Animal Models of Normal and Leukemic Human Hematopoiesis

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Introduction

Over the last 40 years the hematopoietic system has provided many of the important paradigms that guide our understanding of stem cell function. Much of our knowledge of the regulation of the hematopoietic system is derived from experiments in the mouse; these studies have involved identification of various classes of progenitor cells, growth factors that stimulate growth and differentiation, and molecular events that underlie the abnormalities that occur in diseases such as leukemia. This information has derived largely from the development of in vivo transplantation assays for normal stem cells and the ability to establish and grow leukemic cells in vitro and in vivo [1]. In contrast, our understanding of the biology of the human hematopoietic system has suffered relative to that in the mouse because of the lack of similar assays for normal stem cells and leukemic cells. Normal and leukemic human cells often appear to have complex growth factor requirements that are not easy to provide in short- or long-term cultures. Furthermore, the difficulties in growing primary human leukemic cells in culture suggest that there are selective processes that may result in alterations of the properties of such cells over time and the resultant cell lines do not accurately reflect the original disease. In an attempt to develop in vivo animal models for human leukemic cells, a large body of literature has accumulated over the past 20 years on the growth of human tumor xenografts in immune-deficient *nude* mice [2]. However, the growth of human leukemic cells as an ascites or solid subcutaneous tumor in *nude* mice does not reflect the normal course of the disease in humans. In addition to leukemic cells, normal human hematopoietic cells have also been introduced into *nude* mice directly or in diffusion chambers. The transplantation of human bone marrow directly into mice generally yielded inconclusive results [3], while the implantation of diffusion chambers demonstrated the development of human progenitors for as long as 28 days in vivo although it was not possible to distinguish between persistence of progenitors and engraftment of stem cells [4].

The recently described approaches to engraft human cells into two novel strains of immune-deficient mice provide the foundation for in vivo assays to characterize the normal developmental program of human hematopoietic stem cells along the myeloid and lymphoid lineages [5, 6, 7] and to develop models of several human hematopoietic diseases. Already, important preliminary experiments have established the feasibility of such models for leukemia [8], cancer [9, 10, 11], infectious diseases [12, 13, 14], and autoimmunity [15, 16]. Here I will briefly review our attempts to develop animal models for normal and leukemic human hematopoiesis.

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Successful engraftment of human cells into mice is dependent on the development of two particular strains of immune-deficient mice; *scid* and *bg/nu/xid* (abbreviated *bnx*). The *scid* mouse has already played an important role in the characterization of the murine lymphoid system because it is an ideal recipient for transplantation experiments designed to detect lymphoid reconstitution without the complications of lethal irradiation [17]. The *scid* mutation, carried on mouse chromosome 16, prevents the production of nature T and B cells; all other hematopoietic lineages including stem cells, myeloid progenitors, B cell precursors, and natural killer (NK) cells are normal [18]. The action of this gene product is not restricted to normal lymphoid development since *scid* mice have a generalized radiation repair defect that renders the animals at least two times more sensitive to the effects of γ-radiation [19]. In spite of these defects, homozygous *scid* mice have a normal life span and in all other respects appear normal although they are highly susceptible to infection.

The underlying defect and origin of the *bg/nu/xid* mouse is quite different to that of the *scid* mouse. It was constructed by combining three recessive mutations, *beige*, *nude*, and *xid*, to generate a potential recipient mouse for human tumor xenografts that was more immune-deficient then the parental *nude* mouse [20]. The *nude* mutation affects the development of the thymic epithelium, thereby preventing T cell differentiation resulting in an athymic mouse; all other lineages appear normal. It should be noted that some extrathymic processes lead to low levels of mature T cells especially in mice stimulated by exposure to antigens. The *bg* mice have a deficiency in cytotoxic T cells and NK cells [21]. The mutation is a lysosomal storage defect producing abnormally large cytoplasmic granules, so the effect on the immune system is indirect. The *xid* defect is an X chromosome linked gene that affects lymphokine activated killer (LAK) cells as well as B cell responses to certain thymus independent antigens [22]. Animals in which the *nude* and *xid* mutations are combined are very deficient in B cells that appear to be blocked in development at some point between pro-B cells and before cytoplasmic immunoglobulin expression [23]. In the particular outbred *bg/nu/xid* mouse used in our experiments, the *bg* mutation is not as active as if it was alone and the mice have only slightly reduced levels of NK cells, although they are deficient in LAK activity presumably by the action of the *xid* gene. Although the exact relationship between NK and LAK cells is unknown, both may display anti-tumor activity and appear to play an important role in host resistance to xenografting [24, 25]. It is important to stress that both *scid* and *bg/nu/xid* mice have NK cells and intact non-lymphoid resistance systems such as macrophages, therefore any environmental exposure to antigens stimulate these resistance mechanisms. We have found that animals with even mild subclinical infections are extremely resistant to xenografting [8].

In our initial experiments, human interleukin-3 (IL-3) and granulocyte macrophage–colony-stimulating factor (GM-CSF) were delivered to the animals by implanting an osmotic minipump subcutaneously. The animals were also given sublethal doses of radiation since syngeneic transplantation is faster and more complete if the recipient animals are conditioned with irradiation or chemotherapy prior to transplant. Animals were killed at various lengths of time after transplant and the bone marrow and spleen were analyzed by molecular techniques using human specific probes to determine whether human DNA was present; human cells comprised approximately 0.1%–1.0% of these tissues [6]. Since the goal of these experiments was to determine whether any of the earlier hematopoietic cell types had engrafted the mice, cells from these tissues were plated in in vitro progenitor assays that were selective for the growth of human

colony-forming unit–granulocyte macrophage (CFU-GM). Significant numbers of human progenitors were detected in the spleen and bone marrow. No human DNA or progenitors were detected in wild-type animals prepared the same way and only very low numbers of human progenitors could be detected in the hematopoietic tissues of *scid* mice transplanted with human bone marrow.

There did not appear to be any difference in the level or speed of engraftment in the presence or absence of exogenously added human growth factors. This applies only to the two factors tested and on the level of CFU-GM progenitors. It remains to be seen whether other combinations are more effective and whether the presence of these or other factors affect the differentiation of progenitors into mature cell types. There are several explanations for successful engraftment in the absence of exogenous human factors. The human cells could be secreting their own growth factors or the cells are responding to some cross-reactive murine factor or the murine hematopoietic microenvironment. We have recently found that some human factor-dependent myeloid leukemic cell lines proliferate in immune-deficient mice (C. Sirard and J. E. Dick, unpublished) and others have reported the stimulation of human lymphoid leukemic cell lines on murine stromal cells [26], lending credence to the idea that the murine environment is capable of stimulating human hematopoietic cells.

While these data clearly showed that human progenitors can engraft mice, the more important question was whether human stem cells had also engrafted. This is not an easy question to anwer because stem cells can only be identified by their function; this includes the ability to differentiate into all lineages, high self-renewal capacity, slow cell cycling, ability to engraft for long periods of time, etc. [1]. Two lines of evidence suggest that a cell type at least earlier than CFU-GM is responsible for maintaining the engrafted cells. Examination of the kinetics of engraftment indicated there was a rapid increase, of at least 40-fold, in the number of CFU-GM during the first 14 days of engraftment. CFU-GM have a very low self-renewal capacity and would not be able to generate such a large increase, suggesting that an earlier cell type is responsible for this large increase. Furthermore, human progenitors have been detected in animals 7 months after transplantation. Finally we have preliminary evidence that human bone marrow, highly enriched for earlier cell types, can engraft the mice. Taken together this suggests that some earlier cell type can engraft immune-deficient mice, although the exact nature of this cell type is unknown.

Gene transfer provides a powerful tool to more conclusively characterize the engrafting cell types. This stem cell marking technology has been powerfully applied to elucidate the stem cell hierarchy in the mouse [27] and a similar strategy in the human system should enable more precise identification of the human cell types responsible for engrafting the mice. Human bone marrow was infected with a retrovirus vector that contained the dominant selectable *neo* gene using the optimized conditions [31]. Infected and preselected cells were transplanted into immune-deficient mice. A large proportion of the human progenitors detected in the bone marrow for at least 4 months after transplantation contained the retrovirus. In combination with polymerase chain reaction (PCR) technology to clone out the virus integration site in small numbers of cells [29], these gene transfer experiments lay the foundation to determine whether the different progenitors engrafting the mice arise from a common progenitor. The engraftment of mice with both myeloid and lymphoid cells should permit the detection of a pluripotent human stem cell using these approaches.

Models of Human Leukemic Diseases

The growth of normal human hematopoietic cells in *scid* or *bg/nu/xid* mice suggested that these mice may also be useful recipients in which to grow human leukemic cells that often are difficult to establish in culture and to provide a system to study the growth of human leukemia in vivo. Non-T acute lymphoblastic leukemia (ALL) is the most prevalent childhood leukemia and is characterized by a pre-B cell phenotype [30]. Our initial studies have focussed on bone marrow taken directly from patients with non-T ALL and cell lines recently established from relapse patients. One such cell line (A-1) is Epstein–Barr virus (EBV) free, has a normal karyotype, and grows autonomously, producing an unidentified factor which augments its growth in semi-solid clonogenic assays and suspension cultures. Not only did A-1 grow in *scid* mice transplanted according to the same procedures as normal bone marrow but they showed a pattern of infiltration reminiscent of that observed in many children with ALL [8]. For the first month post-transplant, the A-1 cells could be detected only in the bone marrow, and only after an additional 8 weeks were large numbers (>75%) of leukemic cells present in the spleen and bone marrow. Small infiltrates were also present in the kidney and liver at this time. High numbers of A-1 cells were found in the peripheral circulation several weeks later, coinciding with widely disseminated leukemic infiltrates in many organs, including the brain. The animals began to die at 12 weeks after transplant. The widely disseminated growth particularly in the central nervous system is a feature of the terminal disease in children.

Cell lines with different growth factor requirements proliferate differently in *scid* mice. Another cell line (G-2) which responds to different growth factors than A-1 and grows slower in culture, proliferates extremely rapidly in mice, killing animals in 6 weeks at comparable cell doses to A-1. Limiting dilution experiments indicate as few as 100 G-2 cells will produce leukemic growth in *scid* mice. An additional observation has been the infiltration of G-2 into the thymic remnant present in *scid* mice and elevated levels of the CALLA antigen on the cells that infiltrated the thymus. CALLA is a differentiation antigen expressed on early human B cells and is a marker for non-T ALL. It was expressed on the cells originally obtained from the patient but was completely lost during in vitro cell culture. Independent clones and limiting dilution experiments all suggest that CALLA expression is being modulated in vivo as opposed to selection of rare positive cells in the population. The growth and modulation of differentiation antigens of non-T ALL cells in immune-deficient mice implies that they are responding to some murine growth factor or microenvironmental influence. Interestingly, Gluck et al. found that murine stromal cells can support the growth of factor dependent non-T ALL cell lines, lending further credence to this idea [26].

The ability to engraft bone marrow directly from patients with leukemia, either before or after treatment, into *scid* mice could be a valuable tool for predicting the clinical course of the disease, detecting residual leukemias, and for developing individualized therapeutic strategies. Toward this objective, bone marrow cells taken directly from patients at diagnosis with non-T ALL were injected into *scid* mice. Samples taken directly from patients at diagnosis grew little or not at all in the bone marrow and spleen of *scid* mice even after 8 months of observation. In contrast, all of the bone marrow samples taken from patients with recurrent disease in first or second relapse proliferated extensively into a widely disseminated leukemia in mice. Cells from patients who have relapsed several times and who are not responding well to therapy were the most aggressive in mice with the shortest latency. These data show that some biological parameters associated with poor clinical outcome,

such as rapid and widely disseminated proliferation, can be reproduced in SCID mice.

In addition to lymphoid cells, acute myeloblastic leukemia (AML) and chronic myelocytic leukemia (CML) cell lines also grow in immune-deficient mice (C. Sirard and J. E. Dick, unpublished). Some of the well-established lines such as K 562 which do grow in *nude* mice also grew very rapidly in *scid* mice. As noted earlier, one of the AML cell lines (MO7E) which is human growth factor (IL-3, GM-CSF) dependent grew extensively in the bone marrow of *scid* mice without an exogenously added factor.

The establishment of an in vivo model for human leukemia presents a unique system in which to address experimentally a number of biological questions governing the clinical outcome and the growth of leukemic cells in vivo. For example, the identification of leukemic cells in bone marrow usually dictates the course of chemotherapy; however, histological methods for detecting low numbers of residual cells are neither sensitive nor precise. Further refinement of the animal model could offer a sensitive method by which to study residual cells from patients undergoing chemotherapy. Furthermore, new chemotherapeutic and immunotherapeutic protocols, combinations of biological response modifiers, or new unconventional therapies that are difficult to develop and evaluate by human experimentation can be tested in an in vivo situation which mimics the progression of human leukemia. Using high-efficiency gene transfer technology, individual leukemic cells can be marked to follow the growth and development of clones during the multistage progression of the disease. Gene transfer in conjunction with this model system should allow the introduction of key growth regulatory genes, such as oncogenes or tumor suppressor genes, into normal human bone marrow, to determine how their aberrant expression affects normal hematopoiesis and leukemic transformation and progression. CML, in particular, is a good candidate for these gene transfer experiments because some of the genes involved in its etiology (e.g., *bcr/abl*) and progression (e.g., *p 53*) have been identified. It should be possible to directly test the role of these genes in the multistage progression of CML using gene transfer with the long-term goal of developing an animal model for CML.

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