GRAFT PROCESSING

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MOBILIZED PERIPHERAL BLOOD CONTAINS PRIMITIVE HEMATOPOIETIC CELLS THAT CAN BE ENUMERATED AND ISOLATED USING A FLUORESCENT SUBSTRATE FOR ALDEHYDE DEHYDROGENASE ACTIVITY

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High dose therapy followed by autologous or allogeneic transplantation with peripheral blood stem cells (PBSCs) has been used to treat patients with a variety of diseases. Selection of primitive stem cells and progenitors from PBSC collections is useful for reducing the transplant volume and decreasing the number of contaminating tumor cells or T-cells. We have developed a novel approach for enumerating and enriching primitive mobilized peripheral blood cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH). Mobilized cells were stained with a fluorescent ALDH substrate, termed BODIPY-aminoacetaldehyde (BAAA), and then analyzed or sorted using flow cytometry. A population of cells, termed SSCloALDHbr cells, was readily discriminated and comprised a mean of 3.1 +4.8% of the collected events. A mean of 73.4 +11.7% of the SSCloALDHbr population expressed CD34 and 56 + 24.5% of all the mobilized CD34+ cells resided within the SSCloALDHbr population. The SSCloALDHbr population was largely depleted of cells with mature phenotypes and enriched for cells with immature phenotypes. The BAAA staining procedure did not diminish the viability or clonogenic activity of hematopoietic progenitors and caused no toxicity to cells or animals in a variety of pre-clinical toxicology studies. Sorted SSCloALDHbr and SSCloALDHbr CD34+ cells were enriched for progenitors with the ability to 1) generate CFUs and LTC derived CFUs, 2) expand in primary and secondary LTCs and 3) generate multiple cell lineages. In order to test whether the number of PBSC SSCloALDHbr cells would predict engraftment in actual human transplants, the total number of SSCloALDHbr cells infused per kg in 21 cancer patients who had undergone autoPBSC transplantation were compared to the times to an ANC>500 and a platelet count >20, 000 using a Cox proportional hazard analysis. The time to neutrophil and platelet engraftment were both highly correlated with the number of SSCloALDHbr cells/kg ($p<0.015$ and 0.003 respectively) and the number of SSCloALDHbr CD34+ cells/kg ($p<0.013$ and 0.0016 respectively). In summary, PBSC SSCloALDHbr cells have the phenotypic and functional properties of primitive hematopoietic cells and their number correlates with short term engraftment following autoPBSCT transplantation.

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NORMAL AND NEOPLASTIC B CELLS ARE UNDULY SUSCEPTIBLE TO PHOTODYNAMIC CELL THERAPY

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The favorable effect associated with the infusion of a graft purged of its neoplastic cells might be jeopardized by the concomitant elimination of cell populations responsible for (1) the eradication of residual lymphoma cells, (2) the prevention of viral and fungal infections, and (3) rapid hematologic reconstitution post-transplantation. In previous studies, we have shown that TH9402, a rhodamine-derived photosensitizer, selectively eliminates activated T cells, and spares resting T cells. Interestingly, P-glycoprotein (Pgp) modulates TH9402-mediated cytotoxicity by extruding this photosensitizer from the intracellular milieu. The absence of Pgp expression in most NHL cells prompted us to evaluate photodynamic cell therapy (PDCT) with TH9402 for the selective elimination of B-lineage lymphoma cells. To identify optimum treatment conditions, the uptake/retention kinetics and cytotoxicity profile of TH9402 were evaluated with B-lineage cell lines, NHL patient cells harboring or not a bcl-2/IgH rearrangement, and normal B lymphocytes. In clonogenic assays, more than 4 logs of indolent bcl-2/IgH rearranged (RL and DHL-16) and aggressive (Namalwa and Raji) NHL cell lines and patient (n=27) NHL cells were eradicated following PDCT using TH9402. Although normal B lymphocytes retained TH9402 in amounts similar to T cells, as measured by flow-cytometry, PDCT eliminated 4 logs of B cells but only 1 log of T lymphocytes, which were spared for immune reactivity. Importantly, PDCT conditions of maximum intensity that preserved more than 50% of CFU-GM, BFU-E and CFU-Mix colonies, and greater than 75% of LTC-IC progenitors for hematopoietic reconstitution, demonstrated lower levels of retention of TH9402 in CD34+ cells. The discrepancy between TH9402 retention levels and cytotoxicity indicates that the exquisite sensitivity of B lineage cells to TH9402 phototherapy must rely on a retention-independent mechanism of target cell elimination. The specificity of TH9402 for B lymphocytes indicates that this strategy should be evaluated for ex vivo purging of autologous stem cell grafts from patients with NHL and also for the treatment of autoimmune B cell disorders.

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MOBILIZATION OF TYPE 1 AND TYPE 2 DENDRITIC CELLS BY GM-CSF ALONE OR IN COMBINATION WITH G-CSF

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Dendritic cells (DC) are the only antigen-presenting cells capable of priming naive T cells with antigens. Generally, DC does not express lineage-specific markers (lineage negative), but express HLA-DR. There are two subsets of DC: one of lymphoid lineage that induces Th1-like responses and expresses CD11c (DC1) and another of myeloid lineage that induces Th2-like responses and expresses CD123 (DC2). Previous studies reported that G-CSF preferentially mobilized DC2, and not DC1, in normal subjects. To determine the effect of GM-CSF alone or in combination with G-CSF on the mobilization of DC1 and DC2, we studied DC markers in 7 subjects received 10 mcg/kg/day of GM-CSF for 4 days and another 5 subjects received 10 mcg/kg/day of GM-CSF and 10 mcg/kg/day of G-CSF for 4 days. Peripheral blood was obtained from all subjects before treatment and 24 hours after the final dose of the mobilization regimen. The numbers of DC1 and DC2 were determined by staining blood with lineage cocktail antibodies, anti-HLA-DR, anti-CD11c, and anti-CD123, and analyzed by 4-color flow cytometry. Compared with the number of total DC prior to treatment with G-CSF plus GM-CSF, the post-treatment number of DC was significantly higher (32 +/- 6 per mcl versus 143 +/- 35 per mcl; $p = 0.020$). The increase in total DC post-mobilization with G-CSF and GM-CSF was due to a significant increase in DC2 (8 +/- 3 per mcl versus 37 +/- 19 per mcl; $p = 0.046$), and not in the number of DC1. By comparison, mobilization of DC, DC1, and DC2, with GM-CSF alone was only marginal. The preferential expansion of DC2 by the combination of G-CSF and GM-CSF as with G-CSF alone may have important consequences on sensitization of the host and on the engraftment.

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EFFECTIVENESS OF METRONIDAZOLE ON CYCLOSPORINE-INDUCED GINGIVIAL HYPERPLASIA IN BONE MARROW AND KIDNEY TRANSPLANT PATIENTS:A PROSPECTIVE, DOUBLE-BLINDED CONTROLLED CLINICAL TRIAL

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Background: Cyclosporine-induced gingival hyperplasia (CIGH) regression has been reported with some antibiotic therapies, especially Metronidazole and Azithromicine. **Object**: To evaluate the efficacy of metronidazole on CIGH regression, **Method**: we performed a prospective double-blinded study on 22 patients with CIGH, following kidney or bone marrow transplantation. None of the patients received calcium-channel blockers or anticonvulsants. Other causes of gingival enlargement were ruled