49

EARLY PRODUCTION OF DONOR-DERIVED CD4+ CD25+ REGULATORY T CELLS IN PATIENTS GIVEN HEMATOPOIETIC STEM CELL TRANSPLANTS

Woodliff, J.[1], Taylor, C.[1], Douek, D.[2], Margolis, D.[1], Drobyski, W.[1], Johnson, B.[1], Truitt, R.[1] 1. Departments of Pediatrics and Medicine, Medical College of Wisconsin, Milwaukee, WI; 2. NIH Vaccine Research Center, Bethesda, MD

In previous studies using murine models of BMT/DLI therapy, we found that donor-BM-derived CD4+ regulatory T cells (T-REG) produced in the host thymus were responsible for suppression of DLI-associated GVHD. Based on our mouse data, we hypothesized that failure of the host thymus to produce T-REG cells after allogeneic BMT in humans would result in an increased risk of GVHD after DLI therapy and an increased risk of chronic GVHD. Here, we sought to identify, expand and functionally characterize T-cells over time in patients given hematopoietic stem cell transplants (HSCT). Peripheral blood (PB) was collected from 19 adult and 8 pediatric patients at 2, 4, 6 and 12 mo. post-transplant. T-REG cells were identified by staining ficoll-separated PB cells for coexpression of CD4 and CD25. T-REG cells were defined phenotypically as CD4+ cells with high levels of CD25 staining. 88% (22/25) of patients analyzed at 2 mo. had detectable levels of CD4+ CD25+ cells. These cells persisted over time; only two patients with detectable T-REG cells at 2 mo. lost them by 6 mo. Some, but not all, CD4+ CD25+ cells had high TREC levels suggesting that they were recent thymic emigrants. We purified CD4+ CD25+ cells and CD4+ CD25- cells by cell sorting when sufficient PB was available and expanded them ex vivo using anti-CD3/anti-CD28 conjugated Dynal beads and rIL-2. The expanded CD4+ CD25+ cells were confirmed as being of donor-origin by VNTR analysis. Most were functional as shown by their ability to suppress T cell proliferation when titrated into MLR assays. Expanded CD4+ CD25+ cells from 5 of 8 patients suppressed proliferation of T cells from the original HSC donor (i.e., autologous T cells) by 26-94% (R : T-REG = 1:1). When donor T cells were not available, unrelated T cells were used in the MLR assays. Ex vivo expanded cells from 6 of 7 patients suppressed the proliferation of the allogeneic responder T cells by 34-77% (R : T-REG = 1:1). Our results indicate that functional CD4+ CD25+ regulatory T cells of donor origin are present early after HSCT in most patients. The patients, tissue source and histocompatibility of the HSCs used in this study were heterogeneous. Determining whether the de novo generated T-REG cells are sufficient to mitigate GVHD will require a more homogeneous patient group and longer follow-up. Adoptive transfer of T-REG cells isolated from the HSCT donor and expanded ex vivo may be necessary to more effectively modulate GVHD.

50

LOW CIRCULATING DENDRITIC CELL COUNT AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) PREDICTS DEATH, RELAPSE, AND ACUTE GRAFT-VERSUS-HOST DISEASE (AGVHD)

Reddy, V.[1], Iturraspe, J.A.[2], Tzolas, A.C.[1], Meier-Kriesche, H.-U.[1], Schold, J.[1], Greene, S.[1], Wingard, J.R.[1] 1. University of Florida, Department of Medicine, Gainesville, FL; 2. Lifesouth Community Blood Centers, Department of Flow Cytometry, Gainesville, FL

Dendritic cells (DC) are key antigen presenting cells, with a potential role in tumor vaccines. We investigated the hypothesis that early reconstitution of DC around the time of engraftment after allogeneic SCT improves survival. We also correlated DC reconstitution with complications of relapse and AGVHD. Fifty patients were transplanted between February 2000 and March 2003 with a median follow up of 501 days (range 136-1263). Most patients received blood stem cells (92%), and the remainder bone marrow, from HLA matched sibling donors for predominantly high risk hematologic malignancies. Around the time of sustained neutrophil engraftment of >500 cells/mm^3, peripheral blood was analyzed by flow cytometry for DC as lineage negative, HLA-DR positive, and further divided as DC1 (CD11c$^+$) and DC2 (CD123$^+$). Patients were divided into two groups of either low (n = 23) or high (n = 27) absolute DC count based on clustering technique determination. Patients with lower DC count (<4.97 cells/mm^3) had a significantly worse survival by Kaplan-Meier analysis (p = 0.009), increased incidence of relapse (p = 0.002), a higher incidence of AGVHD onset (p = 0.0005) and a composite end point of relapse or death (p = 0.002). A Cox-proportional Hazard multivariate model adjusted for important covariates confirmed that low DC count is independently associated with death (HR 3.8; 95% CI 1.3, 11.3; p = 0.02), time to relapse (HR 10.2; 95% CI 2.2, 47.2; p = 0.003) and in the development of severe AGVHD (HR = 2.7; 95% CI 1.2, 6.4; p = 0.02). The sensitivity and specificity of low DC count in predicting death or relapse after transplantation is 73% and 75% respectively with a positive predictive value (PPV) of 70% and negative predictive value (NPV) of 78%. To further delineate DC functionally, we studied DC1 and DC2 phenotypes, and the failure rates remained higher in the low cell count groups. In addition, DC recovery was independent of DC infused or patient DC counts prior to transplant. Our data suggest that, at least for peripheral blood SCT, the number of DC in the graft has no impact while the numbers of DC ultimately reconstituted in the recipient make the difference. In summary, our findings are unique for describing an early easily reproducible clinical indicator for adverse clinical outcomes such as relapse, death, and AGVHD after hematopoietic SCT. This finding should allow for early recognition of patients and studies for potential therapeutic interventions.

LATE EFFECTS/QUALITY OF LIFE

51

REDUCED-INTENSITY CONDITIONING (RIC) REGIMENS RESULT IN LOW PREVALENCE OF PREMATURE OVARIAN FAILURE (POF) IN WOMEN UNDERWENT HEMATOPOIETIC STEM-CELL TRANSPLANTATION (HST)

Cheng, Y.C., Saliba, R.M., Rondon, G., Giralt, S.A., Lu, K.H., Bodurka, D.C., Gershenson, D.M., Champlin, R.E., Ueno, N.T. The University of Texas M. D. Anderson Cancer Center, Houston, TX

High-dose conditioning (HDC) regimens used for HST can cause high incidence of POF among female transplant survivors. POF results in uncomfortable postmenopausal symptoms and infertility. In contrary, RIC regimens have resulted generally less treatment-related toxicity and GVHD. Therefore, RIC regimens are expected to have lower rate of POF in theory. However, the actual prevalence is unknown. Of the 3,945 patients who underwent HST between January 1987 and September 2001 at our institution, 488 were female, aged 16–40 years at the transplant, and had no history of breast or ovarian cancer. Of those patients, 413 (85%) had been given HDC regimens and 75 (15%) had been given RIC regimens. Two hundred twelve patients (179 [43%] in the HDC group and 33 [44%] in the RIC group) were alive at the time of this cross-sectional study. Questionnaires on menstruation history before and after HST were sent to 166 patients with addresses on file; 89 (64%) of the 138 patients given HDC and 20 (71%) of the 28 given RIC replied. Age, disease status, and treatment variables were statistically similar in patients who replied and those who did not. Median follow-up among women who replied was 75 months for the HDC group and 22 months for the RIC group. Age, disease status, and treatment variables were also statistically similar in the HDC and RIC groups. Similar proportions of women in each group (12 of 89 HDC and 4 of 20 RIC) reported loss of ovarian function (defined as loss of menstruation for ≥12 months or an FSH/LH level >20 IU/L) before HST. Ovarian failure after HST was reported by 61 (79%) of those given HDC vs. only 6 (37.5%) of those given RIC (P = 0.007). Among patients who retained their ovarian function after transplant, 4 in HDC and