diverse cell types distinguishes MAPC from Mesenchymal Stem Cells (MSC), and suggests that MAPCs may therefore be an ideal cell for in vivo therapies for tissue repair or regeneration in multiple organ systems (Jiang Y et al Nature, 2002; 418:41–49). We have developed the technology for the large-scale expansion of MAPC with stable phenotype and biological properties. Under these conditions, cell surface marker analysis of MAPC revealed that these cells are positive for CD10, CD90, and CD49c, they are weakly positive for MHC class I, and are negative for MHC class II, CD45, and CD106, indicating that MAPC are not derived from the hematopoietic lineage. However, to optimize in vivo utilization, the understanding of immunological properties of MAPC is critical. Thus, we undertook an analysis of MAPC immunogenicity. First, these stem cells were shown to be non-immunogenic as MAPC from two different rat strains did not stimulate allogeneic T cell proliferation, while splenocytes of the same rat strains elicited strong proliferative responses in a mixed lymphocytic culture. Second, MAPC displayed immunosuppressive properties. Addition of MAPC at the initiation of a mixed lymphocyte reaction (MLR) suppressed T cell proliferation in a dose dependent manner. The inhibition was detectable when number as low as 3000 MAPC/well were added to 1 × 105 T cell responder. Lymphocyte proliferation in MAPC-containing cultures was inhibited by up to 80% when compared to cultures without MAPC. The ability to inhibit T cell alloresponse was independent of the MHC, allowing the use of third party MAPC as inhibitory cells. MAPC also inhibited proliferative responses to the T cell mitogen Concanavalin A (50%), although inhibition required higher MAPC:responder cell ratios. Taken together, these data suggest that MAPC are progenitor cells that do not express markers of the hematopoietic lineage; they are non-immunogenic to T cells, suggesting that universal MAPC donors may be used for tissue repair or regeneration; and MAPC exhibit potent immunosuppressive properties, a result which suggests that these calls may be useful in the management and/or prevention of GVHD.

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CYTOKINE-INDUCED IN VIVO EXPANSION AND MOBILIZATION OF MARROW MESENCHYMAL STEM CELLS IN NONHUMAN PRIMATES

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Mesenchymal stem cells (MSC), as well as showing promise for tissue regeneration and gene therapies, have the potential to facilitate bone marrow transplantation. First, they can provide stromal support for engrafted cells, and second, their immunologic properties may facilitate engraftment and reduce graft versus host disease. Much is made of the ease with which MSC can be cultured and expanded many-fold in vitro, however there is evidence that this can alter the fundamental properties of these cells. Therefore, we have explored the potential to increase the number of marrow MSC in vivo using various cytokine regimens in a nonhuman primate (NHP) model. Male baboons between 7 and 11 years of age received subcutaneous cytokines as follows: (1) G-CSF 100 mcg/kg/day for 5 days; (2) pegylated G-CSF (pegG-CSF), single dose 300 mcg/kg day −5; (3) G-CSF 100 mcg/kg/day + stem cell factor (SCF) 50 mcg/kg/day for 5 days; and (4) pegylated megakaryocyte growth and development factor (pegMGDF) 1 mcg/kg second daily for 10 days + G-CSF 100 mcg/kg/day for 5 days starting day −5 before the harvest. Bone marrow was aspirated from the iliac crest at baseline and on the final day of cytokine administration. Mononuclear cells were isolated on Ficoll-Paque Plus, and plated in triplicate in alpha-MEM plus 20% FCS for fibroblast colony forming cells (CFU-F). Cells were fixed, stained, and scored on day 7, and the number of CFU-F/mL of bone marrow was then calculated. The mean baseline CFU-F marrow concentration was 2 032/mL (n = 15). There was an increase in CFU-Fs following each of the cytokine regimens to 11 427/mL, 8 430/mL, 20 662/mL, and 15 745/mL after G-CSF (n = 3, $P < .001$), pegG-CSF (n = 4, $P > .05$), G-CSF+SCF (n = 5, $P < .001$), and G-CSF+pegMGDF (n = 4, $P < .01$) compared to baseline, respectively. We also explored the potential for the mobilization of MSC into peripheral blood. CFU-F were not detected in baseline peripheral blood mononuclear cells (PBMNC) samples from any animal. However, CFU-F were detected in 3 animals after G-CSF+SCF at a frequency of 0.8/mL to 1.5/mL, but no other cytokine regimen. In conclusion, these data confirm that cytokine regimens used to mobilize hemopoietic stem cells can be used to induce in vivo expansion and mobilization of MSC and that the combination of G-CSF and SCF may be the most effective.

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MODULATION OF ALDEHYDE DEHYDROGENASE ACTIVITIY AND RETINOID SIGNALING INDUCES HEMATOPOIETIC STEM CELL SELF RENEWAL

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Aldehyde dehydrogenases are cytosolic enzymes that convert aldehydes into carboxylic acids. Human aldehyde dehydrogenase 1 (ALDH1) is highly expressed in the liver and in hematopoietic stem cells (HSCs). Although ALDH1 is a selectable marker of HSCs, its HSC-specific function is unknown. We hypothesized that ALDH might play a critical role in HSC fate determinations since it is required for the production of retinoic acids, which are broadly implicated in tissue differentiation, tissue patterning, and embryonic development in vertebrates. In this study, highly purified human CD34+CD38−lin− HSCs were cultivated with early acting cytokines, thrombopoietin, stem cell factor, and Flt-3 ligand (TSF) in the presence or absence of the competitive ALDH inhibitor, diethylaminobenzaldehyde (DEAB). Treatment of human BM and CB HSCs with TSF caused a loss of CD34+CD38− cells in culture, morphologic differentiation, amplification of committed colony forming cells (CFCs), and a loss of primitive cells capable of repopulating non-obese diabetic/severe combined immunedeficient mice (SCID-Repopulating Cells, SRCs). Conversely, culture of BM and CB HSCs with TSF plus 100 μM DEAB blocked the morphologic differentiation and lineage commitment of HSCs in culture, expanded the CD34+CD38− population, and amplified SRCs 2- to 4-fold compared to input, indicating a fundamental role for ALDH activity in HSC differentiation. The effects of DEAB could be reversed by the co-administration of the retinoic acid receptor (RAR) agonist, all-trans retinoic acid (ATRA), suggesting that the ability of ALDH to produce retinoic acids is important in determining HSC fate. Via screening studies of direct ligands of RAR and RXR, we also identified a selective RXR modulator, LGD101506, which functioned similarly to DEAB in impeding HSC differentiation and causing the 4-fold expansion of SRCs. Interestingly, both DEAB treatment and LGD101506 reversed the down-modulation of HOXB4 transcription that was otherwise observed in CD34+CD38−lin− cells during culture with cytokines, suggesting that inhibition of ALDH or RXR modulation may promote HSC self-renewal via discrete interactions with other regulatory pathways. Modulation of ALDH activity and retinoid signaling is a novel and effective strategy to amplify human HSCs.

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DEXAMETHASONE ENHANCES ERYTHROPOIETIC DIFFERENTIATION OF EMBRYONIC STEM CELLS

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We have studied the in vitro differentiation of murine embryonic stem cells (ES) towards erythropoiesis and expression of genes during this process. It has been reported that dexamethasone directs ES cells towards erythrocytic differentiation but the mechanism of gene regulation induced by dexamethasone is not well understood. We hypothesized that dexamethasone induces upregulation of erythropoietic genes such as GATA-1, FLK-1, EPO-R, and directs ES cells towards erythropoietic differentiation.