12p70 secretion, naive CD4+ T cell expansion, Th1 and Tc1 cells differentiation) in the context of RIC allo-SCT. The fine functions of immune effectors would tend to be more evident in such less toxic regimens, offering new opportunities for a better understanding of aGVHD pathophysiology and therapy.

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RAPAMYCIN ADDED TO HUMAN CD25+ CELL CULTURES ACTIVATED THROUGH CD3/CD28 ENRICHES FOR CD4+CD25+CD27+Foxp3+ REGULATORY T CELLS

Keever-Taylor, C.A., Browning, M.B., Johnson, B.D., Truitt, R.L., Behn, B.A. Medical College of Wisconsin, Milwaukee, WI.

CD4+ T cells that constitutively express high levels of CD25 inhibit T-cell responses in a dose-dependent manner. Methods to isolate and expand CD4+CD25+ regulatory T-cells (Tregs) have been developed to allow for potential clinical use in regulating undesired T cell-mediated responses such as autoimmune disease or GVHD. We have enriched for Tregs by CD25 positive selection followed by culture in ex vivo 15-5% HS and autologous feeder cells. Activation is through a single addition of CD3/CD28 Ab-linked beads (4:1 bead:cell) and IL-2 (100 U/mL added d 3). Cultures with potent suppressor activity expanded on average 450 fold in 14 d when maintained at 5×10^5/mL in medium + IL-2. However, by 21 d suppressor activity greatly diminished and the % of CD25-bright cells decreased, indicating activated non-Treg cells were dominating the cultures. To increase the purity of Tregs we explored the use of rapamycin, a T cell immunosuppressive agent recently shown to selectively spare murine Tregs. Rapamycin (20-0.01 ng/mL) added to CD25-enriched cells significantly inhibited cell expansion at doses $\geq$1 ng/mL that was inversely correlated with enhanced suppressor activity at days 10 and 21. Cultures without rapamycin expanded on average 17,000 fold by day 21 vs 350 fold with 1 ng/mL rapamycin. By 21 d cells from untreated cultures suppressed allo-proliferation by only 28% at a 1:1 suppressor:responder ratio compared to 63 $\pm$ 20% by cells grown in $\geq$1 ng/mL rapamycin (P = .007). The % CD4+CD25bright cells at 10 d and 21 d was higher in rapamycin-containing cultures than untreated cultures, $P < .003$. Intracellular FACS staining for the transcription factor Foxp3, a marker for Tregs, showed significantly higher expression on CD4+ cells from 10 d rapamycin containing cultures with 23 $\pm$ 6% Foxp3+CD4+ cells vs 5 $\pm$ 3% in cultures without rapamycin (P = .03, n = 4). Foxp3 was predominately expressed on CD4+CD25-bright cells that also co-expressed CD27-bright. By day 21 cultures grown in rapamycin contained 1000 to 5000 fold more CD4+Foxp3+ cells than 10 d cultures without rapamycin. Only 0.7% of CD4+ cells from CD4+CD25-neg cells cultured with rapamycin expressed Foxp3. Optimal Foxp3 expression required continued presence of rapamycin in culture and addition at culture initiation was superior to addition at day 3. In summary, addition of rapamycin at doses from 1-20 ng/mL to CD25-enriched cell cultures increased the purity of cells with the phenotype and function of Tregs.

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PROSPECTIVE EVALUATION OF A GvHD-SPECIFIC PROTEOMICS PATTERN AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

Weissinger, E.M.[1], Hillmann, M.[2], Dammann, E.[1], Kolb, H.-J.[3], Holler, E.[4], Mischak, H.[2], Ganser, A.[1], Hertenstein, B.[5] [1]Hannover Medical School, Hannover, Germany; [2]Mosaiques Diagnostics GmbH, Hannover, Germany; [3]Ludwig-Maximilians-University, Munich, Germany; [4]University of Regensburg, Regensburg, Germany; [5]Klinikum Bremen Mitte gGmbH, Bremen, Germany.

We have recently published a polypeptide pattern specific for the early diagnosis of acute graft versus host disease (aGvHD), based on the application of capillary electrophoresis (CE) and mass spectrometry (MS). Here we report the application of aGvHD-specific patterns to prospectively collected samples from 86 patients (45 AML, 14 ALL, 8 MM, 5 CLL, 5 SAA, 3 MDS, 3CML, 3 NHL). Fifty-three patients were transplanted from matched unrelated donors (MUD), 29 received stem cells from matched related donors (MRD), 3 from haplo-identical donors and 1 was transplanted from a syngeneic sibling. In the majority of the patients the GvHD prophylaxis was methotrexate or mycophenolate and cyclosporin A. Urine samples were collected on ice prior to conditioning, weekly until discharge from the ward and monthly thereafter. Immediate freezing of the samples avoids degradation of the proteins/peptides. After thawing and removal of confounding substances like salts and of all molecules larger than 30 kDa, the samples were loaded onto the CE, separated according to their charge and, after ionization, directly analyzed in an electrospray-ionization time-of-flight (ESI-TOF)-MS. Between 500 and 2500 peptides and proteins were detected in individual samples. All data generated are stored in a Microsoft MS database. The polypeptide patterns specific for the early detection of acute GvHD were applied to the data from the prospectively and blinded collected samples. The outcome of these analyses was compared to the clinical diagnosis of aGvHD, sepsis and CMV-reactivation. In 362 samples screened, 112 scored positive with the GvHD pattern. Twenty-eight of those were false positive, mainly at the time of conditioning, but only 3 were scored false negative. Thus the sensitivity of the aGvHD pattern is about 96% with a 75% positive prediction value, the specificity is about 89% with a negative prediction value of 98%. Thus the application of the aGvHD pattern for early recognition of acute GvHD is very useful for predicting the development of aGvHD. Seven patients in the prospective cohort have developed cGvHD so far and samples were also scored with the aGvHD pattern. First results show that in the majority of the patients the polypeptides excreted do not correspond to those forming the aGvHD pattern. Taken together our results demonstrate that the proteome analysis of body fluids collected from patients after HSCT maybe extremely useful for diagnosis of complications.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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UMBILICAL CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS: RESULTS OF THE PROSPECTIVE, MULTI-INSTITUTIONAL CORD BLOOD TRANSPLANTATION STUDY (COBLT)

Kernan, N.A.[1], Carter, S.L.[2], Wagner, J.E.[3], Baxter-Lowe, L.[4], Wall, D.[5], Kapoor, N.[6], Guinan, E.[7], Wagner, E.[8], Geller, N.[8], Kurtzberg, J.[9] [1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2]The EMMES Corporation, Rockville, MD; [3]University of Minnesota, Minneapolis, MN; [4]University of California, San Francisco, CA; [5]Texas Transplant Institute, San Antonio, TX; [6]Children's Hospital of Los Angeles, Los Angeles, CA; [7]Dana Farber Cancer Institute, Boston, MA; [8]The National Heart, Lung and Blood Institute, Bethesda, MD; [9]Duke University Medical Center, Durham, NC.

In 1996, NHLBI sponsored COBLT, a prospective study that established 3 cord blood banks that banked ~8000 CBUs, and funded 28 transplant centers to participate in a clinical trial with the primary endpoint of 180-day post-transplant overall survival (OS). The trial enrolled 316 pediatric pts with median age 4.6 years (range 0.1-17.9), 61% M, 37% minorities, 44% CMV+ and 71% with a malignant (malign) disease, who received a single CBU. CBUs had to be at least 4 of 6 HLA match (intermediate resolution A and B and high resolution [HR] DRB1) and provide a total nucleated cell dose (TNC) $\geq 1.0 \times 10^7$/kg. Approximately 50% of Caucasian and Hispanic pts received a CBU matched at HLA 5-6/6, but only 15% of African-Americans and 36% of Asians were matched at HLA 5-6/6. HR HLA types were retrospectively determined for HLA -A, -B, -DRB1 (292 pairs) and -C and -DQB1 (270 pairs). Only 30% of pairs were HR HLA matched at 8-10 alleles. The median pre-cryopreservation TNC and CD34+ cell dose were 6.8×10^7/kg (range 1.5-50.4) and 2.3×10^5/kg (range 0.1-20.1), respectively. The cumulative incidence (CINC) of neutrophil recovery (>500, day 42) and of platelet engraftment (>50000, day 180) was 81% and 54%, respectively. By day +100, the CINC of grades II-IV aGVHD was 40%. The CINC of cGVHD was 21% at 2 yrs. The probability of OS at 180 days