reporter-gene system applied in this study contributed to reduction of required ex vivo BMPC culture maintenance time and more flexible study design.

230

ROLE OF GRAFT-FACILITATORY CELLS IN HUMAN ENGRAFTMENT IN THE NOD-SCID MOUSE MODEL

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We used the NOD-SCID mouse model to study factors that effect engraftment of human cord blood (CB) stem cells. Previous work showed that non-engrafting CD34$^-$ filler cells must be co-transplanted with unexpanded CB CD34$^+$ cells to detect human cells in the NOD-SCID mouse. MACS enriched CB CD34$^+$ cells were incubated for 7 days with SCF, FLT3 Ligand and MGDF in serum free media. Irradiated NOD-SCID mice were transplanted with CB CD34$^+$ cells ± irradiated CD34$^-$ filler cells. Bone marrow (BM) and spleen were analysed by flow cytometry for human engraftment 6 weeks post transplant. The level of human engraftment is significantly reduced when more than 10^7 filler cells are co-transplanted with unexpanded CD34$^+$ cells. We transplanted mice with 10^7 irradiated filler cells and increasing numbers of unmanipulated or expanded CB CD34$^+$ cells. Engraftment was not increased in mice transplanted with unexpanded CB CD34$^+$ input cells; however human engraftment increased when mice were transplanted with increasing numbers of expanded CB CD34$^+$ input cells. We tested the effect of increasing numbers of irradiated filler cells on the levels of human engraftment in mice transplanted with unexpanded and expanded CD34$^+$ cells and found that expanded cells dont require filler cells for engraftment. Phenotypic analysis of irradiated filler cells and expanded CD34$^+$ cells did not define a specific cell type that could explain the graft facilitatory effect. Filler cells facilitate human engraftment when mice are transplanted soon after irradiation but are not required when transplantation is delayed for 48 hrs, suggesting that engraftment of unexpanded CD34$^+$ cells is facilitated by cytokines. Unexpanded and expanded CB CD34$^+$ cells have different requirements for engraftment in the NOD-SCID mouse. We hypothesize that expanded cells generate cells or factors that facilitate engraftment. Definition of the underlying mechanism for this phenomenon may lead to strategies to facilitate engraftment for transplant recipients.

231

ABC-TRANSPORTER MEDIATED EFFLUX OF ALDEHYDE DEHYDROGENASE REACTION PRODUCT: IMPLICATIONS FOR ENUMERATION AND ISOLATION OF FUNCTIONAL BLOOD PROGENITOR CELLS

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Storms et al [PNAS 96, 9118-9123, 1999] used the aldehyde dehydrogenase [ALDH] substrate BAAA to enrich cord blood [UCB] hematopoeitic stem and progenitor cells [HSPs]. They found that the efflux of BAA, the ALDH reaction product, from UCB cells was slowed by verapamil; this is consistent with the high activity of ABC-transporters in HSPs. We now report that the BAAA method can be used to detect and isolate functional peripheral blood [PB] HSPs. Intracellular accumulation of BAA was stabilized by maintaining labeled PB HSPs on ice or by using probencid instead of verapamil to inhibit ABC-mediated efflux. BAA efflux was the major source of ALDH assay variability in the absence of probenecid. At 2.5mM probenecid, efflux of BAA was reduced >95%, and enumeration of PB HSPs could be readily accomplished at room temperature. Using probenecid decreased the variability of the ADLH assay at least two-fold and allowed robust ALDH-based enumeration of PB HSCs over the entire dynamic range of clinical stem cell determinations; standards yielded an assay CV ranging from 20.4% in samples containing 0.1% CD34+ cells to 5.6% for samples with 6% CD34+ cells. UCB or PB HSPs could be sorted on the basis of ALDH activity without exposure to ABC transport inhibitors by carefully maintaining cells on ice. After sorting, HSPs were incubated at room temperature or 37C to allow efflux of BAA; no residual BAA fluorescence was detected in sorted PB HSP after 30 minutes at room temperature. The ALDH reaction had no effect on the short term clonogenic activity of HSPs, and BAAA had no detectable toxic or mutagenic effects in standard preclinical assays. No more than 1% of cells expressing high ALDH activity, but as many as 40% of the CD34+ cells, in frozen and thawed UCB or PB HSPs were stained by 7AAD. BAA must be retained in cells that stain brightly for ALDH, but CD34 may be accessible to antibody on damaged or apoptotic cells that do not retain BAA. Thus, if ABC-transporter mediated efflux is inhibited, the ALDH method detects functional HSPs with high ALDH activity using a single channel of the flow cytometer. Efflux of the ALDH product can be controlled during HSP enumeration and isolation and can be used to produce functional cell populations with low levels of residual BAA for research and future clinical applications.

SUPPORTIVE CARE

236

EVOLVING EPIDEMIOLOGY OF CANDIDA IN THE BONE MARROW TRANSPLANT UNIT: A LOOK TWELVE YEARS AFTER FLUCONAZOLE

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The increasing incidence of non-*albicans Candida* species, especially *C. glabrata* has been frequently associated with the increase in antifungal use, specifically fluconazole. This point prevalence study evaluated the current epidemiology and evolution of *Candida* species in the era of fluconazole prophylaxis and compared the results to a similar study done in 1989/1990, prior to fluconazole use. Methods: 92 cultures were obtained in the bone marrow transplant unit (BMTU), of these, 42 hand cultures from 21 healthcare workers (HCW) and 50 environmental samples (ES) taken from patient care areas. Hand cultures were obtained by handwashing technique in YEPD broth. ES were obtained using Sabouraud dextrose Rodac plates. Yeast specimens were evaluated for genus/species identification and in vitro susceptibilities were done using the disk diffusion technique for fluconazole (FLZ) and voriconazole (VCZ) and the NCCLS methodology for FLZ, VCZ, amphotericin B (AmB) and micafungin (Mfg). We also reviewed our positive blood cultures for yeast for Jan-Dec 1989 and Jan-Dec 2001. Results: There were 18/42 (43%) positive cultures for yeast from the HCW hand cultures, 4/18 *C. albicans* (Ca), 11/18*C.parapsilosis* (Cp), 1/18 *C.glabrata* (Cg), and 1/18 for *C. kefyr* (Ck). In 1989 only 4/25 (16%)of hand cultures were positive for yeast, all were Cp. Of the 50 ES, all were negative for yeast. Whereas, in 1989, there were 10 ES (+) for yeast, 5 Ca, 1 Cg, 1 *C.guilliermondii*, 3 *C.lusitaniae* (Cl). Blood culture results from 1989 reveal Ca to be the most common, 52%, *C. tropicalis* (Ct) 25%, Cp 12%, Cg 9%, and *C.kefyr* 1.5%. Whereas in 2001, Ca 39%, Cg 34%, Cp 18%, Ct 7%, *C. krusei* and Cl 1%. In-vitro susceptibilities were unchanged in 1989 versus 2002. Discussion: Despite the rise in Cg fungemia, the environment fails to be a culprit in nosocomial Cg colonization and/or in Cg fungemia. Thus, FLZ prophylaxis has had no impact on the nosocomial epidemiology of Cg colonization. In contrast, *Candida* colonization rates especially Cp are much higher on HCW hands today, than before. This study shows that with FLZ prophylaxis, the in-vitro susceptibilities have not changed. It is evident, then, that the nosocomial transmission of *Candida* is species specific. Additional studies are needed to elucidate the origins of Cg increasing role in blood stream infections today.