allografting with fludarabine and ATG after failed engraftment of autologous peripheral blood stem cells

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High-dose myeloablative regimens with autologous stem cell transplantation have been pursued as a consolidation strategy in first remission AML patients. We report two patients with AML who received autologous stem cell transplants in first CR. Both patients received similar regimens and failed to engraft their autologous blood stem cells. Both were then given Fludarabine and ATG with subsequent engraftment of cells from an HLA matched sibling. Patient A.S. is a 32 y.o male diagnosed with AML, FAB subtype M2, with normal cytogenetics who received induction chemotherapy with Ara-C 100mg/m² x 7 days, Daunorubicin 40mg/m² x 3 and VP16 40mg/m² x 3. His intensification/mobilization included Ara-C 2gm/m² x 8 and VP16 10mg/kg x 4. The regimen used for autologous stem cell transplant was Busulfan p.o. 1mg/kg x 6 doses and VP16 60mg/kg x 1. He received 15.6 x 10⁶ CD34 cells/kg bodyweight. On day 32 there was no sign of engraftment. He was then given Fludarabine 30mg/m² x 4 doses and ATG 15mg/kg x 6 doses. Peripheral blood stem cells, 4.2 x 10⁶ E6 CD34 cells/kg bodyweight. On day 32 there was no sign of engraftment. The patient achieved <50% donor chimerism which was managed by ceasing MMF; >90% donor chimerism rapidly developed accompanying the protocol to include ATGAM 15 mg/kg/day IV alone as GVHD prophylaxis in an attempt to not excessively inhibit a potential GVT effect. An unacceptable high incidence of severe acute GVHD and TRM occurred with a low relapse rate. Therefore, we modified the CSA/ATGAM/MMF cohort but not the earlier CSA alone regimen. Of 14 evaluable patients 13 showed sustained >90-95% chimerism using VNTR polymorphism analysis was assessed in 942 (865-1089) days. CHIMERIC: T cell and myeloid chimerism increases of normalized BCR-ABL amount and subsequent PCR positivity with a 3 or 4-log increases of normalized BCR-ABL amount and subsequent hematologic relapse, which occurred 2 and 4 months later, respectively. Although our data should be interpreted cautiously, the presence of chronic GVHD may reduce the risk of relapse in Ph+ ALL. Real-time quantitative RT-PCR appears to be a useful test for BCR-ABL transcript monitoring.

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THE CD4 PEPTIDE ANALOGUE 802-2 INHIBITS PROLIFERATION OF BOTH TH1 AND TH2 CELLS IN RESPONSE TO ALLOGENIC STIMULATION

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Engagement of CD4 and the T cell receptor (TCR)-CD3 complex generates signals which lead, in part, to the activation of helper T (Th) cells. Activated Th cells contribute to graft-versus-host disease (GVHD) arising from allogeneic MHC class II and minor histocompatibility antigenic differences. The 802-2 synthetic cyclic heptapeptide, designed and confirmed by NMR spectroscopy to mimic the D1-CC' loop of CD4, inhibits Th cell activation in human and murine systems. Since Th1 cells appear to be more important in the pathogenesis of acute GVHD than their Th2 counterparts, the efficacy of 802-2 in modulating allogeneic responses of both Th1 and Th2 cells was therefore tested. Primary polarization of Th1 cells was performed by stimulating purified CD4+ cells from healthy donors with irradiated EBV transformed B cells (EBV-B) obtained from unrelated, HLA mismatched donors in the presence of Th1 (IL-2 + IL-12) or Th2 (IL-4) polarizing cytokines. After similar stimulation on days 3 and 6, cells were subsequently propagated in the presence irradiated autologous mononuclear cells through weekly allogeneic restimulation and biweekly cytokine supplementation. Th1 and Th2 cells from 3-4 week old cultures were harvested and stimulated with irradiated EBV-B in the presence of IL-2 with and without 802-2 peptide. The presence of 802-2 (200μM) reduced the proliferation of both Th1 and Th2 cells to near background levels. Delaying exposure to 802-2 by more than 72 hours after allogeneic stimulation substantially reduced or eliminated its ability to inhibit proliferation. While murine Th cells, activated in vivo, in the presence of 802-2 appear to undergo apoptosis, it has not been possible to similarly demonstrate apoptosis of human Th cells activated in vivo in the presence of 802-2. The lack of inhibitory effects of 802-2 when added at later time points after allogeneic stimulation rules out a non-specific inhibition of cellular proliferation or toxicity. We hypothesize that 802-2 inhibits multitimerization of the CD4/MHC class-II/antigen/TCR complex during allogostimulation. The resultant spatial disruption of the associated signaling molecules leads to abrogation of the signals for cell proliferation and may render the Th cells anergic, as occurs in other situations where lymphocytes receive partial or disordered activation signals.
A fluorescence-based assay suitable for monitoring NK activity in clinical settings

Storaro, R.W.; Green, P.D.; Chen, N.J.; Smith, C.A.; Nikcevic, D.A.1 1. Department of Medicine, Duke University Medical Center, Durham, NC; 2. Moffitt Cancer Center, Tampa, FL.

Natural killer (NK) cells are increasing important within the context of cellular therapies, to include allogeneic bone marrow transplantation and donor lymphocyte infusions. Historically, 51Cr release has been the standard in vitro measurement for NK activity. However, 51Cr release has poor sensitivity for NK activity if effector cell numbers are limiting and patients undergoing allogeneic transplantation frequently have very low blood cell counts. To support our ongoing clinical protocols in allogeneic bone marrow transplantation, we have developed a fluorescence-based assay to monitor NK function that is accurate even with limiting effector cell numbers. In this assay the target cells are labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE). After incubations with NK cells, the killing of target cells is monitored by their membrane permeability as detected with the nucleic acid stain 7-amino actinomycin D (7AAD). In parallel assays, the CFSE-based assay generated dose-response curves similar to those achieved with 51Cr release assays. However, the fluorescence-based assay remained sensitive at lower target cell numbers where the results of 51Cr-release assays were not interpretable. As would be anticipated, an immunomagnetic enrichment of CD56+ cells greatly increased the sensitivity of the assay. More importantly, with CD56+-selected cells as effectors the CFSE-based lysis assay yields classic sigmoidal dose-response curves in assays performed in as little as one hour. Taken together, these data demonstrate this fluorescence-based lysis assay is sensitive, easy to conduct and time effective, yet the materials are inexpensive, making it suitable for clinical use.

FAC TORS ASSOCIATED WITH AMBIVALENCE ABOUT BONE MARROW DONATION AMONG NEWLY RECRUITED UNRELATED POTENTIAL DONORS

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Our previous research indicated that feelings of ambivalence or reluctance about donation were associated with donors’ decisions not to donate, and with less positive physical and psychosocial outcomes among donors who donated despite feeling ambivalent. The current study examines the prevalence of ambivalence among newly recruited potential bone marrow donors, and identifies factors associated with greater ambivalence. Using a cross-sectional design, questionnaires were mailed to a stratified random sample of individuals newly recruited to the NMDP registry at 71 local collection centers. Of the 426 (63%) of new recruits completed and returned the questionnaire, Bivariate analyses indicated that multiple recruitment experience and donor perception variables were significantly associated with higher levels of ambivalence among donors who donated despite feeling ambivalent. These associations suggest the need for recruitment strategies that reduce donor ambivalence about bone marrow donation.

Poster Presentations - Session I

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