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MOLECULAR BIOLOGY OF HEMATOPOIESIS. I. Lemischka. Princeton University, Princeton, NJ, USA.

The hematopoietic stem cell has the ability to self-renew as well as the ability to commit to a number of differentiation pathways. Much is known about the *in vivo* developmental behavior of the stem cell population through many years of mouse and human transplantation studies. In addition, the physical cellular phenotype of the stem cell has been described through numerous purification strategies. In spite of the large amount of available information, the exact molecular mechanisms which mediate the choice between self-renewal and commitment which occurs during stem cell division remain almost completely obscure. As a comprehensive approach to shed light on this issue we have: 1) embarked on a broad wide ranging effort to identify the gene expression patterns which exist in undifferentiated and highly purified mouse and human stem cells, 2) begun to analyze how these gene expression profiles are modified in response to cytokine-induced proliferation and differentiation, 3) developed an *in vitro* correlate of the hematopoietic microenvironment and have shown that this culture system can effectively support the establishment and long-term maintenance of the entire primitive stem/ progenitor cell hierarchy and 4) analyzed the panel of gene products specifically expressed in a stromal cell line which represents a candidate stem cell microenvironmental niche. To date our efforts have yielded approximately 1,000 cDNA sequences which are candidates for genes specifically expressed in stem/progenitor cells but not in more mature blood cell lineages. In addition, over 500 sequences have been analyzed from the stromal cell line. Collectively these studies, while far from complete, have already yielded a considerable number of interesting molecules. The predicted amino acid sequences of these molecules strongly suggest important roles in the biology of the hematopoietic stem cell and microenvironment.

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ASSAYING NORMAL AND LEUKEMIC HUMAN STEM CELLS IN NOD/SCID MICE. J. E. Dick Dept. of Genetics, Hospital for Sick Children and Dept. of Molecular and Medical Genetics, Univ. of Toronto, Toronto,

The only conclusive method to assay stem cells is to follow their ability to repopulate conditioned recipients making it difficult to study human stem cells. The development of systems to transplant human hematopoietic cells into immune-deficient mice lays the foundation for such an experimental repopulation assay for primitive human cells. Cell purification and gene marking studies have shown that the repopulating cells, termed SCID-repopulating cells (SRC), are primitive and distinct from most of the progenitors that are detected using short and long-term in vitro culture assays. The SRC are exclusively $CD34^+CD38^-$ and poorly infected with retrovirus vectors. This gene marking data is reminiscent of the human clinical trials establishing that the SRC assay is a good surrogate to develop improved transduction methods. Limiting dilution analysis has been used to establish a quantitative assay for SRC that can be used to precisely determine the effect of various cytokine cocktails on the proliferation and differentiation of SRC during in vitro culture. We have also used this system to identify a cell capable of initiating human AML by transplantation into NOD/SCID mice, termed the SCID Leukemia-Initiating Cell (SL-IC). In contrast to SCID recipients, these mice could be engrafted at limiting doses and with purified cells with AML samples with a myelomonocytic phenotype (AML-M4/M5). We found that the SL-IC were always found in the $CD34^+CD38^-$ fraction. The uniformity of the leukemic stem cell phenotype in these diverse samples and the similarity to normal stem cells suggests that the target for leukemic transformation is a primitive normal stem for most cases of AML regardless of the phenotype of the leukemic blast cells. These data are consistent with the idea that the leukemic clone is organized as a hierarchy with SL-IC detecting a primitive cell.

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INTERACTIONS BETWEEN PRIMITIVE HEMATOPOIETIC CELLS AND BONE MARROW STROMA. C.J. Eaves, J.D. Cashman*, L. Ponchio*, A.C. Eaves, Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC.

The longterm culture (LTC) system has proven a useful in vitro model of the bone marrow microenvironment because it reproduces many of the features of stromal cell-mediated regulation of primitive normal and leukemic cell behaviour in vivo. This includes effects on the viability, proliferation, self-renewal and differentiation of transplantable stem cells and LTC-IC, as well as interactions that can reversibly promote or arrest the cell cycle progression of normal, high proliferative potential colony-forming cells (HPP-CFC) of both erythroid and granulopoietic types. We have focussed on an analysis of the latter mechanism because it is one that CML HPP-CFC are able to evade both in vivo and in the LTC system. Its delineation might, therefore, help explain how the CML clone acquires a selective and deregulated growth advantage in vivo at the level of the CFC compartment. Our initial studies demonstrated an ability of normal LTC adherent layer cells to exert a strong localized anti-proliferative effect on normal (but not CML) HPP-CFC which could be manipulated in 3 important ways: (1) by the addition of agents that activate endogenous production of various hematopoietic growth factors (or inhibitors), (2) by the addition of candidate direct-acting mediators, and (3) by the addition of antibodies or antagonists of endogenous mediators. From such studies we have identified 2 types of endogenous inhibitors that cooperate in forcing normal HPP-CFC to enter G_o in the LTC model. These are certain β (-CC-) chemokines (ie, MIP-1α and MCP-1) which are active on normal but not CML HPP-CFC, and TGF-β, which is active on both normal and CML HPP-CFC. Other β (RANTES, MCP-2, MCP-3 and MIP-1β) and α (-CXC-) chemokines (IL-8 and IP-10) were found to be inactive even on normal HPP-CFC. Interestingly, thus far MCP-1 is the only active chemokine found to contribute to the endogenous cycling control of normal HPP-CFC in LTC. We have also demonstrated a marked difference in the cycling activity of normal and CML LTC-IC in vivo. However, studies of normal LTC-IC cycling in the LTC system suggest that the inhibition of these more primitive cells, like their activation may involve different types or concentrations of factors than those that regulate HPP-CFC proliferation.

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MECHANISMS OF STEM CELL MOBILIZATION IN MICE. W.E. Fibbe, J.F.M. Pruijt*, L. Laterveer*, C.G. Figdor, G. Opdenakker*, R. Willemze. Dept. of Hematology, Leiden University Medical Center, Leiden, Dept. of Tumor Immunology, Nijmegen University, Nijmegen, The Netherlands and Rega Institute, Leuven, Belgium.

While mobilization is a property of most growth factors, relatively prolonged administration is required to induce mobilization. Previously, we have demonstrated that Interleukin-8 (IL-8) induces rapid (30 minutes) mobilization of hematopoietic progenitor cells (HPC) from the bone marrow (BM) of mice (Blood 85: 2269, 1995) and rhesus monkeys (Blood 87: 781, 1996). In an attempt to explain the mechanisms underlying IL-8 induced-mobilization we have studied the role of β2 integrins and matrix metalloproteinasis (MMP). Balb-C mice were treated with intraperitoneal injections of anti-LFA-1 antibody (H154.163). IL-8-induced mobilization of HPC was completely blocked by treatment with the anti-LFA-1 antibody, while mobilization induced by G-CSF or IL-1 was not. Addition of anti-LFA-1 antibody to colony cultures in semi solid media had no inhibitory effect. Transplantation of blood derived mononuclear cells derived from IL-8 mobilized animals pretreated with anti-LFA-1 or saline protected 95% and 19% of lethally irradiated recipient mice respectively. These results indicate that anti-LFA-1 antibodies completely prevent the rapid mobilization of colony forming cells and of cells exhibiting radioprotective capacity and indicate a major role for the β2 integrin LFA-1 in the mechanism of mobilization. To study a possible role for MMP in stem cell mobilization, circulating levels of gelatinase-B were determined by zymographic analysis in rhesus monkeys injected with IL-8. Enzyme levels increased up to 1000-fold concomitant with the increase in numbers of HPC. Rhesus monkeys were then injected with inhibitory monoclonal anti-gelatinase-B antibodies (Rega 3G12) prior to IL-8 injection. A dose on 1 mg/kg anti-gelatinase-B antibody completely inhibited the IL-8-induced mobilization of progenitor cells. Zymographic analysis indicated the induction of gelatinase-B protein. Thus, IL-8 induced the rapid release of gelatinase-B with concurrent mobilization of HPC that could be prevented by blocking gelatinase-B enzyme activity. These data indicate the involvement of gelatinase-B as a mediator of the IL-8 induced mobilization of HPC.