Poster Session II

ALLOGENEIC TRANSPLANTS

275

GRAFT REJECTION FOLLOWING DOG LEUKOCYTE ANTIGEN (DLA)-IDENTICAL NONMYELOABLATIVE HEMATOPOIETIC CELL TRANSPLANTATION (HCT) RESULTS IN LONG-TERM INCREASES IN HOST T REGULATORY (T_{REG}) CELLS

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In the canine HCT model, graft rejection occurred immediately with no evidence of transient donor chimerism among all recipients who were sensitized with whole blood transfusions given prior to conditioning from their respective DLA-identical HCT donor. In a separate study, in order to identify alternative nonmyeloablative conditioning regimens such as 1 Gy total body irradiation (TBI) with post-grafting pharmacological immunosuppression, we observed a cohort of recipient dogs (not previously sensitized with transfusions), who had transient donor/host mixed chimerism followed by graft rejection. To evaluate if these recipients were now sensitized to donor antigens, 27 dogs who had transient mixed chimerism followed by graft rejection after 1st HCT were re-conditioned with 2 Gy TBI and given a second HCT from the original donor. Recipient dogs developed either transient or stable mixed chimerism in 37% and 19%, respectively. We hypothesized that the mechanism for donor-specific tolerance seen following rejection of a hematopoietic cell graft was due to a sustained increase of host T_{reg} cells. We evaluated the induction of T_{reg} in the peripheral blood of 10 dogs conditioned with 0.5, 1 or 2 Gy TBI that had transient donor chimerism followed by rejection of a DLA-identical HCT compared to 10 normal (not transplanted) dogs. We developed a quantitative PCR assay, using canine specific *foxP3* primers to amplify cDNA from sorted T cells. Recipient T cells were cultured with the respective DLA-identical donor dendritic cells to induce allostimulation and were assessed for $CD4^+CD25^+FoxP3^+$ by flow cytometry. Also, intracellular T cell interferon-γ and TNF-α was measured by flow cytometry. We determined that independent of the time after HCT rejection (range, 4–96 weeks) or duration/degree of transient mixed chimerism, T_{reg} cells were significantly increased in dogs that had graft rejection compared to untreated dogs. There was a 4-fold increase in *foxP3* transcripts/cell, consistent with the induction of $CD4^+CD25^+FoxP3^+$ T_{reg} cells in dogs with prior transient mixed chimerism and graft rejection. However $CD4^+TNF\alpha^+$ and Inf-γ^+ cells were also increased, reflecting activation of allo-immune effector cells induced by HCT rejection. The results are consistent with the hypothesis that acceptance of second HCT grafts may occur after 1st transient mixed chimerism because of immune mechanisms favoring T_{reg} induction over pro-inflammatory cytokine producing immune cells.

T Cell Regulatory and Effector Phenotype in Dogs

	Previously Rejected HCT*	Untreated	*p*-value
T Cell Assay	**Mean ± Standard Deviation**		***t*-test**
foxP3 transcripts/10^5 T cells	14,208 ± 6886	3,475 ± 1973	≤0.0001
% $CD4^+CD25^+$	6.9 ± 5.4	1.1 ± 0.3	≤0.047
% $CD4^+CD25^+FoxP3^+$	7.2 ± 6.1	0.8 ± 0.4	≤0.048
% $CD4^+TNF\alpha^+$	18.1 ± 5.1	7.7 ± 4.7	≤0.001
% $CD4^+Inf\gamma^+$	17.7 ± 5.1	12.6 ± 4.0	≤0.031
% $CD8^+Inf\gamma^+$	25.3 ± 7.8	17.1 ± 4.6	≤0.014

*Dogs with graft rejection after transient mixed chimerism following 1st nonmyeloablative HCT.

276

MYELOABLATIVE TRANSPLANTATION FROM MATCHED SIBLINGS (MSD) USING A DAILY INTRAVENOUS BUSULFAN (BU)/FLUDARABINE (FLU) REGIMEN WITH THYMOGLOBULIN (TG): ANALYSIS INVOLVING 200 PATIENTS INDICATES LOW TRANSPLANT-RELATED MORTALITY (TRM) IN ALL BUT OLDER PATIENTS WITH HIGH-RISK DISEASE

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We aimed to identify MSD transplant recipients given Flu/Bu and GVHD prophylaxis incorporating TG who may be at particular risk of TRM and perhaps merit some modification of the protocol. Between 1999 and 2005 200 patients (pts) aged 18–65 (median 46) received Flu 50 mg/m^2 daily × 5 and IV Bu 3.2 mg/kg daily × 4. 46 had additional total body irradiation 200 cGy × 2. GVHD prophylaxis was CSA, methotrexate and TG (Genzyme) 4.5 mg/kg total dose. Cell source was mobilized blood cells in 172 and marrow in 28. At follow-up of survivors of 13–87 months (median 42) low-risk (LR, acute leukemia CR1/CR2, CML CP1) pts had projected 5 year TRM and overall survival (OS) of 4% and 76% for pts ≤45 years old (n = 54) vs 6% and 83% for those >45 (n = 31). For high-risk (HR) pts TRM was 6% vs 27% (18% at one year) (p = 0.04) and OS 64% vs 37% (p = 0.47) in younger (n = 40) and older (n = 75) pts respectively. To correct for imbalance in HR diagnoses we matched each of 17 younger HR pts with 2 older HR (OHR) pts by diagnosis and details of stage and thereafter for other risk factors. OS was 70% vs 37% (p = 0.02), TRM 0 vs 34% (p = 0.02) and relapse 63% (48% at 3 years) vs 46% at 5 years (p = ns) respectively. For a more robust analysis OHR pts were compared with the other 3 groups combined giving TRM of 27% vs 5% respectively (p = 0.002). Incidence of aGVHD grade II-IV, aGVHD grade III-IV and cGVHD was 23% vs 10% (p = 0.02), 4% vs 2% (p = ns) and 66% vs 41% (p = 0.001) respectively. Nine of 14 non-relapse deaths in the OHR group were related to GVHD or its treatment compared with 3 of 6 in all others (p value for GHVD related death = 0.01). Multivariate analysis of OS and DFS correcting for potentially confounding pretransplant factors idenitified only the HRO patients as having significantly increased risk (RR 3.32, CI 1.71–6.47, p < 0.0001 and RR 3.32, CI 1.71–6.43, p < 0.0001 respectively). We conclude that the effect of age on TRM is only apparent in HR pts and is not explained by heterogeneity in diagnoses. HRO pts experience more GVHD and more GVHD related death than others. TRM is no higher than reported with many non-myeloablative regimens, justification for our practice of treating all pts with the same regimen. Additional GVHD prophylaxis might help the HRO pts.

277

A PROSPECTIVE EVALUATION OF THE EFFECT OF POLYOMA (BK) VIRUS INFECTION ON THE INCIDENCE OF HEMORRHAGIC CYSTITIS (HC) AFTER UNRELATED DONOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (UD HSCT)

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Background: BK virus infection is highly prevalent in humans, and has been associated with development of HC after UD HSCT. Previously we determined that UD HSCT is independently associated with higher prevalence of HC (El-Zimaity et al. Blood 2004). In order to further investigate the association of BK with HC, we hypothesized that the presence of BK virus in the urine as