TNF-alpha in the two treatment arms and further study is needed to determine the significance of this observation.

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ANALYSIS OF CD4+CD25+ T REGULATORY CELL SURVIVAL AND FUNCTION FOLLOWING IN VITRO AND IN VIVO IRRADIATION

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Manipulating the host regulatory T cell compartment may be useful in BMT strategies for successful engraftment and anti-tumor responses. In order to investigate the effect of conditioning regimens on host CD4+CD25+ T regulatory cell function prior to BMT, highly purified populations of Tregs were produced from several mouse strains and exposed to varying levels of gamma irradiation *ex vivo*. Following treatment, Tregs were co-cultured with CD4+CD25- responders, accessory cells, and anti-CD3 mAb. At doses as low as 3.0 Gy, Tregs exhibited diminished capacity to regulate CD4+ T cell proliferation *in vitro*. To assess the effect of the radiation on Treg survival, purified populations were exposed to doses from 3.0Gy to 9.0Gy and assessed by Annexin V staining. After 24 hrs., >90% of the Tregs were positive regardless of dose. Co-culture with accessory cells and stimulation with anti-CD3 during this time period could not rescue these cells from death. To examine cell survival following *in vivo* irradiation, splenic CD4+CD25+ cells were purified from mice immediately following 3.0 or 9.0Gy TBI and cultured for 24 hrs. Annexin V staining was equivalently as high as in Tregs irradiated *ex vivo*. Further analysis following *ex vivo* irradiation indicated that apoptotic cells were evident between 4 and 8 hours post-exposure. Since >90% of Tregs exposed to a dose of 3.0Gy are undergoing apoptosis by 24 hrs, and these cells did not lose the entirety of their regulatory function, four hours after 3.0 Gy, irradiated Tregs were co-cultured with anti-CD3 and CD25- responding cells to determine the kinetics of function post-irradiation. These Tregs failed to exhibit any regulatory activity. It may be notable that 24 hrs following busulfan treatment (30mg/kg) of B6 mice, CD4+CD25+ Treg cells maintained their ability to regulate CD4 T cell proliferation. In total, these observations suggest that immediately following doses of ≥3.0Gy Treg survival and function appear to be compromised. Additionally, alternative conditioning regimens may differentially affect the functional capacity of host Treg cells following BMT.

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THE INFLUENCE OF THE GRAFT MONOCYTES IN THE OUTCOME OF ALLOGENEIC BONE MARROW TRANSPLANTATION

Penteado Aranha, F.J.; Vigorito, A.C.; De Souza, C.A.; Borba Oliveira, G.; Brito Eid, K.A.; Zulli, R.; Lorand-Metze, I. Bone Marrow Transplantation, State University of Campinas, Campinas, Sao Paulo, Brazil.

Francisco J P Aranha, Afonso C Vigorito, Carmino A De Sousa, Gislaine B Oliveira, Katia A Eid, Roberto Zulli, Irene Lorand-Metze Bone Marrow Transplantation Unit State University of Campinas, SP/Brazil The influence of graft monocytes on GVHD has not yet been established in clinical trials.We evaluated the influence of bone marrow graft monocytes aiming, primarily, to analyse the correlation with aGVHD and cGVHD, and secondarily with engraftment and survival. Eligibility criteria age <60 years; patients with primary malignant or non-malignant hematological disease receiving BM from an HLA-identical sibling. We analyzed 83 patients. Conditioning was mainly BuCy2 and GVHD prophylaxis CSA-MTX. The median day to reach peripheral leukocytes ≥ 0.5x109/l and platelet count >20x109 /l was 20 (11-34) and 18.5 (10-60) respectively. In univariate analysis, any parameter was correlated with a faster engrafment. The frequency of a-GVHD, grades 2-4 was 12/83 (14.5%). In univariate analysis, TNC ≥2.31x108/Kg and CD14+ cells ≥4.78x106/Kg were correlated significantly with lower rates of a-GVHD (p=0.04, p=0.02, respectively). Furthermore, patients >27 years old and donor gender mismatch had higher rates of aGVHD (p=0.03 and p=0.04, respectively). In a multivariate analysis, both TNC and age maintain significance for lower risk of a-GVHD. The probability was 3.2% when age < 27 years and TNC infused ≥ 2.31 X 108/Kg. A higher risk of a-GVHD was found (51.5%) when age > 27 years and TNC infused ≤ 2.31 X 108/Kg (P<0.001).The number of CD14+ cells showed a correlation with TNC. This interaction might be the cause for the loss of significance for monocytes in the multivariate analysis. Clinical c-GVHD of all grades developed in 31/77 (40%) available patients. It was extensive in 20 cases and limited in 11 cases. In univariate analyses there was a correlation between previous a-GVHD and a higher risk of c-GVHD (p<0.001). CD14+ cells did not influence c-GVHD. The estimate of 6-year overall survival (OS) was 66% (95% CI: 55%-79%). In univariate analyses, the absence of a-GVHD was correlated with a higher survival (p<0.001). Furthermore, there was a trend for a better survival in patients receiving more CD34+ cells (p=0.06). The CD14 cells had no impact on overall survival. These preliminary data suggest that CD14+ cells may have a protective effect in allogeneic BMT.

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TNF TYPE II RECEPTOR GENOTYPE AFFECTS SOLUBLE RECEPTOR LEVELS IN NORMAL SUBJECTS AND ASSOCIATES WITH CHRONIC GVHD SEVERITY

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A single nucleotide polymorphism in the TNF type II receptor(TNFRII) gene, at codon 196, results in the substitution of arginine (R allele) for methionine (M allele). The R allele is reported to associate with susceptibility to autoimmune disease and donor R allele carriage correlates with acute graft-versus-host disease (GVHD) severity after MUD BMT. To investigate the functional impact of this polymorphism, 81 volunteer blood donors were genotyped by PCR and SSCP analysis and soluble TNFRII (sTNFRII) levels were measured by ELISA. Mean (+/- SEM) plasma sTNFRII levels were 1225+/-26 and 1063+/-65 for individuals carrying at least 1 M allele (M+) and RR homozygotes respectively (p=0.02). We examined the impact of this polymorphism on acute and chronic GVHD incidence/severity after non-T cell-depleted sibling BMT. TNFRII 196 M/R genotype was determined amongst 106 transplant recipients, 106 donors and 131 control subjects. R allele frequency amongst recipients, donors and controls was 0.28. In univariate analysis there was a trend towards increased acute GVHD incidence [odds ratio (OR) 2.9] amongst R+ recipients (p=0.1). In multivariate analysis by logistic regression, correcting for established clinical and genetic GVHD risk factors, the impact of recipient TNFRII R+ status was reduced (p=0.13). In univariate analysis, donor RR homozygosity associated with a trend to increased cGVHD incidence (OR 4.6; p=0.1) and an increased risk of extensive cGVHD (OR 8.5; p=0.01). In multivariate analysis donor RR homozygosity associated with an increased risk of extensive cGVHD (relative risk 11.3; p=0.02) but not cGVHD incidence per se. Since sTNFRIIs can act as TNF antagonists, the association between donor RR homozygosity and cGVHD may reflect reduced circulating sTNFRII. The role for pharmacological interventions, particularly the use of recombinant sTNFRII (Etanercept) requires further investigation, but may be influenced by genotype.

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RAPID ENGRAFTMENT IS ASSOCIATED WITH AN INCREASED INCIDENCE OF GRAFT VS HOST DISEASE (GVHD) IN CHILDREN FOLLOWING REDUCED-INTENSITY HEMATOPOIETIC STEM CELL TRANSPLANTATION (RI-HSCT)

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We conducted a retrospective chart review of 19 children who had undergone RI-HSCT at Childrens Memorial Hospital over