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EXPANSION OF COMMMON MYELOID PROGENITORS IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Chronic myelomonocytic leukemia (CMML) is a unique myeloproliferative disease characterized by marrow dysplasia and an increase in monocytes. The median survival of patients with CMML is short (approximately 2 years), in part, because CMML is frequently resistant to therapy including allogeneic peripheral blood progenitor transplantation. In order to reduce regimen related toxicity and provide more effective CMML targeted therapies, a better understanding of the basic biology of CMML progenitors is required. We used FACS analysis and recently identified phenotypic markers to identify phenotypic and functional differences between normal and CMML bone marrow hematopoietic stem cells and myeloid progenitors. CMML marrow was typified by a reduction in CD34+CD38-CD90+Lin-hematopoietic stem cells and an expansion of CD34+CD38-CD90-Flk2+Lin-cells relative to normal bone marrow. In addition, there was a two-fold expansion in common myeloid progenitors (CMPs) and a corresponding decrease in megakaryocyte-erythroid progenitors (MEPs) suggesting that there was a skew in differentiation toward the myeloid lineage. In contrast to normal bone marrow derived CMPs, CMML CMPs gave rise to myeloid but not erythroid colonies. Moreover, real time quantitative RT-PCR analysis of highly purified FACS-sorted CMML CMPs demonstrated an increase in expression of two key regulators of myelomonocytic differentiation, PU.1 and c-jun, compared with normal bone marrow. A more detailed understanding of the basic biology of CMML myeloid progenitors and the genes that work in concert to expand them may aid in identifying novel molecular targets for CMML therapy.

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[Abstract Withdrawn]

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3-DIMENSIONAL MULTI-MODALITY NON-INVASIVE IMAGING OF THE BONE MARROW ENGRAFTMENT MODEL

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The studies of the stem cell biology and engraftment often have to address the question of the cell homing, migration and localization. We tested feasibility of applying a multi-modality imaging approach for a non-invasive localization of a genetically modified graft. The bone marrow cells of C57BL/6 mice were transduced using retrovirus bearing *hsv-tk/egfp/luc* reporter fusion gene, characterized by the GFP expression and the whole bone marrow graft, including up to 38% GFP-positive cells was transfused to the lethally irradiated B6.SJL-Ptprc mice. The preliminary information on the engraftment and location of the cells over a 4 week period from the transplant was obtained by bio-luminescent imaging (BLI) continuously and the 3-dimentinally resolved data on the bone-marrow homing were obtained by a combination of MicroCT and MicroPET using [^{18}F]FEAU as a radiotracer. The data were confirmed morphologically by GFP microscopy of the tissue sections. It was also possible to obtain *ex vivo* bioluminescence from the excised organs and tissues incubated with D-luciferin *in vitro*. 3-Dimensional reconstruction and processing of MicroPET and MicroCT images allowed for coregistration of the major foci of bone marrow transplanted cells within the bone marrow homing areas. The primary sites of the transplanted cell targeting were in the epiphyses of the ling bones, spongeous parts of the skull bones and vertebrae. This analysis allowed differentiating the specific signal originating from the lumar spine from a non-specific radiotracer cleared through the gut. There was a transient signal noted in the projection of spleen over the second week post-transplant observed by the BLI. MicroPET images on days 6 and tissue sample analysis confirmed this finding. Slightly decreased radiotracer accumulation discordant to the continuously increasing BLI signal over the time of observation may denote specific changes in susceptibility of the bone marrow cells towards nucleoside-based radiotracer imaging. MicroCT without contrast agent appeared to be poorly applicable for soft-tissue imaging, but crucial for the bone locations. The study proved feasibility of multi-modality approach for obtaining the information on the bone-marrow cell localization to specific organs non-invasively in live recipients over time combining sensitivity and structural specificity of different methods. Multiple imaging sessions with intravenous administration of the probes were well tolerated by the animals.

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BOTH TISSUE DERIVED AND BONE MARROW DERIVED STROMAL CELLS ARE IMMUNOREGULATORY

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Bone marrow derived mesenchymal stem cells have been demonstrated to have immunosuppressive effects in mice and humans. These finding suggest that they may be exploited in organ transplantation settings. Here, we investigated immunoregulatory aspects of rat intestinal derived and BM MSCs. Bone marrow was extracted from six to eight week old DA rats and plated in DMEM containing 10% Fetal Bovine Serum, 2% L-Glutamine, 1% Penicillin-Streptomycin, and 1% Sodium Pyruvate (Gibco). Every 48 hours, non-adherent cells are discarded. The immunosuppressive effects of these cells in a allogenic mixed lymphocyte reaction were investigated. Intestinal derived mesenchymal cells were obtained by enzymatic (collagenase, dispase) digestion followed by plastic adherence. Mesenteric lymph nodes from both DA and Lewis rats were extracted and passed through a metal filter to extract lymphocytes. Irradiated DA lymphocytes were used as stimulators. Lymphocytes were cultured in IMDM Media containing 1% glutamine, 1% Rat Serum, 1% Non-Essential Amino Acids, 0.1% Beta-Mercapthanol, 1% Penicillin-Streptomycin, 1% Sodium Pyruvate, 1% HEPES, and 1% vitamin (Gibco). DA MSCs were added at gradual concentrations of 10^5, 10^4, 10^3, 10^2, and 10^1. After a four day incubation, 1uCi of tritiated thymidine was added. Lymphocytes were also cultured with 2 mcg/ml ConA. In MLR cultures, MSC dramatically suppressed MLR responses (99%) when added at concentration of 10^5 MSCs. Suppression was dose dependent. In ConA cultures, MSC suppression of lymphoid cells was maximal at 10^5 cells. MSC suppression was not MHC restricted as Lewis derived MSCs also suppressed MLR and ConA responses (>90%). Intestinal MSCs also suppressed MLR responses (>85%). MSCs suppressed skin graft rejection in an in-vivo model. The results suggest that BM and tissue derived MSCs may have clinical utility in transplantation settings.

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COMMON LYMPHOID PROGENITORS REQUIRE COMMON GAMMA (γ_c) AND C-KIT SIGNALS FOR SURVIVAL *IN VIVO*

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CLP are rare, highly proliferative bone marrow cells which are committed to T, B, and NK cell maturation, but lack myeloid or erythroid potential. CLP are phenotypically identified in marrow as IL-7R$^{(+)}$ Lin$^{(-)}$ Sca-1$^{(lo)}$ C-kit$^{(lo)}$ cells. Co-transplantation of small numbers of CLP in hematopoietic stem cell transplant recipients results in rapid improvements in antigen-specific immune function without causing graft-versus-host disease. The mechanisms of CLP survival, proliferation, and development *in vivo* are not well understood. Because the IL-7 and c-kit receptors are simultaneously expressed on CLP, genetic models were used to determine the role of these two receptor pathways in the mainte-