 families were uniquely involved in the anti-leukemia response, whereas Vβ 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 Vβ 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 including 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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DENLEUKIN 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 cytocidal 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.

Previous studies on human T lymphocytes have shown that the degranulation marker CD107a can be used to define T cells, which show tumor specific cytolysis. To determine whether CD107a can be used in the murine setting as a marker for cytolysis, we first tested the degranulation activity on splenocytes from C57BL/6 (H-2b), FVB (H-2q) or Balb/c (H-2d) against Balb/c splenocytes. Results from the mixed lymphocyte cytolytic assays (MLCA) and cytolytic assays [^{51}Cr] showed a high degree of correlation with CD107a expression by FACS analyses. We next analyzed the degranulation activity of murine CIK cells by evaluating their capacity to mobilize CD107a to the cell membrane as a parameter of tumor specific cytolysis. CIK cells showed a high cytolytic capacity in ^{51}Cr assays when directed against syngeneic and allogeneic tumor cell lines (A20, P3X63, EL4, Yac-1 and P815). CIK cytotoxicity correlated with CD107a expression by FACS analyses. Immunofluorescence microscopy could confirm the existence of CD107a positive cytolytic granules in CIK cells from tumor cytotoxic assays. Using in vivo Bioluminescence Imaging (BLI) we characterized the migration pattern of CIK cells derived from a luciferase expressing transgenic mouse in tumor-bearing mice as compared to healthy controls. Furthermore, Balb/c mice bearing a sub-cutaneous A20 lymphoma, revealed tumor-specific homing of CIK cells and reduction of the tumor size within 3 days after i.v. injection. To identify tumor-specific CIK in vivo we are currently combining imaging techniques with CD107a degranulation analysis. These data will help to elucidate the complex cellular interaction mounting a graft-versus-tumor response, which depends on an effective combination of efficient trafficking, recognition and elimination of the tumor cells.

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METHOTREXATE ALTERS HEMATOPOIETIC RECOVERY AND ENGRAFTMENT KINETICS WHEN ADDED TO CYCLOSPORINE FOR ACUTE GVHD PROPHYLAXIS AFTER REDUCED-INTENSITY STEM CELL TRANSPLANTATION

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Methotrexate (MTX) is a standard drug for graft-versus-host disease (GVHD) prophylaxis, but its effects on hematopoietic recovery and donor engraftment in the setting of reduced-intensity stem cell transplantation (RIST) are not well described. We compared these parameters and acute GVHD in patients with hematologic malignancies undergoing RIST on 2 consecutive clinical trials using identical reduced-intensity conditioning (fludarabine and cyclophosphamide). Group 1 included 50 patients receiving cyclosporine (CSA) alone for GVHD prophylaxis after RIST. Group 2 included 24 patients receiving CSA + MTX (5 mg/m^2 on days +1, +3, +6, +11) for GVHD prophylaxis. The groups were similar with respect to host immune status before RIST, age, and allograft dose of CD34$^+$ and CD3$^+$ cells. All patients in each group received salvage therapy with etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus fludarabine (EPOCH-F) prior to RIST. Fourteen patients in the CSA/MTX group also received rituximab with EPOCH-F. Hematopoietic recovery was delayed in the CSA/MTX group, with median neutrophil recovery (>500/μL) and platelet recovery (>100K/μL) occurring 3 and 6.5 days later, respectively, than in the CSA group. Donor engraftment was rapid and complete for most patients in each group (median total mononuclear cell chimerism 100% at day +28 for both groups), and no patient experienced graft rejection. However, full donor chimerism (>95%) was less common for CSA/MTX (75% at day +28, 71% at day +100) than for CSA (90% at day +28, 97% at day +100). Adding MTX to CSA decreased the incidence and severity of acute GVHD. Grade 2–4 acute GVHD occurred in 33/50 (66%) CSA patients, versus 9/24 (38%) CSA/MTX patients. Grade 3–4 GVHD affected 17/50 (34%) CSA patients, including 6 with grade 4 GVHD and 8 with steroid-refractory disease. In contrast, only 5/24 (21%) of CSA/MTX patients had grade 3 GVHD, while none had grade 4 or steroid-refractory GVHD. No treatment-related deaths occurred in the CSA/MTX group, but 14 CSA patients died of treatment-related complications. Thus, the addition of MTX to CSA markedly attenuates the GVH response post RIST, slowing donor engraftment and delaying hematopoietic recovery. These data illustrate the sensitivity of engraftment kinetics to the intensity of GVHD prophylaxis following RIST. Further studies should determine if CSA/MTX will be associated with an increased risk of disease progression after RIST.

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GRADE 2–4 ACUTE GRAFT-VERSUS-HOST DISEASE AND EXTENSIVE CHRONIC GRAFT VERSUS HOST DISEASE ARE ASSOCIATED WITH SIGNIFICANTLY DECREASED SURVIVAL FOLLOWING REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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Grade 2–4 acute graft-versus-host-disease (aGVHD) and extensive chronic GVHD (cGVHD) are associated with decreased overall survival (OS) following conventional allogeneic stem cell transplantation but their impact on reduced intensity transplantation remains controversial. We evaluated the impact of GVHD on survival in 112 high-risk patients, median age 50 years (range 18–70), with AML (n=29), MDS (n=19), CML (n=9), CLL (n=5), ALL (n=3), HD (n=10), NHL (n=16), MM (n=9), MMM (n=7), PNH (n=2), or renal cell carcinoma (n=3), who underwent a reduced intensity preparative regimen of extracorporeal photopheresis day −7, −6, pentostatin 8 mg/m^2 by continuous intravenous infusion day −5 through −4, and total body irradiation in three 200 cGy fractions day −3, −2, followed by allogeneic bone marrow stem cell infusion from 6/6 HLA matched related (n=70), 5/6 HLA matched related (n=10), or matched unrelated (n=32) donors. Thirty patients had prior autologous stem cell transplantation and 5 patients had prior conventional allogeneic stem cell transplantation. Full donor chimerism occurred in 89% of patients. Day 100 transplant related mortality (TRM) was 20%. The disease relapse rate was 22%. Grade 2, 3 or 4 aGVHD occurred in 7%, 6%, and 6% of patients respectively. The one-year OS by aGVHD grade was 70% for grade 0, 69% for grade 1, 29% for grade 2, 17% for grade 3, and 0% for grade 4. Grade 2–4 aGVHD was associated with higher day 100 TRM (37% vs 14%: p=0.03) and decreased median OS (5 months vs "not reached": p=0.001). Median OS was lower among patients with grade 2–4 aGVHD as compared to patients with grade 0–1 aGVHD in matched related donor transplants (5 months vs "not reached": p=0.002) and in mismatched related or matched unrelated donor transplants (6 months vs 35 months: p=0.0004). Patients with grade 2 or grade 3–4 aGVHD had similar median OS (6 months vs 3 months: p=0.24). Patients with limited or no cGVHD had similar one-year OS (90% vs 79%) but patients with extensive cGVHD had a significantly worse one-year OS (56% vs 82% p=0.0007). In conclusion, high-risk patients who undergo reduced intensity transplantation and develop grade 2–4 aGVHD or extensive cGVHD tolerate GVHD poorly, leading to significantly higher TRM and decreased overall survival. Patients with grade 2 aGVHD or grade 3–4 aGVHD have similarly poor OS. To improve survival, reduced intensity transplantation regimens that decrease the incidence of grade 2–4 aGVHD or extensive cGVHD need to be developed.

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SIGNIFICANCE OF ONE HUMAN LEUKOCYTE ANTIGEN MISMATCH ON OUTCOME OF NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION FROM RELATED DONORS USING THE MEXICAN SCHEDULE

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